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DOI
10.1088/2057-1976/ad865d

Publication date
2024

Document Version
Final published version

Published in
Biomedical Physics & Engineering Express

Biomedical Physics & Engineering Express

CrossMark

OPEN ACCESS

RECEIVED
8 March 2024

REVISED
7 October 2024

ACCEPTED FOR PUBLICATION
14 October 2024

PUBLISHED
30 October 2024

PAPER

Assessing the influence of visual stimulus properties on steady-state visually evoked potentials and pupil diameter

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Keywords: brain-computer interfaces, flicker frequency, visual stimulus, pupillary light reflex

Supplementary material for this article is available online

Abstract

Steady-State Visual Evoked Potentials (SSVEPs) are brain responses measurable via electroencephalography (EEG) in response to continuous visual stimulation at a constant frequency. SSVEPs have been instrumental in advancing our understanding of human vision and attention, as well as in the development of brain-computer interfaces (BCIs). Ongoing questions remain about which type of visual stimulus causes the most potent SSVEP response. The current study investigated the effects of color, size, and flicker frequency on the signal-to-noise ratio of SSVEPs, complemented by pupillary light reflex measurements obtained through an eye-tracker. Six participants were presented with visual stimuli that differed in terms of color (white, red, green), shape (circles, squares, triangles), size (10,000 to 30,000 pixels), flicker frequency (8 to 25 Hz), and grouping (one stimulus at a time versus four stimuli presented in a 2×2 matrix to simulate a BCI). The results indicated that larger stimuli elicited stronger SSVEP responses and more pronounced pupil constriction. Additionally, the results revealed an interaction between stimulus color and flicker frequency, with red being more effective at lower frequencies and white at higher frequencies. Future SSVEP research could focus on the recommended waveform, interactions between SSVEP and power grid frequency, a wider range of flicker frequencies, a larger sample of participants, and a systematic comparison of the information transfer obtained through SSVEPs, pupil diameter, and eye movements.

Introduction

Steady-state visually evoked potentials (SSVEPs) are electrical responses of the brain that match the frequency of a visual stimulus. This effect, which can be measured through electroencephalography (EEG), is elicited when an observer views a stimulus that flickers at a certain frequency, usually between 5 and 40 Hz (Pastor *et al* 2003). The SSVEP response can be observed as a peak in magnitude within the EEG spectrum that is identical to the frequency of the visual stimulus or its harmonics.

Scientific interest in SSVEPs spans several areas, including assessing visual acuity and colorblindness (e.g., Zheng *et al* 2020, Hamilton *et al* 2021), enhancing our understanding of the visual system (Nguyen *et al* 2019, Kohler and Clarke 2021), investigating attentional mechanisms (Vialatte *et al* 2010, Christodoulou *et al* 2018, Chinchani *et al* 2022), and exploring possibilities in brain-computer interfaces (Kalunga *et al* 2016, İşcan and Nikulin 2018, Chen *et al* 2022). In particular, SSVEPs can be used for typing, selecting tasks, or controlling robotic devices by looking at flickering icons or characters. In the context of spellers, users select a character by directing their gaze toward it, and the corresponding frequency of the user's brain waves is detected. This selection is processed by the computer system, allowing for non-traditional typing or command input. Similarly, for human-robot interfaces, different actions or directions can be assigned unique flickering patterns. As the user focuses on the desired action, the SSVEP response is interpreted as a specific command, enabling control of the robotic device (e.g., Chen *et al* 2015, Chen *et al* 2019b, Shao *et al* 2020, Li *et al* 2021).

The magnitude of the SSVEP response is influenced by various stimulus characteristics. In Duszyk *et al* (2014), an effect of color on the SSVEP response was demonstrated. Among the colors tested (blue, red, green, white, and yellow), blue yielded the weakest response for all flicker frequencies (14, 17, 25, 30 Hz). Conversely, white and yellow light evoked the strongest responses. The authors attributed this variation to the differences in luminance and contrast with the background, with brighter colors like white and yellow being more distinguishable against the black background. Duszyk *et al* also showed a consistent effect of stimulus size on the SSVEP responses, with larger stimuli inducing a stronger response. The shape of the stimuli (squares versus circles) did not affect SSVEP magnitude in a statistically significant manner. The findings of Duszyk *et al* are consistent with Cao *et al* (2012), who studied the effect of five colors (white, gray, red, green, and blue) on SSVEPs at frequencies between 7.5 and 17 Hz. Their findings indicated that white color yielded the strongest SSVEP response, followed by gray, red, green, and blue in descending order of effectiveness. Similarly, in Chu *et al* (2017), ten stimulus colors were tested for their effect on SSVEPs. The results showed that violet and blue-like colors produced the weakest responses. However, a limitation of their work was that only one flicker frequency (10 Hz) was used.

More recently, Duart *et al* (2020) investigated how different colors (red, green, white) and flicker frequencies (low, middle, high) of visual stimuli affect SSVEPs. The results showed that the color and frequency both significantly influenced the signal-to-noise ratio (SNR) of the evoked potentials. Middle frequencies (12 Hz) generally produced the best SNR, followed by low (5 Hz) and high (30 Hz) frequencies. At 5 Hz, red and green yielded a higher SNR than white; at 12 Hz, there was no significant difference between red and white, both outperforming green; and at 30 Hz, the SNRs for the three colors did not differ significantly. These results suggest that while red is often used for its attention-grabbing properties, green and white can be viable alternatives, especially at low or middle frequencies, respectively (Duart *et al* 2020). This is a useful finding because flickering of the color red (or red in combination with blue) should possibly be avoided as it is said to potentially induce epileptic seizures (for further findings and discussion, see Ishida *et al* 1998, Drew *et al* 2001, Rubboli *et al* 2004, Fisher *et al* 2005, Parra *et al* 2007).

In summary, in SSVEP research, there is still some ambiguity about whether color may interact with flicker frequency, and whether it is stimulus color or stimulus size that affects the SSVEP response, operationalized as a SNR. The current study aims to gain insight into this issue by replicating elements of the work of Duszyk *et al* (2014). We presented stimuli of three colors (white, red, green) at different frequencies (8, 13, 19, 25 Hz). We also changed the shape (square, circle, triangle) and the size of the stimulus. Given the fact that stimuli of higher luminance result in both a stronger SSVEP response and a stronger pupillary constriction (Thigpen *et al* 2018), we have included pupil diameter measurements in this study to assess whether this covariation also applies to color. Furthermore, our stimuli were presented in singular form or in a 2 × 2 matrix where a participant was instructed to focus on one target stimulus out of four, in order to simulate a brain-computer interface (BCI) speller context. The purpose of this was to determine whether such a multiple presentation, where four stimuli simultaneously flicker at slightly different frequencies and the participant looks at one of these four stimuli, adversely affects the SNR compared to more ideal circumstances where only one stimulus flickers.

