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Single-session gamma sensory stimulation entrains real-time electroencephalography but does not enhance perception, attention, short-term memory, or long-term memory

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Abstract

Background: Studies have shown that gamma (40 Hz) audiovisual stimulation can enhance gamma oscillations and improve cognitive functioning in patients with Alzheimer's disease. However, despite promising long-term results, the efficacy of short-duration or single-session 40 Hz entrainment in humans has been questioned by behavioral studies that fail to find observable cognitive aftereffects, for two possible reasons: 1) lack of validated gamma entrainment, as most studies lacked concurrent electroencephalography (EEG) data to verify that gamma neural entrainment did take place, and 2) lack of diverse cognitive tests, as most studies did not test a wide range of cognitive factors.

Objective: This study aimed to increase sensitivity for detecting single-session gamma entrainment. We employed 1) mid- and post-stimulation EEG monitoring to ensure entrainment worked, and 2) a comprehensive cognitive battery that probes perception, attention, working memory, and long-term memory.

Methods: Participants received 30 min of synchronized 40 Hz light and sound stimulation, followed by a visual perceptual task, attentional network task, change detection working memory task, and long-term picture memory task, with concurrent EEG.

Results: We observed robust 40 Hz EEG entrainment during stimulation but no significant 40 Hz oscillation after stimulation, and no significant cognitive improvements.

Conclusions: Despite robust 40 Hz online entrainment, gamma sensory entrainment requires consistent long-term exposure to induce cognitive and neurological changes. Future research should determine the optimal duration and frequency of 40 Hz stimulation to maximize its therapeutic potential.

Keywords

Alzheimer's disease, audiovisual stimulation, gamma entrainment, gamma stimulation, GENUS, sensory entrainment

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Introduction

High-frequency gamma oscillations ranging from 30 to 100 Hz play an important role in human cognitive functions such as perception, attention, memory consolidation, and the processing of information. These oscillations have been shown to mediate synchronization of neuronal activity across different brain regions, thereby facilitating cognitive processes[1] to support working memory[2,3] as well as long-term memory learning and consolidation.[4] As such, disturbances in gamma oscillatory activity have been linked to a range of neurological and psychiatric disorders, including

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Alzheimer's disease (AD) and schizophrenia, suggesting their essential role in maintaining cognitive integrity.[5,6]

Effect of 40 Hz stimulation in animal models

Recently, studies have attempted sensory stimulation at 40 Hz to boost gamma oscillations in the brain through neural entrainment (for a comprehensive review, see study by Sahu and Tseng[7]). One specific type of sensory entrainment, known as Gamma Entrainment Using Sensory Stimuli (GENUS), combines both auditory and visual stimuli at 40 Hz bursts/pulses to boost such oscillatory activity.[8–11] In animal models, particularly in those engineered to mimic AD, GENUS has been shown to significantly reduce amyloid-β (Aβ) plaques and tau phosphorylation, and induce improvements in cognitive functions, and enhanced neural connectivity across various brain regions, including the hippocampus and prefrontal cortex.[8–10] Notably, changes in microglia, astrocytes, and vasculature were significantly greater with combined auditory and visual stimulation as the multimodal approach, but not visual or auditory stimulation alone, produced decreased amyloid in medial prefrontal cortex.[10] These effects were mediated by mechanisms such as microglial activation and increased phagocytosis,[8] as well as increased glymphatic cleansing.[12]

Effect of 40 Hz stimulation in humans

Translating the findings from animal models, early clinical studies on AD patients have shown that 40 Hz sensory stimulation can be safe and potentially effective in humans. Studies involving AD patients have reported audiovisual gamma entrainment increased functional connectivity, and in some cases, reductions in disease markers such as ventricular dilation and hippocampal shrinkage.[11,13,14] These improvements were associated with enhanced cognitive functions, particularly in mild to moderate stages of the disease.[14,15] Moreover, extended exposure to 40 Hz stimulation was shown to alter neuroinflammatory markers and support network connectivity in the brain, indicating potential therapeutic benefits in slowing the progression of AD.[16] Furthermore, studies using 40 Hz auditory stimulation alone, which has been shown to induce stronger gamma oscillation than visual stimulation alone,[17] have also observed improved cognitive functioning and even mood in patients after an 8-week session, with prolonged improvement for up to 1 year.[15,18]

The search for optimal entrainment parameters

Given the promising findings reviewed above, recent studies have begun to explore the different parameters that can maximize the effect of gamma entrainment. For example, in a study by Lee et al., organic LEDs attached to eyeglasses were utilized to emit gamma flickering light (ranging from 32 Hz to 50 Hz) to assess the effectiveness of different colors, intensities, and frequencies on gamma synchronization in healthy adults.[19] The research measured event-related synchronization of gamma waves and found that red light was the most effective in entraining gamma waves, surpassing the effects seen with white light, while green and blue lights were least effective. Additionally, the study observed that higher luminance levels (700 and 400 cd/m^2) produced more pronounced gamma waves compared to lower luminance levels (100 and 10 cd/m^2).

In another study, it was found that auditory 40 Hz stimulation works better than visual stimulation in terms of immediate electroencephalography (EEG) entrainment,[17] which correlates well with the visual and auditory discrepancies from long-term behavioral follow-up studies, where auditory stimulation led to more positive behavioral and neuroanatomical changes.[8,10] Indeed, acknowledging methodological and parameter differences may perhaps be the key to promote converging evidence, as well as resolving some of the inconsistent findings in the field. Recently, despite some of the positive evidence reviewed above, Soula et al. used APP/PS1 and 5xFAD animal models and showed that application of both single-session (1-h stimulation) and multi-session (1-h stimulation for consecutive 7 days) 40 Hz visual flickering did not have a positive impact on deeper brain structures.[20] The results further showed no obvious changes in Aβ load or microglia morphology in the animals (also see Wilson et al.'s study).[20,21] However, this lack of positive finding has been argued to be attributable to differences in methodologies, such as 1) pooling of multiple AD mouse models, sexes, and ages, and 2) measuring amyloid with enzyme-linked immunosorbent assay (ELISA) based analysis on fixed tissue.[22]

Short versus long stimulation duration in humans

Given the importance of methodological consistency across studies, one important but underexplored aspect of 40 Hz entrainment in humans is time/duration of stimulation. That is, studies in animal models have shown positive findings in neuronal health and behavioral testing, with both single-session (e.g. 1 h: Iaccarino et al.[8]) and multi-day stimulation (e.g. 7 days: Martorell et al.;[10] 6 weeks: Adaikkan et al.[9]) protocols. However, we know mice and humans rely on different sensory modalities, and have different processes in sensory integration.[23,24] Therefore, the amount of time that is necessary for the positive effects of 40 Hz stimulation to emerge in humans still requires further investigation. This is especially true for shorter durations (e.g. single session/day) as long-term effects of gamma entrainment in humans are quite well-documented both in cognitive/behavioral measures[14,15] and neuroimaging.[14,16]

In terms of short durations in humans, there are very few studies that have tested the effects of single-session entrainment. Most studies have used real-time EEG to measure the magnitude of 40 Hz entrainment, but without any cognitive/behavioral outcome measure. For example, the study by Park et al.[25] presented flicker light stimuli for 2 s each to assess the effectiveness of different luminance and colors. Similarly, Agger et al.[26] presented different lighting conditions such as invisible spectral flicker, continuous light, and stroboscopic light for 30 s each. Lastly, Han et al.[27] presented 40 Hz auditory stimulation of various waveforms to identify the optimal auditory parameter, and in their study each entrainment session was only 120 s. However, none of these studies have utilized any kind of cognitive/behavioral testing, and thus the question of whether single-session 40 Hz entrainment can readily translate to improvement of cognition remains unanswered.

To our knowledge, the only short-duration study that has incorporated cognitive/behavioral testing is a recent study by Hsiung and Hsieh.[28] This study used a variety of entrainment methods (e.g. flickering monitor, binaural beats) to test the possible effects of 40 Hz entrainment on participants' perceptual sensitivity and short-term memory using a visual threshold task and visuospatial change detection task, respectively. The authors found no perceptual or memory improvement with 40 Hz entrainment, which possibly suggests that the positive short-term effects in mice cannot be readily translated to humans yet. However, there are two important methodological differences that are worth noting, which may have contributed to the study's null findings. First, the authors used a *concurrent* stimulation design, meaning that participants received 40 Hz stimulation at the same time as they performed the cognitive tasks. This is somewhat unconventional in the field of brain stimulation because 1) such design leaves no time for stimulation aftereffects to occur, and importantly, 2) neural entrainment has been shown to be highly interactive with participants' concurrent cognitive/neural state and task at hand (e.g. Juan et al.),[29] which can go as far as to *erase* or *reverse* the effect of stimulation from a positive one to negative (e.g. Huang et al.; Suppa et al.).[30,31] Second, the study did not utilize any EEG recording to verify whether 40 Hz entrainment did in fact take place. This verification is especially important given that the study employed some highly unconventional entrainment procedures (e.g. flickering monitor).

The present study

Taken together, unlike studies using animal models, there is insufficient evidence that short-duration (especially single session 40 Hz entrainment) can work equally well in humans as it is in mice. To properly test this translational possibility, it is necessary to use 1) consistent experiment design to enable generalization with the literature, 2) concurrent EEG to verify the presence of 40 Hz entrainment, and 3) comprehensive behavioral testing that covers a broad range of cognitive functioning to avoid Type II error. To this end, here we employ a single-session experiment with pre- and post-stimulation design, concurrent EEG for all stages of the experiment (i.e. pre-stim rest, stimulation, post-stim rest, pre-stim task, post-stim task), as well as a comprehensive cognitive/behavioral battery that includes perception, attention, working memory, and long-term memory.

Methods

Participants

Twenty-two participants were recruited for this experiment (8 females, 14 males; age range: 20 to 27 years; mean age: 21.45 years). The inclusion criteria were an age between 20 and 60, with normal or corrected-to-normal vision. Exclusion criteria included having psychiatric or neurological conditions, being on prescription medication, having a personal or family history of epilepsy, having Tourette syndrome or allergies, and being students with a grading relationship with the host or co-host of the experiment. One participant was excluded due to intolerance to the light stimulation and dropped out midway through the experiment. Another participant was excluded after noticing the display pattern of repeated images in the visual working memory task. In the end, twenty participants (7 females, 13 males; age range: 20 to 27 years; mean age: 21.5 years) were included in the analysis, which either matched or exceeded the sample size from previous studies that used the same perceptual task ($n = 15$),[32] long-term memory task ($n = 20$),[33] attentional task ($n = 14$),[34] and working memory task ($n = 20$),[35] to ensure sufficient statistical power. All participants gave informed consent in written form prior to their participation in the study. All procedures were approved by local ethics committee.

Procedure

Participants performed two sessions of the experiment: a control session and a 40 Hz experimental session. In the experimental session, participants received 30 min of 40 Hz visual and auditory stimulation, while in the control session, they received 30 min of constant light and sound. The two sessions were conducted at least one week apart, with the order randomized using MATLAB's number generation.

In each session, participants sat in front of a screen with their chins fixed on a chinrest and followed these steps sequentially. After the encoding phase of the long-term memory (LTM) task, each participant received non-invasive light and auditory stimulation simultaneously for

30 min (either 40 Hz or constant sensory stimulation), followed by the first retrieval phase of the LTM task. Next, participants performed the attention, visual perception, and visual working memory tasks in a random order, followed by the second retrieval phase of the LTM task. The stimuli for the four cognitive tasks were presented on a monitor at a viewing distance of approximately 57 cm. Each session took about 100 min, during which participants' EEG was recorded continuously. To avoid fatigue of the participants, we opted for a post-test-only design to keep each session at under 100 min. Therefore, both the control and 40 Hz session consisted of post-tests only, without pre-test before the stimulation. This design gives up on participants' baseline performance (i.e. no stimulation versus constant stimulation), but is still able to compare performance between control and 40 Hz (i.e. constant stimulation versus 40 Hz stimulation). Furthermore, this design is still able to control for possible practice effect by randomizing the order of control versus active stimulation sessions, such that half of our participants performed the control session first, and another half performed the 40 Hz session first. Thus, if there indeed is systematic practice effect in the first or second session, they would cancel each other out as the number of subjects who experienced control versus 40 Hz session first is the same.

