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Exploring the Effects of Transcranial Electrical Stimulation on Natural Vision and Cortical Activity

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CERTIFICA

Que el presente trabajo titulado:

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y que constituye la Memoria del Trabajo Fin de Máster en Biotecnología y Bioingeniería, que presenta:

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Y para que así conste, se expide y firma el presente certificado en Elche a 30 de junio de 2025

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ABSTRACT

Transcranial alternating current stimulation (tACS) is а non-invasive neuromodulation technique that can elicit visual phenomena known as phosphenes and potentially modulate visual cortical processing. This study systematically investigated how tACS parameters—frequency, current intensity, and electrode montage—affect subjective phosphene perception and objective visual evoked potentials (VEPs) in healthy adults. Using a within-subject design, eight participants underwent 40 stimulation conditions while reporting phosphene characteristics and completing pre/post VEP recordings. Results showed that phosphene perception increased with current intensity, peaked at 16 Hz, and was significantly higher with a fronto-central (FPz-Cz) montage compared to occipital (Oz–Cz) stimulation. Phosphene features such as brightness, shape, and spatial location varied systematically with stimulation parameters, while discomfort remained minimal. Although VEP analyses did not reveal statistically significant changes, trends suggested reduced N2 latency post-stimulation, indicating potential enhancement of cortical processing. These findings support the feasibility of tACS as a tool for modulating visual perception and cortical activity, with implications for non-invasive visual rehabilitation.

KEY WORDS: transcranial electrical stimulation (tES), tACS, phosphenes, visual perception, visual evoked potentials (VEPs).

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INTRODUCTION

Transcranial electrical stimulation (tES) represents a class of non-invasive brain stimulation (NIBS) techniques (Reed et al. 2018) in which weak electrical currents are delivered through scalp electrodes to modulate cortical excitability and neural dynamics (Liu et al. 2018). TES comprises different techniques, including transcranial direct current stimulation (tDCS), alternating current stimulation (tACS) and random noise stimulation (tRNS). While these techniques share a common mode of delivery—via electrodes placed on the scalp—their stimulation patterns differ significantly, leading to distinct neurophysiological and perceptual outcomes (Reed et al. 2018). By modulating cortical activity, tES enables causal investigations into various cognitive and perceptual functions, such as attention, learning, and visual perception.

The earliest recorded application of electrical stimulation for visual purposes dates back to 1755, when Charles LeRoy applied an electric discharge to the surface of the eye in a cataract-blind patient, eliciting light sensations, called phosphenes, via transorbital stimulation targeting the optic nerve (Sehic et al. 2016). Nearly two centuries later, Brindley and Lewin (1968), and Dobelle and Mladejovsky (1974) demonstrated that direct electrical stimulation of the primary visual cortex induces phosphenes—illusory flashes or geometric shapes perceived in the absence of real visual input. Antal et al. (2004) showed that non-invasive tACS over occipital cortex can reliably elicit phosphenes, enabling systematic exploration of their cortical generators (Perin et al. 2020).

Phosphenes are described as illusory visual sensations such as flashes or geometric shapes perceived in the absence of actual visual stimuli (Kvasnak al., 2022). While they may occur spontaneously, such as during migraines or meditative states, phosphenes can also be reliably evoked via electrical stimulation of the retina, visual cortex, or related visual structures (Evans et al., 2021). Despite their subjective nature, these percepts provide valuable insights into visual system excitability and have promising implications for the development of visual prosthetics. Such technologies may offer partial visual restoration for individuals with various forms of blindness, including amblyopia, amaurosis, glaucoma, retinochoroiditis, and white optic atrophy.

Phosphene characteristics

The appearance of phosphenes—including their brightness, shape, location, and color—varies according to stimulation parameters such as frequency, intensity, and electrode montage. Recent findings suggest that under mesopic (dim) lighting, tES-induced phosphenes can be elicited with lower currents, particularly at 16 Hz (Evans et al., 2022). However, at frequencies above 40 Hz, phosphene perception tends to diminish or disappear (Moliadze et al., 2010). Changes in stimulation intensity have been shown to modify phosphene attributes (Kvasnak et al., 2022), and phosphene size tends to increase with higher stimulation current and with increasing eccentricity from the central visual field, as demonstrated using implanted subdural surface electrodes for direct cortical stimulation (Bosking et al., 2017). Moreover, anterior montages and higher frequencies are associated with more consistent and intense phosphene perception (Matsumoto et al., 2017). Although many tES studies focus solely on whether phosphenes can be perceived, relatively few have systematically examined how variations in frequency, current intensity, and stimulation site influence the qualitative characteristics of those phosphenes.

tACS effects on natural vision

Beyond eliciting phosphenes, tACS has demonstrated the potential to modulate visual perception and acuity. Reinhart et al. (2016) observed improvements in visual acuity and contrast sensitivity, particularly at high spatial frequencies, following stimulation. In studies involving healthy individuals, tES has been shown to produce perceptual effects that reach conscious awareness, accompanied by changes in ocular neurophysiology and enhanced cortical connectivity, as revealed by EEG (Perin et al., 2020). These findings support the hypothesis that electrical stimulation may positively influence natural visual processing, offering a noninvasive alternative for visual rehabilitation.

Although current treatments for major causes of blindness—such as glaucoma, age-related macular degeneration (AMD), diabetic retinopathy, and retinitis pigmentosa (RP)—are primarily focused on slowing disease progression, they generally do not restore

lost vision. Visual prosthetics, while promising, often involve invasive procedures and carry significant risk. In contrast, non-invasive approaches like tES offer a potentially safer means of modulating neural activity to enhance residual visual function (Sehic et al., 2016). However, despite these promising findings, tES techniques have not yet been integrated into routine clinical practice for vision rehabilitation, as evidence remains insufficient to support standardized treatment protocols (Park & Thompson, 2024). Hence, further empirical studies and a deeper understanding of how tES alters visual processing are needed.

Aims

The present study aims to systematically investigate how variations in transcranial alternating current stimulation (tACS) parameters—specifically frequency, intensity, and electrode montage—affect the subjective perception of phosphenes. These perceptual features will be evaluated using structured questionnaires to capture brightness, shape, spatial location, and somatosensory experiences. This goes beyond a simple "yes/no" phosphene threshold to a richer, multidimensional characterization of the induced percepts. Additionally, in order to evaluate the effects of tACS on natural visual processing, visual evoked potentials (VEPs) were recorded before and after the stimulation session. VEPs provide a sensitive and objective measure of early-stage cortical responses to transient visual stimuli and are widely used in both clinical diagnostics and research (Odom et al. 2016); accordingly, they serve as a valuable tool for clinical assessment of visual acuity, particularly when behavioral testing is not feasible or reliable (Hamilton et al., 2021). Changes in VEP components (e.g., latency shifts, amplitude modulations) following tACS can reveal how natural vision is affected by electrical stimulation. This multimodal approach—integrating subjective reports and VEPs—seeks to elucidate the mechanisms through which tACS influences the visual system.

