NEUROSCIENCE

The peripheral effect of direct current stimulation on brain circuits involving memory

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An ongoing debate surrounding transcranial direct current stimulation (tDCS) of the scalp is whether it modulates brain activity both directly and in a regionally constrained manner enough to positively affect symptoms in patients with neurological disorders. One alternative explanation is that direct current stimulation affects neural circuits mainly indirectly, i.e., via peripheral nerves. Here, we report that noninvasive direct current stimulation indirectly affects neural circuits via peripheral nerves. In a series of studies, we show that direct current stimulation can cause activation of the greater occipital nerve (ON-tDCS) and augments memory via the ascending fibers of the occipital nerve to the locus coeruleus, promoting noradrenaline release. This noradrenergic pathway plays a key role in driving hippocampal activity by modifying functional connectivity supporting the consolidation of a memory event.

INTRODUCTION

The therapeutic potential of transcranial direct current (DC) stimulation (tDCS) has been examined under more than 70 diverse conditions, including major depression, epilepsy, pain, stroke, and Parkinson's disease (*1*). However, the assumption that electrical stimulation of the scalp modulates brain activity directly and in a regionally constrained manner to positively affect symptoms is the subject of extensive and ongoing debate (*2*, *3*). The skin and skull attenuate most of the current, with only a fraction reaching the brain. Recent research suggests that up to 75% of the applied current does not reach the brain (*1*). A possible explanation is that tDCS affects neural circuits mainly indirectly, i.e., via peripheral nerves (*2*, *3*). Research has shown that the locus coeruleus–noradrenergic (LC-NAc) system can be activated by stimulation of peripheral nerves such as the vagus (*4*). The modulatory influence of the LC-NA projection on synaptic plasticity within hippocampal neural assemblies (*5*) supports rapid formation of a novel episode in memory (*6*). Specific afferent activity from peripheral nerves through the nucleus tractus solitarius (NTS) can influence central NA activity both directly, via synapses on neurons in the LC, and indirectly, via connections linking the LC to the amygdala and hippocampus (*7*). Consistent with this, direct stimulation of the vagus nerve in epileptic patients augments memory formation through the LC-NA pathway (*8*). Less well known is the fact that another peripheral pathway, the greater occipital nerve, has similar effects to those of the vagus (*9*). Both pathways influence bottom-up regulation of cortical gain, psychological arousal, and neurobiological responses to environmental stimuli and stressors via the LC-NA system (*5*). The occipital nerve can be targeted noninvasively using noninvasive electrical stimulation of the scalp using DC (*10*).

The present study tests the hypothesis that transcranial electrical stimulation (tES), using DC, can activate the greater occipital nerve (ON-tDCS) and up-regulate memory performance via activation of the LC-NA pathway. We assess the hypothesis that ON-tDCS induces LC-NA activity changes and enhances communication between the LC, amygdala, and hippocampus that coincide with specific neural oscillations. We further hypothesize that ON-tDCS modulates memory with lasting effects and memory due to enhanced consolidation after learning.

RESULTS

Experiment 1: LC-NA pathway

We tested the effect of ON-tDCS on the LC-NA pathway using three measures, namely, pupil diameter, salivary α-amylase (sAA), and neurophysiology [event-related potentials (ERPs)]. Pupil diameter indexes LC activity in both monkeys and humans (*11*), findings confirmed by intracranial recording and pharmacological challenge studies (*12*). The electrodes are placed over the left and right cervical nerve 2 (C2) dermatome (see fig. S1), and a constant current of 1.5 mA was applied for 20 min. As predicted, ON-tDCS increased mean pupil diameter from a baseline of 12.67 ± 3.32 mm^2, 95% confidence interval (CI)(12.16 to 13.18) up to 13.55 ± 3.11 mm^2, 95% CI(13.00 to 14.09) ($P = 0.022$), in contrast to sham stimulation where the mean size decreased from 12.73 ± 3.94 mm^2, 95% CI(12.18 to 13.29) to 11.96 ± 4.06 mm^2, 95% CI(11.42 to 12.48) [$P = 0.047$; interaction effect: $F_{3,722} = 9.15$, $P = 0.003$, Cohen's $d = 1.29$, Bayesian Factor (BF_{10}) = 6.79; Fig. 1A]. It is known that sAA levels covary with circulating NA levels, with human functional magnetic resonance imaging (fMRI) showing LC activity rising concomitantly with sAA levels during the viewing of emotionally arousing slides (*13*). Levels of sAA, marker of endogenous NA activity, also increased during and immediately following ON-tDCS. During stimulation, sAA was 262.55 ± 166.92 U/ml for active group in comparison to sham group, 120.81 ± 70.94 U/ml, 95% CI(80.71 to 160.91) ($P < 0.001$). After stimulation, sAA levels rose to 304.96 ± 172 U/ml, 95% CI(207.66 to 402.26) ($P < 0.001$) for active group, while for the sham group, sAA level was 138.28 ± 90.96 U/ml, 95% CI(86.8 to 189.78)

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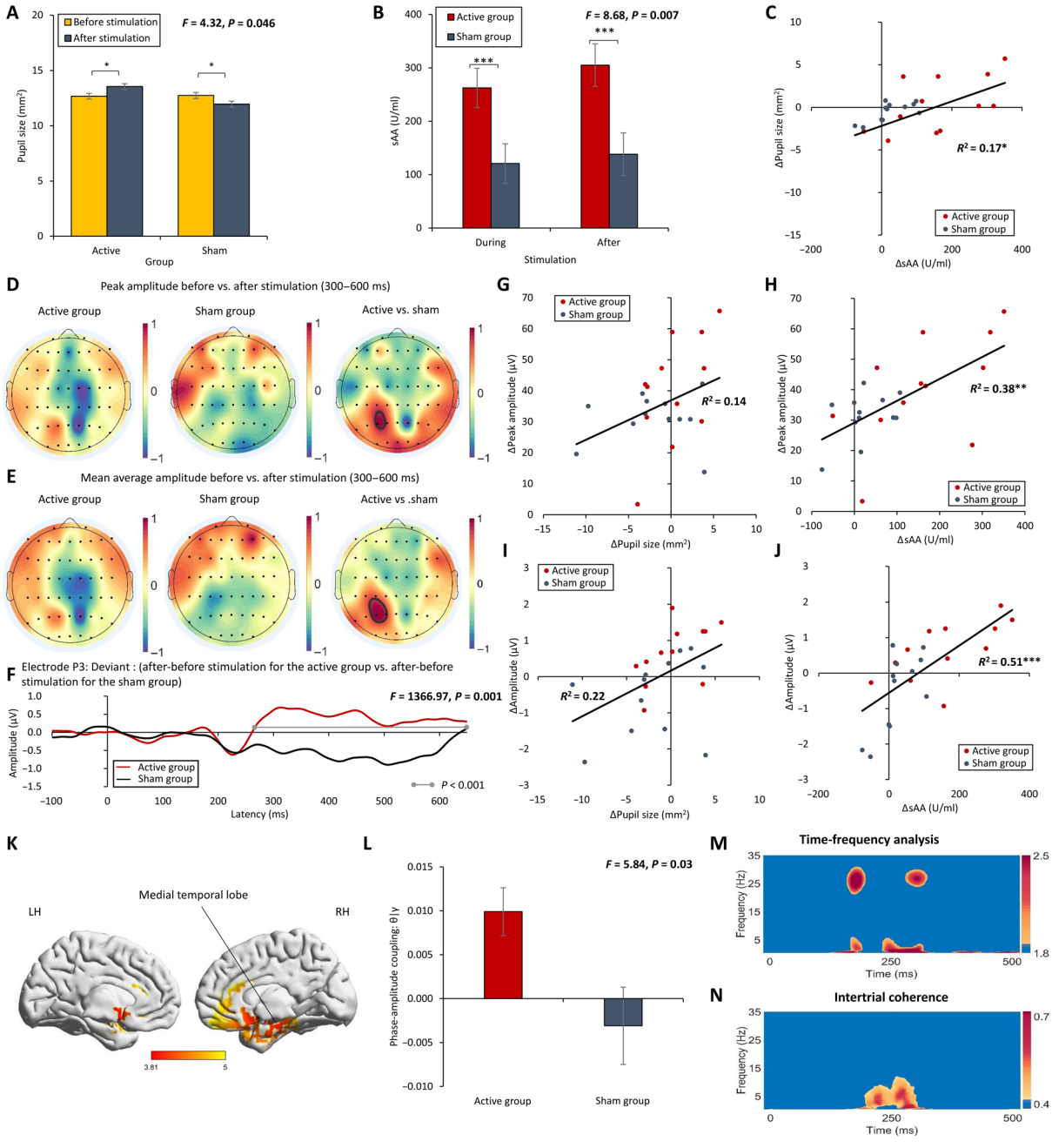

Fig. 1. ON-tDCS modulates the LC-NA pathway. (**A**) ON-tDCS increases average pupil size for the active stimulation group, while participants who received sham stimulation had a decreased average pupil size. (**B**) ON-tDCS increases sAA during and after stimulation from baseline for the active group, while for the sham group, sAA levels remain similar during and after stimulation relative to baseline. (**C**) Mean average pupil diameter correlates with sAA. (**D** to **F**) Difference over the left parietal electrode side (black circle) for the active group relative to the sham group after ON-tDCS for (D) the peak amplitude and (E) mean amplitude over 300 to 600 ms with (F) a maximum difference for scalp electrode P3. (**G** to **J**) The difference in (G and I) pupil size and (H and J) sAA (after-before) correlates with the difference peak amplitude (after-before) and difference mean amplitude (after-before) for electrode P3. Error bars, SEM. Asterisks represent significant differences (*$P < 0.05$ and ***$P < 0.001$). (**K**) Source-reconstructed resting-state EEG analysis shows increased synchronization for the theta frequency band in the hippocampus for the active group relative to the sham group after ON-tDCS (corrected for baseline activity; permutation test, $t_{20} = 3.81$, $P = 0.009$). LH; left hemisphere, RH; right hemisphere. (**L**) Increased theta-gamma phase-amplitude coupling in the hippocampus after active ON-tDCS relative to sham stimulation. (**M**) A time-frequency analysis and (**N**) intertrial coherence for the auditory oddball task demonstrates increased theta and gamma power between 150 and 350 ms [see black circles in (D) and (E)] after active ON-tDCS relative to sham ON-tDCS (baseline- and bootstrap-corrected). See fig. S1 for ERP analysis for the standard.

(overall effect: $F_{1,22} = 8.68$, $P = 0.007$, $\eta^2 = 0.28$, $BF_{10} = 5.79$ after baseline correction; Fig. 1B). The difference between pupil diameter size before and after stimulation correlates positively with the difference in sAA ($R^2 = 0.17$, $P = 0.032$; Fig. 1C). The third proxy measure of LC-NA activity was the P3b ERP, which peaks at 300 to 600 ms after a task-relevant stimulus (*14*, *15*) and is a strong cortical electrophysiological correlate of the LC-NA response (*16*). A standard P3b-evoking auditory oddball task showed that active ON-tDCS led

to a significant difference over the left parietal electrode side (with a max difference for scalp electrode P3) in comparison to sham for the oddball stimulus (peak amplitude: $F_{1,239} = 1.46$, $P < 0.001$, Cohen's $d = 0.52$, $BF_{10} = 1.15$; mean amplitude: $F_{1,239} = 2.55$, $P < 0.001$, Cohen's $d = 0.68$, $BF_{10} = 1.25$; ERP: $F_{1,351} = 4.56$, $P < 0.001$, Cohen's $d = 0.91$, $BF_{10} = 1.99$; Fig. 1, D to F, and see also figs. S1 and S2). All three-proxy measures of LC-NA activity were significantly intercorrelated. The differences in peak P3b amplitude immediately before and after stimulation correlates with the difference in pupil size only before correction for multiple comparison (grand average: $R^2 = 0.22$, $P = 0.021$; peak amplitude: $R^2 = 0.14$, $P = 0.068$, Cohen's $f^2 = 0.12$, $BF_{10} = 4.21$), and difference in sAA with differences in P3b remained after correction for multiple comparison (grand average: $R^2 = 0.51$, $P < 0.001$, Cohen's $f^2 = 1.04$, $BF_{10} = 1326$; peak amplitude: $R^2 = 0.38$, $P = 0.001$, Cohen's $f^2 = 0.61$, $BF_{10} = 133$; Fig. 1, G to J, and see fig. S2).

Experiment 2: Electrophysiology

In a second study, we tested whether ON-tDCS can modulate the medial temporal cortex, through the LC-NA pathway. Previous research already suggested that neural oscillations in the theta (3 to 8 Hz) and gamma (30 to 50 Hz) frequencies in the corticohippocampal network are an index for human memory formation (*17*). Gamma rhythms bind perceptual features in the hippocampus with perceptual and contextual information from diverse brain regions to form episodic representations (*17*). Theta oscillations act to temporally order these individual episodic memory representations and allow for top-down control of the hippocampus by the frontal cortex, modulating the encoding of memories (*17*). The interaction (i.e., phase-amplitude coupling) between theta and gamma oscillations further encodes and temporally orders memory representations (*17*). Furthermore, animal research demonstrated that increased neural discharge from the LC is followed by theta rhythm change in the hippocampus (*18*) and that LC discharge is essential for an increase in gamma power (*19*). Hence, we hypothesized that if ON-tDCS activates the LC-NA pathway, then it would induce electrophysiological changes within the theta-gamma range in the medial temporal cortex. Electrode placement and stimulation parameters were similar to experiment 1.

A source reconstructed resting-state electroencephalography (EEG) analysis in our study showed increased synchronization for the theta frequency band at the medial temporal cortex for the active group relative to the sham group after tDCS in comparison to before stimulation ($t = 3.81$, $P = 0.009$, Cohen's $d = 1.20$, $BF_{10} = 87.65$; Fig. 1K). In addition, increased theta-gamma phase-amplitude coupling was obtained in the right medial temporal cortex after active tDCS relative to sham stimulation ($F_{1,39} = 5.84$, $P = 0.031$, Cohen's $d = 0.72$, $BF_{10} = 4.01$; Fig. 1L). If we look at our ERP data obtained in study 1, then these findings are supported by a time-frequency analysis of our auditory oddball task, demonstrating increased theta and high beta/gamma power between 200 to 350 ms after active tDCS relative to sham tDCS for the oddball stimulus (Fig. 1, M and N).

