Forty-Hertz audiovisual stimulation does not have a promoting effect on visual threshold and visual spatial memory

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Previous research has demonstrated that 40-Hz audiovisual stimulation can improve pathological conditions and promote cognitive function in mouse models of Alzheimer's disease. However, limited research has been conducted on humans, and the results have been inconsistent. In our study, we divided participants into an experimental group and a control group to investigate whether 40-Hz stimulation could enhance performance in visual threshold tasks and working memory task. In Experiment 1, we used a light bulb as the stimulus source and found a general practice effect, but no difference between the groups. In Experiment 2, we used a computer screen as the stimulus source and set the stimulation frequency to 48 Hz. In Experiment 3 , we used a computer screen and audio as stimulus sources, simultaneously applying a 40-Hz stimulation to both visual and auditory modalities. Both experiments only revealed the disappearance of practice effects in the 40-Hz (48-Hz) group. Experiment 4 focused on testing visual spatial memory, but did not identify any significant differences between or within groups. In Experiment 5, we tested various visual spatial frequencies; yet again, no significant differences were found. Based on the comprehensive results, we conclude that a 40-Hz stimulation does not have a promoting effect on visual threshold or visual spatial memory.

Introduction

Forty-Hertz and Alzheimer's disease mouse models

Gamma waves, also referred to as gamma rhythms, are brain waves with frequencies ranging from 30 Hz to 100 Hz in the electroencephalogram signal, with 40 Hz being the most prominent frequency (McDermott et al., 2018). In the past, 40-Hz brain waves were believed to be associated with cognitive and perceptual functions such as vision (Başar-Eroglu, Strüber, Schürmann, Stadler, & Başar, 1996). Furthermore, it has been observed that physical stimuli flickering at the gamma frequency can capture attention (Bauer, Cheadle, Parton, Müller, & Usher, 2009) and induce synchronization of corresponding brain waves (Jones et al., 2019). Recent research has discovered cognitive-enhancing effects of 40 Hz in patients with dementia (Chan et al., 2021) and even in cognitively healthy individuals (Sharpe, Mahmud, Kaiser, & Chen, 2020). However, findings from studies conducted on healthy adults have been inconsistent.

Given the close relationship between gamma frequency brain waves and cognitive function, previous studies have focused on abnormalities in gamma frequency brain waves in patients with cognitive impairments, particularly in those with Alzheimer's disease. For instance, it has been observed that patients with Alzheimer's disease exhibit slower gamma frequency brain wave responses during cognitive tasks compared with healthy adults (Başar, Emek-Savaş, Güntekin, & Yener, 2016). Additionally, patients with Alzheimer's disease have shown greater gamma frequency responses compared with individuals with mild cognitive impairment and healthy individuals during rest, visual tasks, and while listening to stories or music (Van Deursen, Vuurman, Verhey, van Kranen-Mastenbroek, & Riedel, 2008). Subsequent research efforts have explored the use of 40-Hz stimulation as a potential therapeutic approach for Alzheimer's disease in animal models (e.g., Iaccarino et al., 2016; Singer et al., 2018; Etter et al., 2019; Martorell et al., 2019; Park et al., 2020; but see Soula et al., 2023 and Schneider et al., 2023) that 40-Hz stimulation does not affect innate gamma oscillations and does not affect deeper structures (like the hippocampus) that are relevant for memory.

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Forty-Hertz stimulation in studies of patients with Alzheimer's disease

Similar to the findings in animal models, studies conducted on patients with Alzheimer's disease have reported positive effects of 40-Hz stimulation. Patients who received several months of 40-Hz audiovisual stimulation therapy showed decreased brain atrophy, improved memory performance, and sleep improvement (Chan et al., 2021; Cimenser et al., 2021; He et al., 2021). Furthermore, studies focusing on auditory 40-Hz stimulation alone have reported improved mood, cognition, and happiness in patients after 8 weeks of treatment, with sustained improvement observed in long term treatment for up to 1 year (Clements-Cortes, Ahonen, Evans, Freedman, & Bartel, 2016; Clement & Bartel, 2022). However, it is important to note that the effects of this treatment may require longer durations to become evident; Ismail et al. (2018) found no significant differences in brain volume when treating patients with Alzheimer's disease with 10 days of 40-Hz light stimulation.