STUDY AIMS

- 1. Describe the subjective perceptual characteristics of phosphenes induced by tACS, including brightness, shape, spatial location, size, color, and somatosensory experiences, through detailed self-report questionnaires.
- 2. Examine how variations in tACS parameters—specifically frequency, intensity, and electrode montage—influence the qualitative attributes of phosphene perception, applying repeated-measures analysis and multinomial regression.
- Measure changes in visual evoked potentials (VEPs) before and after tACS to objectively assess alterations in natural vision through latency and amplitude variations in response to visual stimuli.
- 4. Describe trends in VEPs before and after tACS to provide preliminary insights into how tACS may influence visual cortical processing.



METHODS

Participants

Eight healthy adults (3 males, 5 females), aged 19 to 35 years (mean age 26.2 ± 5.0 years), participated in the study after meeting the inclusion criteria. Participants were required to be at least 18 years old, have no history of neurological conditions or epilepsy, not currently be taking any psychoactive substances or medications, and possess normal or corrected-to-normal vision. Inclusion and exclusion criteria were verified through an online questionnaire prior to the experiment. Written informed consent was obtained from all participants. Recruitment was conducted via the Institute of Bioengineering, Miguel Hernández University of Elche. The study was approved by the Human Research Ethics Committee of the University (Approval #241125080958) and complied with the Declaration of Helsinki. Participant demographics are summarized in Table 1.

Participant	Sex	Age
1	F	Age 26
2	F	29
3	M	19
4	F	35
5	F	27
6	F	25
7	М	27
8	М	26

Table 1. Participant demographics (sex and age).

Experimental Design

A comprehensive dataset was collected, comprising 40 unique stimulation conditions (4 frequencies × 5 intensities × 2 montages) tested within a repeated-measures, within-subject design involving eight participants. This design allowed for the systematic examination of tACS-induced neuromodulatory effects on visual cortical activity and phosphene perception. Participants underwent randomized blocks of stimulation and assessment while keeping their eyes open throughout the sessions. A double-blind procedure was employed to ensure that both participants and experimenters remained unaware of the condition order and stimulation intensity. Each stimulation

condition was presented once per participant in a randomized sequence to minimize potential carryover and habituation effects.

Stimulation Equipment and Parameters

tACS was delivered using a battery-powered stimulator manufactured by Ripple Neuro, interfaced through a 126-channel EEG cap. EEG data acquisition and stimulation control were implemented using Trellis software combined with custom Python scripts. Electrode placement adhered to the international 10–20 system, employing two montages: FPz–Cz (frontal–central) and Oz–Cz (occipital–central). Each montage was tested across four carrier frequencies (8, 16, 28, and 50 Hz) and five current intensities (0, 0.125, 0.25, 0.375, and 0.5 mA peak-to-peak), resulting in a total of 40 conditions. All stimulation parameters complied with ICNIRP safety guidelines to ensure participant safety. Prior to stimulation, electrodes were prepared by applying conductive gel with a syringe, carefully parting the hair and spreading the gel in circular motions to optimize scalp contact and minimize impedance. Electrode impedances were monitored at the start and end of each experimental session, maintaining levels below 10 k Ω to ensure signal quality and stimulation efficacy.

Subjective Assessment of Phosphenes

Following each stimulation block, participants verbally completed a standardized questionnaire assessing their phosphene perception. The questionnaire evaluated multiple dimensions, including presence, number, size, shape, spatial location within the visual field, brightness (rated on a 1–5 scale, where 1 = very dim, 2 = dim, 3 = medium bright, 4 = bright, and 5 = very bright), color, and any associated somatosensory sensations. These subjective reports were subsequently analyzed in conjunction with EEG data to investigate correlations between perceptual experiences and physiological responses.

Objective EEG Measures

Visual Evoked Potentials (VEP): To assess early-stage visual cortical responses, VEPs were recorded in response to a high-contrast full-field checkerboard reversal stimulus, presented under either binocular or monocular viewing conditions, depending on the participant. Stimuli adhered to ISCEV standards (JV et al., 2016). Each block consisted of 200 trials, with a check size subtending approximately 1° of visual angle. The stimuli were displayed on an LCD screen measuring 62 × 38 cm (horizontal × vertical), with a diagonal of 72 cm. Participants viewed the screen from a distance of 50 cm. The luminance of the white and black checks was 208 and 0.07 cd/m², respectively, resulting in a Michelson contrast of approximately 99.9%. Data analysis focused on canonical VEP components—N1 (~75 ms), P1 (~100 ms), and N2 (~150 ms)—recorded from occipital electrodes (Oz, O1, O2).

Latencies were defined as the time point of the maximum negative or positive peak within standard time windows. Peak-to-peak amplitudes were calculated by measuring the voltage difference between the local maximum and minimum of adjacent components (e.g., P1–N1 amplitude was obtained by subtracting the voltage at the N1 trough from the preceding P1 peak; similarly, P1–N2 amplitude was calculated as the voltage difference between the P1 peak and the following N2 trough). All signals were baseline-corrected prior to peak detection to ensure consistency across participants.

Statistical Analysis

Behavioral Data Analysis: Repeated-measures ANOVA was used to assess the effects of stimulation frequency and current intensity on subjective phosphene perception (e.g., presence, brightness) within participants. For perceived brightness, a three-way repeated-measures ANOVA examined the interaction between current amplitude, frequency, and electrode montage. Subjective ratings of phosphene features were further explored using quadratic and cubic regression analyses to capture potential non-linear relationships. Mixed-effects models were conducted with frequency, intensity, and montage as fixed effects, and participant as a random intercept, to account for interindividual variability in perception. Categorical perceptual features such as phosphene

shape and spatial location were analyzed using multinomial logistic regression to test the influence of stimulation parameters.

To quantify perceptual sensitivity for distinguishing active vs. sham stimulation, signal detection theory (SDT) metrics were calculated and d-prime (d') scores were computed for each participant. Somatosensory experiences were binarized (present vs. absent) and analyzed separately for each montage across all combinations of current intensity and frequency. All repeated-measures analyses were corrected for multiple comparisons using Bonferroni or Holm adjustments to control for Type I error.