Note that for both control and experimental sessions, the LTM encoding stage took place *before* the 30-min 40 Hz entrainment (Figure 1). This is because memory encoding is heavily dependent on attentional processing, and if 40 Hz entrainment improved participants' attentional processing, then it inevitably would also improve the LTM encoding process and lead to better LTM performance, though the locus of the effect is not LTM per se. In other words, if 40 Hz entrainment truly improved attention, then our attention network task (ANT) task would have revealed this effect, and we therefore did not want LTM task to show the same improvement due to attentional enhancement instead of LTM enhancement. As such, if both ANT and LTM tasks show positive entrainment-induced improvement, we would be more confident to conclude that the improvement was more than just attentional enhancement, but also memory consolidation or retrieval. Therefore, to somewhat dissociate the distinct roles of ANT and LTM tasks, LTM encoding was implemented before the 40 Hz entrainment.

Sensory stimulation

In the 40 Hz experimental session, synchronized visual and auditory stimulation at 40 Hz was delivered. The visual stimulation was provided by a white light-emitting diode (LED) panel (13.2 cm diameter, 5000 K). Auditory stimulation was delivered through headphones (MDR7506, Sony) at the same frequency. The combined auditory-visual stimulation approach was chosen because it has been shown to be more effective than either modality alone in animal model,[10] and also with positive results in long-term stimulation studies in humans.[11,14] In the control session, participants received constant light and sound from the same LED panel and headphones for 30 min. Participants were seated 57 cm from the LED panel throughout the entire sensory stimulation period. The average volume of the auditory stimulation was around 60 dB.

Cognitive tasks

Long term memory. The LTM task was adapted from Köster et al.'s study[33] and comprised three phases: an encoding phase and two retrieval phases. The experimental stimuli were presented using E-Prime, version 2.0 (Psychology Software Tools Inc., USA).

During the encoding phase, 100 photos were displayed in random order, each lasting 2 s with a 1-s interstimulus interval. Participants were instructed to memorize as many photos as possible. The time interval between the encoding phase and the first retrieval phase was 30 min, corresponding to the duration of the stimulation.

During the first retrieval phase, 50 new photos and 50 photos from the encoding phase were presented in random order. Participants were instructed to determine whether each photo had been seen during the encoding phase. If the photo had been displayed in the encoding phase, participants pressed "m" with their right index finger; if not, they pressed "v" with their left index finger. Each photo remained on screen until the participant responded.

In the second retrieval phase, there were 150 trials, consisting of 50 new photos and 100 old photos. The new photos were the same as those presented in the first retrieval phase, while the old photos were from the encoding phase. Fifty of the old photos were presented in both retrieval phases (old2), and the other 50 were presented only in the second retrieval phase (old1). Participants were instructed to identify whether each photo had been previously presented in the encoding phase. To prevent practice effects, distinct sets of images were used in the two sessions (experimental and control), counterbalanced among participants. All image contents primarily featured daily necessities or common animals. The images were sourced from publicdomainvectors.org and hemera-photo-clip-art.software.informer.com.

Participants' hit rate, false alarm rate, d' value, and the accuracy of each photo category (old1, old2, new) were measured separately for both the first and second retrieval phases.

Visual perception. The perception task was adapted from Antal et al.'s study.[32] The experiment utilized E-Prime, version 3.0 (Psychology Software Tools Inc., USA), and

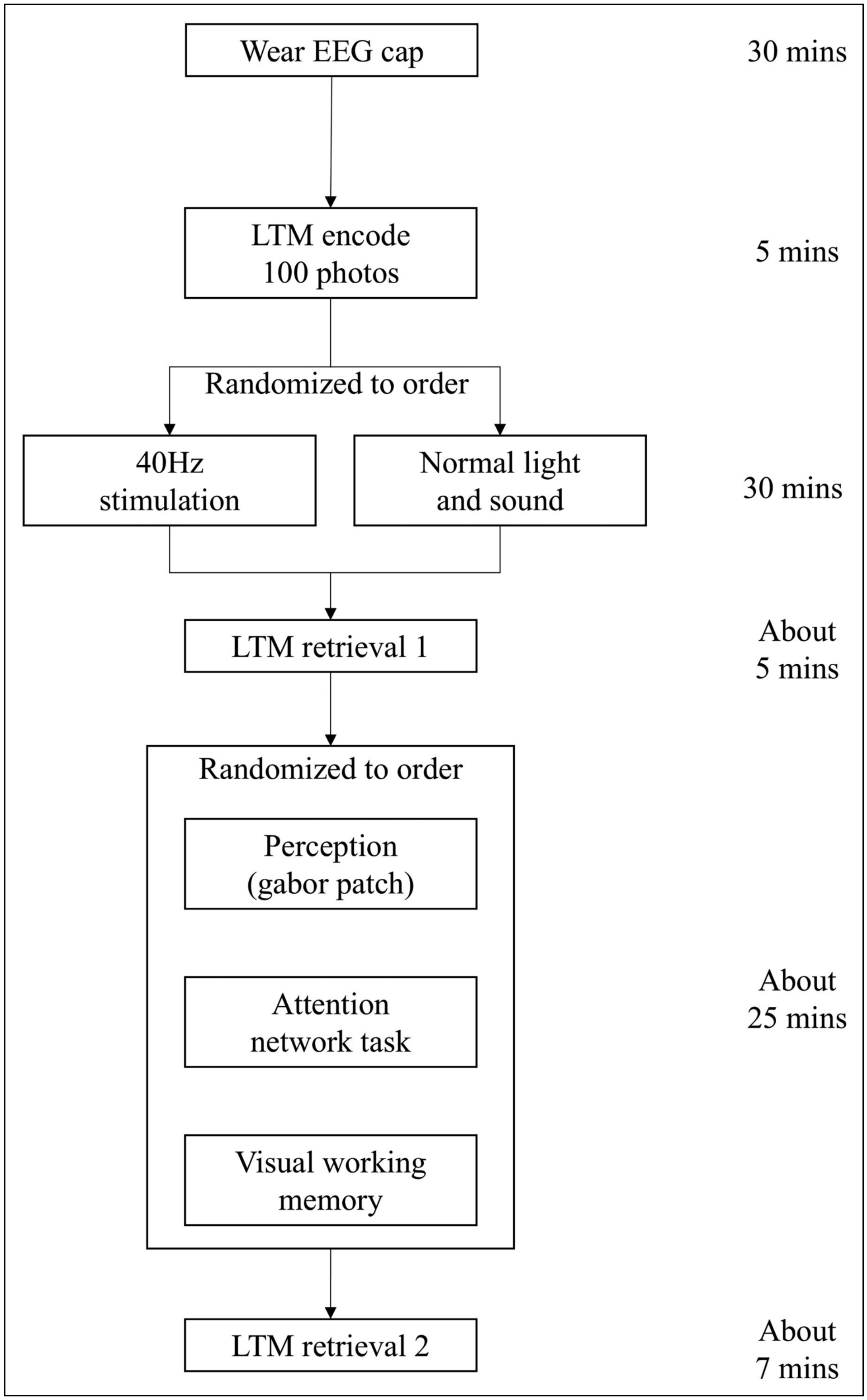


Figure 1. Experimental procedure diagram.
Participants completed two sessions, a control session with 30 min of constant light and sound and an experimental session with 30 min of 40 Hz visual and auditory stimulation. The order of sessions was randomized, with at least a one-week interval between sessions. Each session included an encoding phase of a long-term memory task (LTM), followed by the respective sensory stimulation, then the first retrieval phase of the LTM task, and finally, attention, visual perception, and visual working memory tasks in random order, concluding with a second LTM retrieval phase. EEG was recorded throughout the approximately 100-min experiment.

the Gabor patches were generated using an online Gabor patch generator created by Sebastiaan Mathot (http://www.cogsci.nl/software/online-gabor-patch-generator). Gaussian patches were used, with a size of 256 pixels. The standard deviation was set to 32 pixels, the frequency to 0.10 cycles/pixel, and the phase to 0.00 cycles. Four angles were generated (30, 60, 120, 150 degrees). Each orientation had 20 difficulty levels. The RGB values for the background color and color 2 were fixed at 128-128-128, while the RGB values for color 1 ranged from 128.2-128.2-128.2 to 132-132-132 in intervals of 0.2 (Figure 2).

In total, this task consisted of 80 trials displayed in random order. Participants had to distinguish the orientation of the patches. If the bars were oriented to the right (30 and 60 degrees), they pressed "m" with their right index finger; if the bars were oriented to the left (120 and 150 degrees), they pressed "v" with their left index finger. If participants couldn't distinguish the orientation, a time limit of 5000 ms was set. The total accuracy, d' value, and the highest difficulty level each participant could achieve were measured. Participants' highest difficulty level was determined based on the highest difficulty level at which they achieved a 100% accuracy rate.

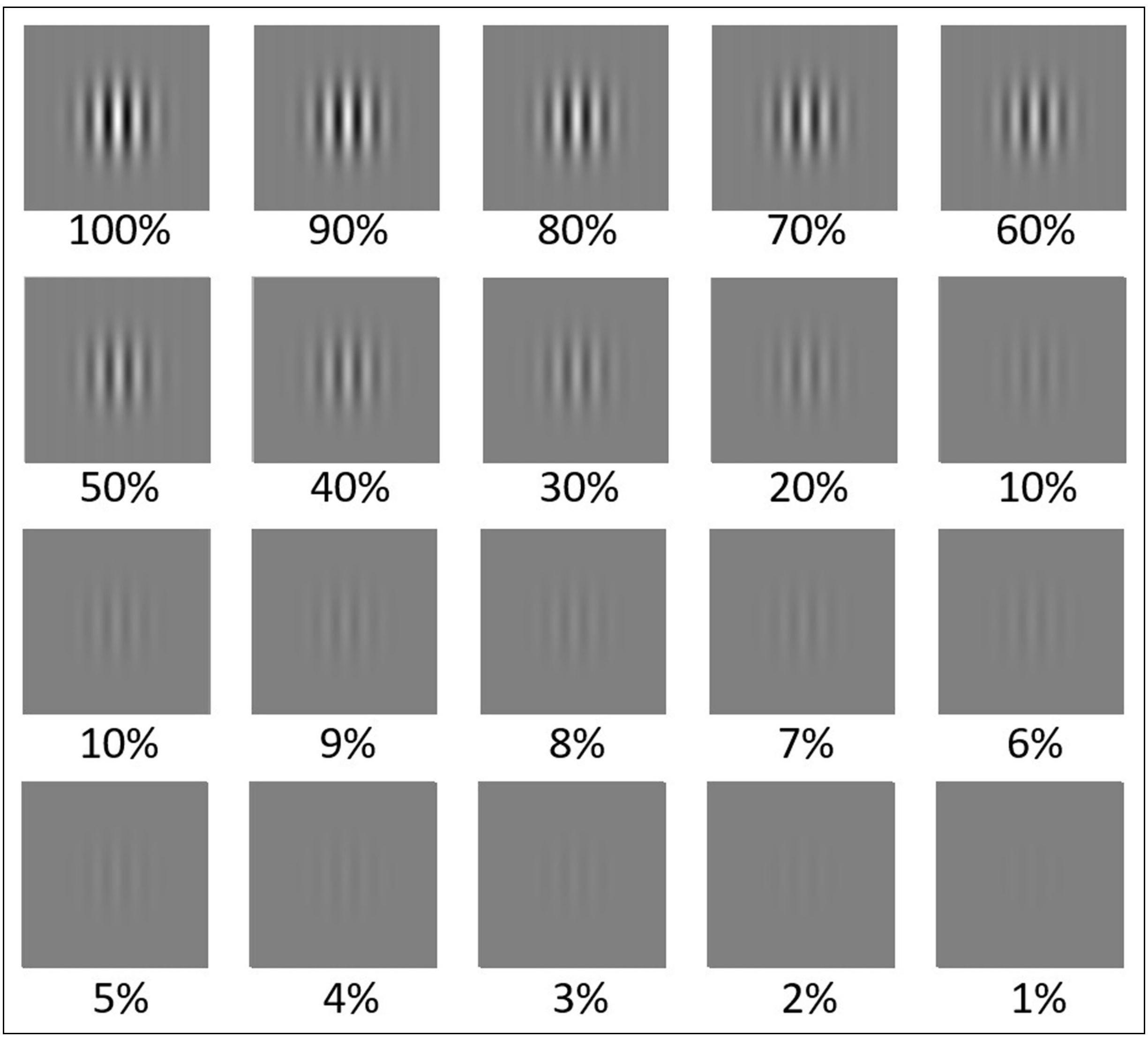


Figure 2. Experimental stimuli: four orientations of Gabor patches.
Sample stimuli from the perception task. The task was adapted from Antal et al.,[32] utilized Gabor patches generated with Sebastiaan Mathot's online tool and E-Prime 3.0 software. Gaussian patches of 256 pixels were displayed in four orientations (this figure shows 90 degrees for illustrative purposes, the actual experiment used 30, 60, 120, and 150 degrees) with 20 difficulty levels (1% to 100%, see above) each, totaling 80 trials. The RGB values of the background color and color 2 were fixed at 128-128-128, and the RGB values of color 1 from level 1 to level 20 ranged from 128.2-128.2-128.2 to 132-132-132 with an interval of 0.2.