Forty-Hertz stimulation in healthy individuals

Research on the effects of 40-Hz stimulation in healthy individuals remains limited, and the behavioral outcomes have shown more divergent results. However, it has been consistently observed that both auditory and visual stimulation at 40 Hz can induce brain wave synchronization or elicit corresponding responses in the brain (Jones et al., 2019; Ross & Lopez, 2020; Chan et al., 2021; Lee et al., 2021; Noda et al., 2021; Lin et al., 2021; Agger et al., 2022; Khachatryan et al., 2022). Lin et al. (2021) investigated effects of 40-Hz visual stimulation on memory test performance in healthy adults. Although differences were observed in regions such as the hippocampus through functional magnetic resonance imaging, no behavioral differences were found. To date, this study was the only one that has examined the behavioral effects of single visual 40-Hz stimulation, and no short-term cognitive enhancement effects of 40-Hz light stimulation have been identified. Conversely, there have been more studies on auditory 40-Hz stimulation, providing more support for its short-term cognitive enhancement effects. These studies have used binaural beats as a means of stimulation presentation, where participants listen to different audio frequencies in each ear to create an auditory illusion of a 40-Hz tone. Colzato, Barone, Sellaro, & Hommel (2017) found that participants who listened to 40-Hz binaural beats showed an interaction effect between group and stimulus type (global or local) in a global–local task, suggesting that the 40-Hz group exhibited a smaller increase in response time in the local condition compared with the control group. Similarly, Wang, Zhang, Li, & Yang (2022) observed improved memory performance in participants who listened to 40-Hz binaural beats after being exposed to white noise in a pretest. Shekar, Suryavanshi, & Nayak (2018) reported faster visual and auditory simple reaction times in the 40-Hz group, whereas Sharpe et al. (2020) found greater long-term emotional improvement. Nevertheless, these effects could potentially be attributed to practice effects, and there have also been studies that did not find short-term cognitive enhancement effects of auditory 40-Hz stimulation. Jirakittayakorn and Wongsawat (2017) discovered no overall difference in word recall accuracy in participants who listened to 40-Hz binaural beats. Similarly, other studies have reported no short-term memory enhancement effects of 40-Hz stimulation (Borges, Arantes, & Naves, 2023; Shekar et al., 2018). Ross and Lopez (2020) found that participants who listened to 40-Hz binaural beats on the first day and 16 Hz on the second day performed better in an attentional blink-related task compared with the group with the reverse order, but there were no differences on the third day. Although the authors interpreted this as a delayed gain effect of 40-Hz stimulation, it could also be explained as a short-term inhibition effect of 40 Hz. Additionally, Hommel, Sellaro, Fischer, Borg, & Colzato (2016) found that in a continuous number magnitude judgment task, the interaction between group and the consistency between the first and second target answers showed that the 40-Hz group had longer reaction times when the answers were inconsistent. In summary, among studies conducted on healthy adults, only auditory 40-Hz binaural beats, but not visual 40 Hz, have shown promoting effects, and multiple studies have not found such effects. Therefore, the cognitive enhancement effects of 40-Hz stimulation remain unclear. Here we aimed to investigate the cognitive enhancement effects of 40-Hz stimulation, including both less-explored low-level perceptual functions such as visual threshold and commonly studied higher level cognitive functions like short-term memory.

Experiment 1

Participants

University students were recruited from the NTU community Facebook group. In Experiments 1 and 2, a total of 40 participants were recruited in each experiment, and in Experiments 3, 4, and 5, 30 participants were recruited in each experiment. The majority of participants were university students, aged between 18 and 22 years. The overall average age of participants was approximately 20 years, with a nearly

equal male-to-female ratio. Every participant had normal or corrected-to-normal vision. This work was approved by the Ethics Committee of Behavioral and Social Science at the National Taiwan University and was conducted according to its guidelines. Before the experiment, written informed consent was given by all participants.

Experiment set up

Participants underwent the experiment in a soundproof dark room. The only light source in the room was the computer screen to ensure that there were no surrounding environmental light sources affecting the participants' visual perception. The background brightness in the dark room was less than 0.1 lux. During the experiment, the participants' chin was fixed to maintain a consistent viewing distance and height. Experiments were carried out within the time frame of 10:00 AM to 9:00 PM. It is important to note that the timing of these experiments was balanced on average across both groups. Consequently, the time of day should not introduce any confounding factors when assessing and comparing the outcomes between the two groups.

Computer screen

Participants were positioned at a distance of 57 cm from the screen, and the height was adjusted so that the participants could view the center of the screen at eye level. The size of the screen was 60.0 × 34.5 cm. The screen refresh rate was 60 Hz.

LED bulb and signal generator

The LED bulb was driven by a signal generator generating a 40-Hz sinusoidal signal with a voltage of 10 volts. The bulb used was an E10 type and was placed at the middle top edge of the screen. The continuous light source, also known as the steady light, was produced by the same signal generator. The frequency used for the steady light was the highest frequency of the signal generator, which was 1,250 Hz, approximately equivalent to a continuous light source. The illuminance measured at the participants' viewing distance (57 cm away) was approximately 6 lux.