Experiment 3: Resting-state fMRI

In a third study, we conducted a resting-state functional connectivity MRI study to further explore the relationship between changes in LC-NA activity and the hippocampus. LC-NA neuron activity reportedly induces brain network reconfigurations to flexibly regulate the cognitive functions critical for adaptive behavior (*20*–*22*). Prior work has shown that modulating NA transmission via intramuscular injections of atomoxetine (thus increasing extracellular levels of NA) in monkeys, as well as reboxetine (an NA modulator) intake in humans, led to the reorganization of brain activity in the brainstem that included the LC and led to increased functional connectivity between regions within the NA pathway, including the LC, amygdala, and hippocampus (*23*). On the basis of these findings, we hypothesized that individuals receiving ON-tDCS would show increased functional connectivity between the LC and the hippocampus. Electrode placement and stimulation parameters were similar to experiment 1. We scanned in three consecutive blocks: immediately before stimulation, during stimulation, and immediately after stimulation.

A seed-based analysis targeting the LC revealed a significant interaction effect between before and during stimulation depending if participants received active or sham ON-tES ($F_{1,28} = 5.12$, $P = 0.031$, $\eta^2 = 0.15$, $BF_{10} = 2.98$). A post hoc analysis revealed an increased correlation strength with the dorsal anterior cingulate cortex and the temporoparietal junction during stimulation for the active group in comparison to the sham group ($P = 0.041$; Fig. 2A). No significant effect was obtained between the active and sham groups before stimulation ($P = 0.42$). A seed-based analysis targeting the LC yielded a significant interaction effect between before and after stimulation depending whether participants received active or sham ON-tES ($F_{1,28} = 4.57$, $P = 0.041$, $\eta^2 = 0.14$; $BF_{10} = 2.53$). After stimulation, an increased correlation strength between LC and the right hippocampus was obtained, as well as the left and right dorsolateral prefrontal cortex, left and right precuneus, and the right angular cortex for the active group in comparison to the sham group ($P = 0.043$; Fig. 2B and see also table S1). No significant effect was obtained between the active and sham groups before stimulation.

A region of interest (ROI)–ROI analysis (Fig. 2C) of our resting-state functional connectivity MRI data further indicates that during stimulation, after baseline correction (before stimulation), the right amygdala ($F_{1,28} = 5.45$, $P = 0.027$, $\eta^2 = 0.16$, $BF_{10} = 2.54$) and the right hippocampus ($F_{1,28} = 3.81$, $P = 0.045$, $\eta^2 = 0.12$, $BF_{10} = 1.68$) showed an increase in connectivity strength with the LC relative to the sham group that was not demonstrated for the left amygdala and left hippocampus (Fig. 2, D and E). After stimulation, an ROI-ROI analysis revealed an increase connectivity strength (after baseline correction) between the LC and the right hippocampus relative to the sham group, as well as for the left hippocampus ($F_{1,28} = 3.81$, $P = 0.045$, $\eta^2 = 0.14$, $BF_{10} = 1.68$; Fig. 2G). No effect was obtained for the amygdala ($F_{1,28} = 0.84$, $P = 0.37$; (Fig. 2F). These findings go together with an increase in active for the right amygdala ($F_{1,28} = 4.95$, $P = 0.035$, $\eta^2 = 0.15$, $BF_{10} = 2.53$; Fig. 2H) and the right hippocampus ($F_{1,28} = 5.10$, $P = 0.032$, $\eta^2 = 0.15$, $BF_{10} = 2.31$; Fig. 2I) during ON-tDCS for the active group in comparison to the sham group.

Experiment 4: Face-name association memory task

We investigated whether ON-tDCS during training can modulate memory. On the basis of our previous findings that ON-tDCS modulates the hippocampal LC-NA pathway, we hypothesized that stimulating the greater occipital nerve to target the LC-NA system could noninvasively modulate memory. To test this hypothesis, we performed a fourth experiment, where participants had to learn an association between a face and a name. The face-name association memory task was divided into (i) an encoding phase, (ii) a consolidation phase, and (iii) a retrieval phase. During the encoding phase, participants studied 60 successively presented face-name pairs. Participants

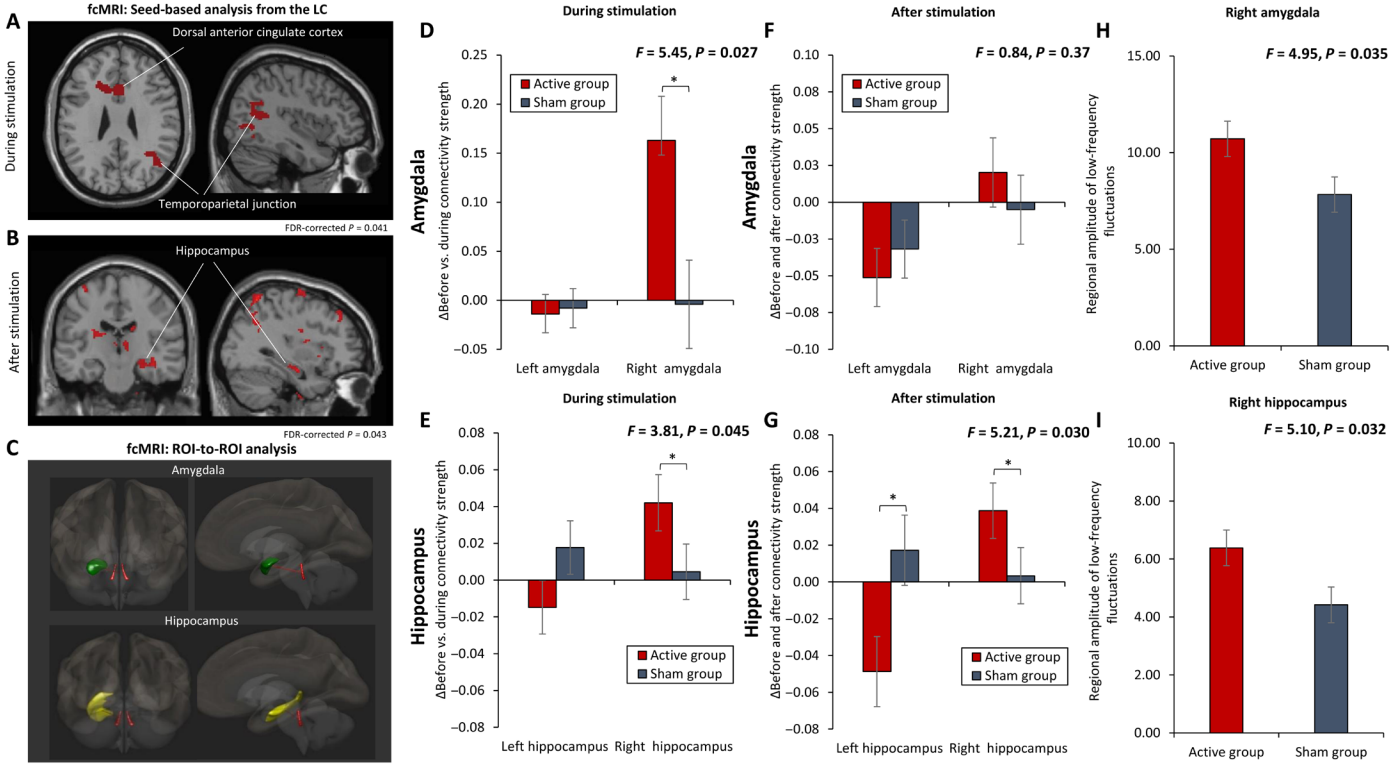

Fig. 2. ON-tDCS modulates the amygdala-hippocampal region through the LC-NA pathway. (**A**) A seed-based approach revealed increased connectivity between LC, dorsal anterior cingulate cortex, and the temporoparietal junction during stimulation (see also table S1) and (**B**) an increased correlation strength between the LC and the hippocampus after stimulation (see also table S1). FDR, false discovery rate; fcMRI, functional connectivity MRI. (**C**) A region of interest (ROI)–to-ROI analysis shows an increased correlation strength during stimulation between LC and both the amygdala and the hippocampus (**D** and **E**), as well as correlation strength between the LC and the hippocampus after stimulation (**F** and **G**) for the active group relative to the sham group (corrected for baseline). (**H** and **I**) Differences were obtained for the active ON-tDCS group relative to sham ON-tDCS for the right amygdala and right hippocampus during stimulation. Error bars, SEM. Asterisks represent significant differences (*$P < 0.05$).

were instructed to assess the gender of each face shown to keep participants focused on the task. The encoding phase lasted approximately 5 min. This is followed by a consolidation phase where participants were instructed to "sit still, relax, and think about nothing in particular" for 10 min. During the retrieval phase, participants were presented with 60 old and 60 new faces and were instructed to assess whether they had seen this face during the encoding phase or if it was a "new" face. For faces that were judged as old, participants were to indicate the correct name out of four options. Participants received ON-tDCS for the 5 min during encoding phase and 10 min during consolidation phase before they conducted the retrieval phase without ON-tDCS. Electrode placement and current used was similar to experiment 1.

In a face-name association memory task (Fig. 3A), participants recognized old faces in both the active ON-tDCS [68.44 ± 11.71%, 95% CI(62.79 to 74.10); $t_{14} = 7.00$, $P < 0.001$, Cohen's $d = 1.57$, $BF_{10} = 813.7$] and sham ON-tDCS [57.78 ± 10.21%, 95% CI(52.30 to 66.59); $t_{14} = 2.75$, $P = 0.022$, Cohen's $d = 0.76$, $BF_{10} = 5.32$] above chance level. For new faces, the participants in the active ON-tDCS [75.00 ± 13.09%, 95% CI(62.79 to 74.10); $t_{14} = 7.40$, $P < 0.001$, Cohen's $d = 1.91$, $BF_{10} = 1760$] and sham ON-tDCS [77.67 ± 12.83%, 95% CI(70.56 to 84.77); $t_{14} = 8.35$, $P < 0.001$, Cohen's $d = 2.16$, $BF_{10} = 4275$] correctly classified faces above chance level. Participants recognized more faces after active ON-tDCS [68.44 ± 11.71%, 95% CI(62.79 to 74.10)] relative to sham tDCS [57.78 ±10.21%, 95% CI(67.75 to 82.25)] ($P = 0.013$; Fig. 3B). For faces that participants had not seen before, no significant difference was obtained between active tDCS (75.00 ± 13.09%) and sham tDCS (77.67 ± 12.83%; $P = 0.58$), suggesting that ON-tDCS augments memory (interaction effect: $F_{2,27} = 3.42$, $P = 0.047$, $\eta^2 = 0.20$, $BF_{10} = 1.77$; Fig. 3B). For name recognition, we also verified whether participants were able to recognize the name above chance for both the active ON-tDCS [44.22 ± 11.54%, 95% CI(37.83 to 50.61); $t_{14} = 6.45$, $P < 0.005$, Cohen's $d = 1.67$] and sham ON-tDCS [35.44 ± 12.04%, 95% CI(28.78 to 42.11); $t_{14} = 3.36$, $P = 0.005$, Cohen's $d = 0.87$]. Participants were more likely to associate the correct name to a face for the active group [44.22 ± 11.54%, 95% CI(37.83 to 50.61)] relative to the sham group [35.44 ± 12.04%, 95% CI(28.78 to 42.11)] ($F_{1,28} = 4.17$, $P = 0.049$, $\eta^2 = 0.23$, $BF_{10} = 1.57$; Fig. 3C), which further corroborates these findings. In addition to the hit rate, we also looked at the reaction times (RTs) for correctly classifying a face as old and associating the correct name to a face. No significant difference was obtained for either faces ($F_{1,28} = 1.58$, $P = 0.22$; Fig. 3D) or names ($F_{1,28} = 0.001$, $P = 0.97$; Fig. 3E) when comparing the active group with the sham group.

Experiment 5: word association memory task I

The size effect obtained for the face-name association task is rather low. This might be because during this task, people were asked whether they recognized rather than recalled items. Previous research already suggests important differences between recognition

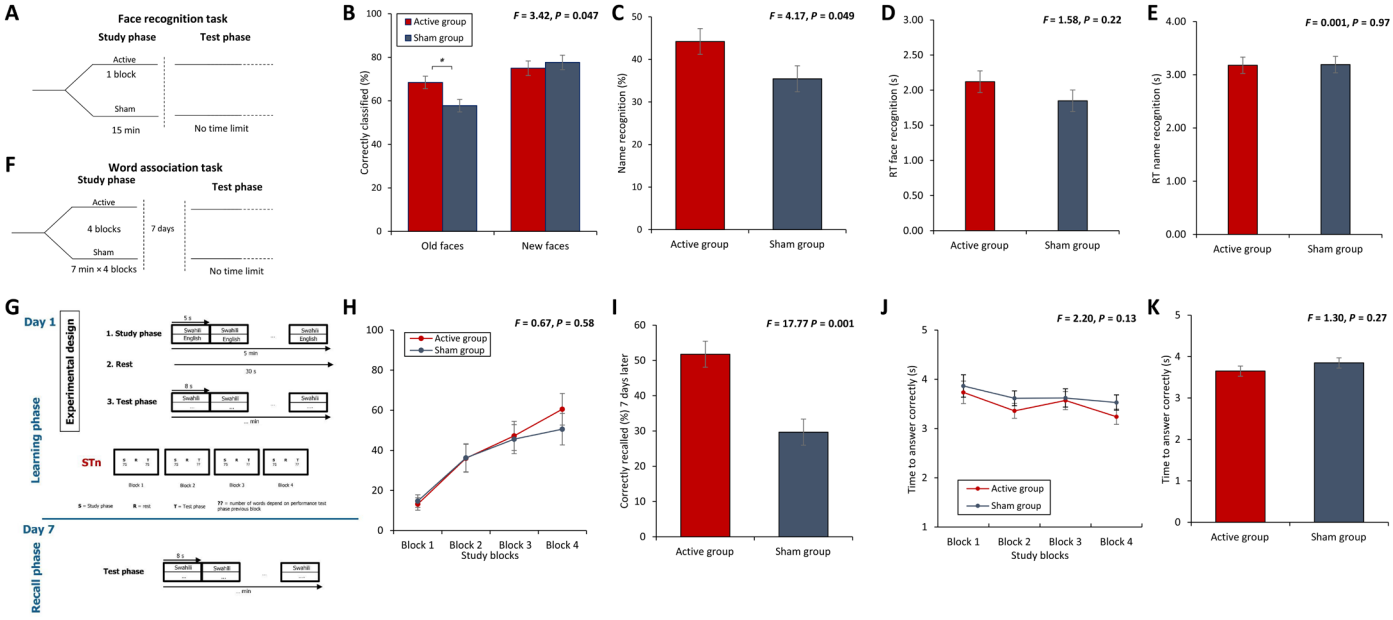

Fig. 3. ON-tDCS paired with training can enhance memory encoding 1. (**A**) The timeline for the face recognition task. (**B**) For the face-name association memory task, participants recognized more old faces but no new faces after active ON-tDCS relative to sham ON-tDCS. (**C**) Participants who received active ON-tDCS were better at associating the correct name to a face than sham group. (**D** and **E**) RTs for correctly classifying a face as old and for correctly associating a name to a face for the active and sham groups. (**F**) The timeline for the word association memory task. (**G**) Study design for the word association memory task. (**H**) During the study phase of the word association memory task, participants in the sham and active groups learn similarly. (**I**) ON-tDCS during a word association memory task can improve memory recall 7 days after the study phase for the active ON-tDCS group relative to the sham group. (**J** and **K**) No differences are obtained between the participants assigned to active or sham group for the time to answer during the study phase or 7 days after the study phase.

and recall memory tasks, where recall might be more challenging (*24*) and involves more hippocampal activity (*25*), that might also have an influence on our findings. To further explore the effect of ON-tDCS on memory, we conduct a follow-up experiment using a recall memory task (Fig. 3, F and G) to investigate the long-term effects of ON-tDCS. The strength of using this latter task is that this is not based on recognition but on recall, which requires more processing resources than recognition, and, hence, is more challenging.

