Heliyon 10 (2024) e39358

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Binocular imbalance measured by SSVEP predicts impaired stereoacuity in amblyopia

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ARTICLE INFO

Keywords:
Amblyopia
SSVEP
Binocular imbalance
Stereoacuity
Binocular suppression
Interocular interaction

ABSTRACT

Purpose: The current study aims to implement steady-state visual evoked potentials (SSVEPs) in quantifying the binocular imbalance of amblyopia and to assess the predictive value of SSVEP-derived indices for amblyopic stereoacuity.

Methods: We measure frequency-tagged SSVEP responses elicited by each eye ($F1 = 6$ Hz through the fellow eye; $F2 = 7.5$ Hz through the amblyopic eye) within a binocular rivalry paradigm among a cohort of anisometropic amblyopic observers ($n = 29$, mean age: 12 years). Binocular suppression was quantified by assessing the disparity in SSVEP amplitudes between the eyes, while the strength of interocular interaction was evaluated through the intermodulation response at $F1+F2 = 13.5$ Hz. Subsequent analyses explored the associations between these neural indices and relevant behavioral metrics in amblyopia.

Results: Results reveal a significant difference in SSVEP amplitudes elicited from the fellow eye and the amblyopic eye, with the former exhibiting notably higher responses. Moreover, the fellow eye demonstrated prolonged dominance duration compared to its amblyopic counterpart. Furthermore, a negative correlation between binocular suppression and interocular interaction was observed, with stereoacuity showing a significant correlation with binocular suppression. Utilizing stepwise mulptiple linear regression analysis, we established that a predictive model combining binocular suppression and visual acuity of the amblyopic eye provided the best prediction of stereoacuity.

Conclusions: These results highlight the potential of binocular suppression, as assessed by SSVEPs within a binocular rivalry paradigm, as a promising neural predictor of stereopsis in amblyopia.

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https://doi.org/10.1016/j.heliyon.2024.e39358
Received 16 April 2024; Received in revised form 25 September 2024; Accepted 12 October 2024

1. Introduction

Amblyopia is a neurodevelopmental disorder characterized by dysfunctions in the amblyopic eye, which impair visual processing and affect daily activities [1,2]. Typical deficits associated with amblyopia include a reduction in visual acuity in the amblyopic eye and abnormalities in binocular functions, particularly stereoacuity. Traditionally, visual acuity in the amblyopic eye has been considered the primary concern in amblyopia, with impaired stereovision seen as a secondary consequence. These conventional clinical assessments, evaluating both monocular and binocular vision, have been widely employed as essential behavioral screening measures to gauge the extent of visual impairment in individuals with amblyopia [3,4].

In contrast, recent studies have proposed that developmental deficits in the visual cortex disrupt the normal communication between the two eyes in amblyopia, resulting in imbalanced binocular interactions among visual neurons [5–8]. These findings suggest that the primary concern in amblyopia should be imbalanced binocular interactions, rather than the visual acuity of the amblyopic eye. In fact, novel binocular treatment approaches aimed at rebalancing the inputs presented to both eyes have shown promising results in improving visual acuity and stereoacuity in adult amblyopia [9–11]. These lead to a reasonable assumption that directly assessing binocular imbalances using neural signals in the visual cortex may offer a potent method for evaluating the deficits and plasticity associated with amblyopia.

Binocular imbalance encompasses both binocular suppression and interocular interaction. Behavioral studies have indicated that stronger inhibition between the two eyes is associated with the severity of visual deficits [5,12]. These inhibitory interactions lead to suppression from the fellow eye toward the amblyopic eye, ultimately resulting in suboptimal performance in both monocular and binocular visual tasks [13–15]. Previous neurophysiological studies have reported reduced neural responses to stimuli presented to the amblyopic eye compared to those presented to the fellow eye [16–20]. Meanwhile, steady-state visual evoked potential (SSVEP) is a brain response synchronized with periodic visual stimuli, primarily originating from the primary visual cortex [21]. It is a reliable and less time-consuming method [22,23] widely implemented in the field of brain computer interface (BCI) [24] and cognitive neuroscience [25]. Neural activity assessed through SSVEPs has revealed deficient binocular visual functions and abnormal activation in the visual cortices of amblyopic adults [20,22,26]. Nevertheless, whether SSVEPs can quantitatively measure the degree of binocular suppression and interocular interaction in amblyopic children and young adults has remained uncertain.