Procedures

Participants were divided into two groups: the 40-Hz group and the control group. The former received steady light stimulation during the pretest, followed by 3 minutes of exposure to 40-Hz light, and continued to receive light stimulation until the end of the post-test. The latter only received steady light throughout the entire experiment.

Visibility task

The visibility task was a sandwich masking two-alternative forced choice task including 100 trials. In each trial, participants were asked to indicate whether they saw the target and to indicate whether it appeared on the left or right side. The contrast of the target decreased if they answer correctly and increase if they answer incorrectly. The experiment was run using PsychoPy. The mask stimulus was a noise image, and the actual image was provided as an attachment, with a size of 9 cm. The target stimulus was generated using the GratingStim function in PsychoPy, with the parameters "tex='sin', mask='gauss'", a size of 3 cm, and a default spatial frequency of approximately 1.25 cycles/cm. The parameter "contrast" was manipulated during the experiment. Each trial started with a fixation point for 2 seconds. In the first trial, there was an additional one-half-second to allow the participants to prepare. Subsequently, a forward mask of 116 ms (7 frames at 60 Hz) was presented, followed by a target stimulus of 17 ms (1 frame at 60 Hz), and finally, a backward mask of 116 ms (7 frames at 60 Hz) was presented. After the backward mask, there was a one-half-second interval, followed by the first question: "Did you see the stimulus?" The participants used the 1, 2, and 3 keys in the upper left of the keyboard with their left hand to indicate "yes," "no," or "uncertain," respectively. After their response, the second question appeared: "On which side did the stimulus appear?" The participants used the "a" and "d" keys with their left hand to indicate "left" or "right," respectively. After their response, feedback on the correctness of the second question was provided before proceeding to the next trial. The target stimulus always appeared in the middle of the square noise stimulus, and it always appeared on one side only. Whether it appeared on the left or right side was determined by a pregenerated random sequence that includes 50 left and 50 right appearances. If the participant answered "yes" to the "Did you see the stimulus?" question and correctly answered the location of the stimulus, the contrast of the target decreased in the next trial. In all other cases, the contrast increased (Figure 1).

There were two blocks for each pretest and post-test. One of the initial contrasts (ICs) would begin at 0.7, and the other would begin at 0.3. The initial increment or decrement in contrast value for the first trial was 0.1, and after every 10 trials, this value decreased by 0.01, meaning that the increment or decrement was 0.1 for trials 1 to 10, 0.09 for trials 11 to 20, and so on, until trials 91 to 100, where it was 0.01.

With this design, participants could approach the visual threshold more quickly in the initial rounds,

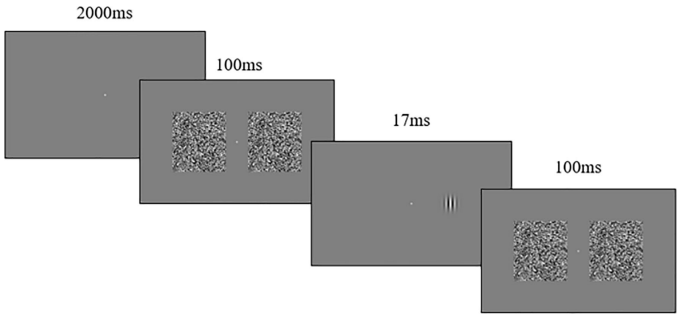

Figure 1. Visibility threshold task.

decreasing the wastage of trials, while also ensuring that the contrast changes were smaller when approaching the visual threshold.

Data analysis

To begin, we computed the average of the last five reversal points for each participant within each block, which served as the block's score. Subsequently, we derived six distinct scores:

1. "Pretest threshold $_{IC=0.7}$" (representing the visual threshold measured with an IC of 0.7 in the pretest).
2. "Pretest threshold $_{IC=0.3}$" (representing the visual threshold measured with an IC of 0.3 in the pretest).
3. "Post-test threshold $_{IC=0.7}$" (representing the visual threshold measured with an IC of 0.7 in the post-test).
4. "Post-test threshold $_{IC=0.3}$" (representing the visual threshold measured with an IC of 0.3 in the post-test).
5. "(Pretest threshold $_{IC=0.7}$) – (pretest threshold $_{IC=0.3}$)" (signifying the difference between the two pretest scores).
6. "(Post-test threshold $_{IC=0.7}$) – (Post-test threshold $_{IC=0.3}$)" (signifying the difference between the two post-test scores).

After computation of these score sets, we identified and removed outliers, characterized by scores deviating by more than two standard deviations from the mean across all participants. Ultimately, we conducted analysis of variance and *t* tests using Python to analyze the data.