Methods

Participants

A total of 6 participants, comprising 3 males and 3 females, participated. The mean age was 24.8 years ($SD = 3.4$ years). All participants were new to SSVEPs, except Participant 1. The research was approved by the TU Delft Human Research Ethics Committee, approval number 2555. The experimental measurements were carried out in an EEG laboratory located in the basement of the University Medical Center Amsterdam, location AMC.

Apparatus

The SR Research EyeLink Portable DUO was used to record eye movements and pupil diameter at a sample rate of 2000 Hz. A head support was used to prevent head movements and to ensure a consistent distance of approximately 68 cm between the participants' eyes and the stimulus monitor.

The EEG recordings were obtained using the actiCHamp Plus amplifier of BrainVision in combination with the standard actiCAP snap, which is a gel-based EEG headset. The sample rate of the EEG recordings was 2500 Hz. The conductive gel used was ECI Electro-Gel. The system used active electrodes. A total of 63 electrodes were used for the recordings.

The 17.3-inch 1920 × 1080-pixel display in use belonged to an ASUS GX701LV-DS76 laptop. The width of the screen was 383 mm, and its height was 215 mm. This laptop was equipped with an Intel Core i7-10750H CPU @ 2.60 GHz and an NVIDIA GeForce RTX 2060. The screen refresh rate was set to 60 Hz. The experimental setup is shown in figure 1.

Software

The stimuli, consisting of videos at a frame rate of 59.94 fps, were shown using Experiment Builder (v2.3.38) by SR Research Ltd. This software, which enables concurrent eye-tracking and EEG recording, was used alongside BrainVision Recorder by Brain Products GmbH for EEG data acquisition.

Figure 1. The experimental setup.

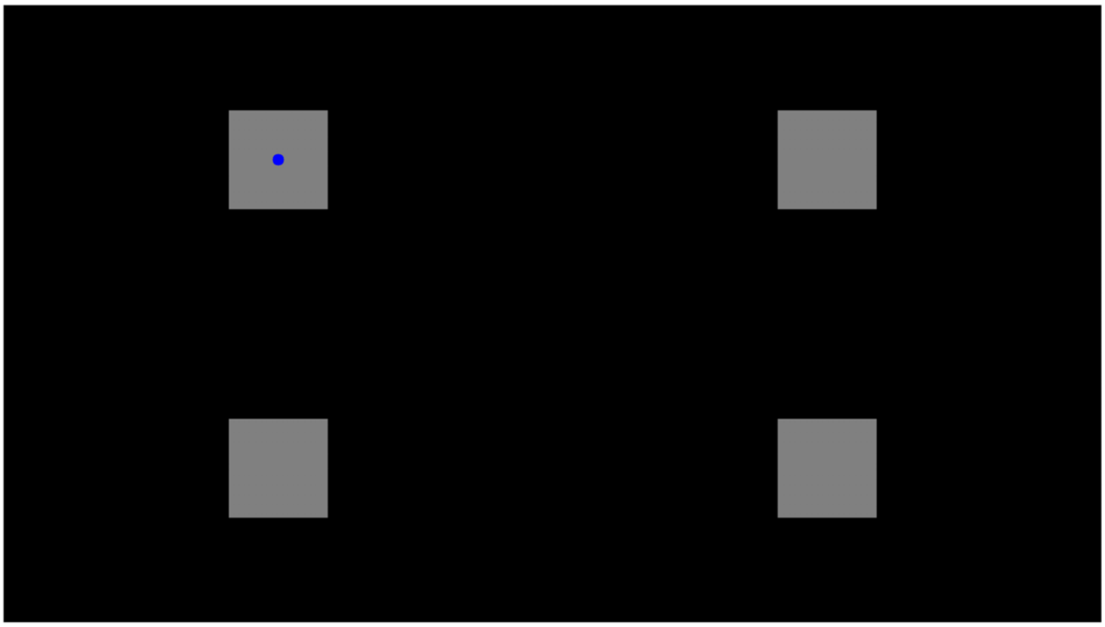

Figure 2. Two-second static screen with a blue dot to indicate the target stimulus in Experiment 2 × 2. After this, the blue dot disappeared, and all four squares started flickering at a slightly different frequency.

Stimuli

Experiment 1, also referred to as Experiment 1 × 1, involved presenting a single stimulus at a time. These stimuli had different shapes, sizes, colors, and flicker frequencies.

Experiment 2, also referred to as Experiment 2 × 2, presented four stimuli simultaneously, one in each quadrant of the screen, with center positions at (480, 270), (480, 810), (1440, 270), and (1440, 810), as illustrated in figure 2. One of the four was designated as the target stimulus, which flickered at a frequency identical to those in Experiment 1 × 1. The other three stimuli were shown at frequencies close to the target frequency. In Experiment 2 × 2, only the size, color, and frequency of the stimuli were varied.

Frequency. We used a sinusoidal waveform because of its ease of implementation at specific frequencies when compared to a square waveform. That is, the sinusoidal form is more adaptable to various frequencies, and avoids the complexities of matching the on–off cycles of a square wave with the given refresh rate of a computer screen. Four target frequencies were used: 8, 13, 19, and 25 Hz. In Experiment 1 × 1, a single stimulus was presented at one of these frequencies. In Experiment 2 × 2, four stimuli were shown simultaneously, with the target stimulus being one of the specified frequencies and the other three having frequencies close to the target, deviating by 0.3 Hz. Similar frequency intervals have been used in BCI research, such as spellers, in which the user glances at a keyboard with flickering characters (Chen *et al* 2015, Abdelnabi *et al* 2019, Sun *et al* 2021). One of the four squares always flickered at a lower frequency than the target frequency, and two squares flickered at a higher frequency. For example, if the target frequency was 25 Hz, the three other squares flickered at 24.7, 25.3, and 25.6 Hz.

Size. Three stimulus sizes were used: 10,000, 20,000, and 30,000 pixels.

Shapes. In Experiment 1 × 1, three shapes were used: circles, squares, and equilateral triangles. The smallest circle (area: 10,000 pixels) had a diameter of 113 pixels, which, given the viewing distance of 68 cm, corresponded to an angle of 1.9° in both horizontal and vertical directions. For the largest circle (area: 30,000 pixels), the diameter was 196 pixels, with a corresponding angle of 3.3°. The smallest square had sides of 100 pixels, and the largest square had sides of 173 pixels. The smallest triangle had sides of 152 pixels, and the largest triangle had sides of 263 pixels. In Experiment 2 × 2, only squares were used.