In the current study, we conducted measurements of frequency-tagged SSVEP responses for each eye within a binocular rivalry paradigm involving a group of amblyopic individuals. The objective was to investigate the utility of SSVEP signals in quantifying binocular suppression and interocular interaction in the context of amblyopia. Additionally, we aimed to assess whether these SSVEP-based indices could serve as predictors for both monocular and binocular impairments in amblyopic eyes.

Table 1
Clinical information on patients with anisometropic amblyopia.

Subjects	Age	Sex	BCVA of Fellow eye (logMAR)	BCVA of Amblyopic eye (logMAR)	Stereoacuity (log arcsec)
1	8	M	0	0.7	3.845098
2	14	F	0	0.2	3.845098
3	17	M	0	0.3	2.39794
4	16	M	0	0.8	3.845098
5	9	M	−0.1	0.2	2.69897
6	7	M	0	0.7	3.845098
7	9	M	0	0.2	1.845098
8	7	M	0	0.2	2.30103
9	7	M	0	0.4	2.30103
10	12	F	0	1	3.845098
11	10	M	0	1	3.845098
12	9	M	0	0.8	3.845098
13	16	F	0	0.7	3.845098
14	12	F	0	0.3	3.845098
15	8	F	0	0.4	3.845098
16	7	M	0	0.3	2.39794
17	12	M	0	0.3	2.60206
18	8	M	0	0.2	1.845098
19	8	M	0	0.2	1.845098
20	29	M	0	0.7	3.845098
21	28	M	0	0.4	2
22	21	F	0	0.2	2.30103
23	10	F	−0.1	0.2	3.845098
24	13	F	0	0.2	3.845098
25	11	M	0	0.2	3.845098
26	11	M	0	1	2.69897
27	12	M	0	0.2	3.845098
28	7	M	0	0.2	1.845098
29	11	F	0	0.3	2.69897

2. Methods

2.1. Participants

The study involved children and young adults, with subjects ranging in age from 7 to 29 years. This included ten healthy adults (7 females and 3 males; Age: Mean ± SD: 20.30 ± 0.82) and twenty-nine individuals diagnosed with anisometropic amblyopia (9 females and 20 males; Age: Mean ± SD: 12.03 ± 5.72). The study received ethical approval from the Zhongshan Ophthalmic Center Ethics Committee (V 20191017.1), and was conducted in compliance with the principles outlined in the Declaration of Helsinki. Prior to data collection, all subjects, or their legal guardians, provided informed consent by signing consent forms.

Participants diagnosed with anisometropic amblyopia had to satisfy the following criteria for inclusion: (1) an interocular BCVA (Best Corrected Visual Acuity) difference of 0.2 logarithms of the minimum angle of resolution (logMAR) or greater (equivalent to 2 lines); (2) a spherical equivalent or interocular astigmatism difference of 1.0 diopter (D) or more; (3) the absence of any prior systemic or ocular disorders, except for anisometropic amblyopia. Healthy participants without any systemic or ocular disorders were required to have normal BCVA in both eyes (0 or −1 logMAR) and normal stereoacuity (at least 40 arcseconds). The dominant and nondominant eyes in the control group were determined using the hole-in-the card test [27].

All participants were provided with refractive correction that adhered to the following criteria, as compared to their cycloplegic refraction: (1) Hyperopia was undercorrected by no more than 1.5 diopters (D); (2) The spherical equivalent (SE) was within 0.5 D of full anisometropia; (3) Cylinder power was within 0.5 D of full astigmatism; and (4) The cylinder axis was within 6° when the cylinder power was equal to or greater than 1.0 D. Participants who met these requirements continued to use their customary refractive correction as usual.