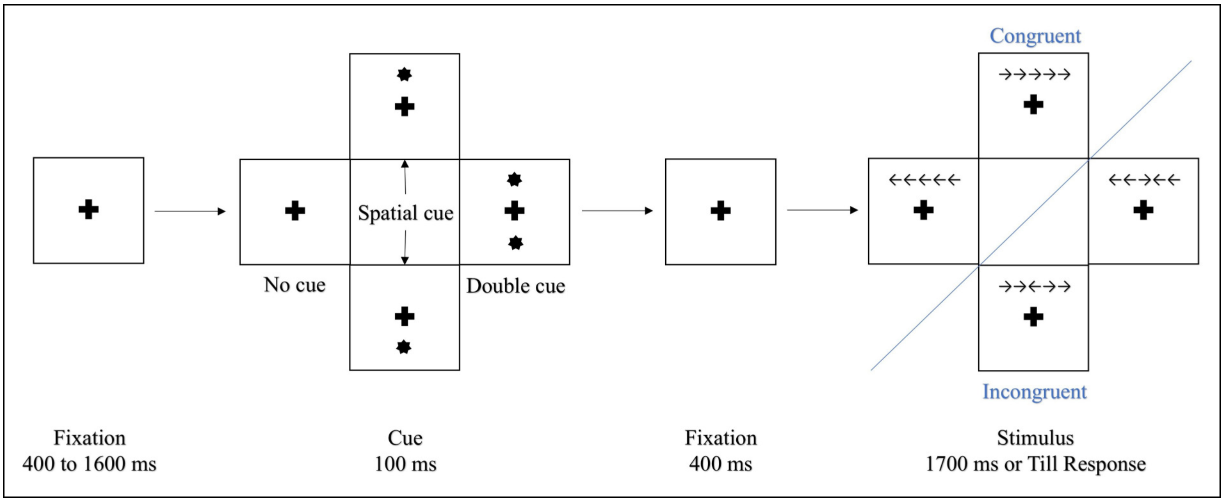


Figure 3. Stimuli timeline for attention network test.
This experiment, conducted using E-Prime 3.0, consists of 96 trials where each trial begins with a fixation cross (+) shown for 400 to 1600 milliseconds randomly, followed by a 100 milliseconds cue (*). Then, 400 milliseconds after the cue's offset, a target appears until a response is made or for a maximum of 1700 ms. Participants must respond to the direction of the middle arrow in a string of five arrows, pressing "v" for left and "m" for right. The task includes cue conditions (no cue, double cue, spatial cue) and target conditions (congruent, incongruent, neutral), requiring quick and accurate identification of the target arrow's direction.

Attention network task. The ANT is a cognitive function test designed to assess attentional abilities by measuring response times to different cues and stimuli under various attentional conditions. Researchers typically analyze participants' sub-scores for attention, including Alerting, Orienting, and Conflict Effect.[36,37]

This experiment utilized E-Prime, version 3.0 (Psychology Software Tools Inc., USA), and consisted of 96 trials. Each trial began with a fixation cross (+) for a variable interval ranging from 400 to 1600 ms. Following the fixation cross, a cue (*) was presented for 100 ms. Four hundred milliseconds after the cue's offset, a target appeared and remained on until a response was made or for 1700 ms if no response was detected (Figure 3). Participants were instructed to respond based on the direction of the target arrow, specifically the middle arrow. Before the formal task, there were 12 practice trials.

The experiment comprised two main parts: cue condition and target condition. The cue condition included three categories: no cue, double cue, and spatial cue. The double cue provided temporal information but no spatial information, while the spatial cue, presented either above or below the fixation point, provided spatial information with 100% validity, indicating where the impending target would appear. The target, a central arrow within a string of 5 arrows, appeared either above or below the fixation cross. The target condition included three categories: congruent, incongruent, and neutral. In the congruent condition, all 5 arrows pointed in the same direction, whereas in the incongruent condition, the arrows flanking the target pointed in the opposite direction (e.g. the target points left, and flankers point right, or vice versa). In the neutral condition, only the central arrow was shown with non-directional diamond flankers. Participants were instructed to respond quickly and accurately to identify the direction of the target arrow. If the arrow pointed left, participants pressed "v" with their left index finger; if it pointed right, they pressed "m" with their right index finger. The total duration of the experiment was approximately 5.12 min for the 96 formal trials.

Participants' response accuracy and reaction time (RT) for each trial were recorded. The mean RT was calculated for each combination of the six cue and target conditions, considering only correct trials. Subsequently, the mean RTs were computed for each of the three cue conditions, averaging across the three target conditions. Additionally, the mean RTs were calculated for the congruent and incongruent target conditions, averaging across the cue conditions.

To calculate the three functions of attention scores, the following subtractions were conducted: alerting effect (RT no cue minus RT double cue), orienting effect (RT double cue minus RT spatial cue) and conflict effect (RT incongruent minus RT congruent). Both alerting and orienting efficiencies denote benefits (i.e. decreases in RT) resulting from information present in the cues. Alerting efficiency reflects the advantage gained by knowing the timing of the target's appearance. Orienting efficiency signifies the advantage derived from knowing both the timing and location of the target compared to knowing only the timing (i.e. double cue). On the other hand, conflict efficiency (or executive function) represents the incurred cost, reflected in increased RT, when the incongruent flanking arrows demand a different response than the central target arrow.

Visual working memory. Participants' visual working memory performance was measured using a change detection task, which has been widely employed to study visual working memory (VWM) and provides various quantitative measures to estimate memory accuracy. The change detection task utilized E-Prime, version 2.0 (Psychology Software Tools Inc., USA), and comprised 50 trials.

Each trial began with a black fixation cross at the center of the screen for 250 ms, followed by a memory array for 250 ms and a retention interval of 1000 ms. Finally, a test array was presented until the participant responded. Participants were required to memorize an array of 6 colored solid circles in the memory array and compare it with a subsequent test array to indicate whether any circle had changed color. If all six colors were the same, participants pressed "v" with their left index finger, indicating the memory and test arrays were identical. If there was a color change, they pressed "m" with their right index finger, indicating a difference (Figure 4).

Stimuli in each trial were displayed on a gray background measuring 15 cm by 15 cm. During both the memory array and the test array phases, six solid circles (1.6° visual angle) of various colors were presented, scattered within the gray background. These six colors were randomly selected from a set of nine easily distinguishable colors: black, blue, brown, green, orange, purple, pink, red, and yellow. Each trial presented six non-repeating colors. In these 50 randomly ordered trials, 50% of the memory and test array pairs were identical, while the other 50% had one of the six solid circles in the test array replaced with another color. The replacement color was also non-repeating, and the circle's shape, size, and position remained unchanged. The entire task took approximately 5 min to complete.

Participants' accuracy and reaction times for each trial were recorded by the computer. We calculated participants' d' and Cowan's K in both control and 40 Hz stimulation sessions. Cowan's K formula was used to estimate each participant's visual working memory capacity.[38,39] Cowan's K formula assumes that an individual's capacity to retain information in memory (K) out of a set of items (S) can be calculated as: $K = \text{Set Size} \times (\text{Hit Rate} - \text{False Alarm Rate})$. This formula accommodates guessing by subtracting incorrect guesses from the correct responses.

Statistical analysis for cognitive tasks

To examine whether participants' cognitive performance improved after 40 Hz sensory stimulation, we conducted paired samples t-tests to compare the dependent variables between the 40 Hz experimental session and the control session for each cognitive task. For the visual perception task, we compared accuracy and d' values between the two sessions. For the attention network task, we compared overall accuracy and reaction time differences across the three networks (alerting, orienting, and conflict) between the two sessions. For the visual working memory task, we compared visual working memory capacity measures, including d' and Cowan's K values, between the sessions. For the long-term memory task, we compared d' values between the 40 Hz experimental session and the control session during the first and second retrieval phases.

Electroencephalography

EEG activity was recorded from 32 Ag/AgCl electrodes mounted in a BrainCap electrode cap (Brain Products GmbH, Munich, Germany), following the 10–20 system. The online reference electrode was positioned between Fz

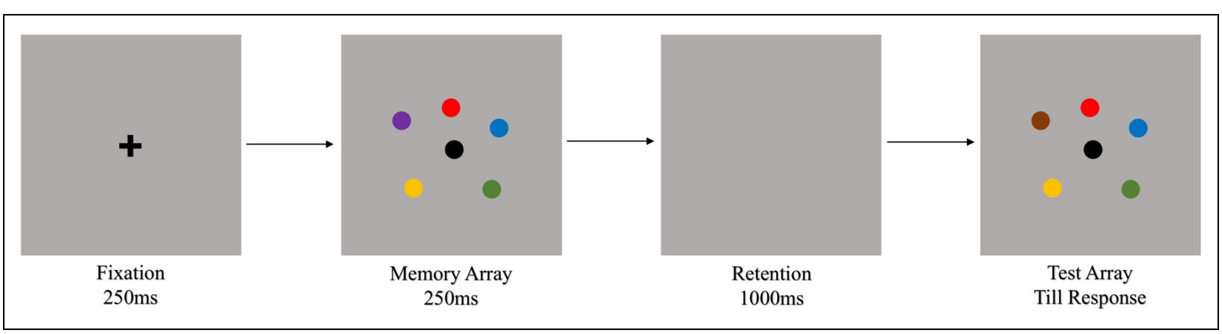


Figure 4. Stimuli timeline for visual working memory task.
Visual working memory was assessed using a change detection task implemented in E-Prime 2.0, consisting of 50 trials. Each trial began with a black fixation cross for 250 milliseconds, followed by a memory array of 6 colored circles for 250 milliseconds, a retention interval for 1000 milliseconds, and then a test array presented until a response was made. Each stimulus was displayed on a 15 cm × 15 cm gray background, with 6 colors randomly chosen non-repetitively from 9 distinguishable options, and the circle's shape, size, and position remained unchanged. Participants indicated whether the test array matched the memory array by pressing "v" for identical arrays and "m" for a change in one circle's color.

and Cz. To measure participants' vertical and horizontal eye movements, two sets of electrodes were placed on the upper and lower sides of the right eye and on the canthi of both eyes. All electrode impedances were kept below 10 kΩ. All signals were amplified using the BrainAmp amplifier (Brain Products GmbH, Munich, Germany). Data were recorded with Brainvision Recorder version 1.22.0001 at a sampling rate of 1000 Hz.

All preprocessing and analysis procedures were conducted using EEGLAB[40] and custom MATLAB scripts. The continuous EEG data were first offline re-referenced to the average of the electrodes at the left and right mastoids (A1 and A2). A digital band-pass filter between 1 and 100 Hz was applied. Ocular and muscle artifacts were identified and removed using independent component analysis (ICA). Following ICA decomposition, we utilized the ICLabel toolbox in EEGLAB to assist in classifying ocular and muscle artifacts. This process incorporated multiple features, including scalp topography, ERP images (representing the activity of the independent component across the entire dataset), component time series, and activity power spectrum.[41] For the EEG data recorded during 30-min 40 Hz sensory stimulation and control sessions, we applied the Clean Rawdata plugin in EEGLAB for automated artifact rejection. The algorithm, based on Artifact Subspace Reconstruction (ASR), removes data segments with a standard deviation exceeding 20 times that of the clean data segments.[42]

The Power Spectral Density (PSD) of individual epochs was calculated using the spectopo function of EEGLAB. The spectopo function uses MATLAB's pwelch function, applying a Hamming window. As spectopo computes the logarithm of PSD ($X = 10 * \log10(PSD)$), the PSD values were computed by eliminating the logarithms ($PSD = 10^{(X/10)}$). The frequency range used for analysis was 2 to 80 Hz.