During first visit (day 1), participants learn the English translation of Swahili words in four consecutive study-test blocks. During the study phase, participants received active or sham ON-tDCS. The electrode location was similar to experiment 1 using a constant current of 1.5-mA intensity during the study phase of the experiment. Participants came back 7 days later and were tested to see how many English translations of the Swahili words they could remember.

No difference was obtained between the active and sham groups in how many words participants learned during the first visit of four blocks on visit one ($F_{3,16} = 0.67$, $P = 0.58$; Fig. 3H). After 7 days, we tested participants again revealing that tDCS paired with a word association memory task (Fig. 3J) during training increased memory recall for the active group [51.74 ± 12.39%, 95% CI(43.96 to 59.53)] relative to the sham group [29.66 ± 11.01%, 95% CI(21.88 to 37.45)] ($F_{1,18} = 17.77$, $P = 0.001$, $\eta^2 = 0.50$, $BF_{10} = 50.65$; Fig. 3I). No significant difference in RTs was revealed between the active and sham groups for the four study blocks during the first visit ($F_{3,16} = 2.20$, $P = 0.13$; Fig. 3J). Seven days after the study phase, again, no difference in RTs was obtained between the active and sham groups ($F_{1,18} = 1.30$, $P = 0.27$; Fig. 3K).

Experiment 6: Word association memory task II

To find out whether the memory effect is only related to the occipital nerve, we conducted a similar word association memory task and setup as in experiment 5 but now with two active control conditions stimulating the neck, which included the trigeminal nerve or cervical nerves five and six (see fig. S1 for setup) during study phases on visit one. In addition, there were active and sham ON-tDCS groups. No difference was obtained between the four groups in how many words they learned during the first visit ($F_{3,36} = 0.75$, $P = 0.65$; Fig. 4A). Comparing active ON-tDCS (left or right) to active controls (targeting the head or the neck) showed increased memory recollection for the ON-tDCS group (50.69 ± 16.14%, 95% CI(41.09 to 60.29)] relative to the control groups [31.51 ± 14.68%, 95% CI(21.91 to 41.11)] ($F_{1,38} = 8.19$, $P = 0.007$, $\eta^2 = 0.18$, $BF_{10} = 6.65$; Fig. 4B). This memory recall 7 days later correlates with the difference in sAA levels during training (before versus after; $R^2 = 0.09$, $P = 0.032$, Cohen's $f^2 = 0.10$, $BF_{10} = 5.03$; Fig. 4C). No significant difference in RTs was revealed between the four groups on visit one ($F_{3,36} = 0.83$, $P = 0.59$; Fig. 4D), or 7 days later on visit two ($F_{3,36} = 1.30$, $P = 0.27$; Fig. 4E).

In experiment 3, we were already able to show that ON-tDCS modulates activity and phase amplitude changes in the medial temporal lobe independent of a task. To further explore the effect of ON-tDCS, in this experiment, we collected rest-state EEG data immediately before and after ON-tDCS. Previous research already demonstrate that LC discharge enhances synchronization theta and gamma activity in the hippocampus in rats (*26*) and that gamma oscillations play an important role in long-term memories and could

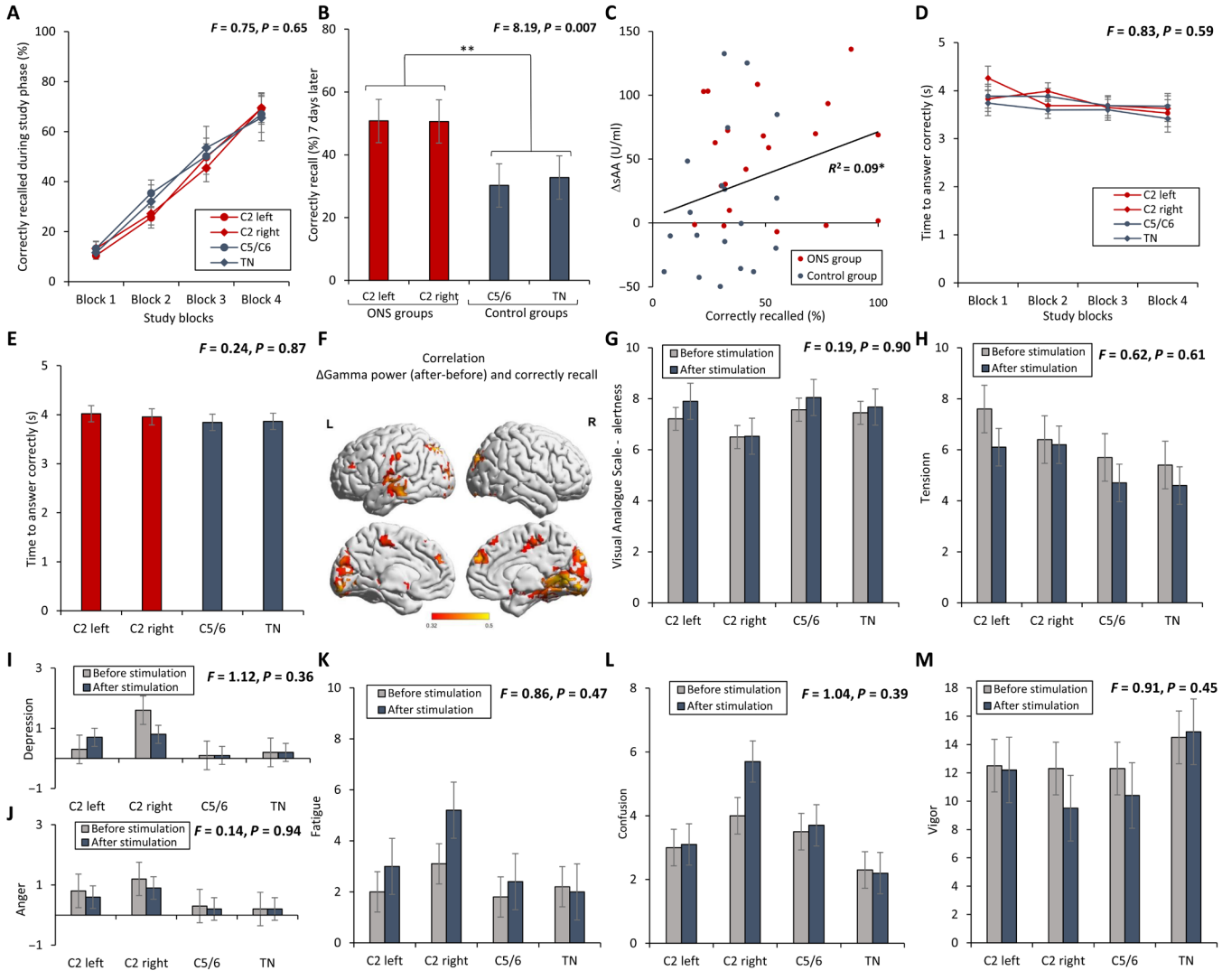

Fig. 4. ON-tDCS paired with training can enhance memory encoding 2. (**A**) tDCS targeting C2, neck (C5/6), or head [trigeminal nerve (TN)] during the study phase of the word association memory task, participants in the different group learning performed similarly. (**B**) ON-tDCS paired with a word association memory task can improve memory recall 7 days after the study phase for the active ON-tDCS group relative to the C5/6 or the trigeminal nerve. (**C**) A positive correlation between memory recall 7 days after the study phase and the difference in sAA before and after stimulation. (**D** and **E**) No differences are obtained between the participants assigned to one of the four conditions (C2 left, C2 right, C5/6, and trigeminal nerve), study phase (left), or 7 days after the study phase (right). (**F**) Memory recall 7 days after the study phase correlates with increased activity in the medial temporal lobe, as well as anterior and posterior cingulate cortex during training for the ON-tDCS group. (**G**) Stimulation of the C2 left, C2 right, C5/6, and trigeminal nerve revealed no significant difference when comparing the outcome for alertness before and after stimulation. (**H** to **M**) POMS questionnaire shows no significant difference before and after stimulation for the active and sham groups for tension-anxiety, anger-hostility, confusion-bewilderment, depression-dejection, fatigue-inertia, and vigor-activity. Error bars, SEM. Asterisks represent significant differences (*$P < 0.05$ and **$P < 0.01$). Photo credit: Wing Ting To.

potentially predict subsequent recall (*27*, *28*). On the basis of these findings, we hypothesize that ON-tDCS will induce gamma changes in the medial temporal lobe that correlate with successful recall. Memory recall 7 days later correlates significantly with increased gamma power in the medial temporal cortex, as well as the precuneus and dorsal lateral prefrontal cortex immediately after stimulation (overall: $R^2 = 0.10$, $P = 0.021$, Cohen's $f^2 = 0.11$, $BF_{10} = 6.09$; Fig. 4F).

To verify the effect of ON-tDCS before and after stimulation was not a sensation effect, the participants were asked to complete the Visual Analogue Scale for alertness (VAS_a) and Profile of Mood States (POMS) questionnaires. No group differences were obtained before and after ON-tDCS on alertness ($F_{3,36} = 0.19$, $P = 0.90$; Fig. 4G). For the POMS, no group differences were demonstrated for tension ($F_{3,36} = 0.62$, $P = 0.61$; Fig. 4H), depression ($F_{3,36} = 1.12$, $P = 0.36$; Fig. 4I), anger ($F_{3,36} = 0.14$, $P = 0.94$; Fig. 4J), fatigue ($F_{3,36} = 0.86$, $P = 0.47$; Fig. 4K), confusion ($F_{3,36} = 1.04$, $P = 0.39$; Fig. 4L), or vigor ($F_{3,36} = 0.91$, $P = 0.45$; Fig. 4M).

Experiment 7: Rodent model

To ensure that stimulation was directed specifically at the greater occipital nerve, i.e., the dorsal primary ramus of cervical spinal nerve 2, we implanted rodents with a cuff electrode around the greater occipital nerve and then applied stimulation following either inhibitory avoidance or object recognition training. For inhibitory avoidance task, animals learned to associate a context with the occurrence of an aversive event, while for the object recognition

task, animals explore and learn objects. Immediately after training, rats were given active or sham occipital nerve stimulation (ONS). Twenty four hours later, animals were tested on the latency to enter the dark compartment for the inhibitory avoidance task or the time spent exploring each of the objects for the object recognition task. Results yield increased inhibitory avoidance [238.75 ± 245.96 s, 95% CI(33.12 to 444.37) versus 68.22 ± 94.21 s, 95% CI(−4.19 to 140.64); U_{17} = 17.00, P = 0.034, Cohen's d = 0.99, BF_{10} = 1.39] and object recognition [10.00 ± 7.09 s, 95% CI(4.07 to 15.93) versus −1.55 ± 10.24 s, 95% CI(−9.42 to 6.31); U_{17} = 12.5, P = 0.011, Cohen's d = 1.31, BF_{10} = 3.51) for the active group relative to the sham group 24 hours after training (Fig. 5, A to D).

Experiment 8: ON-tDCS after training

We cross-validated our memory findings by applying ON-tDCS immediately after the training/encoding phase to avoid learning/performance-related issues such as arousal, attention, sensation, or motivation, in line with the idea that neural processes underlying memory become consolidated after learning (*29*). We used a similar setup as in experiment 5. Participants were randomly assigned to one of three groups: active stimulation during training and sham stimulation immediately after training, sham stimulation during training and active stimulation immediately after the training, or sham stimulation during training and immediately after training.

During visit 1, no effect was obtained between the different conditions in how many words they learned ($F_{6,80}$ = 1.01, P = 0.43; Fig. 6A). Results revealed a significant difference in memory recall 7 days later when tDCS was applied either during training/sham after training [45.25 ± 15.20%, 95% CI(−38.06 to 52.44); P = 0.016] or sham during train/active immediately after training [47.01 ± 13.47%, 95% CI(39.82 to 54.20); P = 0.007] relative to the sham condition both during training and immediately after training [32.60 ± 12.61%, 95% CI(25.41 to 39.79); $F_{1,42}$ = 4.87, P = 0.013, η^2 = 0.19, BF_{10} = 4.39; Fig. 6B]. No difference was obtained between ON-tDCS applied during training or immediately after training (P = 0.73). Memory recall 7 days later correlates with the difference in sAA levels during the first visit (before versus after; R^2 = 0.16, P = 0.006, Cohen's f^2 = 0.19, BF_{10} = 31.36; Fig. 6C). Memory recollection 7 days after stimulation is associated with increased gamma power in the medial temporal cortex, as well as the precuneus and dorsal lateral prefrontal cortex immediately after stimulation (average: R^2 = 0.11, P = 0.011; Fig. 6D). To verify that the memory effect is not due to a better sleep pattern, we asked about sleep quality and how many hours participants slept in the past 7 days after the first visit. No effect or correlation was revealed between the different groups ($F_{1,42}$ = 0.18, P = 0.83; Fig. 6E) and between sleep and correctly recalled words (R^2 = 0.001, P = 0.98; Fig. 6F).