All participants underwent a series of assessments, which included the following: cycloplegic refraction, slit-lamp examination,

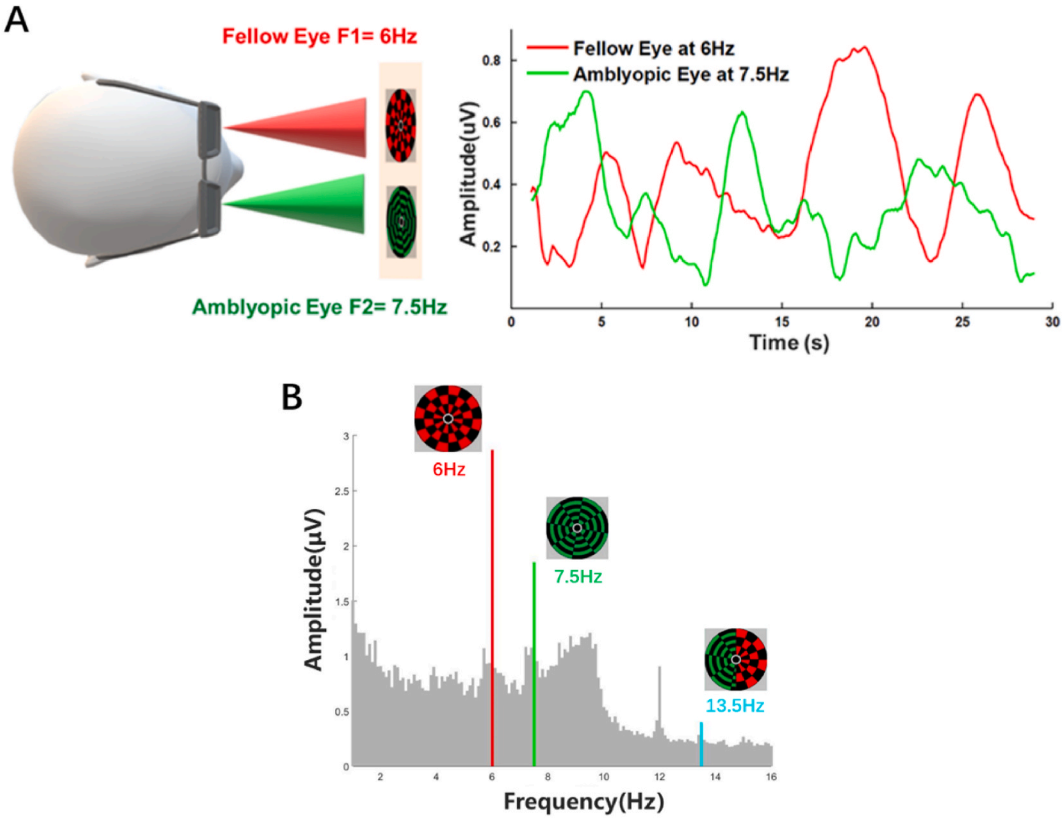

Fig. 1. Experimental Design and Sample SSVEP Responses. **(A)** Binocular stimuli in varying colors were simultaneously presented to each eye. The stimulus directed to the fellow eye exhibited a flicker frequency of 6.0 Hz, while the stimulus presented to the amblyopic eye had a flicker frequency of 7.5 Hz. In a typical trial, the time course illustrates alternating dominant responses to the fellow-eye frequency (depicted by the green line) and the amblyopic-eye frequency (indicated by the red line). It is noteworthy that responses to the fellow-eye frequency, in general, displayed higher amplitude and an extended dominance duration when compared to responses to the amblyopic-eye frequency. **(B)** In this illustrative trial, a red circular checkerboard was presented to the fellow eye, while a green circular checkerboard was presented to the amblyopic eye. Neural responses driven by visual inputs in the fellow eye (F1 = 6 Hz) and the amblyopic eye (F2 = 7.5 Hz) are evident in the amplitude spectrum. When the amblyopic eye dominated, a prominent peak at 7.5 Hz was observed. Notably, our results also revealed a distinct intermodulation response at 13.5 Hz. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

funduscopic examination, measurement of best-corrected distance visual acuity using the Early Treatment Diabetic Retinopathy Study numbers chart, and assessment of stereoacuity using the Randot Stereotests (Stereo Optical Company, Inc, Chicago, IL, USA). Stereoacuity measurements were converted to log arcseconds (arcsec), while best-corrected visual acuity (BCVA) measurements were converted to logMAR. For patients who did not exhibit measurable stereoacuity, a value of 3.845098 log arcsec was assigned for subsequent data analysis. Table 1 provides clinical information for individuals with anisometropic amblyopia.