Statistical analysis for 40 Hz entrainment

The extent of the 40 Hz entrainment elicited by the visual and auditory stimulation was further quantified using the signal-to-noise ratio (SNR). SNR represents the prominence of the 40 Hz amplitude response with respect to the surrounding frequency neighborhood (i.e. baseline, 38 Hz to 42 Hz) during the 30-min 40 Hz and constant visual and auditory stimulation.[43] To analyze our EEG data statistically, we used a cluster-based permutation test to compare the amplitude response at 40 Hz with the surrounding frequency neighborhood (i.e. 38 Hz to 42 Hz) across all channels during the 30-min sensory stimulation in the 40 Hz experimental and control sessions. We used EEGLAB's topoplot function to generate topographic maps of the 40 Hz PSD across all electrodes. These maps depicted the SNR values, which represent the prominence of the 40 Hz amplitude response relative to the surrounding frequency neighborhood (i.e. baseline frequencies from 38 Hz to 42 Hz). This approach provided a spatial distribution of the 40 Hz frequency component's prominence across the scalp.

To investigate the extent of 40 Hz entrainment during cognitive tasks, we analyzed the power spectrum density of EEG data collected during the tasks following the 30-min sensory stimulation and the 30-min control session (i.e. constant light and sound), respectively. During the visual perception task, preprocessed EEG data were segmented into epochs spanning from the onset of the Gabor patch to 1000 ms post-onset. Baseline correction was applied across the entire epoch, and epochs with artifacts exceeding ±100 μV were excluded. Power analysis was conducted on the remaining epochs, with data averaged according to the correctness of participants' responses. In the attention network task, the preprocessed EEG data were epoched from the onset of the target (i.e. a string of 5 arrows) to 500 ms after its onset. Baseline correction was applied across the entire epoch, and epochs with artifacts exceeding ±100 μV were excluded. Power analysis was then conducted on the remaining epoched EEG data, and the data were averaged according to the trial types: congruent (i.e. five arrows pointing in the same direction), incongruent (i.e. the direction of the middle arrow differs from the flankers), and neutral trials (i.e. only the central arrow is shown, with non-directional diamond flankers). In the visual working task, the preprocessed EEG data were epoched from the onset of the fixation cross to 500 ms after the onset of test array. Baseline correction was applied across the entire epoch, and epochs with artifacts exceeding ±150 μV were excluded. Power analysis was then conducted on the remaining epoched EEG data, and the data with correct responses were averaged based on trial types: hit (i.e. memory array and test array are the same) and correct rejection (i.e. memory array and test array differ). In the long term memory task, the preprocessed EEG data were epoched from the onset of the photo to 1000 ms after its onset during the retrieval phase. Baseline correction was applied across the entire epoch, and epochs with artifacts exceeding ±100 μV were excluded. Power analysis was then conducted on the remaining epoched EEG data, and the data with correct responses were averaged based on trial types: hit (i.e. participants correctly responded to the photos shown in the encoding phase.) and correct rejection (i.e. participants correctly responded to the new photos that were not shown in the encoding phase).

Results

To investigate the impact of a single session of 40 Hz sensory stimulation on cognitive benefits, we compared the cognitive performances on four cognitive tasks—

perception, attention, short-term memory, and long-term memory—following a 30-min 40 Hz sensory stimulation with performances following a 30-min constant light and sound stimulation.

Additionally, to observe the effects of 40 Hz neural entrainment, we analyzed the power spectrum of EEG signals during both the stimulation and the execution of cognitive tasks in the 40 Hz experimental and control sessions. The power spectrum analysis allowed us to quantify the 40 Hz entrainment by examining the EEG signal's frequency components and comparing the 40 Hz amplitude response to the surrounding frequency neighborhood (baseline, 38 Hz to 42 Hz). This analysis helped us understand the neural mechanisms underlying the potential cognitive benefits of 40 Hz sensory stimulation.

40 Hz Neural entertainment

The extent of the 40 Hz neural entrainment elicited by the 40 Hz experimental and control conditions is further quantified using the SNR, which represents the prominence of the 40 Hz amplitude response with respect to the surrounding frequencies (i.e. 38 to 42 Hz). Analysis confirmed that 40 Hz stimulation did in fact induced strong 40 Hz oscillation in the visual cortex (Figure 5(b), red), which then propagated to the frontal region (Figure 5(a), red). Figure 5(c) shows the SNR in scalp topography for the 40 Hz experimental and control conditions averaged across subjects.

Our results demonstrate that the 40 Hz amplitude response, compared to surrounding frequencies, shows significant differences across 28 recorded channels under the 40 Hz sensory stimulation condition ($p < 0.025$). However, under the constant light and auditory condition (i.e. control condition), no significant differences are observed. In these analyses, all p-values are corrected for multiple comparisons using a nonparametric cluster-based correction method. Additionally, a summary of 40 Hz entrainment effects for each electrode during the 30-min 40 Hz sensory stimulation and control conditions is provided in Supplemental Table 1.

Visual perception

We conducted a paired samples t-test to examine whether participants' visual perception performance improved under 40 Hz sensory stimulation. The comparison involved accuracy and d' values between the 40 Hz experimental session and the control session.

Our results showed that total accuracy in the control session ($M = 82.44\%$) and the 40 Hz experimental session ($M = 82.44\%$) were not significantly different ($t(19) < 0.001$, $p = 1.000$, Cohen's $d < 0.001$). Similarly, the d' value in the 40 Hz experimental session ($M = 2.187$) and the control session ($M = 2.208$) did not reach a significant difference ($t(19) = -0.163$, $p = 0.872$, Cohen's $d = -0.037$) (Figure 6 and Table 1).

As mentioned in the methods section, there were 20 difficulty levels (from the easiest level 20 to the most difficult level 1). The difficulty limit of what the participants could see was also analyzed. Since the Shapiro-Wilk tests suggested a deviation from normality, the difficulty limit was analyzed using the Wilcoxon signed-rank test. When accuracy equaled 1, the difficulty level participants could achieve in the control session ($M = 6.500$) and the 40 Hz experimental session ($M = 6.600$) were not significantly different ($W = 26.500$, $z = -0.578$, $p = 0.585$). To avoid the possibility of participants accidentally pressing the wrong button, we also analyzed the difficulty level when accuracy equaled 0.75. However, when accuracy equaled 0.75, the difficulty level participants could achieve in the control session ($M = 6.000$) and the 40 Hz experimental session ($M = 5.800$) were also not significantly different ($W = 30.000$, $z = 0.889$, $p = 0.390$), suggesting no improvement in visual acuity after stimulation.

We analyzed the PSD of participants' EEG data while they performed the tasks after a 30-min sensory stimulation, which included both 40 Hz stimulation and control sessions. However, no evidence of any in-task 40 Hz neural entrainment effects was observed (Figure 7). Although 40 Hz entrainment was not observed in the spectral analysis, we provide a summary of its effects, represented by the signal-to-noise ratio of each electrode during the 30-min 40 Hz sensory stimulation and control conditions, in Supplemental Table 2. Additionally, we compared specific frequency bands, including alpha (8–12 Hz) and beta (13–35 Hz), across correct and incorrect trials at the frontal and occipital regions between the 40 Hz sensory stimulation and control conditions. This comparison was conducted to explore their potential role in compensatory neural processes, as shown in Supplemental Table 3.

Attention network task

To assess whether participants' attention performance improves with 40 Hz sensory stimulation, we employed a paired samples t-test. We compared participants' overall accuracy and reaction time differences across three networks (alerting, orienting, and conflict networks) between the 40 Hz experimental session and the control session. Our analysis revealed that there were no significant differences in the alerting, orienting, and conflict networks between the 40 Hz experimental session and the control session, as shown in Figure 8 and Table 2.

We analyzed the PSD of participants' EEG data while they performed tasks after a 30-min sensory stimulation, which included both 40 Hz stimulation and control

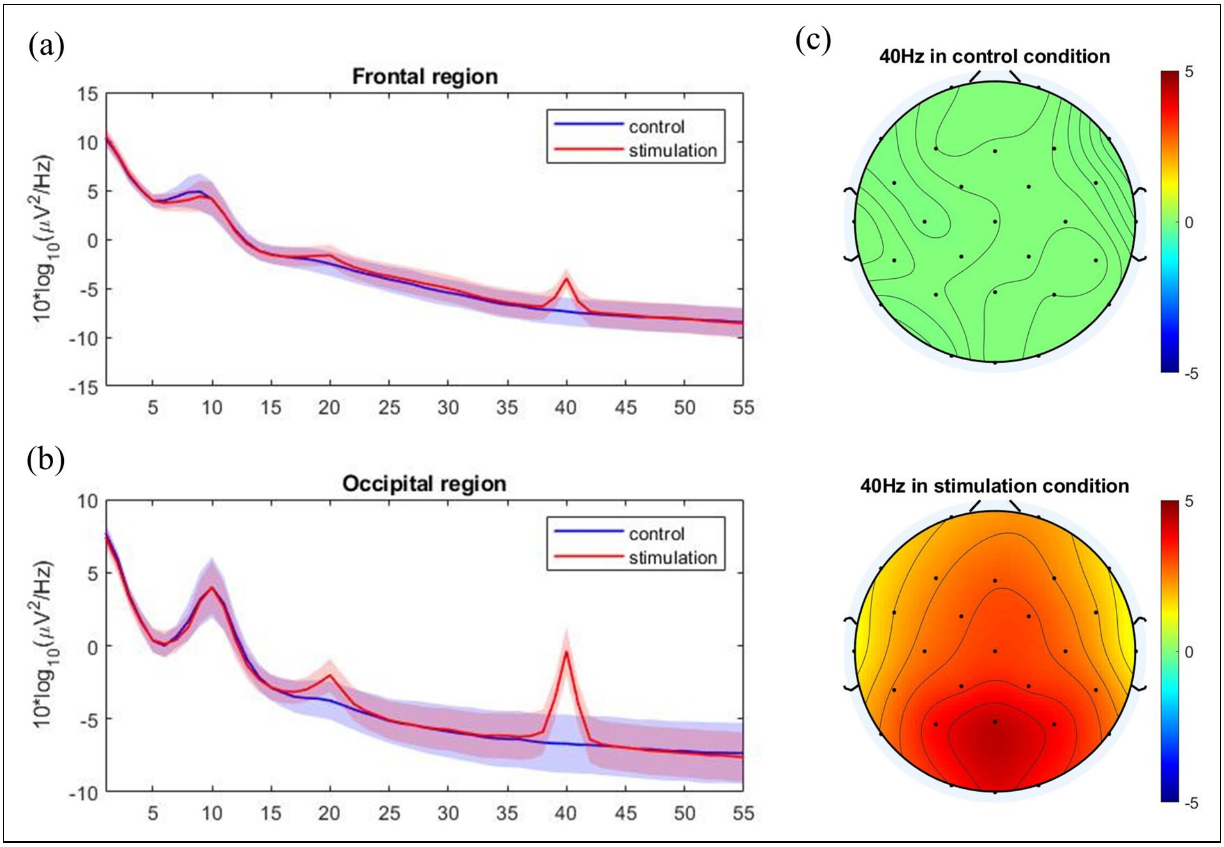


Figure 5. Average frequency spectrum and spatial distribution of 40 Hz neural entrainment across the scalp for both 40 Hz sensory stimulation and control conditions.
Average frequency spectrum for both 40 Hz sensory stimulation (red line) and control conditions (blue line) in frontal region (a) and occipital region (b), aggregated across subjects. The shaded area represents the 95% confidence interval. In (c), topographic distribution of 40 Hz entrainment signal-to-noise ratios in the 40 Hz experimental and control conditions are illustrated across participants, effectively demonstrating the spatial distribution of 40 Hz entrainment over the scalp during 40 Hz sensory stimulation.