Results and discussion

In this experiment, the 40-Hz group, the pretest average is 0.42 with a standard deviation of 0.2, and the post-test average is 0.27 with a standard deviation of 0.13. In the control group, the pretest average is 0.42 with a standard deviation of 0.23, and the post-test average is 0.29 with a standard deviation of 0.21. In this experiment, significant practice effects were observed for both the control group and the 40-Hz group, $t(16) = 3.769$, $p = 0.002$; $t(16) = 5.871$, $p < 0.001$. However, there was no significant interaction effect between the groups, $F(1,32) = 0.414$, $p = 0.524$, and there were no significant differences in scores between pretest and post-test for both groups, $t(32) = -0.27$, $p = 0.789$; $t(32) = 0.23$, $p = 0.819$ (Figure 2).

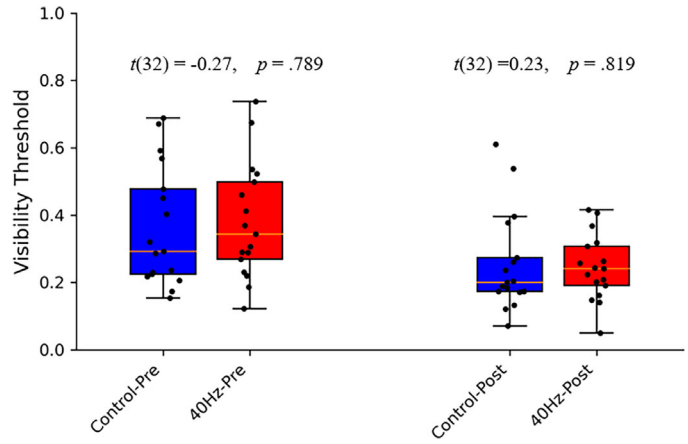

Figure 2. Experiment 1 visibility threshold result.

We also used a linear regression model for Bayesian statistical testing and obtained the following results. The estimated difference in pre- and post-test scores for the control group is 0.57, with a 95% confidence interval ranging from 0.17 to 0.90. The estimated difference for the experimental group is 0.46, with a 95% confidence interval ranging from 0.30 to 0.61. The estimated difference between the two groups in the pretest is 0.02, with a 95% confidence interval ranging from −0.09 to 0.15, and the estimated difference in the post-test is 0.07, with a 95% confidence interval ranging from −0.00 to 0.13. These results are consistent with those obtained using *t* tests.

In this experiment, we only observed practice effects without significant interaction or post-test differences between the two groups, indicating no facilitative effect of the 40-Hz stimulation. We speculated that this lack of effect might be attributed to the insufficient intensity of the light source from the light bulb, which could have hindered the observation of any significant effects. To address this, in Experiment 2, we used a computer screen with stronger intensity light, as the flickering stimulus, aiming to enhance the effectiveness of the stimulation.

Experiment 2

In Experiment 2, we maintained the same conditions as in Experiment 1, with the exception that we replaced the original flickering stimulus with a 48-Hz screen

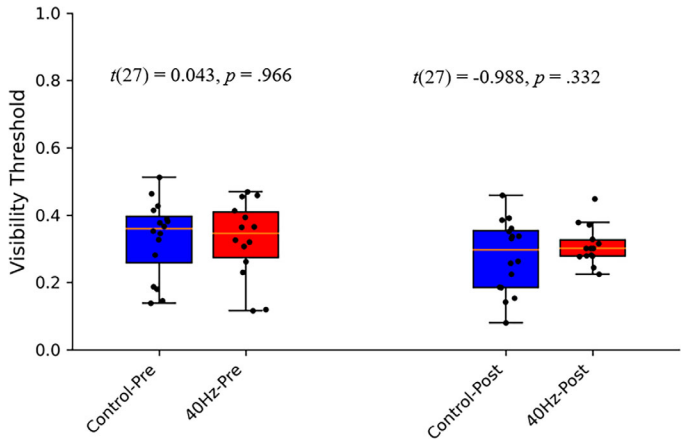

Figure 3. Experiment 2 visibility threshold result.

flickering stimulus to explore whether other gamma frequencies close to, but not exactly 40 Hz, could be effective. The flickering stimulus was displayed in the background, and the monitor operated at a frequency of 144 Hz.