2.2. Apparatus and stimuli

Stimuli were generated and presented using MATLAB (Mathworks, Natick, MA, USA) and Psychtoolbox, following the methods of previous studies employing SSVEP in binocular rivalry [28]. The display screen used was a gamma-corrected 27-inch ASUS VG278HE monitor with an average luminance of 150 cd/m^2 and a resolution of 1920 × 1080 pixels, operating at a refresh rate of 120 Hz. Participants observed binocular rivalry stimuli in a dark, shielded room from a distance of 57 cm, facilitated by the use of stereo-goggles (NVIDIA 3D Vision 2). As part of the binocular rivalry stimuli, each eye simultaneously received incompatible circular checkerboards with a width of 10° visual angle (as illustrated in Fig. 1A). These circular checkerboards were presented in different colors: red ([255,0,0]) for one eye and green ([9,148,11]) for the other, against a whole-screen black background (grey: [195,195, 195]). Before each experimental block, the position of the dichoptic circular checkerboards for each participant was meticulously aligned by gradually adjusting the position of the checkerboard visible to the left eye. Fusion was deemed successful when the participant reported perceiving only one circle-shaped checkerboard. Throughout the experiment, a chinrest was employed to minimize head movements and ensure stable viewing conditions.

The two checkerboard patterns were designed to flicker at different frequencies, inducing SSVEP responses through contrast reversals on each trial. Specifically, the checkerboard presented to the fellow eye flickered at 3 Hz (with a second harmonic frequency F1 = 6 Hz), while the checkerboard presented to the amblyopic eye flickered at 3.75 Hz (with a second harmonic frequency F2 = 7.5 Hz). Participants were instructed to maintain their gaze on the central black spot. For normally sighted individuals, the right eye's checkerboard flickered at 3 Hz, while the left eye's checkerboard flickered at 3.75 Hz. However, due to challenges faced by young children in our study, most of the amblyopic children were not able to report their perceived stimuli. Nevertheless, all the control adult participants, as well as three of the amblyopic participants, were able to provide their reported perceptions by using the keyboard. They pressed the left key for green, the right key for red, and the down key for mixed colors to indicate their perceived stimulus.

2.3. Study procedures

For amblyopic participants who had previously undergone treatment, they were instructed to discontinue any patching treatments for at least one day or atropine use for at least one week before participating in the experiment. Prior to the SSVEP test, each participant's stereoacuity and best-corrected visual acuity (BCVA) were evaluated, and a summary of the data can be found in Table 1. During each trial of the SSVEP test, participants observed binocular rivalry stimuli for a duration of 30 s while their EEGs were recorded. The test included a total of 18 trials. The colors and checkerboard patterns of the binocular stimuli were swapped for each eye and were balanced across the trials.

2.4. EEG acquisition and data analysis

We utilized a 32-channel wireless EEG system (NeuSen32, Neuracle, China) to capture EEG data, with a sampling rate of 1000 Hz. Given that SSVEP responses primarily manifest in occipital regions [21], we limited our electrode placement to occipital sites (Oz, O1, O2, Pz, P3, P4, PO3, PO4) to streamline the setup process. The ground electrode was positioned at AFz, and the reference electrode was situated at CPz. To ensure optimal data quality, all electrode impedances were maintained below 10 kΩ.

The analysis of EEG data was performed using custom MATLAB scripts and functions from EEGLAB [29]. To reduce noise, a Laplacian spatial filter was applied to the raw data [30] by subtracting the signal at Oz from the average of the signals at nearby electrodes (Pz, P3, P4, PO3, PO4, O1, O2). The EEG signals obtained were segmented into 30-s epochs for each trial and subjected to band-pass filtering between 1 and 30 Hz using a Finite Impulse Response (FIR) filter [31]. A small fraction of trials (approximately 2.1 % of all trials) had to be discarded due to technical issues, such as missing recording markers. Consequently, the total number of epochs available for subsequent analysis ranged from 15 to 18 in individual participants.

To extract SSVEP amplitudes at particular frequencies (i.e., F1 = 6 Hz for the fellow-eye frequency, F2 = 7.5 Hz for the amblyopic-eye frequency, and F1+F2 = 13.5 Hz for the intermodulation response) over time, a recursive least-squares adaptive filter (RLS) technique was employed. The analysis time window was set at 2 s, resulting in a frequency resolution of 0.5 Hz.