Visual Evoked Potential (VEP) Analysis: Pre- and post-stimulation VEPs were compared using paired-samples t-tests to examine differences in component latencies and peak-to-peak amplitudes (P1–N1 and P1–N2) across all participants. Percent change in VEP signal measures was calculated using the formula: Percent Change = [(Post – Pre) / Pre] × 100. All VEP data were baseline-corrected prior to peak detection to ensure consistency.

RESULTS

Behavioral Findings

This study tested how transcranial alternating current stimulation (tACS) parameters—specifically current intensity, frequency, and electrode montage—influence the subjective perception of phosphenes in healthy adults. On each trial, participants indicated whether they perceived a phosphene. Whenever they did, we collected ratings of the brightness of the perception, as well as its shape, size, color and spatial location. Additionally, we asked whether the participants felt any discomfort during the stimulation.

Phosphene Perception: Incidence and Distribution

Phosphenes were reported in 36.65% of all stimulation trials. The likelihood of phosphene perception was systematically analyzed as a function of current intensity, frequency, and montage.

Effect of Current Intensity on Phosphene Perception

Phosphene perception increased monotonically with stimulation current (Figure 1). Despite inter-individual variability in perceptual thresholds, the trend was robust across participants. Averaged across subjects, phosphene reports 23% at 125 μ A to approximately 64% at 500 μ A, demonstrating a clear intensity-response relationship.

A repeated-measures ANOVA confirmed a significant main effect of current intensity on phosphene perception, F (3, 21) = 51.57, p < .001. Post-hoc comparisons with Bonferroni correction revealed that stimulation at 375 μ A (t(21) = -6.28, p = .002, 95% CI [-0.44, -0.12]) and 500 μ A (t(21) = -13.57, p < .001, 95% CI [-0.53, -0.30]) led to significantly higher perception rates compared to 125 μ A. No significant difference was found between 250 μ A and 375 μ A (t(21) = -2.05, p = .478, 95% CI [-0.13, 0.04]), and the comparison between 375 μ A and 500 μ A showed a trend toward significance (t(21) = -3.87, p = .037, 95% CI [-0.26, -0.01]), though it did not reach the adjusted threshold for statistical significance.

These results suggests that higher currents increase neural activation in the visual system, which induces the perception of phosphenes.

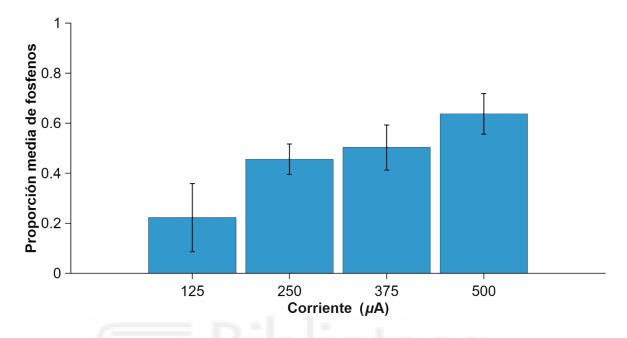


Figure 1. Proportion of phosphene perception as a function of current intensity averaged across participants presented with standard error of the mean (SEM).

Effect of Frequency on Phosphene Perception

Phosphene perception varied non-linearly across stimulation frequencies (Figure 2). The 16 Hz condition elicited the highest average perception (~65%), followed by 8 Hz (~37%) and 28 Hz (~35%), while stimulation at 50 Hz produced the lowest incidence (~12%).

A repeated-measures ANOVA revealed a significant main effect of stimulation frequency on phosphene perception, F (3,21) = 39.41, p < .001, indicating that the proportion of trials in which phosphenes were reported differed significantly across frequencies. Post hoc pairwise comparisons with Bonferroni correction showed that 16 Hz produced significantly higher phosphene perception than 8 Hz (t(21) = 6.07, p = .003, 95% CI [0.11, 0.44]), 28 Hz (t(21) = 6.80, p = .002, 95% CI [0.14, 0.45]), and 50 Hz (t(21) = 12.47, p < .001, 95% CI [0.37, 0.67]). Additionally, 8 Hz elicited significantly more phosphenes than 50 Hz (t(21) = 8.15, p = .0005, 95% CI [0.14, 0.36]). No significant

differences emerged between 8 Hz and 28 Hz (t(21) = 0.41, p = 1.0) or between 28 Hz and 50 Hz (t(21) = 3.33, p = .076).

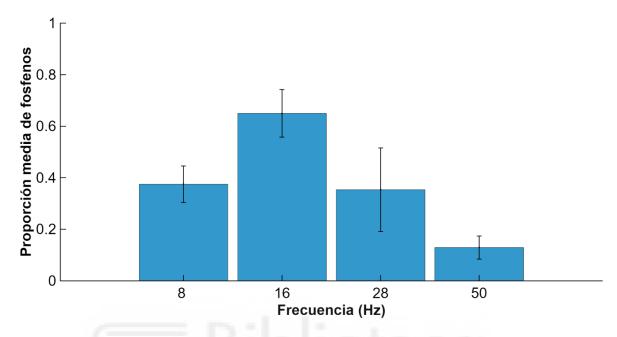


Figure 2. Proportion of phosphene perception as a function of frequency averaged across participants presented with standard error of the mean (SEM).

These results demonstrate that phosphene perception varies systematically with stimulation frequency, with intermediate frequencies (particularly 16 Hz) producing the strongest perceptual effects. This pattern supports the notion of frequency-dependent modulation of retinal or cortical excitability during transcranial alternating current stimulation.

Effect of Electrode Montage on Phosphene Perception

Phosphene perception differed significantly depending on electrode montage (Figure 3). A paired-samples t-test revealed a significant difference in phosphene perception between the two electrode montages (t (7) = 14.17, p < .001). Participants reported a higher proportion of phosphenes with the FPz–Cz montage (mean = 0.55, SD = 0.06) compared to the Oz–Cz montage (mean = 0.17, SD = 0.06).

These findings indicate that fronto-central stimulation (FPz-Cz) is more effective than occipito-central (Oz-Cz) in eliciting phosphenes, suggesting that electrode positioning plays a critical role in modulating subjective phosphene perception.

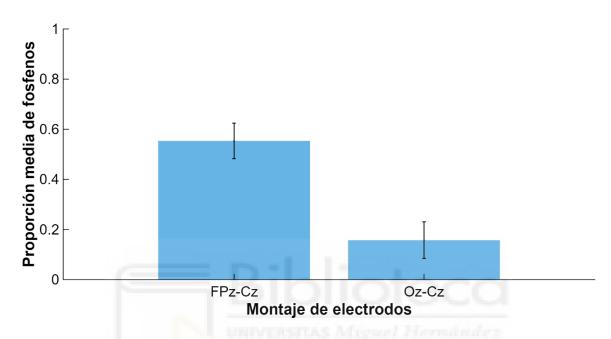


Figure 3. Proportion of phosphene perception as a function of electrode montage averaged across participants (with standard error of the mean).