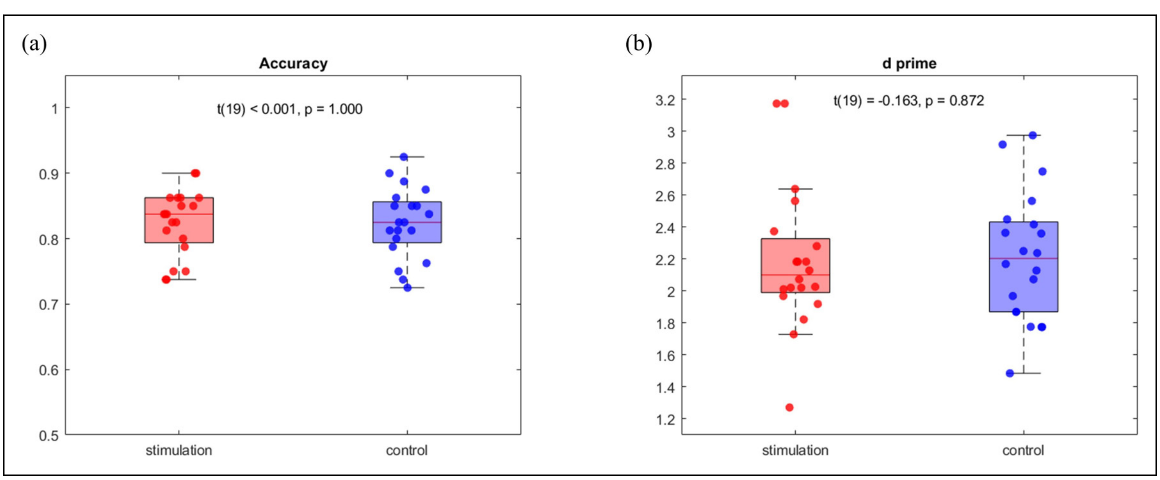


Figure 6. No significant improvement in visual perception performance under 40 Hz sensory stimulation.
A paired samples t-test was conducted to compare participants' visual perception performance between the 40 Hz experimental session and the control session, including accuracy (a) and d' values (b). Total accuracy (a) in the control session ($M = 82.44\%$) and the 40 Hz experimental session ($M = 82.44\%$) were not significantly different ($t(19) < 0.001$, $p = 1.000$, Cohen's $d < 0.001$). Similarly, the d' values (b) between the 40 Hz session ($M = 2.187$) and the control session ($M = 2.208$) did not show a significant difference ($t(19) = -0.163$, $p = 0.872$, Cohen's $d = -0.037$). Each box plot represents data from 20 participants.

Table 1. Accuracy and d' in 40 Hz experimental and control sessions.

	40 Hz experimental session		Control session				
	M	*SD*	*M*	*SD*	*t*(19)	*p*	Cohen's *d*
Accuracy	82.44%	4.99%	82.44%	5.39%	<0.001	1.000	<0.001
d'	2.187	0.445	2.208	0.401	−0.163	0.872	−0.037

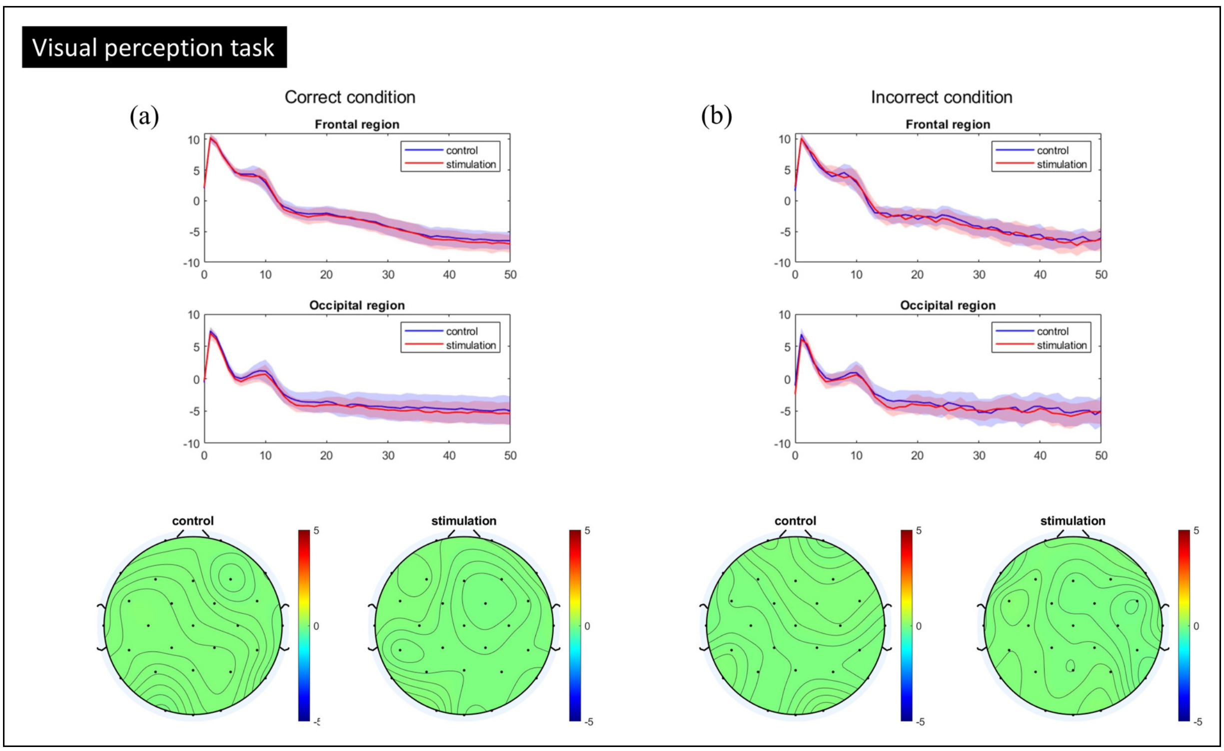


Figure 7. Average frequency spectrum and spatial distribution of 40 Hz neural entrainment across the scalp for visual perception task. The power spectrum density of participants' EEG data was divided into correct (a) and incorrect (b) conditions based on their responses following a 30-min sensory stimulation session comprising both 40 Hz stimulation (red line) and control conditions (blue line). The plots illustrate the average frequency spectrum in frontal and occipital regions, with the shaded area indicating the 95% confidence interval. The bottom row shows the topographic distribution of 40 Hz entrainment signal-to-noise ratios in both the experimental and control conditions. There was no evidence of 40 Hz neural entrainment effects during the task in either condition.

sessions. No evidence of in-task 40 Hz neural entrainment effects was observed, even when we examined different experimental conditions based on manipulations within each task (e.g. congruent and incongruent conditions in the Attention Network Test) (Figure 9). Although 40 Hz entrainment was not evident in the spectral analysis, we provide a summary of its effects, represented by the signal-to-noise ratio of each electrode during the 30-min 40 Hz sensory stimulation and control conditions, in Supplemental Table 4. Additionally, we compared specific frequency bands, including alpha and beta, across three conditions at the frontal and occipital regions between the 40 Hz sensory stimulation and control conditions, as shown in Supplemental Table 5.

Visual working memory

The d' and Cowan's K values were calculated, and a paired samples t-test was conducted to compare participants' visual working memory capacity based on d' and Cowan's K values between the 40 Hz stimulation and control sessions. The results showed no significant differences in both d' and Cowan's K values between the control session (M of d' = 2.049, M of Cowan's K = 3.768) and the 40 Hz experimental session (M of d' = 2.042, M of Cowan's K = 3.768) (d': $t(19) = 0.059$, $p = 0.954$; Cowan's K: $t(19) < 0.001$, $p = 1.000$), as shown in Figure 10 and Table 3.

We analyzed the power spectrum density of participants' EEG data while performing these tasks after a 30-min

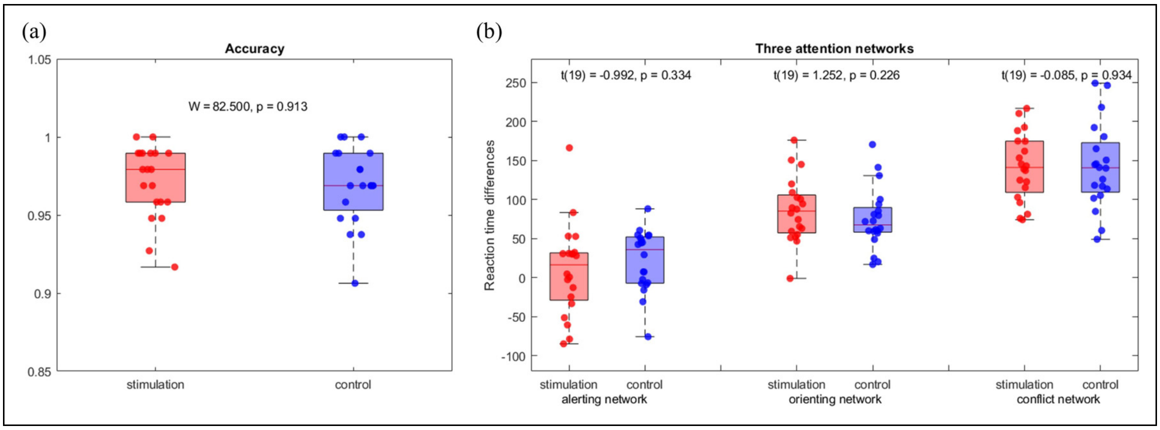


Figure 8. No significant improvement in performance of attention under 40 Hz sensory stimulation.
A paired samples t-test was conducted to compare participants' performance of attention network task between the 40 Hz experimental session and the control session, including accuracy (a) and three attention networks (b). Overall accuracy (a) in the control session (M = 96.88%) and the 40 Hz experimental session (M = 97.14%) were not significantly different (W = 82.500, p = 0.913, Cohen's d = 0.035). Similarly, three attention networks (b) between the 40 Hz session (M = 2.187) and the control session (M = 2.208) did not show a significant difference (alerting: t(19) = −0.992, p = 0.334, Cohen's d = −0.222; orienting: t(19) = 1.252, p = 0.226, Cohen's d = 0.280; conflict: t(19) = −0.085, p = 0.934, Cohen's d = −0.019).

Table 2. Overall accuracy and reaction time differences across three attention networks in 40 Hz experimental and control sessions.

	40 Hz experimental session		Control session				
	M	*SD*	*M*	*SD*	*t*(19)/z[a]	*p*	Cohen's *d*
Overall accuracy	97.14%	2.34%	96.88%	2.44%	82.500[a]	0.913	0.035
Alerting	9.55	58.69	21.72	39.36	−0.992	0.334	−0.222
Orienting	86.05	41.19	74.78	38.85	1.252	0.226	0.280
Conflict	141.36	43.11	142.29	54.55	−0.085	0.934	−0.019

aSince the Normality test (i.e. Shapiro-Wilk tests) suggest a deviation from normality, the overall accuracy was analyzed with Wilcoxon signed-rank test.

sensory stimulation, which included both 40 Hz stimulation and control sessions. However, no evidence of any in-task 40 Hz neural entrainment effects was observed, even when examining different experimental conditions based on manipulations in each task (e.g. hit and correct rejection conditions in the *Visual Working Memory Task*) (Figure 11). Although 40 Hz entrainment was not evident in the spectral analysis, we provide a summary of its effects, represented by the signal-to-noise ratio of each electrode during the 30-min 40 Hz sensory stimulation and control conditions, in Supplemental Table 6. Additionally, we compared specific frequency bands, including alpha and beta, across hit and correct rejection trials at the frontal and occipital regions between the 40 Hz sensory stimulation and control conditions, as shown in Supplemental Table 7.

Long term memory

To investigate whether participants' long-term memory performance improves with 40 Hz sensory stimulation, we conducted a paired samples t-test to compare participants' d' values between the 40 Hz experimental session and the control session. In the first retrieval phase (i.e. right after the 30-min stimulation), we found no significant difference in d' values between the 40 Hz experimental session and the control session (t(19) = −0.478, p = 0.638, Cohen's d = −0.107). Similarly, in the second retrieval phase, there was no significant difference in d' values between the 40 Hz experimental session and the control session (t(19) = 1.050, p = 0.307, Cohen's d = 0.235) (Figure 12 and Table 4).