Experiment 9: Lidocaine/prilocaine study

In a follow-up study, we tested whether the effect is caused by transcranial stimulation of cortical neurons using a topical skin anesthetic (lidocaine/prilocaine cream) to reduce any potential contribution from transcutaneous stimulation of peripheral nerves. This lidocaine/prilocaine preparation blocks sodium channels in peripheral nerves in the skin and thereby stabilizing the membrane potential and increasing the threshold for firing an action potential (*30*). We

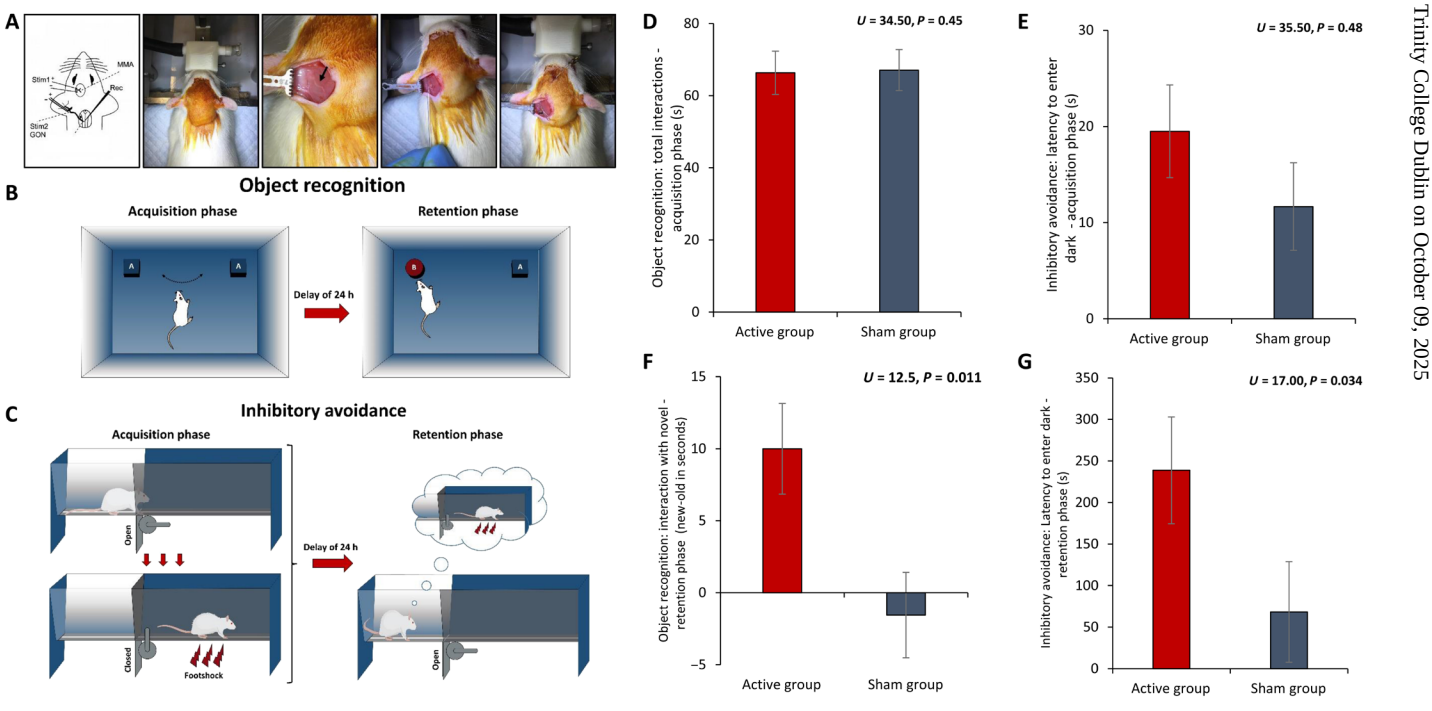

Fig. 5. ON-tDCS immediately after the training in animals. (**A**) Implantation targeting the greater occipital nerve (GON) [dorsal primary ramus of cervical spinal nerve using a cuff electrode (*59*)]. (**B**) Design for the objection recognition task and (**C**) design for the inhibitory avoidance task. (**D**) During the acquisition phase, time spent exploring the objects is not different between the active and sham groups in rats with a cuff electrode around the greater occipital nerve. (**E**) During the retention phase 24 hours later, rats in the active group interact more with novel objects relative to the sham group. (**F**) During acquisition, no difference in latency to enter the dark was obtained between the active and sham groups in rats with a cuff electrode around the greater occipital nerve. (**G**) Rats are given a single footshock in the dark chamber during the acquisition. During the retention phase 24 hours later, animals in the active group avoid the dark more relative to the sham group. Photo credit: Rimenez R. Souza

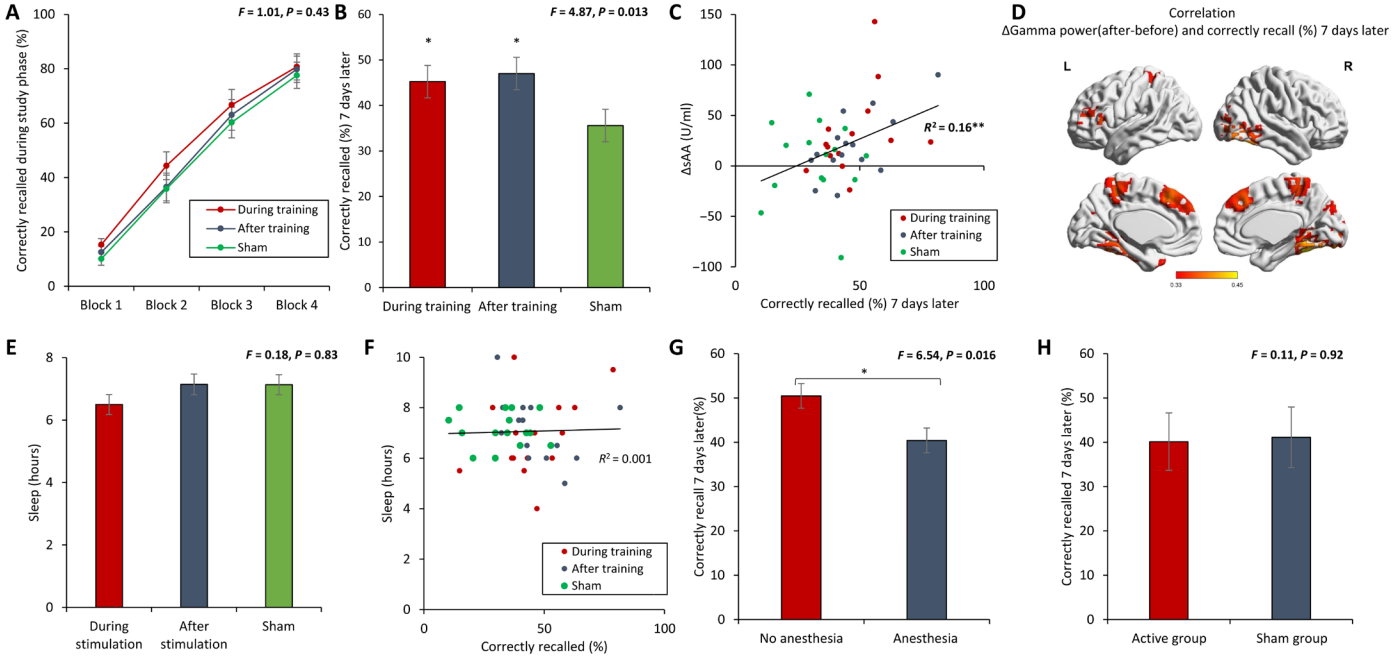

Fig. 6. ON-tDCS paired immediately after training can enhance memory encoding in humans. (**A**) ON-tDCS during or immediately after the study phase of the word association memory task, participants in the sham and active groups perform similarly. (**B**) ON-tDCS during or immediately after a word association memory task can improve memory recall 7 days after the study phase for the active ON-tDCS groups (independent of ON-tDCS during or immediately after the study phase) relative to the sham group. (**C**) Memory recall 7 days later correlates with the difference in sAA levels during the first visit (before versus after study phase) (**D**) Improved memory recall 7 days after stimulation is associated with increased activity in the medial temporal lobe, as well as anterior and posterior cingulate cortex immediately after ON-tDCS. (**E**) No effect was revealed between the active and sham group for the hours of sleep for the past 7 days after ON-tDCS. (**F**) No correlation is obtained between sleep and correctly recalled words. (**G**) Using ON-tDCS for a word association memory task during training did show a difference in memory recall 7 days after the study phase for the no anesthesia group relative to the anesthesia group. (**H**) ON-tDCS during the test phase (retrieval), 7 days after the participants studied the Swahili-English word associations, did not show a difference between the active and the sham group. Error bars, SEM. Asterisks represent significant differences (*$P < 0.05$ and **$P < 0.01$).

used a similar setup as in experiment 5. Using ON-tDCS for a word association memory task did show a difference in memory recall 7 days after the study phase for the no anesthesia group [50.14 ± 15.6%, 95% CI(42.93 to 57.35)] relative to the anesthesia group [40.41 ± 7.02%, 95% CI(37.17 to 43.65); $F_{1,30} = 6.54$, $P = 0.016$, $\eta^2 = 0.18$, $BF_{10} = 3.51$; Fig. 6G].

Experiment 10: ON-tDCS during retrieval

To test whether the effect of ONS is specific to the consolidation of the information, we applied ON-tDCS during the test phase (retrieval), 7 days after the participants studied the Swahili-English word associations using similar setup and stimulation parameters as in experiment 5. Seven days after the study phase, no difference was obtained between the active [40.12 ± 6.48%, 95% CI(26.12 to 53.79)] and the sham group [41.12 ± 6.83%, 95% CI(26.72 to 55.52); $F_{1,18} = 0.11$, $P = 0.92$; Fig. 6H]. These findings further suggest that the effect of stimulating the greater occipital nerve immediately after training enhances memory.

Blinding

Our results show for all experiments that participants were not able to accurately guess whether they were assigned to the sham or active group, suggesting that our sham arm is reliable (Fig. 7).

Meta-analysis

To check for the robust of the memory effect, we applied a meta-analysis including all experiments that test the behavioral effect. This includes experiments 4, 5, 6, 7, and 9. The standardized mean difference (SMD) measure of effect is used to calculate the individual and overall Hedges' *g* factor. Our analysis revealed an overall significant effect $Z = 6.74$, $P < 0.001$, and an overall Hedges' *g* for an SMD of 1.05. This Hedges' *g* indicates a large effect size. Furthermore, the individual Hedges' g values for an SMD for each of the studies separately were between 0.784 and 1.812, indicating a strong effect for all studies separately (Fig. 8). In addition, the Orwin's fail-safe *N* was 37.37 (critical *g* of 0.20), as well as the Rosenberg's fail-safe *N* was 30.66 (critical α of 0.05), further suggesting the strong effect size.

DISCUSSION

Our studies support the hypothesis that ON-tDCS can induce an effect on memory via the peripheral nervous system, potentially mediated through the activation of brainstem nuclei including the LC-NA pathway. We demonstrated that ON-tDCS induces changes in three intercorrelated proxy measures of LC-NA activity: pupil diameter, sAA, and ERP. In a second study, we showed the capability of ON-tDCS to modulate the medial temporal cortex, for the theta and gamma frequency bands that go together with a phase-amplitude coupling between theta and gamma oscillations. Previous research already indicated that gamma rhythms bind perceptual features in the hippocampus with perceptual and contextual information from diverse brain regions to form episodic representations (*17*), while theta oscillations act to temporally order these individual episodic memory representations and allow for top-down control of the hippocampus

Fig. 7. Blinding experiments. For all human experiments (experiments 1 to 7 and experiments 9 and 10), no difference was obtained between the active and sham group whether they anticipated active or sham stimulation.

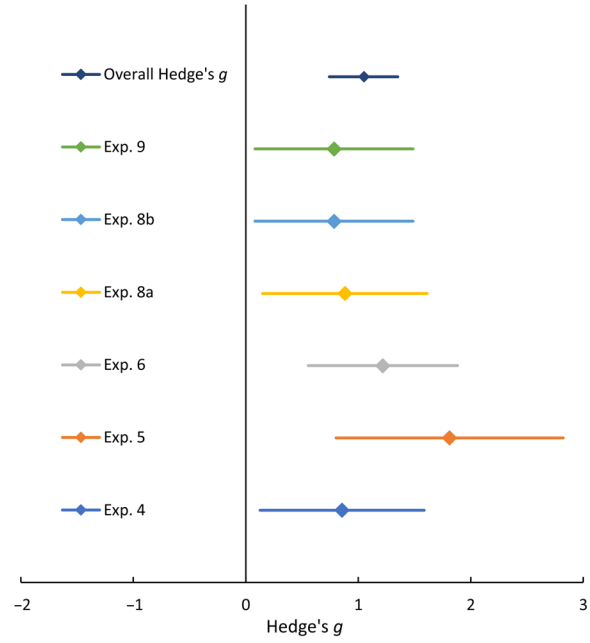

Fig. 8. Meta-analysis. A meta-analysis for the behavioral experiment revealed overall a strong effect size.

by the frontal cortex, modulating the encoding of memories (*17*). The interaction (i.e., phase-amplitude coupling) between theta and gamma oscillations further encodes and temporally orders memory representations (*17*).

Both a seed-based and ROI-ROI analyses on our resting-state fMRI (rsfMRI) data showed increased connectivity strength between the LC, the right amygdala, and right hippocampus during stimulation and between the LC and the right hippocampus after stimulation, respectively. The amygdala plays a well-known role in the modulation of emotional memory consolidation through its interactions with the hippocampus (*31*). Furthermore, a seed-based approach targeting the LC showed increased connectivity with dorsal anterior cingulate cortex and the temporoparietal junction during stimulation and the precuneus, dorsolateral prefrontal cortex, and angular gyrus after stimulation. These areas are known to contribute to bottom-up attention in memory and memory consolidation (*32*, *33*). The hemispheric lateralization of memory within the medial temporal lobe (including the hippocampus) has been discussed for many years. The classic model suggests that the left medial temporal lobe predominates in mediating verbal memory functions, while the right medial temporal lobe is more involved in nonverbal or visual memory functions (*34*). However, this classical view is weakening as more and more studies emerge that document postoperative verbal memory decline in patients after right temporal lobe resection (*35*). For the nonverbal domain, there is even less evidence for a strict lateralization to the right medial temporal lobe (*35*). Evidence suggests that the neurotransmitter noradrenaline (NA) mediates cognitive reserve's protective effects (*36*). These involve a set of interrelated cognitive processes (arousal, sustained attention, response to novelty, and awareness) with a right-hemispheric bias, which is strongly modulated by NA (*37*). It is proposed that this set of processes is one plausible candidate for partially mediating the protective effects of cognitive reserve (*37*). In addition to its biological effects on brain structure and function, NA may also facilitate networks for arousal, novelty, attention, awareness, and working memory, which collectively provide for a set of additional cognitive mechanisms that help the brain adapt to age-related changes and disease (*36*). It is hypothesized that to the extent that the lateral surface of the right hemisphere maintains structural and functional integrity and connectivity, cognitive reserve should benefit, and behavioral expression of pathologic damage should thus be mitigated (*36*, *37*). Overall, our findings indicate that ON-tDCS modulates the amygdala-hippocampal region through the LC-NA pathway, a pattern that is also consistent with our finding of increased theta-gamma coupling, with both shown to be important in memory storage (*4*).