Perceptual Sensitivity Analysis

To quantify participants' ability to distinguish between active and sham stimulation, we computed sensitivity using signal detection theory (SDT) metrics. Trials were classified as "stim" when any current was applied and as "sham" when the current was zero. Participant responses were binary ("yes" or "no") indicating perceived stimulation. Across a standardized set of 40 trials per participant (including 8 sham trials), the hit rate averaged approximately 40%, reflecting correct detection of stimulation during actual stim trials.

Importantly, we observed two distinct response profiles with regard to false alarms (reports of phosphenes during sham trials). Although some participants never reported perceiving phosphenes during sham trials, their false alarm rates were adjusted slightly above zero through the application of the standard log-linear correction. Seven of eight

participants exhibited very low false alarm rates, near 6%, resulting in a mean d' value of approximately 1.4. This indicates a moderate-to-high sensitivity and the ability to reliably discriminate stimulation from sham conditions. Conversely, one participant demonstrated a higher false alarm rate (12.5%), corresponding to a lower d' of around 1.07, suggesting reduced discriminability and a greater tendency to report phosphenes even in the absence of stimulation.

The overall findings support that the majority of participants had strong perceptual sensitivity to transcranial electrical stimulation, consistent with prior evidence of phosphene perception or somatosensory sensations under similar tES protocols. The low false alarm rates in most participants further strengthen the conclusion that the perceptual detection was specific and unlikely driven by guessing or response bias.

Effects of Frequency, Current and Montage on Perceptual Features

5.1 Brightness Ratings

Subjective brightness ratings (scale 1–5) increased with current intensity across all tested frequencies (Figure 4). The highest brightness was consistently reported at 16 Hz, especially at higher current amplitudes, followed by 8 Hz and 28 Hz, whereas 50 Hz stimulation elicited substantially lower brightness ratings regardless of current intensity.

A three-way ANOVA was conducted to examine the effects of stimulation current amplitude, frequency, and electrode layout on perceived brightness during transcranial electrical stimulation. The analysis revealed significant main effects of current (F (3, 233) = 14.72, p < .001, η^2_p = 0.16), frequency (F(3, 233) = 37.16, p < .001, η^2_p = 0.32), and layout (F(1, 233) = 133.70, p < .001, η^2_p = 0.36). These results indicate that each factor independently influences subjective brightness perception, with electrode layout and stimulation frequency accounting for the largest proportion of variance.

In addition, there were significant interactions between the different current intensities and electrode layouts (F (3, 233) = 3.78, p = .011, η^2_p = 0.05); as well as stimulation frequencies and electrode layouts (F(3, 233) = 10.99, p < .001, η^2_p = 0.12). This suggests that the effects of current and frequency on perceived brightness depend on the electrode montage used. The interaction between current and frequency was not significant (F (9, 233) = 0.82, p = .599, η^2_p = 0.03), indicating that the effects of current amplitude and frequency on brightness perception are independent of each other. The pattern observed in the figure supports the magnitude of the layout effect (η^2_p = 0.36) and the frequency × layout interaction (η^2_p = 0.12), indicating that the electrode configuration significantly modulates how stimulation frequency and current intensity affect subjective brightness perception.

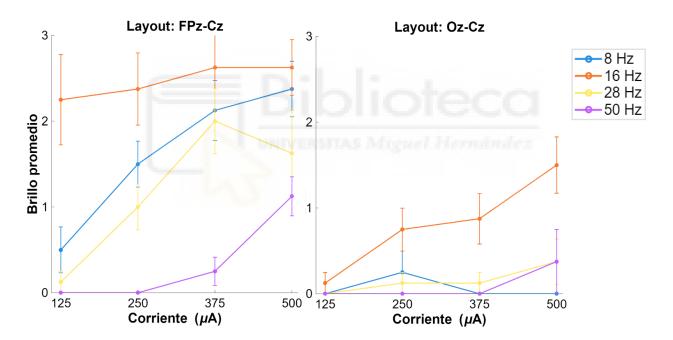


Figure 4. Average brightness rating as a function of current intensity, shown separately for each stimulation frequency and electrode montage across participants. Colored lines represent different stimulation frequencies (8, 16, 28 and 50 Hz). Error bars indicate standard error of the mean (SEM). Overall, perceived brightness increased with current intensity and was consistently higher for the FPz–Cz montage. The frequency effect was more pronounced at lower frequencies (16 Hz and 8 Hz) and at higher current levels, particularly for the FPz–Cz configuration.

5.2 Shape

To assess how transcranial alternating current stimulation (tACS) parameters influence the shape of phosphenes, we analyzed the distribution of reported shape

categories across different combinations of current intensity, stimulation frequency, and electrode montage. A total of eight distinct shape categories were reported by participants, ranging from simple percepts such as a "dot" and "flashing lights" to more structured or dynamic forms like "spiraling lights" or "flashing circle". Figure 5 illustrates the distribution of reported shapes across all stimulation conditions, stratified by electrode configuration (FPz–Cz vs. Oz–Cz). A clear divergence in shape diversity emerged between montages:

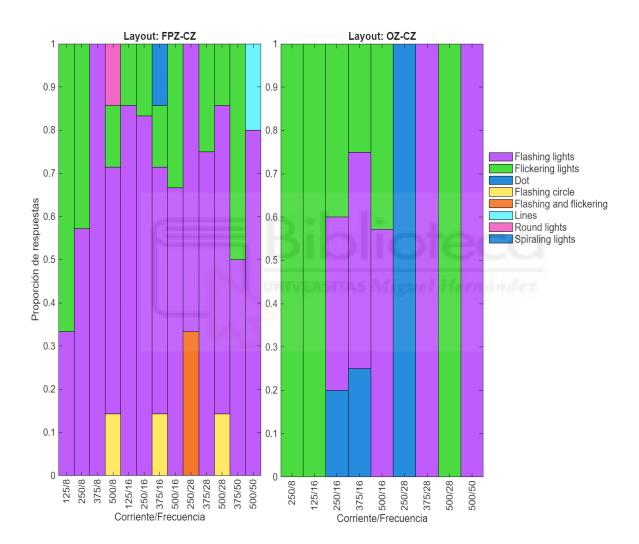


Figure 5. Average proportion of shape as a function of current intensity and frequency, shown separately for each electrode montage across participants.