Moreover, participants' accuracy for the three categories of photos (old1, old2, new) was also compared between the control session and the 40 Hz experimental session. Old2 photos indicate those that appeared in both the first and second retrieval phases, whereas old1 photos represent those appearing solely in the second retrieval phase. New photos were presented in the first retrieval phase but did not appear in the encoding phase. However, there were no significant differences in accuracy for old2, old1, and new photos between the control and the 40 Hz experimental sessions (Figure 13 and Table 5).

We further analyzed the relationship between the sequence of picture presentation during the encoding

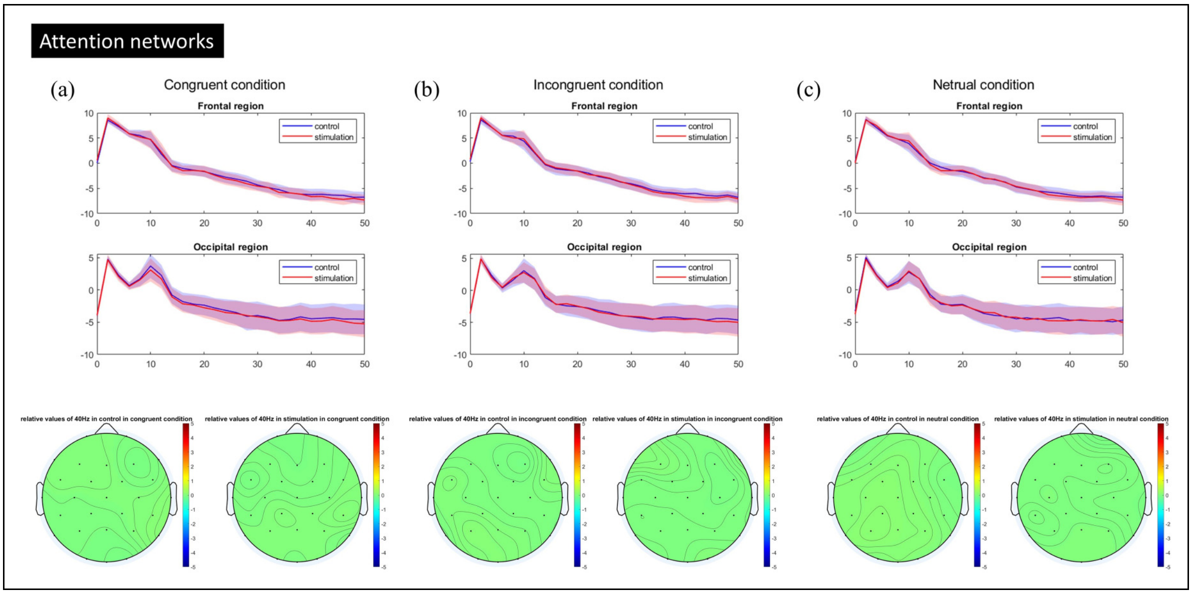


Figure 9. Average frequency spectrum and spatial distribution of 40 Hz neural entrainment across the scalp for attention network task.

The power spectrum density of participants' EEG data was analyzed for three conditions: congruent (a), incongruent (b), and neutral (c), following a 30-min sensory stimulation session that included both 40 Hz stimulation (red line) and control conditions (blue line). The plots illustrate the average frequency spectrum in frontal and occipital regions, with the shaded areas indicating the 95% confidence intervals. The bottom row shows the topographic distribution of 40 Hz entrainment signal-to-noise ratios for both the experimental and control conditions. There was no evidence of 40 Hz neural entrainment effects during the task in either condition.

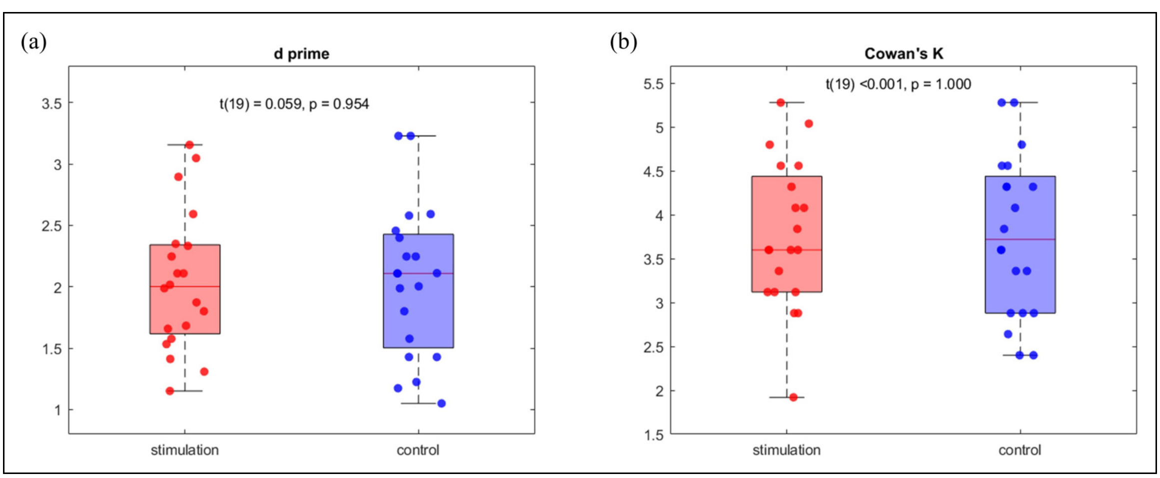


Figure 10. No significant improvement in visual working memory performance under 40 Hz sensory stimulation.

A paired samples t-test was conducted to compare participants' visual working memory performance between the 40 Hz experimental session and the control session, including d' values (a) and Cowan's K (b). The d' values in the control session ($M = 2.049$) and the 40 Hz experimental session ($M = 2.042$) were not significantly different ($t(19) = 0.059$, $p = 0.954$, Cohen's $d = 0.013$). Similarly, Cowan's K values between the control session ($M = 3.768$) and the 40 Hz experimental session ($M = 3.768$) did not show a significant difference ($t(19) < 0.001$, $p = 1.000$, Cohen's $d < 0.001$).

phase and the accuracy rate to explore the presence of a serial-position effect, which includes primacy and recency effects. We divided the 100 pictures presented during the encoding phase into bins of 10 based on their order. The picture encoding sequence for each participant was randomized. From our results, there appears to be a slight primacy effect but no noticeable recency effect in both the first and second retrieval phases (Figure 14(a) and (b)). Overall, the accuracy in the first retrieval phase was higher than that in the second retrieval phase (Figure 14(c)).

Table 3. d' value, and Cowan's K for visual working memory task in 40 Hz experimental and control sessions.

	40 Hz experimental session		Control session				
	M	*SD*	*M*	*SD*	*t*(19)	*p*	Cohen's *d*
d'	2.042	0.566	2.049	0.617	0.059	0.954	0.013
Cowan's K	3.768	0.835	3.768	0.908	<0.001	1.000	<0.001

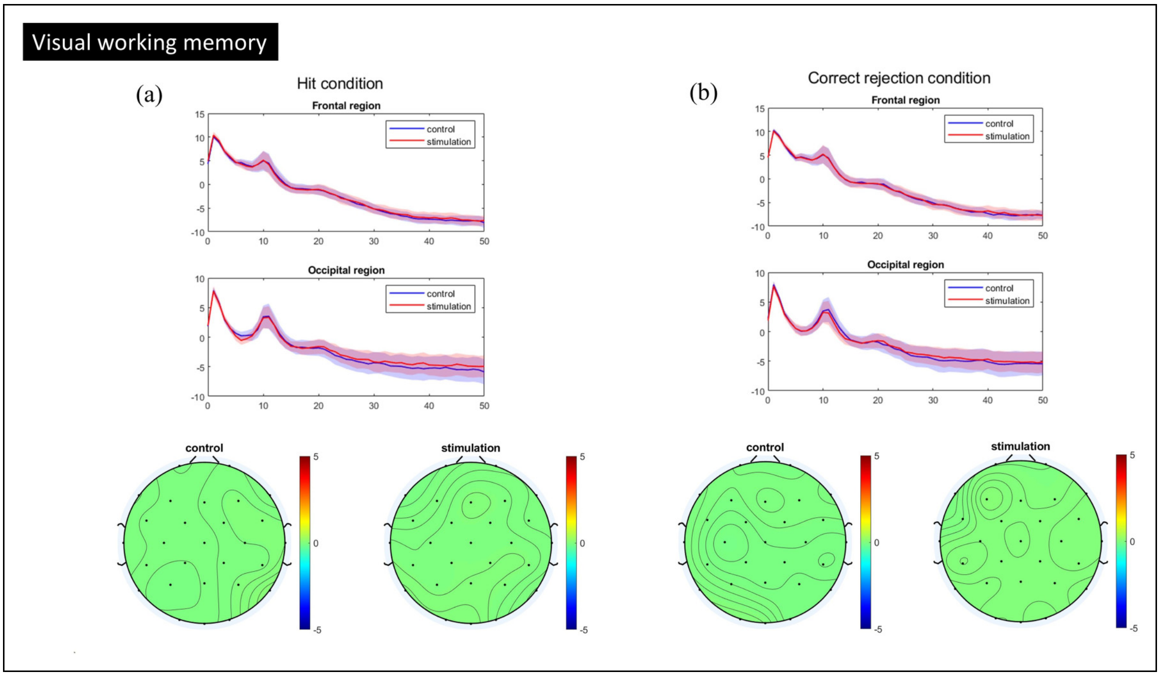


Figure 11. Average frequency spectrum and spatial distribution of 40 Hz neural entrainment across the scalp for visual working memory task.
The power spectrum density of participants' EEG data was divided into hit condition (left) and correct rejection (right) conditions based on their responses following a 30-min sensory stimulation session comprising both 40 Hz stimulation (red line) and control conditions (blue line). The plots illustrate the average frequency spectrum in frontal and occipital regions, with the shaded area indicating the 95% confidence interval. The bottom row shows the topographic distribution of 40 Hz entrainment signal-to-noise ratios in both the experimental and control conditions. There was no evidence of 40 Hz neural entrainment effects during the task in either condition.

We analyzed the power spectrum density of participants' EEG data while performing these tasks after a 30-min sensory stimulation, which included both 40 Hz stimulation and control sessions. However, no evidence of any in-task 40 Hz neural entrainment effects was observed, even when different experimental conditions were examined based on task manipulations (e.g. hit and correct rejection conditions during first and second retrieval phases) (Figures 15 and 16). Although 40 Hz entrainment was not evident in the spectral analysis, we provide a summary of its effects, represented by the signal-to-noise ratio of each electrode during the first and second retrieval phases of the 30-min 40 Hz sensory stimulation and control conditions in Supplemental Table 8. Additionally, we compared specific frequency bands, including alpha and beta, across hit and correct rejection trials at the frontal and occipital regions during the first and second retrieval phases between the 40 Hz sensory stimulation and control conditions. The results are presented in Supplemental Tables 9 and 10, respectively.