Signal-to-noise ratios (SNR) at the fundamental and intermodulation frequency (F1+F2) were found to be significantly greater than 1 ($SNR>1$, $p < 0.01$). In relation to Fig. 1B, the recorded neural signals showed strong second harmonic frequencies (F1 = 6 Hz and F2 = 7.5 Hz), along with distinctive intermodulation frequency (F1 + F2 = 13.5 Hz). Responses at higher harmonic frequencies (2F1 = 12 Hz, 2F2 = 15 Hz, 3F1 = 18 Hz, 3F2 = 22.5 Hz) and other intermodulations (mF1±nF2) were relatively weak. Their amplitudes did not significantly differ from adjacent noise levels and were therefore excluded from this study.

To minimize inter-subject variability, all amplitudes were normalized to the amplitude of the fellow-eye frequency within each participant. Specifically, the binocular suppression index was computed as follows:

Binocular suppression index = $(\text{Amplitude}_{\textit{Fellow/right eye}} - \text{Amplitude}_{\textit{Amblyopic/left eye}})/\text{Amplitude}_{\textit{Fellow/right eye}}$

Additionally, the interocular interaction between the two eyes was calculated using the following formula:

$$\textbf{Interocular interaction index} = \text{Amplitude}_{\textit{Intermodulation}}/\text{Amplitude}_{\textit{Fellow/right eye}}$$

2.5. Statistical analysis

Data analysis was conducted using SPSS Version 22, with a significance level of $p < 0.05$ for all analyses. The following statistical tests and methods were employed:

The differences in SSVEP data, visual acuity, and stereoacuity were assessed using the Wilcoxon signed-rank test.

To investigate the relationships between variables, the Spearman rank correlation was utilized.

To assess the contribution of binocular interactions to stereoacuity, stepwise multiple linear regression analysis were employed. Binocular suppression, interocular interaction, visual acuity, and age were entered into the model step by step to predict stereoacuity.

3. Results

3.1. Neural responses and dominance in anisometropic amblyopia

A total of twenty-nine patients with a history of anisometropic amblyopia participated in this study. The best-corrected visual acuity (BCVA) of their amblyopic eyes ranged from 0.20 to 1.00 logMAR, with a mean of 0.42 ± 0.05 (mean ± SEM), while stereoacuity ranged from 1.85 to 3.85 log arcsec, with a mean of 3.09 ± 0.15.

There was a significantly positive correlation between BCVA and stereoacuity observed across all patients (Spearman rank correlation: Rho = 0.45, $p < 0.05$). During the SSVEP test, participants viewed binocular rivalry stimuli featuring flickering images at different temporal frequencies for each eye (F1 = 6 Hz for the fellow eye and F2 = 7.5 Hz for the amblyopic eye). The recorded neural signals exhibited robust second harmonic responses at the driven frequency of each eye (F1 = 6 Hz for the fellow eye and F2 = 7.5 Hz for the amblyopic eye), along with clear intermodulation (IM) responses at the sum frequency (F1 + F2 = 13.5 Hz), as illustrated in Fig. 1B.

Fig. 2A illustrates the amplitudes of neural responses to the fellow-eye frequency, the amblyopic-eye frequency, and the intermodulation (IM) response for each participant. Notably, the average response from the fellow eye significantly exceeded that from the amblyopic eye (Overall: fellow eye: 1.21 ± 0.12; amblyopic eye: 0.82 ± 0.08; Wilcoxon signed-rank test: $z = -3.45$, $p < 0.001$).

Furthermore, we calculated the duration during which the amplitude of neural responses to one eye's frequency surpassed the other, reflecting the neural dominance of either the fellow eye or the amblyopic eye. The result indicated that the mean dominance of the fellow eye represented a significantly larger proportion than the dominance of the amblyopic eye (Overall: fellow eye: 67.89 ± 3.69 %; amblyopic eye: 32.11 ± 3.69 %; Wilcoxon signed-rank test: $z = -3.69$, $p < 0.0001$), as depicted in Fig. 2B.