FPz-Cz Montage: This configuration yielded the greatest diversity of percepts, particularly at lower stimulation frequencies and currents (e.g., 8 Hz, 125 µA), where

participants reported a wide range of shapes including "round lights", "flashing circle", and "lines". As current increased (e.g., $250-500 \,\mu\text{A}$), the responses converged toward simpler percepts, with "flashing lights" becoming dominant, suggesting a dose-dependent simplification in perceptual content.

Oz–Cz Montage: In contrast, this posterior configuration led to a more homogeneous perceptual profile. At moderate to high stimulation levels, percepts were overwhelmingly dominated by "flashing lights", with very limited reporting of more structured shapes. Only under lower intensity conditions (e.g., 8–16 Hz, ≤250 µA) did alternative forms such as "dot" or "spiraling lights" emerge.

Multinomial logistic regression was used to assess how stimulation parameters — current intensity, frequency, and montage — influenced the perceived shapes of phosphenes. Using "blinking lights" as the reference category, no statistically significant effects were observed in most comparisons (p > 0.1). However, consistent trends emerged that align with visual inspection of the data and support specific hypotheses. Although statistical significance was limited, several robust trends suggest systematic modulation of phosphene perception by stimulation parameters.

Increased current intensity was generally associated with reduced perceptual complexity, favoring simpler forms such as "flashing lights" over more structured shapes like "circles" or linear patterns. Higher stimulation frequencies appeared to increase the likelihood of perceiving dynamic patterns (e.g., "flickering lights") while strongly suppressing other forms, particularly "spiraling lights," which showed a significant negative association (p < 0.001). The use of a frontal montage (versus occipital) tended to promote greater perceptual variability, although this effect did not reach statistical significance in the regression model.

These directional effects, though not conclusive, suggest that increased stimulation intensity and frequency may influence the clarity or structure of phosphene forms. Due to wide confidence intervals and potentially limited statistical power, these findings should be interpreted with caution. See Table 1 for complete model estimates.

Shape	Predictor	β Coefficient	Odds Ratio (e^β)	p-value
Flashing lights	Current	-0.21	0.81	0.726
	Frequency	1.67	5.30	0.314
	Montage	0.95	2.58	0.654
Flickering lights	Current	-8.63	0.00018	0.0006 *
	Frequency	35.41	1.70E+15	<0.001 *
	Montage	2.17	8.75	0.427
Flickering dot	Current	-0.13	0.88	0.898
	Frequency	-2.33	0.10	0.615
	Montage	0.34	1.40	0.902
Flashing circle	Current	-2.08	0.13	0.167
	Frequency	1.98	7.25	0.290
	Montage	1.76	5.80	0.464
Round lights	Current	-0.96	0.38	0.532
	Frequency	7.70	2203.99	0.070
	Montage	-0.71	0.49	0.812
Lines	Current	-3.33	0.036	0.123
	Frequency	-0.32	0.72	0.872
	Montage	1.26	3.54	0.592
Spiraling lights	Current	-0.83	0.43	0.609
	Frequency	-34.10	1.77E-15	<0.001 *
	Montage	-1.99	0.14	0.466

Table 1. Multinomial Logistic Regression Results for the Effect of tACS Parameters on Phosphene Shape Perception (Reference Category: "Blinking Lights"). Statistically significant effects (p < 0.05) are marked with an asterisk and shown in bold. Positive β values indicate an increased likelihood of reporting that phosphene shape relative to "blinking lights"; negative β values indicate a decreased likelihood.

5.3 Location

Phosphene location reports (Figure 6) varied by montage, current, and frequency. FPz-Cz stimulation produced diverse locations including "Upper," "Backward," "Peripheral," "Left," "Central," and "Full Field," with "Upper" predominating, especially at 250 μ A and 50 Hz. Oz-Cz stimulation led to fewer locations, primarily "Upper," "Peripheral," and "Left," with unique "Lower" field reports absent in FPz-Cz.

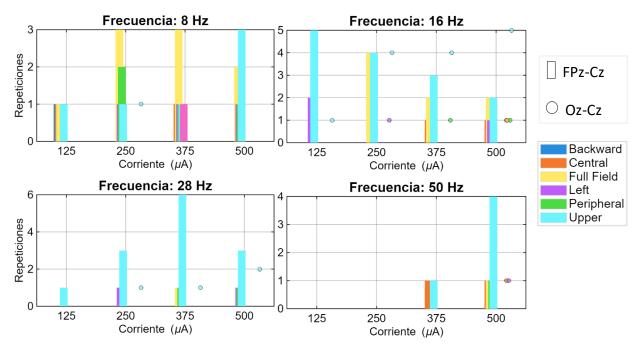


Figure 6. Average location distribution as a function of current intensity, shown separately for each stimulation frequency across participants.

A multinomial logistic regression model was employed to investigate how stimulation parameters — current intensity, frequency, and EEG montage — influence the perceived phosphene locations, using "Full field" as the reference category.

Overall, no statistically significant effects were detected for most predictors across the various phosphene location categories (p > 0.05). However, several notable trends were observed:

Current intensity showed some suggestive effects favoring more localized percepts. For example, the odds of perceiving phosphenes in the Central upper field were reduced with increased current (OR = 0.435, 95% CI [0.209, 0.906], p = 0.0261). Similarly, for the Central left field, current intensity had an OR close to zero (OR ≈ 0.000 , 95% CI [0.000, 0.115], p = 0.0261), suggesting a potential suppressive effect, although this was isolated.

Frequency effects were variable, with some large ORs and very wide confidence intervals, reflecting unstable estimates likely due to limited data. Notably, a significant positive association was observed for the Right central field (OR = 1.065, 95% CI [1.002, 1.132],

p = 0.0439), indicating that higher stimulation frequencies might increase the likelihood of phosphene perception in this location.

Montage layout showed no significant impact on location perception but was associated with increased variability. While most effects lacked statistical significance, these trends suggest stimulation parameters may subtly bias the spatial distribution of phosphenes, potentially reflecting differential cortical engagement. Confirmation with larger sample sizes is recommended.

5.4 Somatosensory Reports

To assess potential discomfort induced by transcranial alternating current stimulation (tACS), participants were asked to report any unpleasant or unusual sensations following each stimulation condition. These responses were binarized and aggregated across participants for each combination of current intensity and frequency, separately for the two electrode montages (Fpz-Cz and Oz-Cz).

As shown in Figure 7, discomfort was not reported in sham trials or at 125 μ A in any condition. At 250 μ A and above, the proportion of trials with discomfort increased, particularly under the Fpz-Cz montage, where rates of 40% were observed as early as 250 μ A. The Oz-Cz montage elicited fewer discomfort reports, with noticeable increases only at 375 μ A and above (Figure 8). Despite this upward trend, discomfort rates remained generally low, with the highest values (60%) occurring only in a minority of high-intensity conditions. These results suggest that the stimulation protocol was well tolerated overall, especially at or below 375 μ A.