Color. Three colors were used: pure white, pure green, and pure red.

Positioning. In Experiment 2 × 2, the target stimulus was a random 1 of 4 squares (left top, right top, left bottom, or right bottom). The other three stimuli flickered at a slightly different frequency, as described above.

Experiment design

In Experiment 1 × 1, participants were exposed to 108 different trials, each trial presenting one stimulus at a time. The 108 different stimuli were created by combining 3 colors, 4 frequencies, 3 shapes, and 3 sizes. The 108 stimuli were presented three times, with the 108 stimuli each time in a different random order. The experiment totaled 324 trials, lasting approximately 44 min.

In Experiment 2 × 2, four stimuli were presented simultaneously, simulating a simple SSVEP speller environment. The experiment involved 144 different trials, created by combining 4 target positions, and the same 4 frequencies, 3 sizes, and 3 colors. The 144 stimuli were presented in a random order. This was repeated three times, with the 144 stimuli each time in a different random order. The experiment totaled 432 trials, lasting approximately 56 min.

Procedure

Half of the participants (Participants 1, 3, and 5) were assigned to begin with Experiment 1 × 1, while the other three participants began with Experiment 2 × 2. There was a break of approximately 10 min between the two experiments.

Before the experiments, participants provided written informed consent. Next, participants were equipped with an EEG headset with conductive gel to get the impedances below acceptable thresholds (10 kΩ), and they placed their head in the head support. Following this, the eye-tracker was calibrated.

Next, participants were presented with an instruction screen, which stated that in the upcoming experiment, participants would see a shape that, after 2 s, begins to flicker for 4 s. It also mentioned that participants would witness a number of shapes (Experiment 1 × 1: 108, Experiment 2 × 2: 144) that vary in size, color, and flicker frequency. The instruction screen indicated that participants would start with 4 practice trials before proceeding to the main trials, which were presented in sets (Experiment 1 × 1: 54 trials per set, Experiment 2 × 2: 72 trials per set). After completing each set, participants were offered a 1 min pause to rest. Each experiment consisted of six such sets. Additionally, participants were requested to minimize blinking and remain as still as possible throughout the process.

By pressing the spacebar, participants commenced the practice trials. Each experiment included four practice trials, randomly selected from all unique combinations of interface characteristics.

Each trial began with a static image of the stimulus that was shown for 2 s. In Experiment 2 × 2, the static image contained a blue dot to indicate the target location on the screen for participants to focus on during the upcoming trial (figure 2). Next, the stimulus began to flicker for 4 s, with three additional stimuli flickering. After this, Experiment Builder prepared the next trial, taking approximately 0.9 s.

Data processing

The impedance of the electrodes was generally noted to be 10 kΩ. An exploratory analysis showed that the SSVEP response could be identified in various electrodes, with the strongest response in the occipital and parietal regions (e.g., O2, Oz, PO4, PO8, POz electrodes), as well as at the inion (Iz electrode). These areas are important for SSVEP research, because they are near the visual cortex, where visual information is processed (Bin *et al* 2009, Marx *et al* 2019, Wang and Yuan 2021). Figure S1 in the Supplementary material provides an overview of the average SNR values (calculation indicated below) for the signals from all 63 electrodes.

For our study, we decided to use only one electrode, namely the Oz electrode. This electrode provided a strong overall response, and in exploratory evaluations, we did not observe any added value from combining the signals of multiple electrodes.

The data was processed in the following steps:

1. Select the 4 s EEG data from the Oz channel of Participant *p* and Trial number *i*.
2. Conduct a discrete Fourier transformation on the 4 s of data. A Fourier transform is a standard recommended method of signal processing within SSVEP research (Norcia *et al* 2015).
3. Calculate the magnitude of the Fourier transform. The result for participant *p* and trial number *i* is 5001 magnitude values corresponding to frequencies from 0 to 1250 Hz in increments of 0.25 Hz.
4. Repeat the above for all participants (*p:* 1 to 6) and all completed trials for that participant (*i*: 1 to 324 for Experiment 1 × 1, *i*: 1 to 432 for Experiment 2 × 2).

For illustrative purposes, the Fourier spectrum for Experiment 1 × 1 and Experiment 2 × 2 were visualized, making a distinction between the four flicker frequencies. For Experiment 1 × 1, the visualized Fourier spectrum represents the average magnitude across 486 trials, (324 trials per participant × 6 participants)/4 flicker frequencies. For Experiment 2 × 2, the Fourier spectrum represents the average magnitude across 648 trials, (432 trials per participant × 6 participants)/4 flicker frequencies.

Next, we extracted, for each trial and for each participant, the magnitude of the discrete Fourier transform at the same frequency (i.e., the first harmonic) and the double frequency (i.e., the second harmonic). For each trial, we calculated the SNR by taking the magnitude at the response frequency and dividing it by the mean magnitude at surrounding frequencies (Norcia *et al* 2015).

The Fourier spectrum is not uniform but exhibits a rising and falling trend (as caused by the alpha band). To account for this, the magnitude at each frequency point was divided by the mean participant-specific baseline value across all trials of that participant, resulting in a normalized magnitude, F_n (see figure S2 in the Supplementary material). We found that considering the Fourier spectrum relative to a baseline spectrum for each participant was more effective than comparing it to reference electrodes. The signal from reference electrodes for the same trials is noisy, and this type of referencing can be detrimental (Delorme 2023).

Next, the 'signal' was calculated as the mean magnitude at the response frequency. The noise signal was taken across 9 frequencies below and 9 frequencies above the response frequency. For example, if the response frequency was 13 Hz, the mean magnitude at [9.0, 9.25, … 11.0, 15.0, 15.25, 15.5, … 17.0 Hz] was taken. We chose to average the noise over a large number of 18 frequency points, in order to obtain a statistically reliable estimate, see equation (1).

$$SNR(f) = \frac{F_n(f)}{(1/18)\left(\sum_{k=8}^{k=16} F_n(f - k\Delta f) + \sum_{k=8}^{k=16} F_n(f + k\Delta f)\right)}, \quad (1)$$

where $\Delta f = 0.25$ Hz

As we are interested in the properties of trials rather than the properties of individuals, we averaged the SNR over the 6 participants. Subsequently, we calculated for each of the response frequencies (i.e., the four flicker frequencies and their second harmonics), the means and 95% confidence intervals (CIs) of the SNR value for the following conditions:

Experiment 1 × 1: (1) Small stimuli, (2) Medium stimuli, (3) Large stimuli, (4) Squares, (5) Circles, (6) Triangles, (7) White stimuli, (8) Green stimuli, (9) Red stimuli. Each mean and 95% CI was based on 27 trials. For example, the mean and CI for small stimuli at a flicker frequency of 8 Hz was based on 3 repetitions × 3 stimulus sizes × 3 colors.