In three behavioral experiments, we investigated whether ON-tDCS during training can enhance memory formation. In a face-name association memory task, participants were able to recognize old faces and were able to recognize the name related to the old face in active groups more accurately in comparison to the sham group. This memory effect was further suggested using a word association

memory task to reveal that 7 days after the studying word pairs, the active ON-tDCS group had increased memory recall in comparison to sham ON-tDCS. In a second word association memory task, we replicate our findings and include two active control conditions (targeting the head or the neck). This memory recall 7 days later correlates with the difference in sAA levels during training and with increased gamma power in the medial temporal cortex in addition to the dorsal anterior cingulate cortex a precuneus immediately after stimulation. These areas are similar to our rsfMRI data and have been associated with memory before (*32*, *33*). Overall, these findings indicate the hypothesis that ON-tDCS during training can enhance memory encoding through the hippocampal LC-NA pathway. Although we see a difference in correctly recognizing faces and names and recalling words, we do not see a difference in the RTs for the active group relative to the sham group. In addition, ON-tDCS during training or immediately after training of the word association task revealed no difference in memory recall 7 days later. These findings suggest that the effect obtained by ON-tDCS might be not related to a general sensation effect but ON-tDCS has a specific effect on memory consolidation.

Using a rodent model, we were able to show that a memory effect using an inhibitory avoidance or object recognition training might be directly related to the greater occipital nerve. This corroborates with experiment 9 where we conducted a word association memory task in combination with lidocaine/prilocaine cream, showing a reduced effect in comparison to the sham condition. This suggest that ON-tDCS affects neural circuits indirectly, i.e., via peripheral nerves, and is in accordance with recent research, which showed that transcranial alternating current stimulation targeting the motor system is mainly driven by trigeminal nerve stimulation (*3*). However, this does not rule out the possibility that some of the current is not going directly to the brain. Research suggests that a small proportion of the current does reach the brain (*1*), and we showed that tES using DC might have a dual working mechanism including both direct and indirect brain modulating mechanisms (*38*). Although our results indicate that the transcutaneous mechanism might be a dominant mechanism driving the memory effect, experiment 9 demonstrated that the anesthesia condition of the greater occipital nerve showed a significant lower effect than the nonanesthesia condition of the greater occipital nerve. However, in comparison to the sham condition in experiment 5 or 6, the results are still better for the anesthesia condition in experiment 9, suggesting that, probably, the current partially reaches the brain directly and causes a transcranial stimulation of cortical neurons. On the basis of our electrode placement, it would suggest that we likely activate the parietal cortex. This would corroborate with research that revealed that a high-frequency transcranial magnetic stimulation targeting parietal cortex activates an episodic memory network (*39*). However, other research suggests that direct brain stimulation is unlikely, as invasive measurements in epilepsy patients revealed that the electrical field generated in the cortex with tES is not strong enough to cause neural entrainment in humans (*40*). In addition, research using a strong focal electrical field while simultaneously blocking the contribution from the peripheral nerve did not induce a tremor entrainment (*40*). It is also possible that the effects that we obtained can be explained by the activation of other neural pathways in addition to the LC-NA pathway. Previous animal research already indicates that peripheral nerve stimulation such as vagus nerve stimulation also activates the dopaminergic (*41*), serotonergic (*42*), and cholinergic (*43*) pathway and that dopamine (*44*), as well as acetylcholine (*45*), plays an important role in inducing long-term plasticity changes related to memory consolidation. Together, our results need to be interpreted with caution as it cannot be excluded that effect is partially driven by activation of other neural pathways or due to direct stimulation of the cortex.

Overall, our studies suggest that part of the effects of electrical stimulation is exerted via the ascending fibers of the occipital nerve that synapse with neurons in the NTS, which then project to the LC and promote NA release that augments functional connectivity with the hippocampus. Unlike pharmaceutical approaches, ON-tDCS offers the possibility of persistent, stimulus-specific changes to neural circuits with minimal side effects. As the LC-NA pathway plays a role in the regulation of memory, attentional stability, responsiveness, and cognitive reserve, tDCS could help to treat insufficient LC-NA function, which would provide an explanation of its therapeutic effect in certain brain diseases including attention deficit hyperactivity disorder, posttraumatic stress disorder, affective disorders, mild cognitive impairment, and chronic pain (*46*). Studies have suggested the memory problems in Alzheimer's are due to ineffective encoding of new information (*47*). Future studies testing whether ON-tDCS could mitigate some degree of memory loss and rate of decline in the earliest stages of Alzheimer's disease would be of interest since no drug to date has significantly modulate memory function. ON-tDCS is a novel approach that could contribute to the armamentaria of solutions to this issue.

METHODS

Experiment 1: Participants

The study design was as a prospective, double-blinded, placebo-controlled randomized parallel group study. Participants were first screened over the phone (e.g., handedness, tDCS contraindications, neurological impairments, and never have participated in a tDCS study) before enrolling into the study. Furthermore, study instructions were emailed to the participants to make sure that they abstained from alcohol 24 hours before the study session, that they did not use any hair products (e.g., hair gel, hair spray, hair conditioner...) on the day of the study session and that they did not consume any caffeinated products or nicotine for at least 16 hours before the study session. On the day of the study session, written informed consent was obtained from all individuals included in the study. The 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 (UT Dallas) (#15-06). Participants were 24 healthy, right-handed adults (12 males and 12 females; mean age was 23.83 years, SD = 2.88 years) with a similar educational background (i.e., enrolled as undergraduate students at UT Dallas). Their handedness was assessed by the Edinburgh Handedness Inventory. All participants had the maximum score on the Mini Mental State Examination. A screening assessment determined that no one had a history of medical, neurological, psychiatric disorders or any tDCS contraindications, including previous history of epileptic insult, head injury, diagnosis of neuropsychiatric disorders, taking neuropsychiatric medications or prescribed stimulants, and chronic use of illicit drugs, i.e., marijuana and cocaine. All participants had normal or corrected-to-normal vision, and no participants were taking medication or other drugs.

Experiment 1: Saliva collection

Participants' saliva was collected three times during the experiment: before, during, and after stimulation. The collection during stimulation was performed 10 min after the start of stimulation. The participants were asked to refrain from dental work at least 48 hours before the experiment. Participants were further requested to refrain from foods with high sugar or acidity, high caffeine content, alcohol, energy drinks, nicotine consumption, prescription drugs, and steroidal/anti-inflammatory drugs and were recommended to have a good night's rest the day before the experiment. Participants were asked to avoid a major meal 60 min before the experiment, avoid brushing their teeth 45 min before the experiment, and avoid indulging in any rigorous exercise and were asked not to drink water or rinse their mouth 10 min before saliva collection. If the study was scheduled for the afternoon, then participants were requested to avoid taking naps during the day. When the participants were ready to collect saliva, they were requested to gently tip their head backward 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.

Experiment 1: Pupil dilation

The response of the pupil to three types of light stimulation (blue, 470 nm; white, 8000-K color temperature; red, 624 nm) was recorded in real time using a binocular Basler dart near-infrared (NIR) cameras. The lenses have a fixed focal length of 8 mm with an M12 × 0.5 body. The images were recorded at a frame rate of 120 Hz. There is a constant NIR illumination of the eye (850 nm), and the cameras are equipped with a "daylight cut filter," which passes NIR and blocks any wavelengths below ~800 nm. Surface-mounted light-emitting diodes were used for light stimulation, and the cameras were all mounted on a single eyepiece, which communicated with a Windows laptop through a Universal Serial Bus 3.0 cable. Each color was shone for 200 ms first in the left eye and then in the right eye with an interstimulus interval (ISI) of 8 s. This left-right trial was repeated three times for each color. The average total duration of the procedure was 2 min per participant. The participant was requested to focus on a point inside the eyepiece and open their eyes as wide as possible. They were asked to avoid rapid and frequent blinking of eyes, to avoid movement of eyes, and specifically to avoid blinking during stimulus presentation. The videos were then postprocessed to obtain the dilation of the pupil. The pupil was extracted as an ellipse from each frame with a segmentation algorithm, and the diameter was calculated from the average of the major and minor axes of the resulting pupil ellipse. The difference between the maximum dilation and maximum constriction of the both pupils in response to each color for every trial was calculated for each person before and after ON-tDCS.

Experiment 1: Electrophysiological recordings

Continuous EEG data were collected from each participant in response to the auditory oddball paradigm, before and after the application of C2 stimulation. The data were collected using a 64-channel Neuroscan SynAmps 2 Quick Cap configured per the International 10-20 placement system with the midline reference located at the vertex and the ground electrode located at AFz using the Neuroscan Scan 4.5 software. The impedance on each electrode was maintained at less than 5 kilohms. The data were sampled using the Neuroscan SynAmps 2 amplifier at 500 Hz with online band-pass filtering at 0.1 to 0.100 Hz. The auditory oddball task is a simple and well-established paradigm for the investigation of robust P3b components (more detailed information can be obtained in the Supplementary Materials). Data were preprocessed using MATLAB and EEGLAB in a manner similar to the original paper that showed a relationship between ERP and LC-NAc arousal function (*48*) (more detailed information can be obtained in the Supplementary Materials).

The peak and mean amplitude of the P3b component of the ERP to the standard and deviant were calculated for every participant using the ERP measurement tool in ERPLAB as in the original paper that showed a relationship between ERP and LC-NAc arousal function (*48*). These data were extracted from every electrode under the prestimulation and poststimulation conditions in the 250- to 600-ms time window after stimulus presentation for the active and sham groups. The peak was identified as the local maximum in the time window that was larger than the average of five sample points (10 ms) on either side of that maximum.

Experiments 1, 2, and 4: ON-tDCS

DC was transmitted via a saline-soaked pair of surface sponges (35 cm^2) and delivered by specially developed, battery-driven, constant current stimulator with a maximum output of 10 mA (Eldith; www.neuroconn.de). For each participant receiving ON-tDCS, the anodal electrode placed over the left C2 nerve dermatome, and cathodal electrode placed over the right C2 dermatome (see fig. S3). A constant current of 1.5 mA was applied for 20 min. For sham ON-tDCS, placement of the electrodes was identical to that of active ON-tDCS. ON-tDCS was first switched on in a ramp-up fashion over 5 s. Current intensity (ramp down) was gradually reduced (over 5 s) as soon as ON-tDCS reached a current flow of 1.5 mA. Hence, sham ON-tDCS only lasted 10 s (as opposed to 20 min in the active group). The rationale behind this sham procedure was to mimic the transient skin sensation at the beginning of active ON-tDCS without producing any conditioning effects on the brain.

Experiment 1: Procedure

Participants performed the pupillometry and auditory oddball task twice, immediately before and immediately after the ON-tDCS session, while saliva was collected before, during, and immediately after the ON-tDCS session. Participants were randomly assigned to the sham or active ON-tDCS condition. The person who controlled the tDCS device was not involved in instructing the participant; this instruction was instead performed by a second person who is blind to the stimulation protocol and not in the room during the stimulation.

Experiment 1: Statistics

Electrophysiological recordings

The amplitude of the mean voltage of the P3b component was computed as the mean of the voltages within the time window. These data were compared using an analysis of variance (ANOVA) with group (active versus sham) × stimulation (before versus after) × electrode (64 channels) as independent variables and the peak amplitude and mean amplitude as dependent variables.

On the basis of the results of the topographic plots, we further compared the waveform of the difference between the poststimulation and prestimulation conditions between the active and the sham groups in the same time window in electrode P3. This was performed by extracting the single trial P3b component for every person under the prestimulation and poststimulation conditions and calculating the single

trial difference in the waveform of the P3b component. The difference in waveforms between the active and sham groups, subtracting poststimulation (after) from prestimulation (before), was statistically compared using an ANOVA for the deviant and the standard with group (active versus sham) × time (250 to 600 ms) as independent variables. In addition, we calculated the effect size and the BF_{10}, which indicate the relative evidence for the alternative hypothesis over the null hypothesis.

To compare the frequency content between the active and sham groups, we performed a time/frequency analysis by decomposing the spectral content of the signal across time. This was performed by considering a sum of windowed sinusoidal functions (i.e., wavelets). The event-related spectral perturbation (ERSP) measures the mean event-related changes in power spectrum of the data at a given channel (*49*). The ERSP was calculated by computing the power spectrum over a sliding window across the entire ERP waveform. The power spectrum was computed using a sinusoidal wavelet transform whose number of cycles expanded with increasing frequency. The epochs (−100 to +700 ms) for the correctly identified deviants and correctly ignored standards from electrode P3 were selected for each group under each condition from which the difference between the poststimulation and prestimulation data were computed for the two groups. The ERSP of this average difference between the two groups was calculated using a 0.1-cycle wavelet with a Hanning-tapered window. This window expands to reach half the number of cycles in the equivalent fast Fourier transformation window at the highest frequency and a sliding window of 100 ms. The comparison of the difference between the active and sham conditions was bootstrapped at 200 permutations and corrected for multiple comparisons using the false discovery rate.

Saliva

Using the saliva collected via passive drooling, sAA levels were measured (see the Supplementary Materials). We conducted an ANOVA with group (active versus sham) × stimulation (during and after) as independent variables, sAA before stimulation as covariate, and sAA as dependent variable. A simple contrast analysis was applied to compare the difference conditions using a Bonferroni correction.

Pupil dilation

The difference between the maximum dilation and maximum constriction of the both pupils in response to each color for every trial was calculated for each person before and after ON-tDCS. A general linear mixed model for the left and right pupil size was with subject and trial number as random factor and group (active versus sham) × stimulation (before versus after) × trial as fixed factors. A simple contrast analysis was applied to compare the different conditions using a Bonferroni correction (see also the Supplementary Materials). A Pearson correlation was used to correlate the pupil size change with the sAA changes. In addition, we calculated the effect size and the BF_{10}.

Pearson correlation was used to correlate the change in Peak amplitude, as well as mean amplitude with pupil size change and sAA changes. A Bonferroni correction was applied to correction for multiple comparison.

Experiment 2: Participants

The study was in accordance with the ethical standards of the Helsinki Declaration (1964) and was approved by the Institutional Review Board of the UT Dallas (#15-06). Participants were 30 healthy, right-handed adults (15 males and 15 females; mean age was 21.41 years, SD = 1.97 years) with a similar education background (i.e., enrolled as undergraduate students at UT Dallas). The inclusion criteria were similar as for study 1.