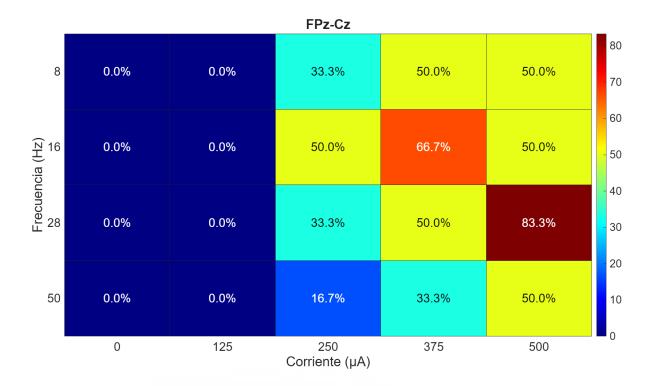


Figure 7. Percentage of somatosensory reports as a function of current intensity and frequency electrode montage in electrode montage Fpz-Cz across participants.

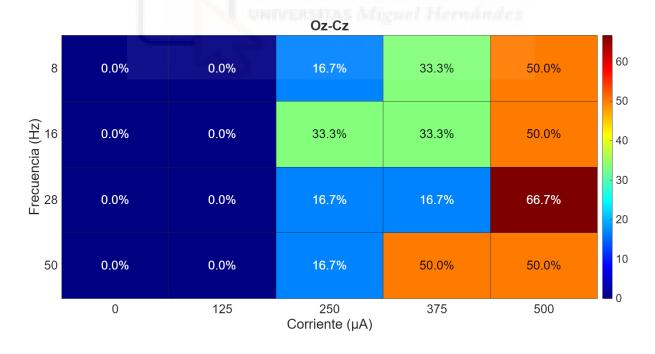


Figure 8. Percentage of somatosensory reports as a function of current intensity and frequency electrode montage in electrode montage Oz-Cz across participants.

Participants also provided qualitative descriptions of the sensations experienced. The most commonly reported sensations associated with discomfort were vibration, tingling, kneading, and knocking, primarily localized under the electrode sites. Vibration and tingling were especially prevalent under the Fpz-Cz montage at higher intensities. The distribution of these sensations supports the idea that tACS can evoke mild cutaneous effects depending on the montage and intensity, with frontal stimulation being more prone to somatosensory discomfort.

5.5 Number of phosphenes, size and color.

The most frequently reported response was "Multiple" phosphenes, accounting for 81.8% of valid trials (Figure 9). Singular experiences such as "One" (15.6%) and "Two" (1.3%) were much less common. An additional category, ">1", also appeared (1.3%), which conceptually overlaps with "Multiple", indicating possible ambiguity or redundancy in participant labeling.

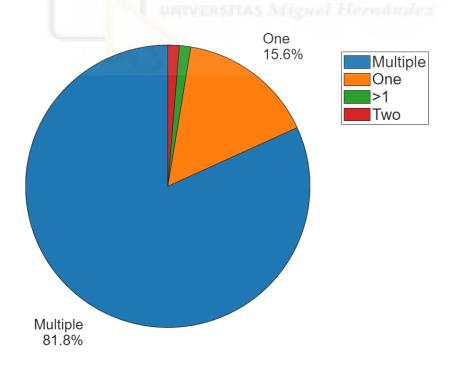


Figure 9. Average number of phosphenes reports across participants.

Responses were heavily skewed toward smaller perceived sizes. The "Small" category dominated, with 92.0% of all valid responses (Figure 10). Both "Medium" and "Big" sizes were reported in only 4.0% of cases each, suggesting that under the stimulation parameters tested, phosphenes were predominantly perceived as compact and localized phenomena

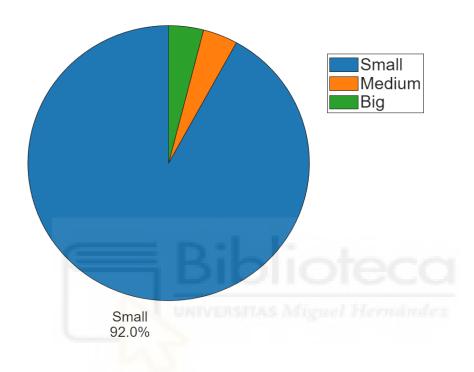


Figure 10. Average sizes of phosphenes report across participants.

Color perception was similarly concentrated. The vast majority of responses identified phosphenes as "White" (84.4%), while "Bright" and "Dark" hues were each reported in only 1.6% of cases (Figure 11). One participant response included a mixed description ("White/light green"; 1.6%), and 10.9% of trials were labeled as "Unspecified," likely indicating that no clear color percept was reported.

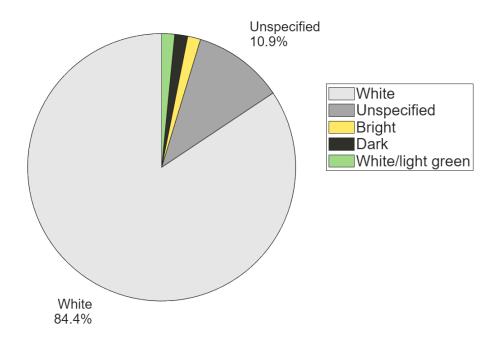


Figure 11. Average color of phosphenes reports across participants.

Analysis of Visual Evoked Potential (VEP) Latency and Amplitude Modulation

This section evaluates the effects of the experimental intervention on the temporal dynamics of visual cortical processing, as indexed by the latencies and amplitude of three key components of the visual evoked potential (VEP): N1, P1, and N2. While the study initially included eight participants, one was excluded from the VEP analysis due to technical issues during EEG data acquisition. Thus, the analyses are based on data from the remaining seven participants, and results are reported as mean ± standard error of the mean (SEM) to reflect inter-individual variability.

1. VEP Latency

Figure 12 illustrates the latencies of the N1, P1, and N2 VEP components before and after the intervention.

N1 Latency: The average N1 latency decreased from 78 ± 2.2 ms (Before) to 71 ± 1.6 ms (After). Despite this downward trend, a paired-samples t-test revealed that this

difference was not statistically significant (t(6) = 1.03, p = 0.343). Hence, there is no statistical evidence for faster early visual processing post-intervention in N1 latency in this sample.