Experiment 2 × 2: (1) Small stimuli, (2) Medium stimuli, (3) Large stimuli, (4) Left top target, (5) Right top target, (6) Left bottom target, (7) Right bottom target, (8) White stimuli, (9) Green stimuli, (10) Red stimuli. Each mean and 95% CI was based on 36 trials (for size and color) or 27 trials (for position).

In addition to means and 95% CIs, we attempted to visually illustrate the interaction between stimulus color and flicker frequency using scatter plots. We displayed the SNR for each red shape individually plotted against each separate white shape, for a flicker frequency of 13 Hz and a flicker frequency of 25 Hz. This combination of color and frequency was selected because they appeared to represent a large interaction effect.

Lastly, we attempted to determine whether luminance was an explanatory factor for the SSVEP response. To this end, we performed an RGB-to-gray transformation $(0.299 \times R + 0.587 \times G + 0.114 \times B)/255$ (International Telecommunication Union 2011) on each video frame and calculated the average across all video frames of that trial to obtain an index of luminance. Additionally, pupil diameter was used as an index of luminance. To process the recorded pupil diameter data, we first applied a median filter and blink gap interpolation (as in De Winter *et al* 2021). Subsequently, we created task-

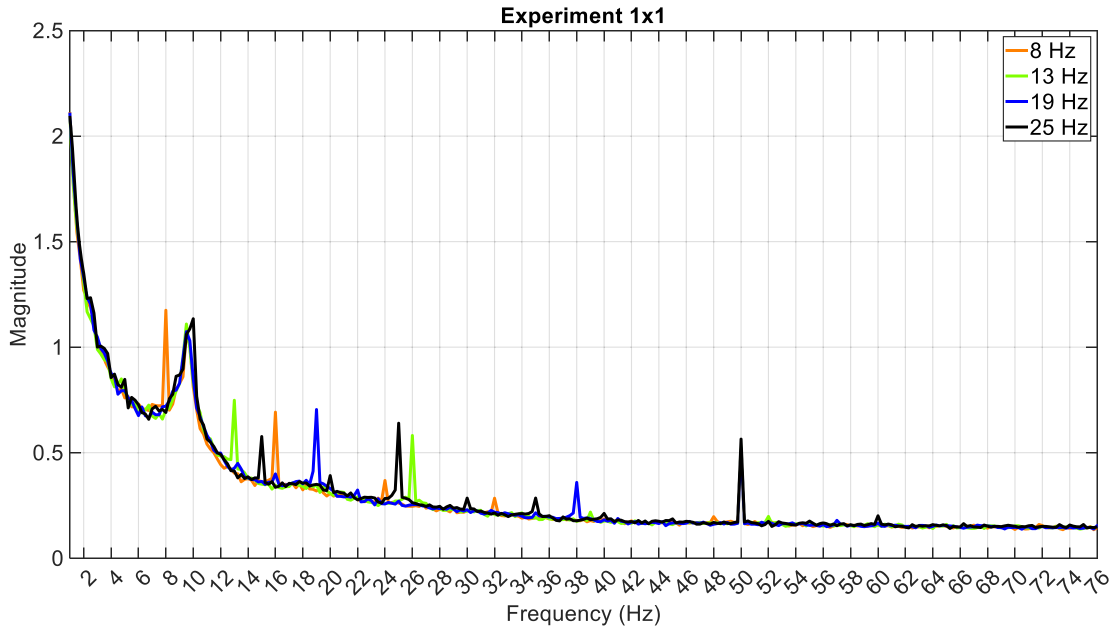

Figure 3. Averaged Fourier spectrum over all trials and participants for Experiment 1 × 1. A distinction is made between the four flicker frequencies.

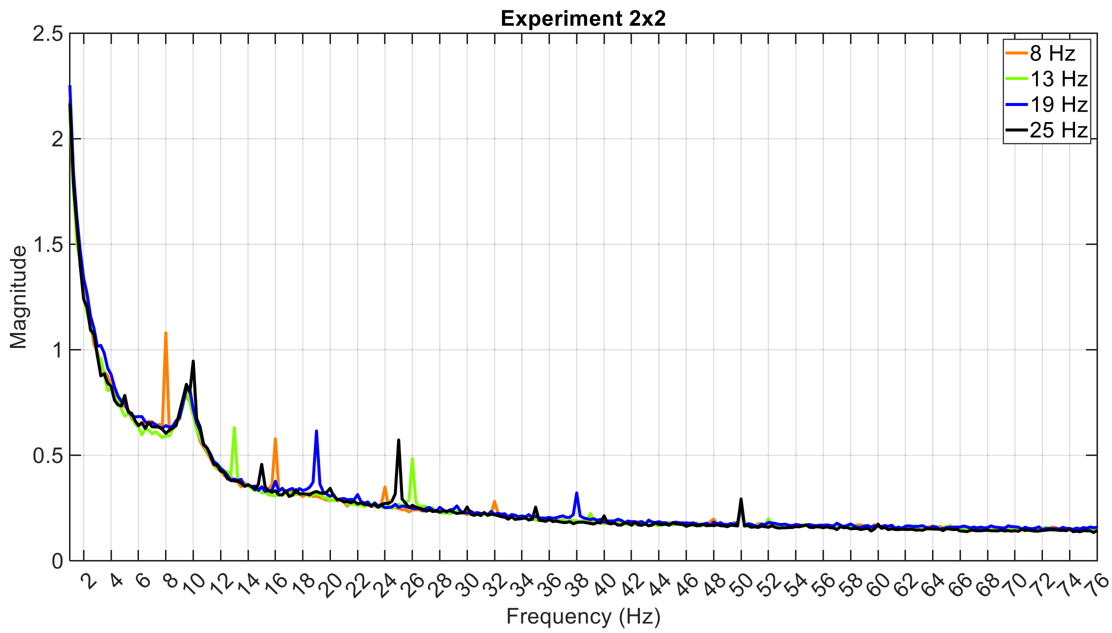

Figure 4. Averaged Fourier spectrum over all trials and participants for Experiment 2 × 2. A distinction is made between the four flicker frequencies.

evoked pupillary response (TEPR) figures with elapsed time (0 to 4 s) on the horizontal axis and percentage changes in pupil diameter relative to the beginning of the trial at $t = 0$ s on the vertical axis. We also calculated the pupil diameter change after 1 s as an index of the pupillary light reflex (De Winter *et al* 2021), and plotted these values in bar graphs with means and 95% CIs, just as we did for the SNR values. We determined whether the stimuli that caused significant pupil constriction were also those with a strong SSVEP response.