Experiment 2: Rest-state electrophysiological recording

System specification can be found in study1 section electrophysiological recordings. Data were collected eyes closed (sampling rate, 1 kHz; band-passed DC, −200 Hz) and lasted approximately 5 min. The midline reference was located at the vertex, and the ground electrode was located at AFZ. Participants were instructed not to drink alcohol 24 hours before EEG recording or caffeinated beverages 1 hour before recording to avoid alcohol- or caffeine-induced changes in the EEG stream. The alertness of participants was checked by monitoring both slowing of the alpha rhythm and appearance of spindles in the EEG stream to prevent possible enhancement of the theta power due to drowsiness during recording. No participants included in the current study showed these EEG changes during measurements. For EEG processing, source reconstruction phase amplitude was applied (see the Supplementary Materials).

Experiment 2: Procedure

We collected the resting EEG twice in all participants, immediately before and immediately after the ON-tDCS session. Participants were randomly assigned to the sham or active ON-tDCS condition. The person who controlled the tDCS device was not involved in instructing the participant; this was performed by a second person who was blind to the stimulation protocol and not in the room during the stimulation.

Experiment 2: Statistics

A comparison between prestimulation and poststimulation for the active and sham groups was conducted. The methodology used is a nonparametric permutation test. It is based on estimating, via randomization, the empirical probability distribution for the maximum statistics under the null hypothesis comparisons (*50*). This methodology corrects for multiple testing (i.e., for the collection of tests performed for all voxels and for all frequency bands). Because of the nonparametric nature of this method, its validity does not rely on any assumption of Gaussianity (*50*). These whole-brain comparisons were performed by sLORETA through multiple voxel-by-voxel comparisons using a logarithm of *F* ratio. The significance threshold for all tests was based on a permutation test with 5000 permutations. In addition, we calculated the effect size and the BF_{10}. For phase-amplitude coupling, we subtracted the poststimulation (after) from the prestimulation (before) for both the active and sham groups. We conducted a one-way ANOVA with group (active versus sham) as independent variable and the difference in theta-gamma coupling between the prestimulation and poststimulation conditions as dependent variable. In addition, we calculated the effect size and the BF_{10}.

Experiment 3: Participants

The study was designed as a prospective, single-blinded, placebo-controlled, randomized parallel group study. The participants were asked to report any history of neuropsychiatric disorders and/or current medications, and those with any current prescriptions were excluded from the study. Possibly pregnant participants were not allowed to take the magnetoresistance (MR) scan, and a urine pregnancy test was given if the participants were unsure. Those who have had surgeries or metallic objects placed inside their bodies were not

permitted to participate. Every participant was asked to fill in the questionnaire screening form before ON-tDCS. The tDCS exclusion criteria included previous history of epileptic insult, head injury, diagnosis of neuropsychiatric disorders, taking neuropsychiatric medications or prescribed stimulants, and chronic use of illicit drugs (i.e., marijuana and cocaine). The study was conducted in accordance with the ethical standards of the Helsinki declaration (1964) and was approved by the Institutional Review Board of the UT Dallas (#17-84) and the Institutional Review Board of the UT at Southwestern (STU 072016-064). Participants were 30 healthy, right-handed adults (15 males and 15 females; mean age was 27.27 years, SD = 2.87 years) with a similar education background (i.e., enrolled as undergraduate students at UT Dallas). Inclusion criteria were similar to study 1 and study 2.

Experiment 3: rsfMRI

The rsfMRI data were collected on a 3-T MR scanner (Achieva, Philips, The Netherlands) using a 32-channel SENSE phased-array head coil. The dimension of the coil was 38 cm (height) by 46 cm (width) by 59 cm (length). During scanning, foam padding and earplugs were used to minimize the head movement and scanner noise. An MR-compatible tDCS system manufactured by MR neuroConn Co. (Germany) was driven by battery power and applied to each participant inside the MR scanner. All of the operating parts and devices that go into the scanner room are MR compatible, and everything else was in the control room, connected via the waveguide. The tDCS system was fully charged before each session, and its impedance level was measured regularly to test whether it is maintained at approximately 5 kilohms on each end, i.e., 10 kilohms total. See fig. S4 for setup.

The MR session with ON-tDCS was divided into three consecutive blocks of scanning: before stimulation, during stimulation, and after stimulation. At the beginning of the prestimulation session, routine survey and T1 anatomical images were acquired for a total of about 5 min. Before acquiring the T1 image, wet ON-tDCS electrodes were positioned on the participant for three consecutive sessions of rsfMRI. For each of the scanning blocks, we acquired 20-min-long rsfMRI images. After the session was completed, participants were asked to inform the experimenter about the sensations of ON-tDCS and any possible adverse effects during the ON-tDCS session using a questionnaire adapted and revised from a previous study (*51*). See fig. S5 for setup.

For the T1 [MPRAGE (magnetization prepared - rapid gradient echo)] anatomical scan, parameters were as follows: a repetition time (TR) of 2300 ms, an echo time (TE) of 2.94 ms, an inversion time of 900 ms, and a flip angle of 9°. A total of 160 sagittal slices were taken, using a matrix size of 256 mm by 256 mm, at a 1 mm by 1 mm by 1 mm resolution.

rsfMRI sequences were acquired with the following imaging parameters (echo planar imaging protocol): TR/TE = 3000/30 ms, field of view = 220 mm by 220 mm, matrix = 64 by 64, and number of slices = 53 with voxel size = 3 mm by 3 mm by 4 mm with no gap between slices. Total number of acquired volumes was 400, counting for 20 min. Preprocessing steps can be found in the Supplementary Materials.

Experiment 3: Statistics

Using the LC ROI as a seed region, connectivity defined by correlation analysis was measured from each seed to all the voxels across the brain. The averaged blood oxygenation level dependent (BOLD) time series of the LC ROI were extracted, and the values were partially correlated to all the other voxels in the brain. That is, a correlation between the ROI and a voxel was calculated controlling for all other voxels and this for all voxels. As a result, a whole-brain map of *Z*-transformed partial correlation coefficients was created, which represents the connectivity weight from each seed ROI to the other voxels. A repeated-measures ANOVA was applied with the connectivity weights before and during (after) stimulation as within variable and active and sham ON-tDCS as between-subject variable. A post hoc analysis was calculated where the weights were compared for the poststimulation condition for active and sham ON-tDCS using two-tailed independent *t* test in CONN toolbox. We limited the statistical analysis to voxels that have the *P* values of less than 0.05; the resulting voxels were corrected for multiple comparisons using false discovery rate correction at the cluster level, $P < 0.05$. Anatomical labeling of significant clusters was performed by means of the anatomical automatic labeling toolbox (*52*).

On the basis of the seed-based analysis, a functional connectivity analysis was performed using the CONN toolbox. The ROI considered in the analysis were the right hippocampus, right amygdala, and LC. The LC was selected using probabilistic LC atlas (as conducted in a study across 44 adults by localizing its peak signal level of LC neurons in high-resolution T1 turbo spin-echo images and verified the location using postmortem brains) (*53*) (www.nitrc.org/frs/shownotes.php?release_id=3337). While the LC has been extensively studied in animals and human postmortem tissue, only recently have advances in neuroimaging techniques that allowed in vivo investigation in humans. The absence of an anatomical description of LC location in standard neuroimaging space has made it difficult to ascertain whether functional imaging results are specific to the LC. The small size of the LC, its amorphous boundary with surrounding tissue, and the vicinity of other brainstem nuclei have limited that accurate and reliable localization of human imaging results to the LC. The probabilistic LC template was created using processing steps specifically designed to address these difficulties (*53*). To remove potential artifact such as head motion, respiration, and other global imaging artifacts including potential stimulation effects, we regressed out the global average brain signal (see above).

The average BOLD time series across all voxels within the ROI were extracted from the smoothed functional images. Partial correlation analysis was performed, and the resulting Fisher's *Z*-transformed coefficients were used for further statistical analyses. The *Z*-transformed connectivity weights were compared between the active and sham groups for the during stimulation block for the amygdala and hippocampus using a multivariate analysis of covariance (ANCOVA) and the *Z*-transformed connectivity weights for the prestimulation as a covariate. A similar analysis was conducted for the poststimulation connectivity. A similar method was used to look at the connectivity between the LC, left hippocampus, and amygdala (see the Supplementary Materials; ON-tDCS induces lateralization toward the right hemisphere).

In addition, we conducted an amplitude of low-frequency fluctuation (ALFF) analysis for the LC. The time series for each voxel of each ROI was transformed to the frequency domain, and the power spectrum was then obtained. Since the power of a given frequency is proportional to the square of the amplitude of this frequency component, the square root was calculated at each frequency of the power spectrum, and the averaged square root was obtained across 0.01 to 0.17 Hz at each voxel. This averaged square root was taken as the ALFF (*54*). The ALFF of each voxel was divided by the individual

global mean of ALFF within a brain mask, which was obtained by removing the tissues outside the brain using software MRIcron. Spatial smoothing was conducted on the maps with an isotropic Gaussian kernel of 8 mm of full width at half maximum. A multivariate ANOVA was used including stimulation (active versus sham) as independent variable and the different ROIs (ALFF for the ventral tegmental area, raphe nucleus, LC, and nucleus basalis) as dependent variable. A simple contrast analysis was included to compare the difference between active and sham stimulations for each ROI, separately. A similar analysis was applied for the right amygdala and right hippocampus during stimulation. A one-way ANOVA was conducted with ALFF as dependent variable and stimulation (active versus sham) as independent variable for the right amygdala and right hippocampus separately.

Experiment 3: ON-tDCS

The setup was similar to study 1. Shielded cables connected the MR-compatible box and tDCS electrodes, and the stimulation data were transferred via the CAT.6 local area network cable that runs throughout the MR scanner room to the non–MR-compatible stimulation devices in the control room (fig. S4).

Experiment 3: Procedure

Participants were scanned immediately before, during, and immediately after the ON-tDCS session. The person controlling the tDCS device is not involved in instructing the participant; this is performed by a second person who is blind to the stimulation protocol and not in the control room during the stimulation.

Experiment 4: Participants

This study was designed similar to study 1, and participants were screened and enrolled similar to study 1. The study was in accordance with the ethical standards of the Helsinki declaration (1964) and was approved by the Institutional Review Board of the UT Dallas (#17-96; NCT03309072). Participants were 30 healthy, right-handed adults (8 males and 22 females; mean age of 20.13 years, SD = 3.26 years) with a similar education background (i.e., enrolled as undergraduate student at UT Dallas). The inclusion criteria were similar as for study 1.

Experiment 4: Task

All participants performed a face-name association memory task that was based on the task of Jacobs and colleagues (*55*), which included learning an association between a face and a name. The face stimuli consisted of 120 grayscale pictures of human faces. Only faces with a neutral expression facing forward were selected. Half of the faces were male, and the other half were female. Furthermore, we attempted to select faces balanced over all age groups. The 120 common names were selected from the study of Cooper and colleagues (*56*). The face-name pairs were created by randomly pairing them with the only restriction that the face and name were gender consistent. The 120 face-name pairs were divided into two lists of 60 face-name pairs (30 males and 30 females). The task was programmed in Visual Studio software using C# and shown on a computer with a 26-inch screen positioned at eye level. The face-name association memory task was divided into (i) an encoding phase, (ii) a consolidation phase, and (iii) a retrieval phase. During the encoding phase, participants studied 60 successively presented face-name pairs and were instructed to assess their gender to keep them focused to the task. Each face-name pair was presented for 5 s to provide sufficient encoding time. The encoding phase lasted approximately 5 min. The encoding phase was followed by a consolidation phase where participants were instructed to "sit still, relax, and think about nothing in particular" for 10 min. Following the procedure of Jacobs and colleagues (*55*), no task was given during this phase as the stimulation or sham procedure was still ongoing, and an active task might activate other cognitive functions or other brain networks. During the retrieval phase, participants were presented with 60 old (i.e., faces presented during the encoding phase; 30 males and 30 females) and 60 new (i.e., faces not presented during the encoding phase; 30 males and 30 females) faces and were instructed to assess whether they have seen this face during the encoding phase. For faces that were judged as old, participants were to indicate the correct name out of four options. There was no time limit set for the retrieval phase. Participants responded to both encoding and retrieval conditions by through designated buttons. Before the actual experiment, all participants completed a practice session with six unique face-name pairs (i.e., not used in the actual experiment; three old and three new) with verbal feedback from the researcher to make sure they practiced well enough on the task components (of assessing old/new and selecting one name) and button pressing but without seeing the face-name pairs tested in the actual experiment.

Experiment 4: Procedure

Participants were randomly assigned to the sham or active ON-tDCS condition. The person who controlled the tDCS device was not involved in instructing the participant; this is performed by a second person who is blind to the stimulation protocol and not in room during the stimulation. Participants received active or sham stimulation for the 5 min during encoding phase and 10 min of consolidation phase before they conducted the retrieval phase without ON-tDCS.

Experiment 4: Statistics

To compare the old and new faces' hit rate, we calculated the true positive rate (TPR; the proportion of positives that are correctly identified as such). A repeated-measures ANOVA was conducted with the TPR for both old and new faces as within-subject variable and group (active versus sham) as between-subject variable. For the name recognition, a univariate ANOVA was conducted with the percentage of correctly recognized names as dependent variable and group (active versus sham) as independent variable. In addition, we calculated the effect size and the BF_{10}.

Experiment 5: Participants

This study was designed similar to study 1, and participants were screened and enrolled similar to study 1. The study was in accordance with the ethical standards of the Helsinki declaration (1964) and was approved by the Institutional Review Board of the UT Dallas (#17-08). Participants were 20 healthy, right-handed adults (8 males and 12 females; mean age of 22.00 years, SD = 3.54 years) with a similar education background (i.e., enrolled as undergraduate student at UT Dallas). The inclusion criteria were similar as for study 1.

Experiments 5, 6, 8, 9, and 10: Task

All participants underwent a word association memory task that was based on the task of Karpicke and Roediger (*57*), which consisted of Swahili-English vocabulary learning. The Swahili-English word pairs

were taken from the study of Nelson and Dunlosky (*58*). We have selected first 75-word pairs from the list, excluding the word pair rafiki-friend, as this word is also the name of a character in the Lion King and therefore familiar to lots of participants. The task was programmed in Visual Studio software using C# and shown on a computer with 27-inch screen positioned at eye level. In the experiment, the participants learned a list of 75 randomly presented Swahili-English word pairs. Participants got the opportunity to learn the list of 75-word pairs across a total of eight alternating study (S) and test (T) periods. Participants studied the entire list of 75 words in each study period of the four blocks, but only the items that they had not yet recalled were tested in the test period (denoted STn, where Tn indicates that only the nonrecalled pairs were repeatedly tested). The verbal paired-association memory task was divided into four blocks with each block consisting of a study phase, followed by a consolidation phase and a test phase. In block 1, participants studied 75 successively presented Swahili-English word pairs in the study phase, followed by 75 test trials. After that, i.e., in blocks 2 to 4, the number of test trials varied according to the test condition. Therefore, the number of word pairs tested diminished across the periods under this condition. 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 enough encoding time. Participants were instructed to memorize as many word pairs as they can, so they could recall the English word given the Swahili word. Each study phase was followed by a consolidation phase where a crosshair appeared in the middle of the screen for 30 s. After each consolidation screen, participants were tested in a test phase of 75 or fewer test trials. During the test phase, participants were instructed to type in the correct English translation of the Swahili word presented for 8 s using a computer key keyboard, after which the computer program automatically advanced to the next item regardless of whether the participant had entered a response. Participants came back for a final test session (all 75 words tested) 7 days later. Participants' responses and their RTs were recorded by the computer program. The word pair sequence was randomized between blocks, conditions, and participants.