P1 Latency: The P1 component showed only minimal change, with latency shifting from 110 ± 3.0 ms (Before) to 98 ± 2.9 ms (After). This small difference was not statistically meaningful (t(6) = 0.35, p = 0.739), and the wide overlap in SEM bars indicates high interindividual variability. The data therefore do not support an effect of the intervention on the timing of mid-latency VEP responses.

N2 Latency: Of all components, N2 latency exhibited the most notable numerical change, decreasing from 153 ± 4.9 ms (Before) to 146 ± 4.6 ms (After). This reduction aligns with a hypothesis of improved late-stage visual processing efficiency. However, statistical testing again yielded a non-significant result (t (6) = 1.81, p = 0.121). Although this trend may reflect an emerging effect, it does not reach statistical significance under the corrected alpha level, nor under the conventional 0.05 threshold.

While the observed reductions in latency, particularly for the N2 component, suggest a possible acceleration of visual processing following the intervention, the current results do not provide statistical evidence to support this conclusion. All comparisons failed to reach significance after correcting for multiple testing, and none were significant even at the conventional alpha level. Nevertheless, the consistent direction of latency changes across components (all showing reductions) is worth noting and may indicate subtle modulations in cortical timing that could not be reliably detected at the group level due to sample size constraints.

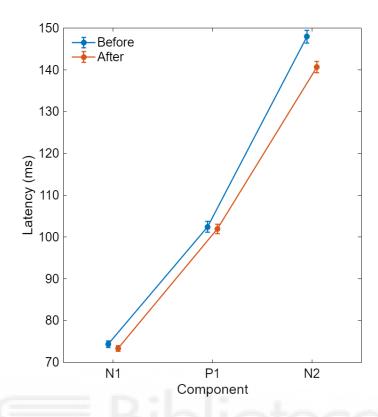


Figure 12: Average of the Latency components (N1, P1, N2) across participants with standard error of the mean.

2. VEP Amplitude

Figure 13 depicts the peak-to-peak amplitudes of the P1-N1 and P1-N2 VEP components before and after the intervention.

P1-N1 Amplitude: Before the intervention, the average P1-N1 amplitude was approximately 11 μ V, decreasing to around 9.7 μ V afterward. This trend suggests a possible attenuation of early visual cortical activity post-intervention. While this decrease may indicate more efficient neural processing, the difference did not reach statistical significance (t(6) = 1.92, p = 0.1038; Bonferroni-corrected α = 0.0250).

P1-N2 Amplitude: The P1-N2 component exhibited a small, statistically non-significant change (t(6) = 0.95, p = 0.3784). The mean amplitude shifted slightly from 8.6 μ V to 8.9 μ V, with overlapping error bars indicating high variability across participants.

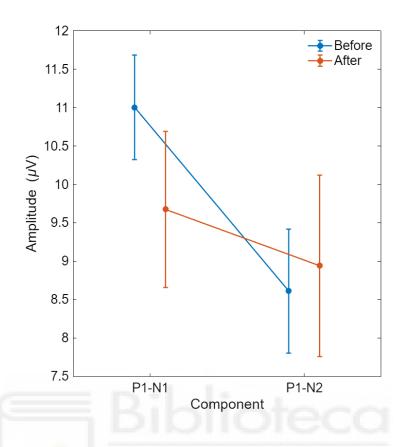


Figure 13: Average peak-to-peak amplitude (μ V) of P1 (P1-N1) and N2 (P1-N2) across participants with standard error of the mean

These findings, although not statistically significant, provide insightful trends. The observed reduction in P1-N1 amplitude—albeit not conclusive—may reflect a more economical neural response during early visual processing. When interpreted alongside the consistent (but non-significant) reductions in latency, particularly for the N2 component, the results suggest subtle modulations in visual cortical activity following the intervention. However, these trends did not reach significance in this sample and should be interpreted with caution.

DISCUSSION

1. Summary and Interpretation of Findings

This study presents a within-subject examination of how transcranial alternating current stimulation (tACS) modulates both subjective phosphene perception and objective visual cortical responses pre and post stimulation. By systematically varying stimulation frequency, current intensity, and electrode montage, and combining high-density EEG with VEP assessments, we provide a multidimensional characterization of tACS effects on the visual system in healthy adults.

Overall, our results show consistent effects of stimulation parameters on both the likelihood and phenomenology of phosphene perception, as well as modest trends suggesting functional modulation of visual cortical dynamics. Although some neural effects did not reach statistical significance, the convergence of behavioral and electrophysiological trends supports the notion that tACS engages frequency- and montage-specific mechanisms that can shape visual perception.

2. tACS-Induced Phosphenes Depend on Stimulation Parameters

Our behavioral findings reaffirm that phosphene induction via tACS is robustly influenced by current intensity, frequency, and electrode configuration. The monotonic increase in phosphene reports with rising current amplitude aligns with previous observations (Kanai et al., 2010; Kvasnak et al., 2022) and reinforces the importance of identifying safe yet effective thresholds. The estimated perceptual threshold near 250 μ A provides a practical reference point for future protocols that aim to balance efficacy with participant comfort.

Interestingly, we observed a non-linear relationship between frequency and phosphene perception. Peak sensitivity around 16 Hz, corresponding to the upper alpha band, supports theories linking alpha oscillations to perceptual gating and retinal-cortical resonance (Kar & Krekelberg, 2012; Evans et al., 2022). Conversely, the low incidence of phosphenes at 50 Hz, despite its higher energy content, suggests reduced cortical

entrainment or potential photoreceptor desensitization at gamma-band frequencies. These frequency-dependent patterns align with emerging models suggesting that tACS interacts with intrinsic network dynamics via resonance mechanisms, whereby externally applied currents are most effective when they match the natural oscillatory frequency of the targeted neuronal population (Helfrich et al., 2014; Ali et al., 2013). This resonance phenomenon is thought to enhance phase alignment and modulate the amplitude of endogenous rhythms, thereby increasing cortical excitability in a frequency-specific manner. For the visual system, this implies that mid-alpha stimulation may reinforce functional oscillatory circuits involved in perceptual gating, while higher frequencies may fail to engage these circuits effectively due to a lack of frequency matching or increased inhibitory feedback.

The montage effect was also pronounced. Stimulation with an FPz–Cz montage consistently produced stronger and more frequent phosphenes than Oz–Cz. This finding is notable, given that the occipital cortex is the canonical target for visual modulation. The enhanced efficacy of frontal placements may reflect increased current penetration through thinner cranial structures (Opitz et al., 2015) or more effective engagement of prefrontal-visual feedback loops. Such anterior–posterior interactions could enhance cortical excitability and perceptual integration, potentially accounting for the broader spatial distribution and increased complexity of phosphene percepts—often extending across a larger portion of the visual field or exhibiting more elaborate shapes—observed during frontal stimulation.