Results and discussion

In Experiment 2, we used the same analysis methods as in Experiment 1. In the 40-Hz group, the pretest average is 0.36 with a standard deviation of 0.12, and the post-test average is 0.31 with a standard deviation of 0.08. In the control group, the pretest average is 0.36 with a standard deviation of 0.14, and the post-test average is 0.31 with a standard deviation of 0.12. In Experiment 2, we used the same analysis methods as in Experiment 1. In the control group, a significant practice effect was observed, $t(15) = 2.981$, $p = 0.009$, but there was no practice effect in the 48-Hz group, $t(12) = 0.765$, $p = 0.458$. Moreover, no significant interaction effect was found between the groups, $F(1,27) = 1.219$, $p = 0.279$, and there were no significant differences in pretest scores, $t(27) = 0.043$, $p = 0.966$, and post-test scores, $t(27) = -0.988$, $p = 0.332$, between the groups (Figure 3).

The Bayesian statistical analysis revealed that the control group's practice effect was estimated at 0.7 with a 95% credible interval between 0.39 and 1.09. In contrast, the practice effect for the experimental group was more modest at 0.29, with a 95% credible interval spanning from 0.04 to 0.53. When comparing the pretest measurements, there was essentially no difference between the groups, with an estimated effect size of 0.00 and a 95% credible interval ranging from −0.08 to 0.09.The post-test measurements suggested a small estimated difference of 0.03, with a 95% credible interval between −0.03 and 0.1, indicating only a slight divergence in outcomes between the two groups following the intervention.

Under the condition of using a 48-Hz flickering stimulus, we observed the disappearance of the practice effect in the 48-Hz group. We had two conjectures for this result. First, it was possible that the facilitative effect of 40-Hz stimulation might only occur when precisely delivered at 40 Hz. Second, it was likely that the visual 40-Hz stimulation might not be potent enough to induce significant behavioral differences, and, therefore, additional auditory stimuli might be required to enhance the effect.

Experiment 3

Experiment 3 was identical to Experiment 2, with the exception that the 48-Hz screen flickering in the surroundings has been eliminated. Instead, a larger screen was used for 40-Hz visual flickering, and participants were asked to wear headphones to listen to a 40-Hz binaural beat. The left ear was presented with an audio frequency of 400 Hz, and the right ear a stimulus at 440 Hz, both at a sound level of 60 dB.

Results and discussion

In the 40-Hz group, the pretest average is 0.43 with a standard deviation of 0.2, and the post-test average is 0.4 with a standard deviation of 0.19. In the control group, the pretest average is 0.43 with a standard deviation of 0.14, and the post-test average is 0.4 with a standard deviation of 0.11. The practice effect in the control group showed significant results, $t(10) = 4.054$, $p = 0.002$, whereas the 40-Hz group did not exhibit significant effects, $t(11) = 1.101$, $p = 0.295$. Furthermore, there was no interaction effect observed, $F(1,21) = 0.327$, $p = 0.573$, and there were no significant differences between the groups, pretest: $t(21) = -0.948$, $p = 0.354$; post-test: $t(21) = -1.430$, $p = 0.168$ (Figure 4).

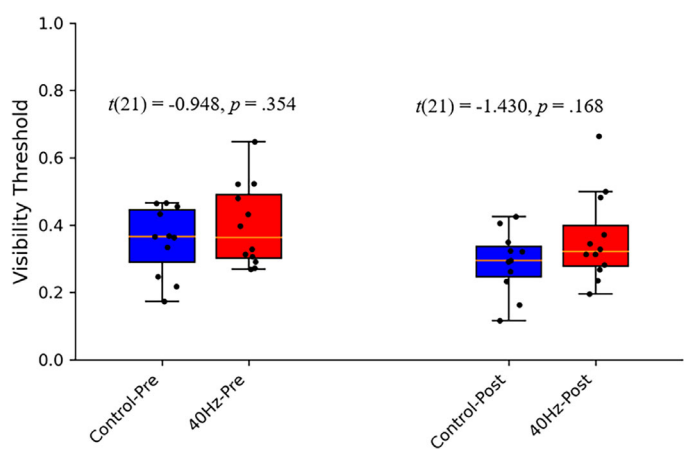

Figure 4. Experiment 3 visibility threshold result.

In the Bayesian statistical analysis, the control group showed a practice effect with an estimated increase of 0.6, and the 95% credible interval ranged from 0.17 to 1.04, indicating a positive effect albeit with a degree of uncertainty. The experimental group demonstrated a slightly higher practice effect, with an estimate of 0.64 and a 95% credible interval from 0.09 to 1.19, suggesting a potentially greater but similarly uncertain improvement.

Looking at the differences between the two groups, the estimated difference in the pretest measurements was minor, at 0.03, with the 95% credible interval stretching from −0.07 to 0.13, which implies there was no significant difference between the groups before the intervention. For the post-test measurements, the groups exhibited a slightly greater estimated difference of 0.07, with a 95% credible interval from −0.02 to 0.17, suggesting a small potential difference in practice effect after the intervention, although the credible interval indicates this result is not definitive.