Discussion

In this study we used 4 tasks that separately examined the effect of gamma sensory entrainment on visual perception, attention, working memory, and long-term memory. Participants received synchronized 40 Hz light and sound stimulation for 30 min, and then performed the 4 cognitive tasks. Previous studies have reported a lack of cognitive aftereffect with sensory stimulation, but did not have real-

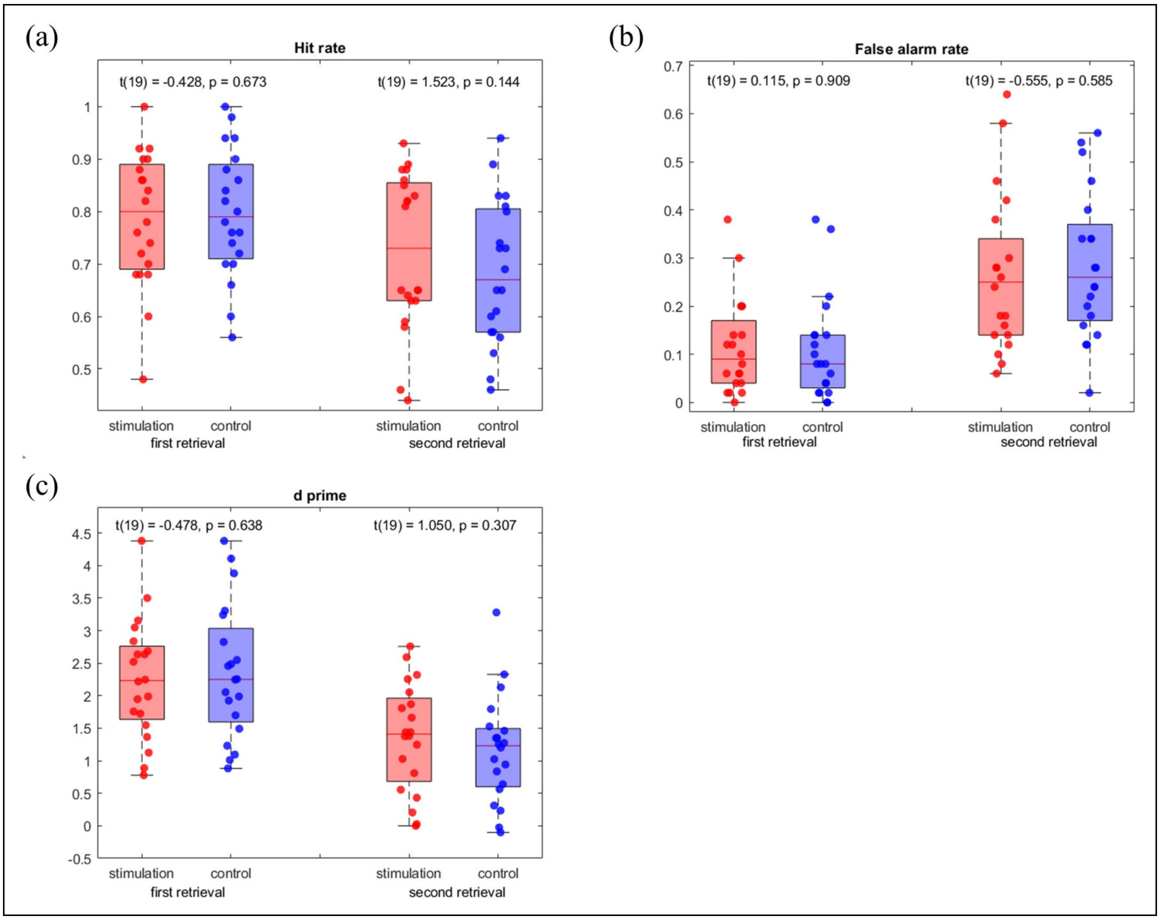


Figure 12. Comparison of long-term memory performance between control and 40 Hz experimental sessions in both the first and second retrieval phases.
Box plots show hit rate (a), false alarm rate (b), and d' values (c) for both the first and second retrieval phases in the control and 40 Hz experimental sessions. A paired samples t-test revealed no significant difference in hit rate (a) between the 40 Hz experimental session and the control session in the first retrieval phase ($t(19) = -0.428$, $p = 0.673$) or in the second retrieval phase ($t(19) = 1.523$, $p = 0.144$). Similarly, false alarm rate (b) between the control session and the 40 Hz experimental session were not significantly different in the first retrieval phase ($t(19) = 0.115$, $p = 0.909$) or in the second retrieval phase ($t(19) = -0.555$, $p = 0.585$). Also, the d' values (c) between the 40 Hz session and the control session did not show a significant difference in the first retrieval phase ($t(19) = -0.478$, $p = 0.638$) or in the second retrieval phase ($t(19) = 1.050$, $p = 0.307$).

Table 4. Hit rate, false alarm rate, and d' value of the first and second retrieval phases for long term memory task in 40 Hz experimental and control sessions.

	40 Hz experimental session		Control session		*t*(19)	*p*	Cohen's *d*
	M	*SD*	*M*	*SD*			
First retrieval phase							
Hit rate	0.786	0.127	0.797	0.122	−0.428	0.673	−0.096
False alarm rate	0.115	0.100	0.112	0.108	0.115	0.909	0.026
d'	2.249	0.909	2.356	1.024	−0.478	0.638	−0.107
Second retrieval phase							
Hit rate	0.725	0.149	0.683	0.137	1.523	0.144	0.341
False alarm rate	0.264	0.162	0.285	0.152	−0.555	0.585	−0.124
d'	1.363	0.831	1.169	0.824	1.050	0.307	0.235

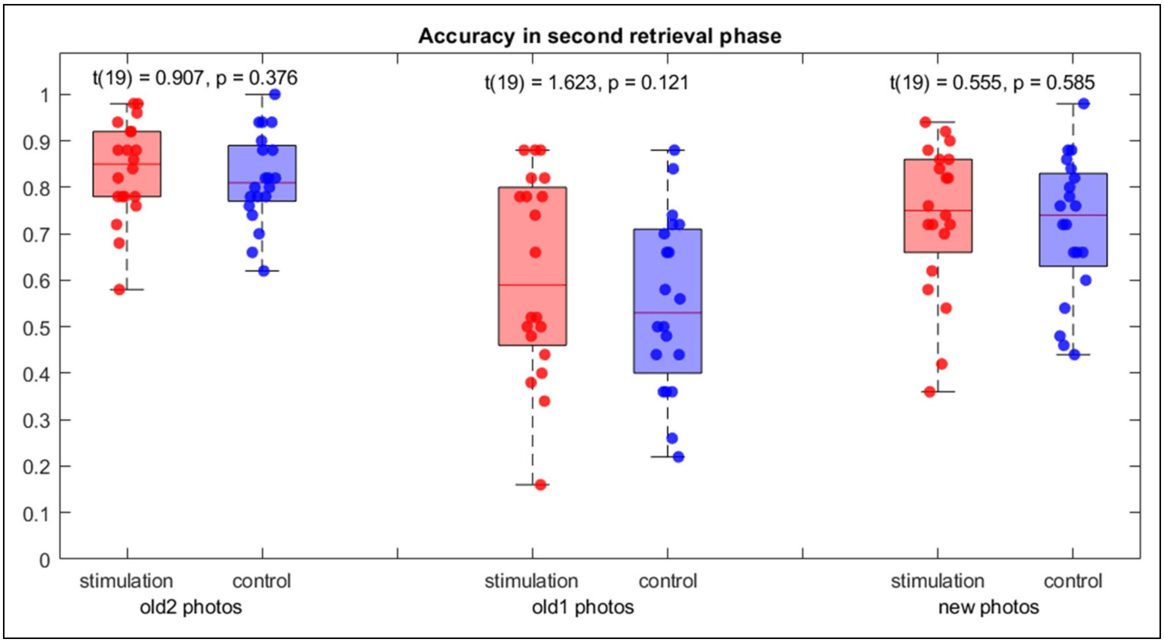


Figure 13. Accuracy in second retrieval phase for different categories of photos.
Box plot comparing participants' accuracy for three categories of photos (old2, old1, new) between the control session and the 40 Hz experimental session during the second retrieval phase. No significant differences in accuracy were observed between the control and 40 Hz experimental sessions for photo categories old2 ($t(19) = 0.907$, $p = 0.376$), old1 ($t(19) = 1.623$, $p = 0.121$), and new ($t(19) = 0.555$, $p = 0.585$).

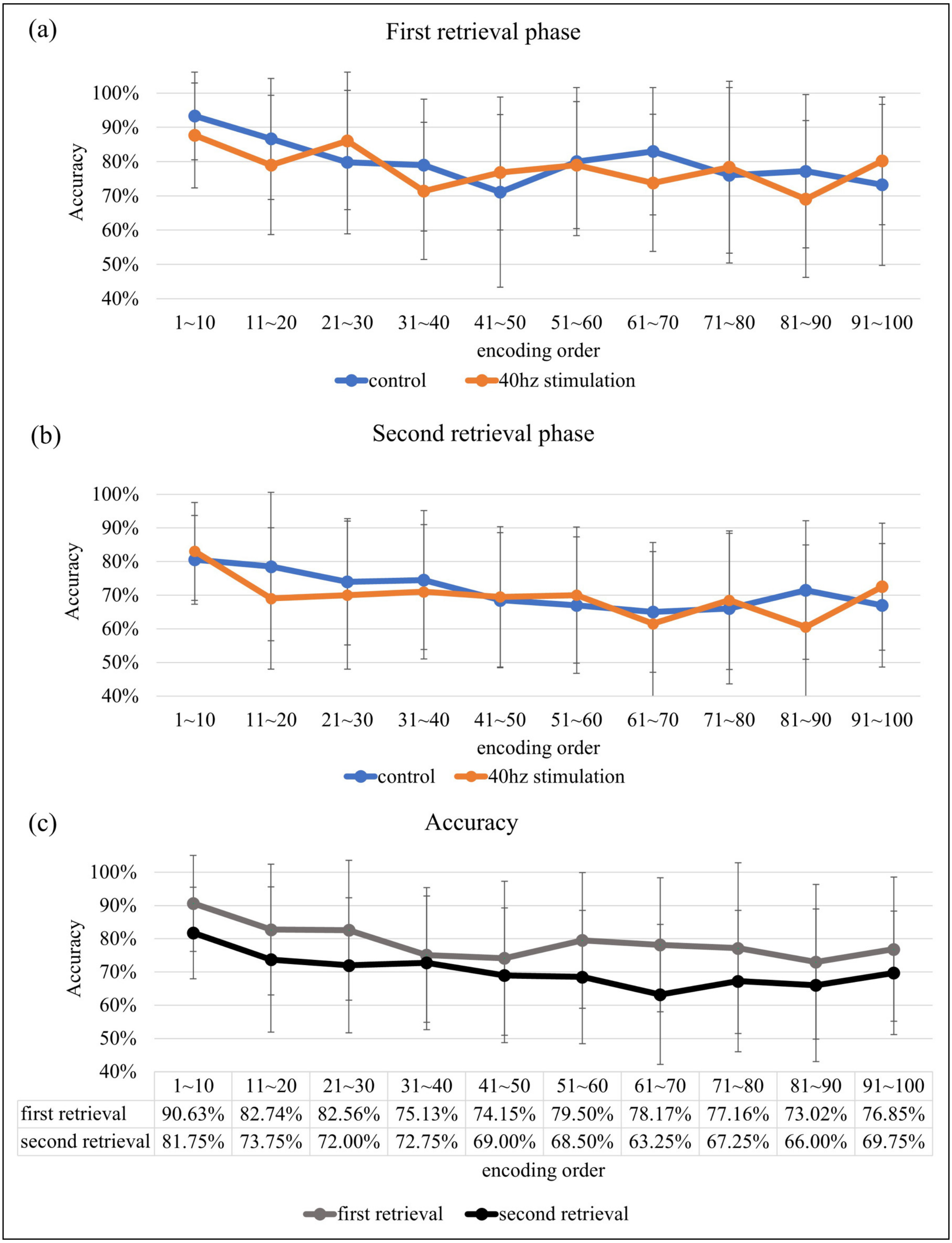
Table 5. Accuracy in old2, old1, and new photos in the second retrieval phase.

	40 Hz experimental session		Control session				
	M	*SD*	*M*	*SD*	*t*(19)	*p*	Cohen's *d*
Second retrieval phase							
Old2 photos	83.60%	10.52%	81.80%	9.86%	0.907	0.376	0.203
Old1 photos	61.30%	21.42%	54.90%	18.78%	1.623	0.121	0.363
New photos	73.60%	16.20%	71.50%	15.16%	0.555	0.585	0.124

time EEG data to suggest that 40 Hz entrainment did in fact take place. To this end, we observed robust 40 Hz entrainment effect in real-time EEG during the 30 min sensory stimulation (Figure 5), thus confirming the validity of our manipulation. However, we observed no improvement in any of the tasks (i.e. visual perception, attention network task, long-term memory task). Post-stimulation in-task EEG data also revealed no difference between control and stimulation sessions. This was true not just for 40 Hz, but for every frequency. Based on these results, we conclude that single-session 40 Hz gamma stimulation can entrain real-time (i.e. online) EEG, but 1) such entrainment is not observable when the stimulation is turned off, and 2) it is not able to induce significant changes in both EEG signals and cognitive performance, including perception, attention, short-term and long-term memory.