Results

Figures 3 and 4 show the Fourier spectra, averaged per flicker frequency over all trials and the six participants for Experiment 1 × 1 and Experiment 2 × 2, respectively. It can be seen that the two figures look similar, with increased magnitudes at the target frequencies and the second harmonics. However, for the 25 Hz stimulus, the second harmonic cannot be seen because it coincides with power line interference at 50 Hz (the

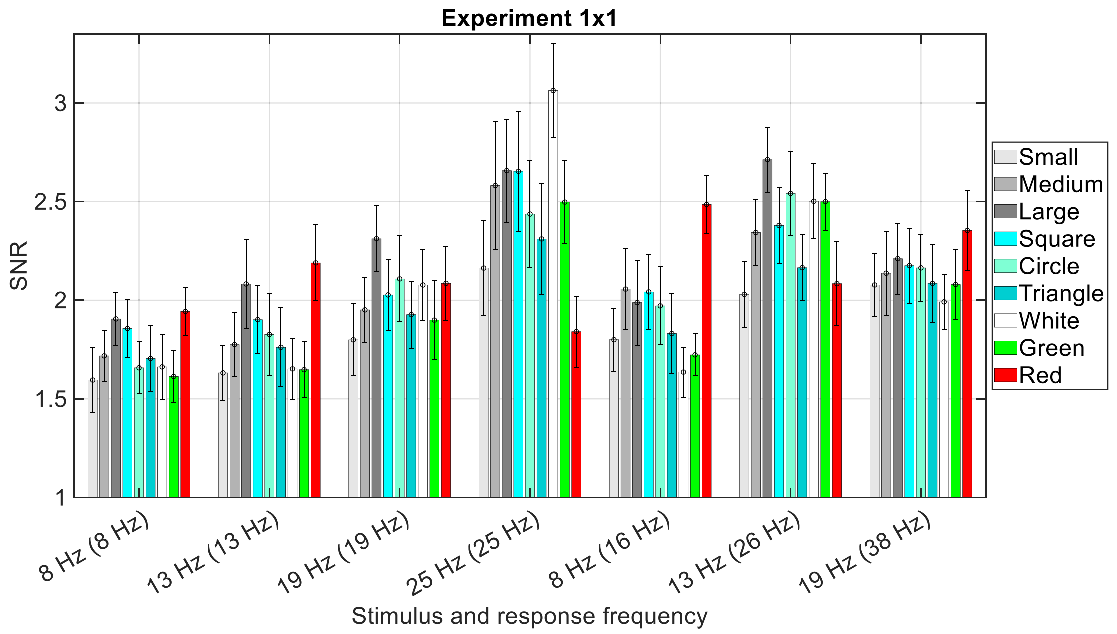

Figure 5. Signal-to-noise ratio (SNR) and 95% confidence intervals for 7 combinations of stimulus and response frequencies, and 9 types of stimulus characteristics (3 sizes, 3 shapes, 3 colors) in Experiment 1 × 1. The horizontal axis depicts the stimulus flicker frequency, with the response frequency listed in parentheses.

electricity in the Netherlands operates at 50 Hz). This phenomenon is more frequently mentioned in SSVEP research and is a reason to avoid the 50 Hz frequency in future research (e.g., Floriano *et al* 2019).

The difference in magnitude at 50 Hz between the two experiments can be traced back to two participants. This may, for example, be related to the placement of the power adapter of the laptop and, therefore, holds no substantive meaning. The bump in the 8–10 Hz range corresponds with the alpha wave frequency band. Note that there were also large individual differences in this case.

Figure 5 shows the main effects of stimulus size, shape, and color for the eight different assessment frequencies in Experiment 1 × 1. Several observations can be made:

- The size of the stimulus has a consistent effect on the SNR, as indicated by the fact that the smallest stimulus demonstrates a lower SNR than the largest stimulus in seven out of seven cases (i.e., all response frequencies, excluding 50 Hz). Averaged across the seven response frequencies, the mean SNR values were 1.871 (95% CI: 1.813–1.929), 2.080 (95% CI: 2.008–2.153), and 2.267 (95% CI: 2.196–2.337) for small, medium, and large stimuli, respectively ($n = 27$).
- Red stimuli exhibited a higher SNR as compared to green and white stimuli for flicker frequencies of 8 and 13 Hz.
- For a flicker frequency of 25 Hz, however, white stimuli exhibited a higher SNR as compared to green, followed by red.
- The SNR for the different shapes showed overlapping 95% CIs. However, for all of the seven response frequencies shown in figure 5, triangles yielded a lower SNR than squares.
- Regarding the second harmonics (bar groups 5–8), red is effective at the lowest frequency, see '8 Hz (16 Hz)'. However, at the second harmonic of 13 Hz, '13 Hz (26 Hz)', red yielded a lower SNR than white and green.

The results of Experiment 2 × 2, shown in figure 6, reveal a similar pattern, where red stimuli yielded the largest magnitude for low flicker frequencies, and white yielded a higher SNR than green and red at a flicker frequency of 25 Hz. Furthermore, the second harmonic of 8 Hz (16 Hz) demonstrated a higher SNR for red as compared to white and green. Averaged across the 7 response frequencies, the mean SNR values were 1.683 (95% CI: 1.643–1.723), 1.865 (95% CI: 1.812–1.918), and 2.029 (95% CI: 1.965–2.094) for small, medium, and large stimuli, respectively ($n = 36$).