In this experiment, we once again observed the practice effect only in the control group, consistent with the results from Experiment 2. No facilitative effects were found for the 40-Hz group. We speculated that the visual threshold task may not be sensitive enough to detect any facilitative effects. As a result, in subsequent experiments, we decided to change the task to a visual spatial memory test.

Experiment 4

The experimental design and procedures remained the same as in Experiment 3, with the only difference being the change in the task to a visual spatial memory task (Figure 5).

Visual spatial memory task

Each trial began with the presentation of a fixation point at the center of the screen for 125 ms, followed by the appearance of five small squares with random colors arranged in a regular pentagon shape around

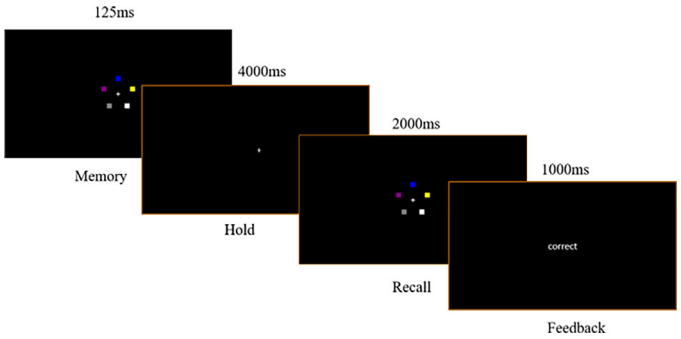

Figure 5. Visual spatial memory task.

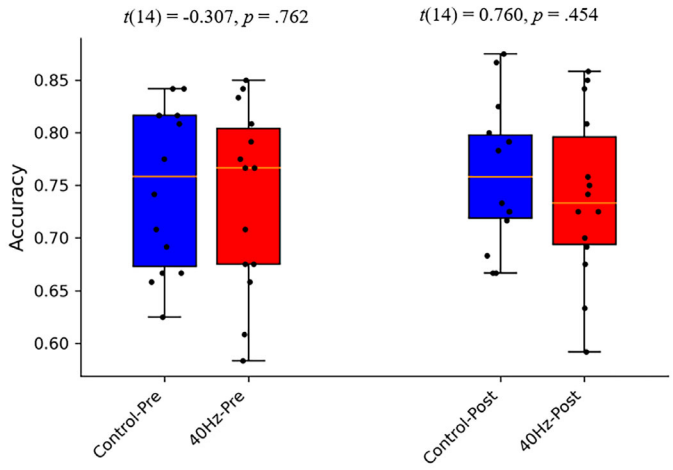

Figure 6. Experiment 4 Visual spatial memory accuracy result.

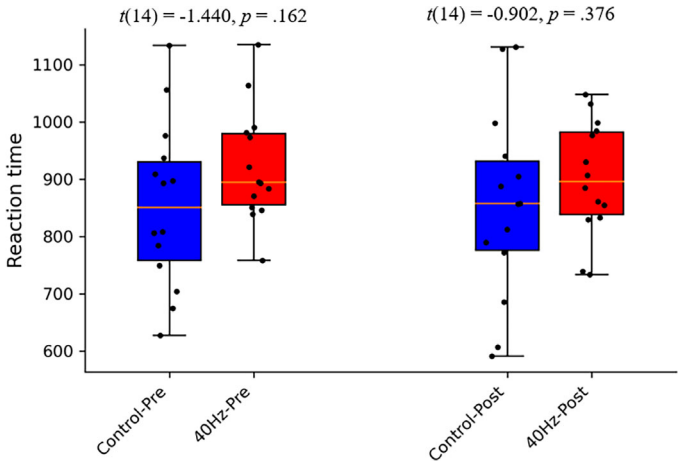

Figure 7. Experiment 4 visual spatial memory reaction time result.

the fixation point. After the initial display, the squares disappeared, leaving only the fixation point visible for a 4-second interval. Subsequently, the squares reappeared, with a 50% chance that they remained unchanged from the previous presentation or a 50% chance that one of the squares had its color altered (without repeating any existing colors). Participants were required to indicate whether the colors of the squares match the previous presentation by pressing "a" for a match or "d" for a mismatch. After their response, feedback was displayed for 1 second to indicate the accuracy of their answer.

Results and discussion

No significant differences between the groups or any interaction effects were found, including accuracy and reaction time (Figures 6 and 7). In the Bayesian statistical analysis for accuracy, the practice effect in the control group was estimated at 0.69 with a 95% credible

interval from 0.42 to 0.94, while the experimental group had an estimated practice effect of 0.73 with a 95% credible interval from 0.36 to 1.15. The estimated difference between the groups at pretest was 0.02 with a 95% credible interval from −0.06 to 0.09, ,and at post-test, the estimated difference was 0.00 with a 95% credible interval from −0.07 to 0.06.