Experiments 5, 6, 8, 9, and 10: Statistics

For learning, a repeated-measures ANOVA was conducted with the four blocks as within-subject variable and group (active versus sham) as between-subject variable. To look at the memory effect (recall) 7 days after learning, we applied a one-way ANOVA with condition as the independent variables and correctly recall words as dependent variables. In addition, we calculated the effect size and the BF_{10}.

Experiment 5: ON-tDCS

The device was the same as the one that was used in the previous studies. A constant current of 1.5 mA intensity was applied during each of the four study blocks (i.e., 375 s × 4 blocks) during visit 1 (day 1). For sham ON-tDCS, placement of the electrodes was identical to active ON-tDCS (see fig. S3). tDCS was first switched on in a ramp-up fashion over 5 s. Current intensity (ramp down) was gradually reduced (over 5 s) as soon as ON-tDCS reached a current flow of 1.5 mA. Hence, sham ON-tDCS only lasted 10 s for each block.

Experiment 5: Procedure

Participants were randomly assigned to the sham or active ON-tDCS condition. The person who controlled the tDCS device was not involved in instructing the participant; this was performed by a second person who was blind to the stimulation protocol and not in the room during the stimulation. Participants received sham or active ON-tDCS during the study phase, but not during the rest and test phases on their first visit. Participants came back 7 days after their first visit to perform one test phase but received no ON-tDCS. A third person, independent of the experimenters responsible for the task and ON-tDCS, conducted the second visit (7 days later).

Experiment 6: Participants

This study was designed similar to study 1, and participants were screened and enrolled similar to study 1. The study was in accordance with the ethical standards of the Helsinki declaration (1964) and was approved by the Institutional Review Board of the UT Dallas (#17-34; NCT03055884). Participants were 40 healthy, right-handed adults (19 males and 21 females; mean age of 21.75 years, SD = 3.67 years) with a similar education background (i.e., enrolled as undergraduate student at UT Dallas). The inclusion criteria were similar as for study 1.

Experiment 6: tDCS

The same device was used as in the previous studies. For the 20 participants receiving ON-tDCS, one electrode was placed over left and right C2 nerves' dermatomes (one group with the anode placed over the right C2 nerve and the cathode over the left C2 nerve; the other group with the anode placed over the left C2 nerve and the cathode over the right C2 nerve) (see fig. S3). A constant current of 1.5-mA intensity was applied during each of the four study blocks (i.e., 375 s × 4 blocks) at the first day. Two control groups received tDCS over the trigeminal nerve dermatomes (left and right temple/jaw) or the C5/C6 nerves dermatomes (lower neck). tDCS was first switched on in a ramp-up fashion over 30 s.

Experiment 6: Procedure

Participants were randomly assigned to one of the four conditions. The person who controlled the tDCS device was not involved in instructing the participant; this was performed by a second person who was blind to the stimulation protocol and not in the room during the stimulation. Participants received active tDCS during the study phase, but not during the rest and test phases on their first visit. Participants came back 7 days after their first visit to perform one test phase but received no ON-tDCS. A third person, independent of the experimenters responsible for the task on the first visit, conducted the second visit (7 days later).

Experiment 6: VAS_a-POMS

The VAS_a and POMS were collected before and after the tDCS procedure. Ten minutes following the completion of ON-tDCS session, participants completed the VAS_a and POMS survey. A one-way ANOVA was applied for both the VAS_a and the subscale POMS as dependent variable and conditions as independent variable.

Experiments 6 and 8: EEG

System specification can be found in the EEG section: data acquisition of study 2. We used the same setup as in study 2 to collect resting-state EEG. We conducted a source reconstruction on our EEG data for gamma frequency band (see study 2). A whole-brain comparison was used to compare between before and after ON-tDCS, and these activity changes were correlated with the words recalled

7 days after training phase using a Pearson correlation. We used nonparametric permutation test similar to study 2 to estimate, via randomization, the empirical probability distribution for the maximum statistics, under the null hypothesis comparisons (*50*). This methodology corrects for multiple testing of tests performed for all voxels. The significance threshold was based on a permutation test with 5000 permutations.

Experiments 6 and 8: Saliva collection

Participants' saliva was collected two times during the experiment: before and after stimulation to look at sAA and cortisol. No saliva was collected during the experiment to not interfere with the word association memory task. For more details, see study 1. A Pearson correlation was calculated to look at the association between sAA and correctly recalled words 7 days after learning the words.

Experiment 7: Animals

Eighteen male Sprague Dawley rats weighing 275 to 350 g were used in experiments, carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the UT Dallas Institutional Animal Care and Use Committee.

Experiment 7: ONS electrodes

Platinum-iridium wire electrodes were affixed to biocompatible microrenathane cuffs (1.25 mm in inner diameter, 2.5 mm in outer diameter, and 3.0 mm in length). Two platinum-iridium wires (7.5 cm in length) from the cuff were plugged into the middle pins of a four-pin connector (PSI-04-AA-LT, Omnetics, USA).

Experiment 7: Surgery

Rats were placed under isoflurane gas anesthesia (2.5% in oxygen, Western Medical Supply, CA, USA), and a 10-mm vertical incision was made from between the ears to the shoulder blades. To find the left greater occipital nerve, the occipital notches were identified, and the left nerve was isolated. The cuff electrode was positioned around the nerve, and the wires were tunneled under the skin to the head cap connector, which was secured to the skull with acrylic cement and bone screws. The cuff electrode was fastened with a nylon suture thread, and the incision was sutured. Antibiotics (ceftriaxone; 4 mg/kg) and anti-inflammatories (ketoprofen; 5 mg/kg) were administered to prevent infection and reduce pain, and lactated ringers' solution (10 ml in 5% dextrose) was injected (intraperitoneally) for hydration and caloric/electrolyte replenishment. Sham-treated animals were subjected to the same surgical and postsurgical procedures. Rats were allowed to recover for 6 days. See Fig. 5A.

Experiment 7: ONS

Stimulation sessions were conducted in acrylic boxes individually housed in sound-attenuated chambers. The stimulation boxes were composed of an irregular hexagon consisting of five clear acrylic walls (17 cm by 25 cm in height) with honeycomb-shaped holes, and a clear solid front door (25 cm by 26 cm in height), differing in shape, color, and flooring from where behavioral tests were performed. Both floor and ceiling were constructed of solid black acrylic. A commutator was installed above the ceiling of the acrylic cage, and a small opening (3 cm in diameter) allowed a swivel to connect the commutator to the head cap. Rats were exposed to two daily 5-min sessions of habituation with a single 30-s stimulation at half of the session. The parameters for ONS were as follows: 0.4-mA intensity, 40 Hz, 10-ms bursts with five pulses at 500 Hz per burst, a pulse width of 1 ms, and 1-ms interpulse interval delivered in constant current mode. The cumulative charge of each 1-ms pulse was balanced during the interpulse interval. Posttraining stimulations were performed immediately after tests, for 6.5 min. Rats were kept in the stimulating chambers for an additional 5 min before they were returned to their home cages.

Experiment 7: Object recognition and posttraining stimulation

After 1 week of recovery from surgery, 18 rats were handled for 5 min per day for 3 days. After habituation to ONS (described above), rats were then trained on an object recognition task. On day 1, rats were habituated to a black chamber (64 cm by 50 cm by 48 cm in height) for 5 min with no objects present. On day 2, two identical objects (an orange pyramid with 6 cm by 6 cm by 6 cm in height) were placed in the chamber at 7 cm to two adjacent corners of the cage. Rats were allowed to explore the apparatus in the presence of the objects for 5 min. Immediately after training, rats were given ONS (n = 9) or sham treatment (n = 9). Long-term memory was tested 24 hours later in the same apparatus. For this test, one of the identical objects was replaced by a novel object (a blue pyramid stacked on top of another blue pyramid; 6 cm by 6 cm by 7 cm in height). Time spent exploring each of the objects was measured by a research assistant who was blind to treatment conditions. Difference in time (in seconds) exploring the new object versus the familiar one was taken as a measure of memory retrieval.

Experiment 7: Inhibitory avoidance and posttraining stimulation

Twenty-four hours after object recognition test, the same rats were trained on the inhibitory avoidance task. The inhibitory avoidance apparatus consisted of a trough-shaped alley (91 cm in length, 15 cm in depth, 20 cm in width at the top, and 6.4 cm in width at the floor). The apparatus was divided into a white and illuminated compartment and a black compartment with low illumination, separated by a manually controlled sliding door. The dark compartment was composed of two dark electrifiable metal floor plates. Rats were placed in the bright compartment that was lined with white Plexiglas (31 cm in length), and upon crossing to the dark compartment, the sliding door was closed, and a single 1-s inescapable foot shock was delivered (0.4 mA). The rat was removed from the apparatus 10 s later, and either ONS or sham stimulation was delivered immediately after. Each rat received the same ONS or sham treatment as in the object recognition. During the retention test 24 hours later, rats were returned to the light compartment of the apparatus. Latency to enter the dark compartment was measured, with a maximum latency of 600 s. Memory was inferred from an increase in latency to enter the dark compartment. After the retention test, each respective rat was treated with ONS or sham stimulation once again.

Experiment 7: Statistics

For both the object recognition and inhibitory avoidance, we applied a Mann-Whitney U test to compare the active and sham groups for the acquisition and retention phase. In addition, we calculated the effect size and the BF_{10}.

Experiment 8: Participants
This study was designed similar to study 1, and participants were screened and enrolled similar to study 1. The study was in accordance with the ethical standards of the Helsinki declaration (1964) and was approved by the Institutional Review Board of the UT Dallas (#18-144). Participants were 45 healthy, right-handed adults (21 males and 24 females; mean age of 19.82 years, SD = 2.35 years) with a similar education background (i.e., enrolled as undergraduate student at UT Dallas). The inclusion criteria were similar as for study 1.

Experiment 8: ON-tDCS
The same device and parameters were used as in study 6. Fifteen people received active stimulation during training (i.e., study phases of the word association task) and sham stimulation immediately after the word association task. Fifteen people received sham stimulation during training and active stimulation immediately after training in the memory consolidation period, and 15 people received sham stimulation both during and after training of the word association task (see fig. S3). Immediately after the training phase, participants in the active consolidation group received stimulation for 25 min keeping the same stimulation setup as during the training phase in which a constant current of 1.5-mA intensity was first switched on in a ramp-up fashion over 30 s. For the sham consolidation group, current intensity (ramp down) was gradually reduced (over 30 s) as soon as tDCS reached a current flow of 1.5 mA. Hence, sham ON-tDCS only lasted 60 s.

Experiment 8: Procedure
Participants were randomly assigned to one of three groups: active stimulation during training and sham stimulation immediately after training, sham stimulation during training and active stimulation immediately after the training, or sham stimulation during training and immediately after training. The person who controlled the tDCS device was not involved in instructing the participant; this was performed by a second person who was blind to the stimulation protocol and not in the room during the stimulation. Participants were randomly assigned to one of the three conditions. Participants came back 7 days after their first visit to perform one test phase but received no ON-tDCS. A third person who was not responsible for the task and ON-tDCS conducted the second visit (7 days later).

Experiment 9: Participants
This study was designed similar to study 1, and participants were screened and enrolled similar to study 1. The study was in accordance with the ethical standards of the Helsinki declaration (1964) and was approved by the Institutional Review Board of the UT Dallas (#18-144). Participants were 32 healthy, right-handed adults (13 males and 19 females; mean age of 19.52 years, SD = 3.01 years) with a similar education background (i.e., enrolled as undergraduate student at UT Dallas). The inclusion criteria were similar as for study 1.

Experiment 9: ON-tDCS
The setup was exactly the same as in experiment 5, with the exception of a sham group. Both groups receive active tDCS (see fig. S3). The anesthesia group received anesthesia using an anesthetic cream that was applied under the tDCS electrodes. For the anesthesia condition, 10 g of topical anesthesia EMLA cream was applied.

Experiment 9: Procedure
Participants were randomly assigned to one of two groups. The person who controlled the tDCS device was not involved in instructing the participant; this was performed by a second person who was blind to the stimulation protocol and not in the room during the stimulation. All participants received active ON-tDCS during the training phase but not during the rest and test phases; participants came back 7 days after their first visit to perform one test phase but received no ON-tDCS. A third person who was not responsible for the task and ON-tDCS conducted the second visit (7 days later).

Experiment 10: Participants
This study was designed similar to study 1, and participants were screened and enrolled similar to study 1. The study was in accordance with the ethical standards of the Helsinki declaration (1964) and was approved by the Institutional Review Board of the UT Dallas (#18-144). Twenty healthy, right-handed adult participants (12 males and 8 females; mean age of 21.03 years, SD = 2.63 years) were enrolled with a similar education background (i.e., enrolled as undergraduate student at UT Dallas).

Experiment 10: ON-tDCS
The setup was exactly the same as in experiment 5 (see fig. S3).

Experiment 10: Procedure
Participants were randomly assigned to the sham (n = 10) or active (n = 10) ON-tDCS condition. The person who controlled the tDCS device was not involved in instructing the participant; this was performed by a second person who was blind to the stimulation protocol and not in the room during the stimulation. Participants received sham or active ON-tDCS during the test phase 7 days after their first visit. A third person, independent of the experimenters responsible for the task and ON-tDCS, conducted the second visit (7 days later).

Blinding
For all experiments, participants were asked after the study whether they thought they were assigned to the sham (no anesthesia) or active (anesthesia) group. We used a χ^2 test to look whether there was a difference between what stimulation people perceived in comparison what people expected.

Meta-analysis
We applied a meta-analysis to include the behavioral data from experiments 4, 5, 6, 7, and 9 and calculated the individual and overall Hedges' g using Meta-Mar (www.meta-mar.com/smd). The Hedges' g is a measure of effect size. A g value of 1 indicates that the two groups differ by 1 SD. The Hedges' g is interpreted in a similar way as Cohens' d, where a large effect is represented by $\geq$0.8. In addition, we calculated the Orwin's fail-safe N and the Rosenberg's fail-safe N. Both are measurements that assess the potential for publication bias by calculating the number of additional "negative" studies that would be needed to increase the P value for the meta-analysis to above 0.05.