Figure 7 illustrates the interaction effect depicted in figures 5 and 6. The top four subfigures show the mean SNR for red versus white shapes, with data from all nine red shapes and all nine white shapes in Experiment 1 × 1, and the three red squares and three white squares in Experiment 2 × 2. The analysis is presented for stimulus and response frequencies of 13 Hz and 25 Hz. The interaction is evident in that, for red 13 Hz stimuli, most SNR values fall above the unity diagonal, while for 25 Hz stimuli, they fall below it. The bottom four subfigures compare green versus white stimuli. At a frequency of 13 Hz, no clear difference is observed

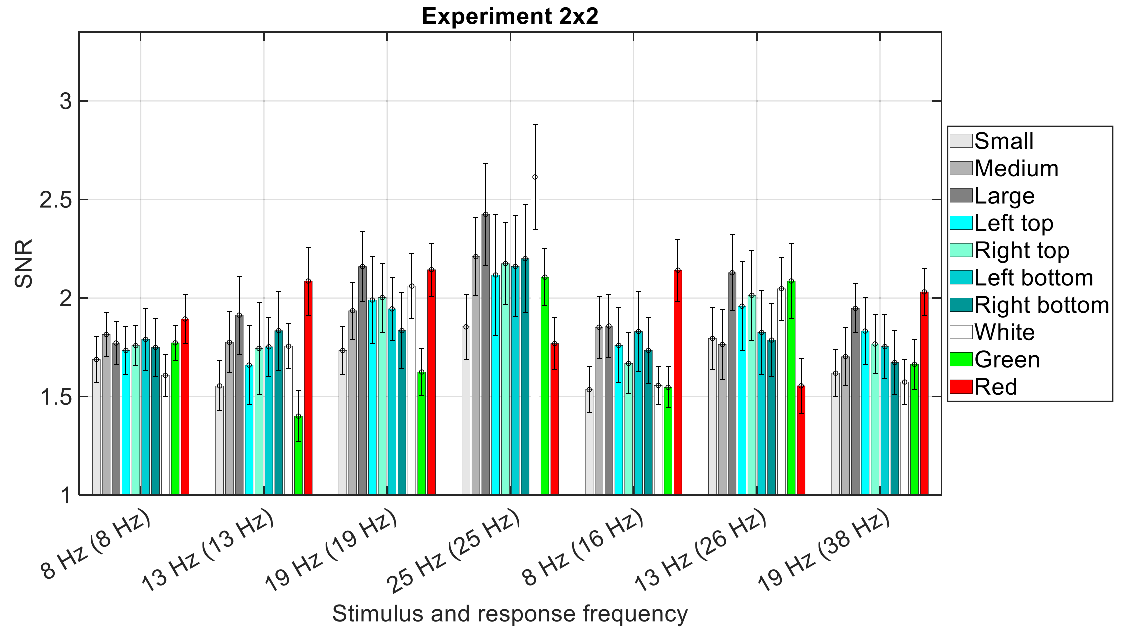

Figure 6. Signal-to-noise ratio (SNR) and 95% confidence intervals for 7 combinations of stimulus and response frequencies, and 10 types of stimulus characteristics (3 sizes, 4 target positions, 3 colors) in Experiment 2 × 2. The horizontal axis depicts the stimulus flicker frequency, with the response frequency listed in parentheses.

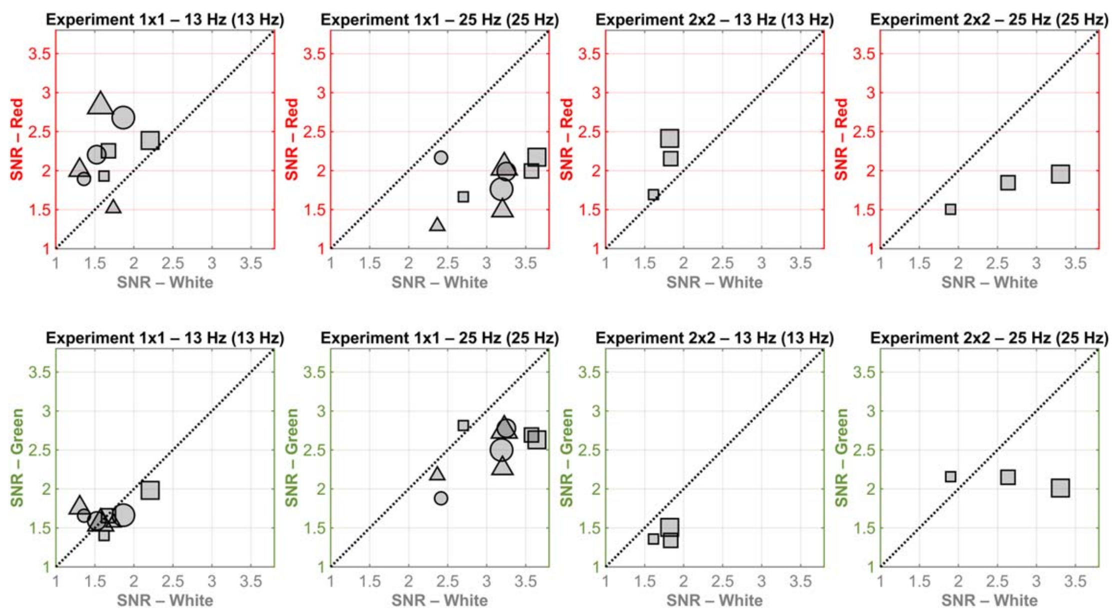

Figure 7. Mean signal-to-noise ratios (SNR) for red versus white shapes (top row) and for green versus white shapes (bottom row). A distinction is made between 13 Hz stimuli (columns 1 and 3) and 25 Hz stimuli (columns 2 and 4), and between Experiment 1 × 1 (columns 1 and 2) and Experiment 2 × 2 (columns 3 and 4). Note that each marker corresponds to a stimulus of a specific size (small, medium, large) and shape (square, circle, triangle). Note that in Experiment 2 × 2, only square stimuli were used. Above each subfigure, the stimulus frequency is shown, with the response frequency in parentheses.

between green and white in Experiment 1 × 1, but in Experiment 2 × 2, white exhibits a higher SNR than green. However, at 25 Hz, white stimuli clearly produce a higher SNR than green stimuli.

Figure 8 shows the task-evoked pupillary response for the different stimulus conditions in Experiment 1 × 1. The graphs show a typical pupil constriction associated with the onset of a stimulus (e.g., Bradley *et al* 2017, De Winter *et al* 2021). It appears that the initial pupillary constriction, i.e., within 1 s, is stronger for larger stimuli (Panel 2), and that white stimuli bring about a stronger constriction than, in turn, green and red stimuli (Panel 4).