3. Blinding Integrity and Perceptual Sensitivity

Signal detection theory (SDT) analyses demonstrated that participants discriminated active tACS from sham with relatively high sensitivity (mean d' = 1.80), despite low false alarm rates. This indicates that subjective detection was unlikely to result from guesswork or response bias, underscoring the challenge of maintaining full blinding in sham-controlled tACS paradigms — an important consideration for clinical trial design.

4. Phosphene Features and Somatosensory Tolerability

The subjective features of phosphenes—shape, brightness, location, and number—varied systematically with stimulation parameters, indicating that tACS can modulate the qualitative nature of visual percepts. Higher intensities were generally associated with simpler, more stereotyped shapes (e.g., "flashing lights"), suggesting perceptual saturation under strong excitatory input. Montage-specific differences in reported location support the idea of topographically distinct activation zones, consistent with intracortical stimulation studies showing eccentricity-dependent phosphene maps (Brindley & Lewin, 1968; Bosking et al., 2017).

Crucially, somatosensory side effects remained minimal across conditions, with discomfort only increasing moderately above 375 μ A. Reported sensations were typical of superficial current spread (e.g., tingling or vibration) and are in line with prior safety studies (Antal et al., 2017). This reinforces the tolerability of sub-500 μ A tACS for both research and potential therapeutic use.

5. Visual Evoked Potentials.

The VEP results provide preliminary evidence that tACS modulates visual cortical excitability beyond subjective phosphene generation. Although none of the amplitude or latency changes reached statistical significance, the consistent trend toward reduced N2 latency may suggest a modest acceleration of visual processing, potentially reflecting increased synaptic efficiency or improved phase alignment of visual networks (Reinhart et al., 2016). These latency shifts align with models positing that tACS can entrain endogenous oscillations and sharpen neural timing (Helfrich et al., 2014).

The observed reductions in P1–N1 and P1–N2 amplitudes, though not statistically significant, should be interpreted cautiously. While some neuromodulation studies link amplitude reductions to more economical processing (Fröhlich & McCormick, 2010), it is equally plausible that lower amplitudes here reflect reduced attentional engagement or participant fatigue — factors known to modulate VEP components (Polich, 2007). As our

recordings occurred during naturalistic viewing without an explicit attentional task, this context likely influenced the observed variability.

Overall, the VEP trends — when combined with the robust perceptual effects — strengthen the interpretation that tACS can subtly modulate cortical dynamics in a frequency- and montage-dependent manner. Future studies employing larger samples, repeated-measures designs, and real-time artifact suppression will be vital to verify these trends and clarify their functional relevance.

6. Limitations and Future Directions

Several limitations warrant consideration. First, the modest sample size limits generalizability and may have reduced power to detect subtle electrophysiological effects. Second, we did not manipulate waveform shape, duration, or cognitive task engagement — all of which could interact with tACS effects on visual perception. Third, our study did not include analyses of ongoing oscillatory activity during stimulation, as real-time EEG recordings are highly susceptible to tACS-induced artifacts. Future studies should prioritize artifact-suppression approaches to enable clearer assessment of neural entrainment in real time.

Despite these constraints, the present findings offer valuable insights into the parameter-specific modulation of visual perception and cortical excitability via tACS. They also underscore the importance of tailoring stimulation protocols to individual neurophysiological profiles. Mid-alpha frequency stimulation over frontal montages, in particular, appears promising for enhancing residual vision or perceptual awareness in clinical populations.

CONCLUSIONS

In summary, our findings demonstrate that tACS at 16 Hz and intensities ≥250 µA reliably elicits phosphenes and modulates visual cortical function in a frequency-, intensity-, and montage-dependent manner. Fronto-central (FPz–Cz) stimulation consistently produced the most vivid and frequent percepts, with perceptual features such as brightness, shape, and location varying systematically with stimulation parameters—supporting spatial and functional specificity in visual pathways. Somatosensory side effects were mild, montage-dependent, and well-tolerated, reinforcing the feasibility of using sub-threshold tACS for visual modulation. VEP results revealed a trend toward reduced N2 latency following stimulation, suggesting a potential enhancement of cortical processing speed, however, this effect did not reach statistical significance.

Collectively, these results outline a robust framework for targeted, non-invasive neuromodulation of visual function and advance our understanding of how non-invasive brain stimulation can influence subjective visual perception and objective electrophysiological markers of visual function.

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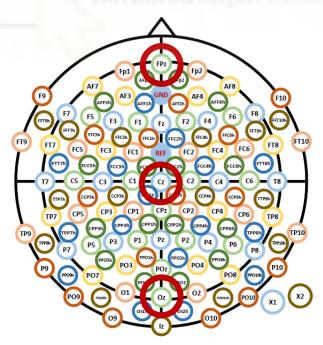
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APPENDICES



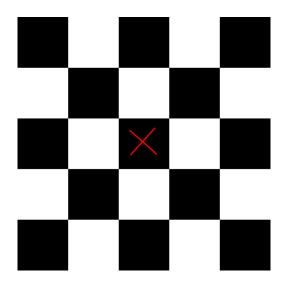
Appendix 1. EGG cap with 126 electrodes.



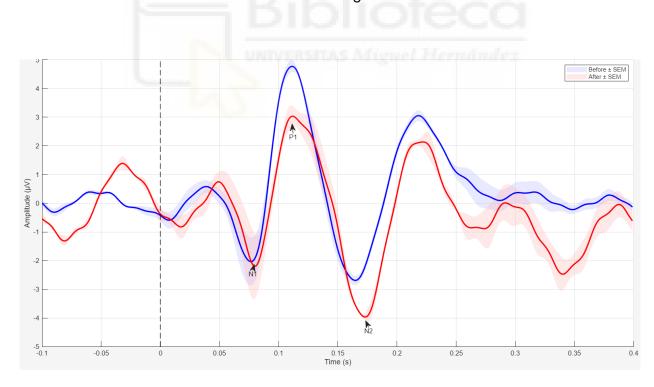
Appendix 2. Channel Naming Conventions and Stimulated Electrode Locations on the EEG Cap (Marked in Red).

Cuestionario: Apariencia de fosfenos								
Ensayo	Sí/No	Número	Forma	Tamaño	Ubicación	Brillo	Color	Molestia
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
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Appendix 3. Template of the Phosphene Appearance Questionnaire.



Appendix 4. Checkerboard Pattern with Central Fixation 'X' Used During VEP Recording.



Appendix 5. VEP Waveform Response of a Participant Before (Blue) and After (Red) Stimulation, with N1, P1, and N2 Components indicated by Arrows.