It is important to note that our observations here do not necessarily contradict with the idea that 40 Hz sensory entrainment can benefit cognitive functioning in the long run. Our results suggest that single session of gamma entrainment is unlikely to be sufficient to induce the desired cognitive changes. Therefore, repeated stimulation in a longitudinal fashion is necessary, and future studies should plan for more time (e.g. 1 to 6 months) to ensure adequate entrainment. Mechanistically, the absence of single-session effect in our study also favors the astrocytic and glymphatic hypothesis.[12]

Many longitudinal studies to date have already documented improved cognitive functioning with gamma sensory entrainment.[13–15] Recently, it was also demonstrated that single-session, real-time, EEG oscillations at 40 Hz can be indicative of future cognitive improvement.[17] In that study, the authors found stronger real-time EEG entrainment response to



	1~10	11~20	21~30	31~40	41~50	51~60	61~70	71~80	81~90	91~100
first retrieval	90.63%	82.74%	82.56%	75.13%	74.15%	79.50%	78.17%	77.16%	73.02%	76.85%
second retrieval	81.75%	73.75%	72.00%	72.75%	69.00%	68.50%	63.25%	67.25%	66.00%	69.75%

Figure 14. Primacy effect and recency effect in the first and second retrieval phases.
We divided the 100 pictures presented during the encoding phase into 10 bins based on their order. Here, we show the accuracy of the 10 images within each bin and list them separately for the 40 Hz stimulation and control sessions during the first retrieval phase in (a) and the second retrieval phase in (b). In (c) panel, we averaged accuracy for 40 Hz stimulation and control sessions, and plot primacy and recency effects for first and second retrieval phases. (Error bars show standard deviations.).

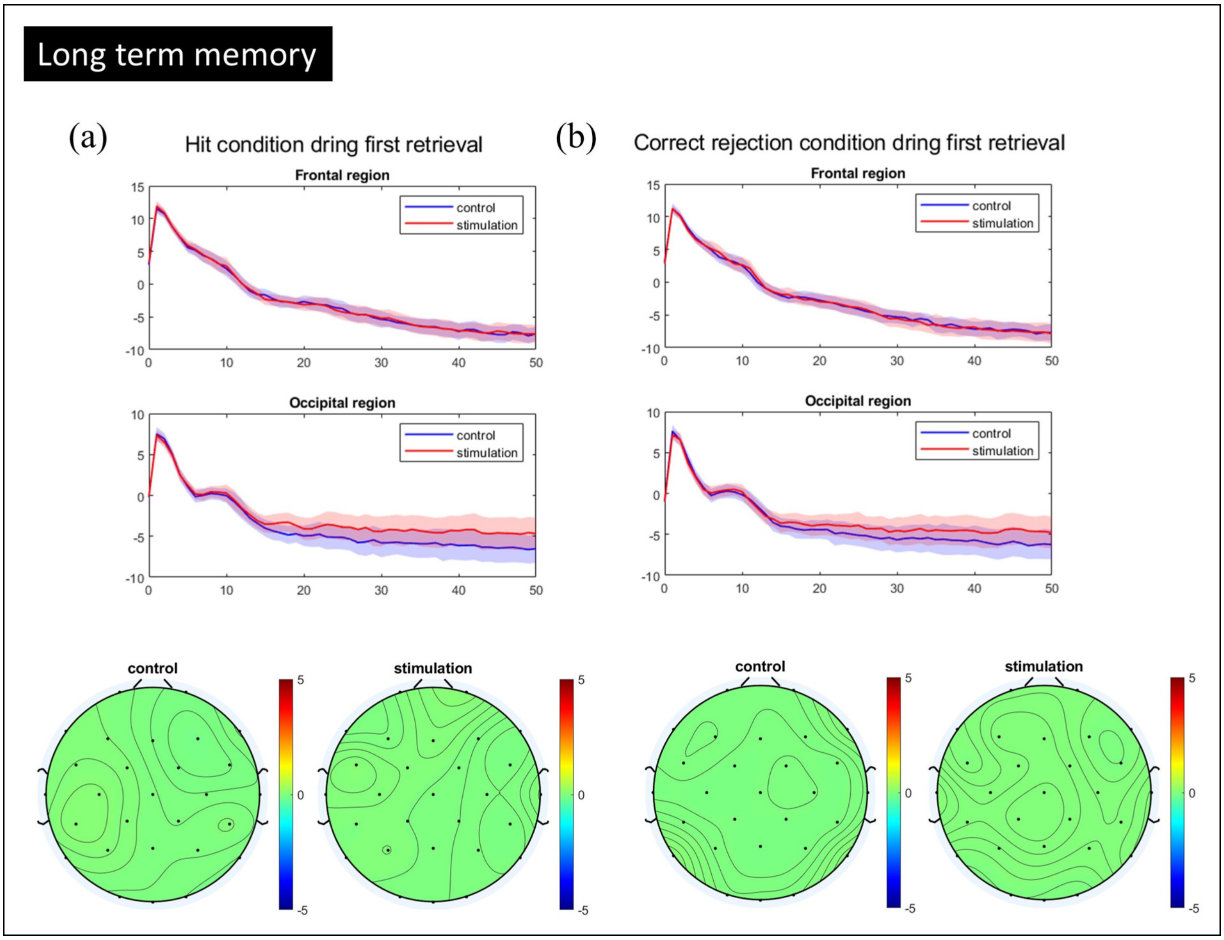


Figure 15. Average frequency spectrum and spatial distribution of 40 Hz neural entrainment across the scalp for first retrieval phase of long-term memory task.

auditory over visual stimulation, which happens to coincide with better behavioral outcome with auditory stimulation.[10] Therefore, rather than contradicting the literature on longitudinal 40 Hz stimulation, the current findings should be considered as complementing evidence, which together suggest that 40 Hz sensory entrainment requires consistent long-term exposure, and cannot be readily tested in one session. This is similar with one recent study by Hsiung and Hsieh,[28] who used a similar set of cognitive tasks as we did here (except for attention and long-term memory) and found no cognitive benefit with one single session of 40 Hz entrainment. Despite not having EEG evidence to validate their entrainment effect, the lack of cognitive effects is consistent with the present study.

There are several limitations worth noting about the current study design. First, the current control condition involves constant light and sound, while a non-rhythmic flickering or alternative-frequency flickering (e.g. 80 Hz) might have served as better control for potential placebo effects (see study by Iaccarino et al.,[8] for use of multiple frequencies as controls). Second, the LTM task was always placed at the end to keep the elapsed time long enough. However, this inevitably introduces fatigue to the task that cannot be counterbalanced out as we did for the other 3 tasks. Therefore, our lack of randomization may have underestimated a potential effect in LTM since the elapsed time is always long, which may confound with fatigue, memory decay, etc. Lastly, it is possible that certain cognitive domains are less responsive to short-term gamma entrainment. For example, cognitive control or decision-making are important functions that patients with AD struggle with but are not directly tested in our study. Therefore, future studies can build on top of our null results here and attempt to test other cognitive functions instead.

In conclusion, the present study suggests that single-session 40 Hz entrainment cannot induce an observable aftereffect in EEG oscillation and cognitive performance. To contextualize our findings with the literature, it is evident that 40 Hz sensory entrainment requires long-term exposure. We already know that 90 days are enough to induce cognitive and neurological changes,[14] and the present study suggests that 1 day is not. Future studies

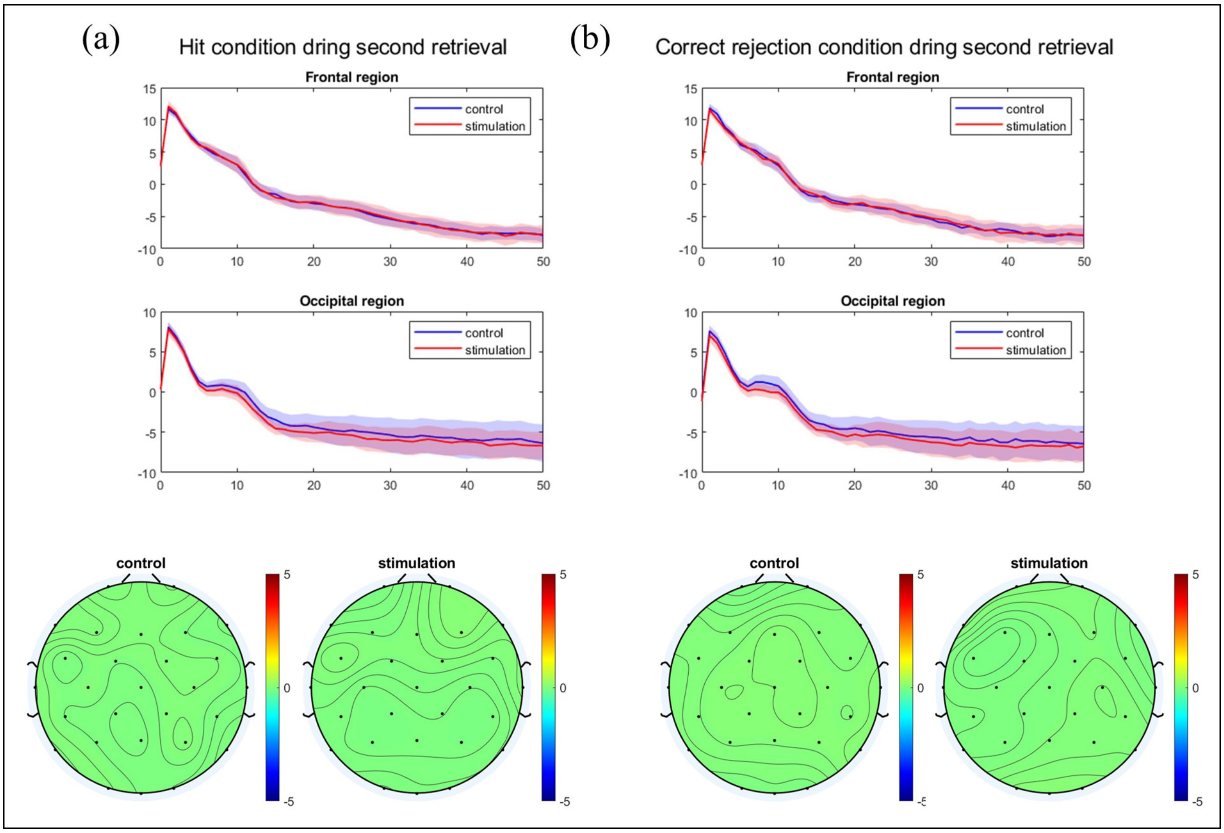


Figure 16. Average frequency spectrum and spatial distribution of 40 Hz neural entrainment across the scalp for second retrieval phase of long-term memory task.
Average frequency spectrum for both hit conditions (a) and correct rejection conditions (b) are shown, aggregated across subjects, with the shaded area representing the 95% confidence interval. The topographic distribution of 40 Hz entrainment signal-to-noise ratios in the 40 Hz experimental and control conditions is illustrated in the bottom row. Analysis of the power spectrum density of participants' EEG data during the tasks after a 30-min sensory stimulation revealed no evidence of in-task 40 Hz neural entrainment effects in either condition.

should explore exactly how long is long enough, both in terms of time (i.e. how much time per day) and sessions (i.e. how many days total), to induce meaningful changes in both EEG oscillation and cognitive functioning.

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Statements and declarations

Author contributions

Cai-Syuan Wu (Conceptualization; Data curation; Formal analysis; Methodology; Writing – original draft); Ting-Xuan Lin (Conceptualization; Data curation; Formal analysis; Methodology; Writing – original draft); Yu-Hui Lo (Conceptualization; Data curation; Formal analysis; Methodology; Writing – original draft); Shih-Chiang Ke (Conceptualization; Methodology); Prangya Parimita Sahu (Methodology; Software); Philip Tseng (Conceptualization; Funding acquisition; Methodology; Supervision; Writing – original draft; Writing – review & editing).

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Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Data availability

The data supporting the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Supplemental material

Supplemental material for this article is available online.

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