For reaction time, the control group's practice effect was estimated at 1.02 with a 95% credible interval from 0.68 to 1.37. The experimental group's estimate was 0.8 with a 95% credible interval from 0.45 to 1.17. The estimated pretest difference between the groups was 74.81 with a 95% credible interval from −29.15 to 172.58, and the post-test difference was 52.66 with a 95% credible interval from −61.51 to 175.68.

In this experiment, no significant effects were found, suggesting that a 40-Hz stimulation may not have any facilitative effect on visual spatial memory. For the next experiment, we intended to further address the measurements related to visual threshold. In the previous experiments, the spatial frequency of the target objects remained consistent. It was possible that the facilitative effect of the 40-Hz stimulation occurs only at specific spatial frequencies. Therefore, we designed an experiment to address this measurement gap. Additionally, we enhanced the illumination by placing a light source in front of the eyes of the participants, increasing the illuminance to 200 lux.

Experiment 5

The experimental design was the same as Experiment 4, but the light source of the screen flicker was changed to a light bulb placed in front of the eyes, with a luminance of approximately 200 lux. Therefore, both binaural beats and visual stimuli

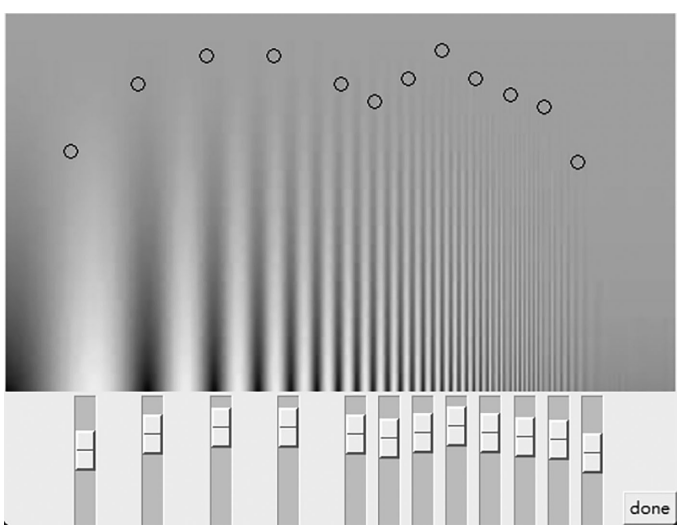

Figure 8. Visual threshold task.

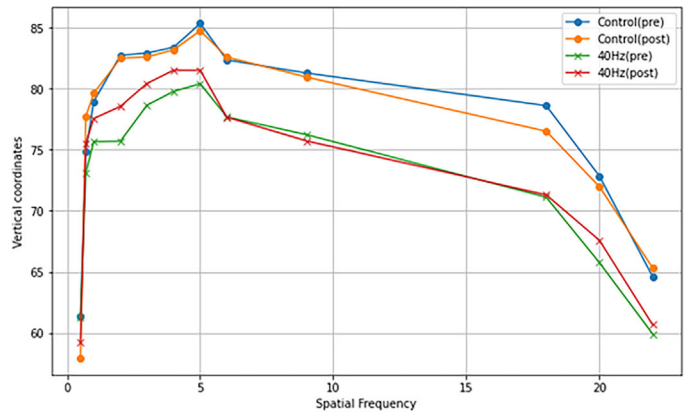

Figure 9. Visual threshold task result.

were used simultaneously. The task was changed to the measurement of spatial frequency and visual threshold.

Participants could vertically move the circles in the upper image using a slider (Figure 8). They are instructed to move 12 circles from left to right until they perceive the boundary between the alternating black and white region and the gray region. The measurement was taken three times, and the average was recorded. The rest of the experimental design remained consistent with the previous experiment.

Pre–post column	Estimate	Q5	Q95
pre_1	0.00287	−7.19	7.29
pre_2	−1.19	−7.08	4.56
pre_3	−2.3	−7.66	3.23
pre_4	−3.58	−10.7	3.97
pre_5	−3.15	−8.43	2.11
pre_6	−2.54	−8.1	2.76
pre_7	−3.41	−8.74	2.16
pre_8	−3.42	−8.41	1.9
pre_9	−3.53	−8.87	1.93
pre_10	−4.76	−10.5	1.49
pre_11	−4.02	−10.5	2.57
pre_12	−3.73	−8.13	0.843
post_1	0.462	−6.98	8.47
post_2	−1.75	−6.37	3.04
post_3	−1.73	−6.1	2.56
post_4	−2.37	−8.59	3.95
post_5	−1.75	−6.28	2.8
post_6	−1.37	−5.54	2.97
post_7	−2.34	−7.56	2.9
post_8	−3.64	−8.58	1.45
post_9	−3.72	−9.04	1.86
post_10	−3.59	−9.01	2.07
post_11	−3.33	−8.33	1.77
post_12	−3.95	−7.75	−0.0752

Table 1. Experiment 5: Bayesian statistical results of pre–post-test differences.