Figure 9 shows the pupil constriction after 1 s with accompanying 95% CIs. It confirms that larger stimuli, thus stimuli with higher luminance, bring about a stronger constriction. The effect of shape on pupil constriction is not clear, with little interpretable

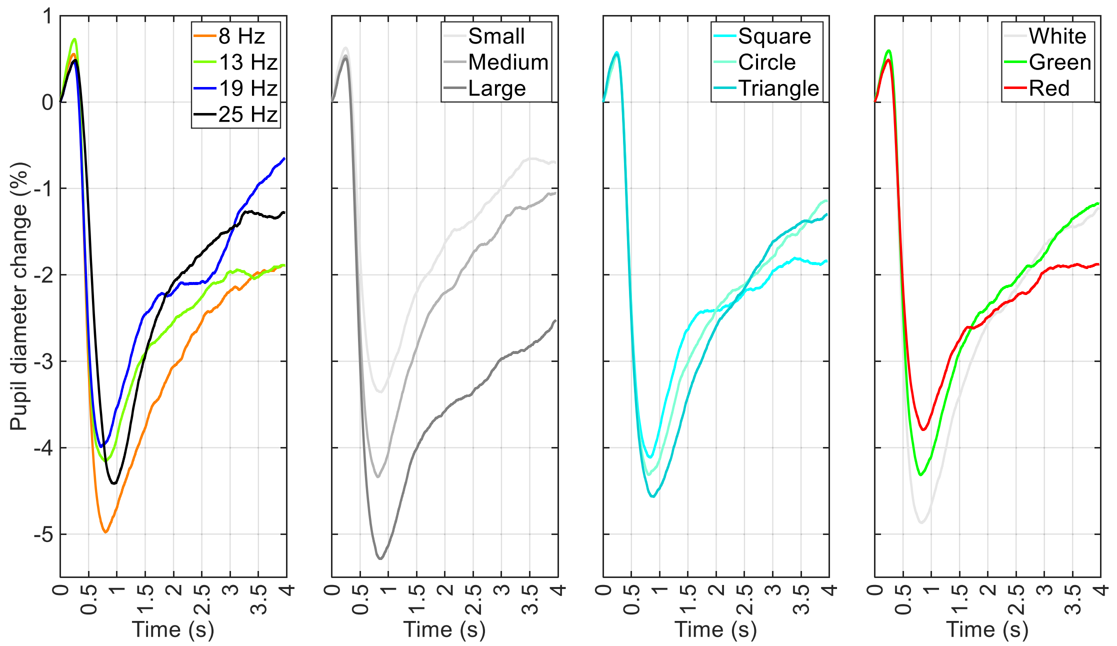

Figure 8. Mean pupil diameter change with respect to the onset of the flickering stimulus (i.e., time = 0 s) for Experiment 1 × 1. A positive value indicates pupil dilation; a negative value indicates pupil constriction.

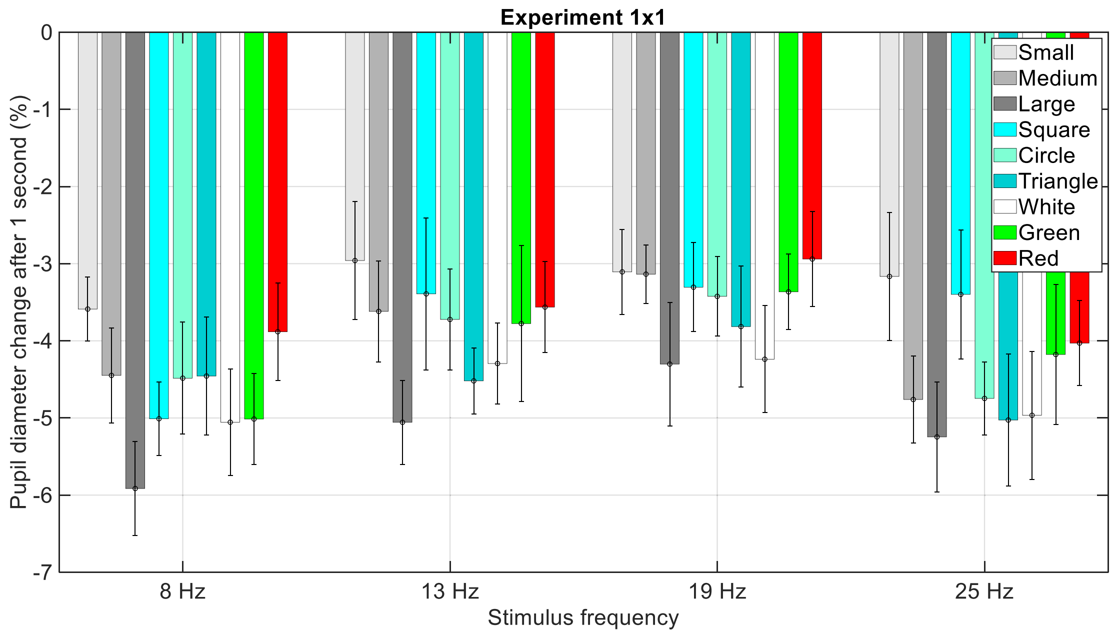

Figure 9. Pupil diameter change at 1 s after the onset of the stimulus and 95% confidence intervals, for 4 flicker frequencies and 9 types of stimulus characteristics (3 sizes, 3 shapes, 3 colors) in Experiment 1 × 1.

differences between the three shapes. Figure 10 confirms that mean pupil diameter was strongly predictable from objective luminance, defined as the mean grayscale value of the 4 s video clips.

Discussion

This study aimed to determine whether the SSVEP response depends on the color of the stimulus or on its size, or on both, and whether there is an interaction between stimulus color and flicker frequency. Additionally, we investigated whether the shape of the stimulus and whether stimuli are presented singly (Experiment 1 × 1) or in a group of four (Experiment 2 × 2) affects the SSVEP.

Our results showed that the intensity of the stimulus is an important factor, with larger stimuli producing a stronger response. The effect of stimulus size was also detectable in pupil diameter. Shape had

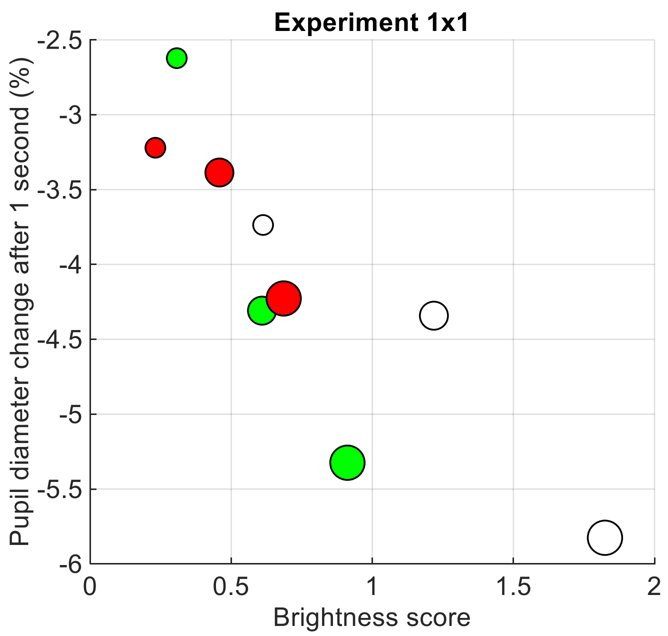

Figure 10. Mean pupil diameter change at 1 s after the onset of the stimulus versus mean brightness score (defined from an RGB-to-gray transformation) for Experiment 1 × 1. A distinction is made between three stimulus colors and three stimulus sizes. The pupil diameter change of each marker represents the mean of 216 trials (6 participants × 3 repetitions × 3 shapes × 4 flicker frequencies).