SUPPLEMENTARY MATERIALS
Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/6/45/eaax9538/DC1

REFERENCES AND NOTES

1. J.-P. Lefaucheur, A. Antal, S. S. Ayache, D. H. Benninger, J. Brunelin, F. Cogiamanian, M. Cotelli, D. De Ridder, R. Ferrucci, B. Langguth, P. Marangolo, V. Mylius, M. A. Nitsche, F. Padberg, U. Palm, E. Poulet, A. Priori, S. Rossi, M. Schecklmann, S. Vanneste, U. Ziemann, L. Garcia-Larrea, W. Paulus, Evidence-based guidelines on the therapeutic use of transcranial direct current stimulation (tDCS). *Clin. Neurophysiol.* **128**, 56–92 (2017).
2. M. Vöröslakos, Y. Takeuchi, K. Brinyiczki, T. Zombori, A. Oliva, A. Fernández-Ruiz, G. Kozák, Z. T. Kincses, B. Iványi, G. Buzsáki, A. Berényi, Direct effects of transcranial electric stimulation on brain circuits in rats and humans. *Nat. Commun.* **9**, 483 (2018).
3. B. Asamoah, A. Khatoun, M. Mc Laughlin, tACS motor system effects can be caused by transcutaneous stimulation of peripheral nerves. *Nat. Commun.* **10**, 266 (2019).
4. C. K. McIntyre, J. L. McGaugh, C. L. Williams, Interacting brain systems modulate memory consolidation. *Neurosci. Biobehav. Rev.* **36**, 1750–1762 (2012).
5. S. J. Sara, The locus coeruleus and noradrenergic modulation of cognition. *Nat. Rev. Neurosci.* **10**, 211–223 (2009).
6. A. Wagatsuma, T. Okuyama, C. Sun, L. M. Smith, K. Abe, S. Tonegawa, Locus coeruleus input to hippocampal CA3 drives single-trial learning of a novel context. *Proc. Natl. Acad. Sci. U.S.A.* **115**, E310–E316 (2018).
7. L. B. Couto, C. R. Moroni, C. M. d. R. Ferreira, D. H. Elias-Filho, C. A. Parada, I. R. Pelá, N. C. Coimbra, Descriptive and functional neuroanatomy of locus coeruleus-noradrenaline-containing neurons involvement in bradykinin-induced antinociception on principal sensory trigeminal nucleus. *J. Chem. Neuroanat.* **32**, 28–45 (2006).
8. K. B. Clark, D. K. Naritoku, D. C. Smith, R. A. Browning, R. A. Jensen, Enhanced recognition memory following vagus nerve stimulation in human subjects. *Nat. Neurosci.* **2**, 94–98 (1999).
9. M. Plazier, S. Vanneste, I. Dekelver, M. Thimineur, D. De Ridder, Peripheral nerve stimulation for fibromyalgia. *Prog. Neurol. Surg.* **24**, 133–146 (2011).
10. T. Kano, G. S. Cowan, R. H. Smith, Electroanesthesia (EA) studies: EA produced by stimulation of sensory nerves of the scalp in Rhesus monkeys. *Anesth. Analg.* **55**, 536–541 (1976).
11. P. R. Murphy, R. G. O'Connell, M. O'Sullivan, I. H. Robertson, J. H. Balsters, Pupil diameter covaries with BOLD activity in human locus coeruleus. *Hum. Brain Mapp.* **35**, 4140–4154 (2014).
12. R. H. Hou, C. Freeman, R. W. Langley, E. Szabadi, C. M. Bradshaw, Does modafinil activate the locus coeruleus in man? Comparison of modafinil and clonidine on arousal and autonomic functions in human volunteers. *Psychopharmacology* **181**, 537–549 (2005).
13. A. H. van Stegeren, O. T. Wolf, W. Everaerd, P. Scheltens, F. Barkhof, S. A. R. B. Rombouts, Endogenous cortisol level interacts with noradrenergic activation in the human amygdala. *Neurobiol. Learn. Mem.* **87**, 57–66 (2007).
14. S. Sutton, M. Braren, J. Zubin, E. R. John, Evoked-potential correlates of stimulus uncertainty. *Science* **150**, 1187–1188 (1965).
15. J. Polich, Updating P300: An integrative theory of P3a and P3b. *Clin. Neurophysiol.* **118**, 2128–2148 (2007).
16. S. Nieuwenhuis, G. Aston-Jones, J. D. Cohen, Decision making, the P3, and the locus coeruleus-norepinephrine system. *Psychol. Bull.* **131**, 510–532 (2005).
17. E. Nyhus, T. Curran, Functional role of gamma and theta oscillations in episodic memory. *Neurosci. Biobehav. Rev.* **34**, 1023–1035 (2010).
18. C. W. Berridge, S. L. Foote, Effects of locus coeruleus activation on electroencephalographic activity in neocortex and hippocampus. *J. Neurosci.* **11**, 3135–3145 (1991).
19. R. M. Neves, S. van Keulen, M. Yang, N. K. Logothetis, O. Eschenko, Locus coeruleus phasic discharge is essential for stimulus-induced gamma oscillations in the prefrontal cortex. *J. Neurophysiol.* **119**, 904–920 (2018).
20. S. Bouret, S. J. Sara, Network reset: A simplified overarching theory of locus coeruleus noradrenaline function. *Trends Neurosci.* **28**, 574–582 (2005).
21. A. J. Yu, P. Dayan, Uncertainty, neuromodulation, and attention. *Neuron* **46**, 681–692 (2005).
22. G. Aston-Jones, J. D. Cohen, An integrative theory of locus coeruleus-norepinephrine function: Adaptive gain and optimal performance. *Annu. Rev. Neurosci.* **28**, 403–450 (2005).
23. C. Guedj, E. Monfardini, A. J. Reynaud, A. Farnè, M. Meunier, F. Hadj-Bouziane, Boosting norepinephrine transmission triggers flexible reconfiguration of brain networks at rest. *Cereb. Cortex* **27**, 4691–4700 (2016).
24. G. Madler, Recognizing: The judgment of previous occurrence. *Psychol. Rev.* **87**, 252–271 (1980).
25. J. S. Holdstock, A. R. Mayes, N. Roberts, E. Cezayirli, C. L. Isaac, R. C. O'Reilly, K. A. Norman, Under what conditions is recognition spared relative to recall after selective hippocampal damage in humans? *Hippocampus* **12**, 341–351 (2002).
26. M. Hajos, W. E. Hoffmann, D. D. Robinson, J. H. Yu, E. Hajos-Korcsok, Norepinephrine but not serotonin reuptake inhibitors enhance theta and gamma activity of the septo-hippocampal system. *Neuropsychopharmacology* **28**, 857–864 (2003).
27. D. Osipova, A. Takashima, R. Oostenveld, G. Fernandez, E. Maris, O. Jensen, Theta and gamma oscillations predict encoding and retrieval of declarative memory. *J. Neurosci.* **26**, 7523–7531 (2006).
28. P. B. Sederberg, M. J. Kahana, M. W. Howard, E. J. Donner, J. R. Madsen, Theta and gamma oscillations during encoding predict subsequent recall. *J. Neurosci.* **23**, 10809–10814 (2003).
29. J. L. McGaugh, Consolidating memories. *Annu. Rev. Psychol.* **66**, 1–24 (2015).
30. M. Kumar, R. Chawla, M. Goyal, Topical anesthesia. *J. Anaesthesiol. Clin. Pharmacol.* **31**, 450–456 (2015).
31. J. R. McReynolds, K. M. Anderson, K. M. Donowho, C. K. McIntyre, Noradrenergic actions in the basolateral complex of the amygdala modulate Arc expression in hippocampal synapses and consolidation of aversive and non-aversive memory. *Neurobiol. Learn. Mem.* **115**, 49–57 (2014).
32. R. Cabeza, E. Ciaramelli, I. R. Olson, M. Moscovitch, The parietal cortex and episodic memory: An attentional account. *Nat. Rev. Neurosci.* **9**, 613–625 (2008).
33. Y. Huang, J. Hullfish, D. De Ridder, S. Vanneste, Meta-analysis of functional subdivisions within human posteromedial cortex. *Brain Struct. Funct.* **224**, 435–452 (2019).
34. W. M. Kelley, F. M. Miezin, K. B. McDermott, R. L. Buckner, M. E. Raichle, N. J. Cohen, J. M. Ollinger, E. Akbudak, T. E. Conturo, A. Z. Snyder, S. E. Petersen, Hemispheric specialization in human dorsal frontal cortex and medial temporal lobe for verbal and nonverbal memory encoding. *Neuron* **20**, 927–936 (1998).
35. M. K. Sidhu, J. Stretton, G. P. Winston, A. W. McEvoy, M. Symms, P. J. Thompson, M. J. Koepp, J. S. Duncan, Memory network plasticity after temporal lobe resection: A longitudinal functional imaging study. *Brain* **139**, 415–430 (2016).
36. I. H. Robertson, A noradrenergic theory of cognitive reserve: Implications for Alzheimer's disease. *Neurobiol. Aging* **34**, 298–308 (2013).
37. I. H. Robertson, Right hemisphere role in cognitive reserve. *Neurobiol. Aging* **35**, 1375–1385 (2014).
38. S. Vanneste, B. Langguth, D. De Ridder, Do tDCS and TMS influence tinnitus transiently via a direct cortical and indirect somatosensory modulating effect? A combined TMS-tDCS and TENS study. *Brain Stimul.* **4**, 242–252 (2011).
39. S. Kim, A. S. Nilakantan, M. S. Hermiller, R. T. Palumbo, S. VanHaerents, J. L. Voss, Selective and coherent activity increases due to stimulation indicate functional distinctions between episodic memory networks. *Sci. Adv.* **4**, eaar2768 (2018).
40. B. Lafon, S. Henin, Y. Huang, D. Friedman, L. Melloni, T. Thesen, W. Doyle, G. Buzsáki, O. Devinsky, L. C. Parra, A. A. Liu, Low frequency transcranial electrical stimulation does not entrain sleep rhythms measured by human intracranial recordings. *Nat. Commun.* **8**, 1199 (2017).
41. S. M. Perez, F. R. Carreno, A. Frazer, D. J. Lodge, Vagal nerve stimulation reverses aberrant dopamine system function in the methylazoxymethanol acetate rodent model of schizophrenia. *J. Neurosci.* **34**, 9261–9267 (2014).
42. D. R. Hulsey, C. M. Shedd, S. F. Sarker, M. P. Kilgard, S. A. Hays, Norepinephrine and serotonin are required for vagus nerve stimulation directed cortical plasticity. *Exp. Neurol.* **320**, 112975 (2019).
43. D. R. Hulsey, S. A. Hays, N. Khodaparast, A. Ruiz, P. Das, R. L. Rennaker II, M. P. Kilgard, Reorganization of motor cortex by vagus nerve stimulation requires cholinergic innervation. *Brain Stimul.* **9**, 174–181 (2016).
44. A. J. Duszkiewicz, C. G. McNamara, T. Takeuchi, L. Genzel, Novelty and dopaminergic modulation of memory persistence: A tale of two systems. *Trends Neurosci.* **42**, 102–114 (2019).
45. S. V. Maurer, C. L. Williams, The cholinergic system modulates memory and hippocampal plasticity via its interactions with non-neuronal cells. *Front. Immunol.* **8**, 1489 (2017).
46. L. A. Schwarz, L. Luo, Organization of the locus coeruleus-norepinephrine system. *Curr. Biol.* **25**, R1051–R1056 (2015).
47. J. R. Hodges, D. P. Salmon, N. Butters, Differential impairment of semantic and episodic memory in Alzheimer's and Huntington's diseases: A controlled prospective study. *J. Neurol. Neurosurg. Psychiatry* **53**, 1089–1095 (1990).
48. P. R. Murphy, I. H. Robertson, J. H. Balsters, R. G. O'connell, Pupillometry and P3 index the locus coeruleus–noradrenergic arousal function in humans. *Psychophysiology* **48**, 1532–1543 (2011).
49. S. Makeig, Auditory event-related dynamics of the EEG spectrum and effects of exposure to tones. *Electroencephalogr. Clin. Neurophysiol.* **86**, 283–293 (1993).
50. T. E. Nichols, A. P. Holmes, Nonparametric permutation tests for functional neuroimaging: A primer with examples. *Hum. Brain Mapp.* **15**, 1–25 (2002).
51. A. Fertonani, C. Ferrari, C. Miniussi, What do you feel if I apply transcranial electric stimulation? Safety, sensations and secondary induced effects. *Clin. Neurophysiol.* **126**, 2181–2188 (2015).
52. N. Tzourio-Mazoyer, B. Landeau, D. Papathanassiou, F. Crivello, O. Etard, N. Delcroix, B. Mazoyer, M. Joliot, Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *NeuroImage* **15**, 273–289 (2002).

53. K. D. Tona, M. C. Keuken, M. de Rover, E. Lakke, B. U. Forstmann, S. Nieuwenhuis, M. J. P. van Osch, In vivo visualization of the locus coeruleus in humans: Quantifying the test-retest reliability. *Brain Struct. Funct.* **222**, 4203–4217 (2017).
54. Y. F. Zang, Y. He, C.-Z. Zhu, Q.-J. Cao, M.-Q. Sui, M. Liang, L.-X. Tian, T.-Z. Jiang, Y.-F. Wang, Altered baseline brain activity in children with ADHD revealed by resting-state functional MRI. *Brain and Development* **29**, 83–91 (2007).
55. H. I. Jacobs, J. M. Riphagen, C. M. Razat, S. Wiese, A. T. Sack, Transcutaneous vagus nerve stimulation boosts associative memory in older individuals. *Neurobiol. Aging* **36**, 1860–1867 (2015).
56. B. Y. Cooper, R. D. Johnson, T. J. Nutter, Exposure to Gulf War Illness chemicals induces functional muscarinic receptor maladaptations in muscle nociceptors. *Neurotoxicology* **54**, 99–110 (2016).
57. J. D. Karpicke, H. L. Roediger III, The critical importance of retrieval for learning. *Science* **319**, 966–968 (2008).
58. T. O. Nelson, J. Dunlosky, Norms of paired-associate recall during multitrial learning of Swahili-English translation equivalents. *Memory* **2**, 325–335 (1994).
59. T. Bartsch, P. J. Goadsby, Stimulation of the greater occipital nerve induces increased central excitability of dural afferent input. *Brain J. Neurol.* **125**, 1496–1509 (2002).

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