Group column	Estimate coefficient	Q5	Q95
control_1	0.77	0.17	1.35
40 Hz _1	0.87	0.3	1.45
control_2	0.64	0.33	0.95
40 Hz _2	0.58	0.32	0.84
control_3	0.5	0.19	0.8
40 Hz _3	0.69	0.47	0.89
control_4	0.85	0.2	1.5
40 Hz _4	0.65	0.36	0.9
control_5	0.39	−0.14	1.08
40 Hz _5	0.74	0.6	0.88
control_6	0.72	0.35	1.14
40 Hz _6	0.51	0.27	0.76
control_7	1.73	1.36	2.07
40 Hz _7	0.67	0.44	0.9
control_8	0.94	0.51	1.34
40 Hz _8	0.88	0.7	1.06
control_9	0.89	0.55	1.26
40 Hz _9	0.9	0.63	1.16
control_10	0.92	0.46	1.36
40 Hz _10	0.83	0.62	1.04
control_11	0.65	0.06	1.25
40 Hz _11	0.55	0.35	0.75
control_12	1.14	0.22	2
40 Hz_12	0.68	0.52	0.83

Table 2. Experiment 5: Bayesian statistical results of group differences.

Results and discussion

There were no significant differences in the vertical coordinates of the 12 circles between the pretest and post-test for both the 40-Hz group and the control group. Similarly, no significant differences were observed between the two groups. No significant difference was found, suggesting that 40 Hz has no effect on the visibility threshold at different spatial frequencies (Figure 9).

The Bayesian statistical results are summarized in Tables 1 and 2. In Table 1, the first column denotes the specific group and column, and the subsequent columns provide the estimated regression coefficients and their 95% credible intervals. Table 2 presents the Bayesian analysis results for the pre–post differences.

General discussion

In this series of experiments, we examined the effects of a 40-Hz stimulus on visual threshold and visual spatial memory as summarized in Table 3 (Bayesian statistics in Table 4). Experiment 1 and subsequent experiments (Experiments 2 and 3), where we enhanced the flashing and added auditory stimuli, failed to produce any promoting effects and, in some cases, even resulted in inhibitory effects. Experiment 5, which explored different spatial frequencies, also yielded no significant differences. In Experiment 4, which investigated visual spatial memory, the 40-Hz stimulus presented using binaural beats did not enhance visual spatial memory.

Based on the comprehensive results of these experiments, we concluded that the 40-Hz stimulus, whether presented through flashing, binaural beats, or audio, does not have a promoting effect on either low-level perceptual functions or higher order cognitive functions.

The visual spatial memory test used in Experiment 4 was similar to that of Wang et al. (2022). However, they did not separate experimental and control groups, which could lead to their observed effects being attributed to practice. In our Experiment 4, neither

Experiment	40-Hz stimulus	Measurement	Result
1	LED light bulb	Visual threshold	Practice effect (both group)
2	Screen flicker (48 Hz)	Visual threshold	Practice effect (only control)
3	Screen flicker, BB	Visual threshold	Practice effect (only control)
4	Screen flicker, BB	Short-term memory	No difference
5	LED light bulb (200 lux)	Visual threshold with difference spatial frequency	No difference

Table 3. Experiment results summary.

Experiment	Group	Pre–post	Pre–post + group	Pre–post + group + interaction
1	−1.18	11.03	10.3	9.44
2	−1.87	4.7	3.44	1.67
3	−0.64	3.67	3.10	1.63
4-ACC	−1.87	12.72	11.72	9.98
4-RT	0.35	81.79	81.48	79.47
5	5.62	114.59	345.78	344.19

Table 4. All experiments Bayesian statistical results of group differences.

group showed practice effects, possibly because of the difficulty grading in Wang et al. (2022) study, resulting in more trials and making small effects significant. Other studies related to short-term memory also showed no significant differences, consistent with our findings.

Although future research could explore whether the 40-Hz audio frequency has promoting effects on other measures, based on the current literature and our experimental results, it suggested that, for healthy adults, the 40-Hz stimulus does not have promoting effects on cognitive and perceptual functions (short-term spatial memory and visual thresholds).

Keywords: 40 Hz, visual threshold, visual memory, cognitive promotion

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