minor effects, although there are indications that triangles yielded a lower SNR than squares. The relationship between color and SSVEP response and pupil diameter was of an interactive nature: Although white stimuli caused a stronger pupillary constriction than red stimuli, the effect of color on the SSVEP response was not consistent with this, but was dependent on flicker frequency. Specifically, red was effective at low flicker frequencies, and white at higher flicker frequencies. This finding was comparable for Experiment 1 × 1 and Experiment 2 × 2. Additionally, it was found that the SNR values in Experiment 2 × 2 were overall somewhat weaker than in Experiment 1 × 1, but not dramatically so. This indicates that the use of multiple stimuli, simulating an SSVEP speller, is not especially disadvantageous compared to a single centrally presented stimulus.

Our results, which attempted to replicate the work of Duszyk *et al* (2014), provide a more refined picture: while Duszyk *et al* showed that stimulus intensity (as determined by color and size) is a primary determining factor, our results confirm stimulus intensity is indeed a factor, but above all, the right color must be chosen in combination with flicker frequency. Our findings partially confirm the pioneering work of Regan (1966, 1975), which demonstrated a color and flicker frequency interaction in SSVEP responses.

This research work constitutes an incremental contribution to the literature, and we cannot fully explain why our results partly differ from those of Duszyk *et al* (2014), who found that white stimuli elicited the strongest response regardless of flicker frequency. A possible explanation is that SSVEP responses are idiosyncratic, and that specific frequencies and colors activate specific photoreceptors, visual pathways, and neural areas. It is also possible that the white color used in Duszyk *et al*'s case was very bright compared to their use of red. Furthermore, our experimental setup may differ from Duszyk *et al*'s experiment, for example due to different lighting conditions or differences in the stimulus presentation screen.

Limitations

While our work demonstrates an interaction effect between color and flicker frequency, the SNR values were relatively low, up to values of around 3 (see figure 5). This is, for example, comparable to Duart *et al* (2020), but much lower than Duszyk *et al* (2014), who showed SNR values above 10. A possible explanation is that we used an LCD screen, and perhaps more intense LEDs would elicit a stronger response.

Our current SNR values may not be sufficient to generate a usable BCI. We built a simple classifier where, for each individual trial, we determined the SNR in four ways: (1) the average value at 8 and 16 Hz, (2) the average value at 13 and 26 Hz, (3) the average value at 19 and 38 Hz, and (4) the value at 25 Hz. We then identified which of these four SNR values was the highest, and if it corresponded to the stimulus flicker frequency, we marked the classification as correct. If one of the other three frequencies produced the highest SNR, we marked the classification as incorrect. In random data, the correct classification percentage would be expected to be 25%. We found that the correct classification percentage varied between 56% and 83% across different stimulus conditions, higher than chance, but still far from 100% accuracy (see figures S3 and S4 in the Supplementary material). The current classification method relies solely on SNR values, and accuracy might improve by incorporating additional features. However, it is clear that near-perfect classification accuracy has not yet been achieved. To approach 100% accuracy, the stimuli might need to be presented for a longer duration or with greater intensity.

Furthermore, we implemented a sinusoidal waveform alongside a screen refresh rate of 60 Hz. This combination and rendering resulted in a periodic repetition of RGB values and the inability to achieve the maximum RGB values. Across all frames, the maximum 'green' was [0 215 0] (instead of the expected [0 255 0]), the maximum 'white' [252 254 252] (instead of the expected [255 255 255]), and the maximum 'red' [255 24 0] (instead of the expected [255 0 0]). For future research, a square waveform is recommended for a stronger SSVEP response (Teng *et al* 2011, Chen *et al* 2019a, Panitz *et al* 2023), although the research community still seems inconsistent regarding the extent to which this provides a higher SNR or better classification accuracy compared to a sinusoidal waveform (Teng *et al* 2011, Wang *et al* 2013, Dreyer and Herrmann 2015, Jukiewicz and Cysewska-Sobusiak 2016, Tanji *et al* 2018, Chen *et al* 2019a, Zheng *et al* 2020, Chailloux Peguero *et al* 2023, Panitz *et al* 2023).

We also observed stimulus presentation issues where, during the first 500 ms, video frames were not displayed correctly, resulting in half of the frames being skipped and the rest displayed at a frame rate of 30 fps instead of 60 fps, which may have attenuated the SNR for the 25 Hz flicker frequency, and which may explain the additional peaks in the Fourier spectrum for the 25 Hz stimuli at 15 Hz, 20 Hz, 30 Hz, 35 Hz, and 60 Hz (see figure 3).

Additionally, inspection of the SSVEP response revealed large individual differences in the EEG response, not only in alpha wave intensity (see figure S2 in the Supplementary material) but also in terms of SSVEPs. One participant reported being colorblind, unable to recognize the colors yellow and green; however, this individual still had an identifiable SSVEP response to green stimuli.

Another final issue is that red stimuli, while found to be effective at low frequencies (13 Hz) and commonly used in SSVEP research (Zhu *et al* 2010), can trigger photoparoxysmal response (PPR), possibly leading to seizures in individuals with photosensitive epilepsy (e.g., Parra *et al* 2007). To mitigate this risk, we recommend white stimuli flickering at 25 Hz as a safer alternative. However, it should be noted that 25 Hz is a subharmonic of the 50 Hz mains frequency. Previous research suggests that interactions may exist between the SSVEP response at 25 Hz and the mains frequency, though this remains a topic for further investigation (Herrmann 2001).

Conclusion

In conclusion, this research has demonstrated that the effectiveness of SSVEPs is strongly influenced by the combination of color and flicker frequency, rather than solely by stimulus intensity. For future studies, it is recommended to investigate different waveforms and a wider range of flicker frequencies. Additionally, it may be worthwhile to explore whether pupillometry and eye-tracking are as effective as EEG for BCIs. Finally, future research could examine individual differences in SSVEP responses across a broader sample of participants.

Data availability statement

The data that support the findings of this study are openly available at: https://doi.org/10.4121/624445db-e312-495c-a9d9-c467d9f98eee.

Conflict of interest

The authors declare that they have no conflict of interest.

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