Despite only three amblyopic participants providing perceptual reports in our study, each of them indicated a longer perceived duration for the fellow eye compared to the amblyopic eye (fellow eye: 11.73 ± 3.09s; amblyopic eye: 6.34 ± 0.34s) based on their reports. Moreover, we observed a significant positive correlation between the amplitude of neural responses to the fellow-eye

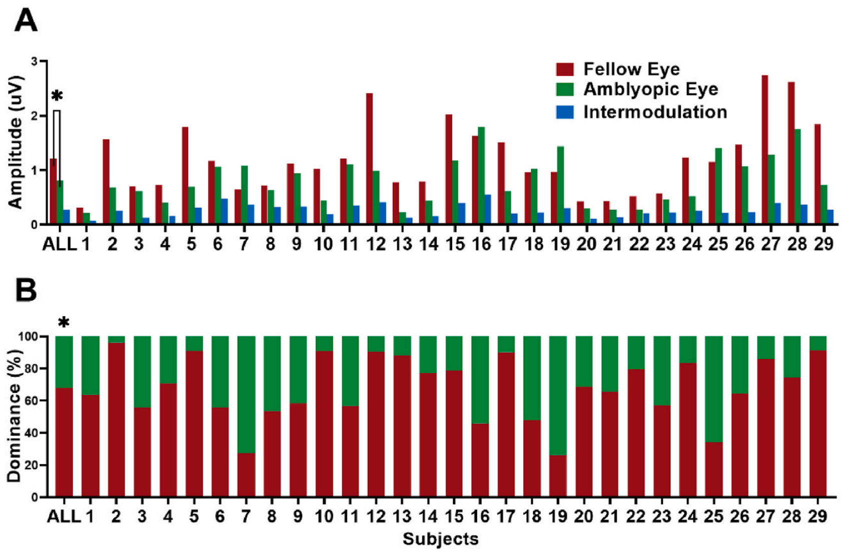

Fig. 2. Neural Responses and Dominance in the Anisometropic Amblyopic Visual Areas. (A) This panel displays the amplitude of neural responses for each participant in response to visual inputs from the fellow eye (red bar), the amblyopic eye (green bar), and the intermodulation term between the two eyes (blue bar). **(B)** The percentages of neural dominance for each participant are presented for the fellow eye (red bar) and the amblyopic eye (green bar). Data for all participants are depicted as the mean; asterisks indicate statistical significance; *$p < 0.001$. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

frequency and the percentage of neural dominance for the fellow-eye frequency across all patients (Spearman rank correlation: $Rho = 0.41$, $p < 0.05$). These results collectively highlight stronger neural responses and dominance of the fellow eye over the amblyopic eye in individuals with anisometropic amblyopia.

3.2. Neural binocular interactions in anisometropic amblyopia

Two indices derived from SSVEP responses were utilized to assess neural interactions in anisometropic amblyopia: binocular suppression and interocular interaction (for additional details, please refer to the Method section). Fig. 3A displays these two indices for each participant and their respective mean values. Notably, a negative correlation was observed between binocular suppression (0.27 ± 0.06) and interocular interaction (0.25 ± 0.02), as depicted in Fig. 3B (Spearman rank correlation: $Rho = -0.65$, $p < 0.001$).

3.3. Predictive effect of neural binocular interactions on stereovision in anisometropic amblyopia

We further examined the relationship between neural binocular interactions and behavioral measurements. Stereoacuity, measured in log arcsec, exhibited correlations with binocular suppression (Spearman rank correlation: $Rho = 0.37$, $p < 0.05$) and the percentage of fellow-eye dominance (Spearman rank correlation: $Rho = 0.40$, $p < 0.05$) in Fig. 4, indicating that stronger binocular suppression is associated with weaker stereovision. However, the visual acuity of the amblyopic eye did not show significant correlations with binocular suppression, interocular interaction, or the dominance of the fellow eye (Spearman rank correlation: Binocular Suppression: $Rho = 0.19$, $p > 0.05$; Interocular Interaction: $Rho = -0.11$, $p > 0.05$; Dominance of Fellow Eye: $Rho = 0.21$, $p > 0.05$).

To further dissect the contributions of neural indices, behavioral measurements, and age to stereoacuity, we employed a stepwise multiple linear regression analysis. This method examined all the variables within a linear model to understand their impact on the chosen dependent variable. The model was significant when binocular suppression (Suppression) and visual acuity of the amblyopic eye (AE) were entered as independent factors to predict stereoacuity as the dependent variable (Stereoacuity = 1.07 * AE + 0.90 *

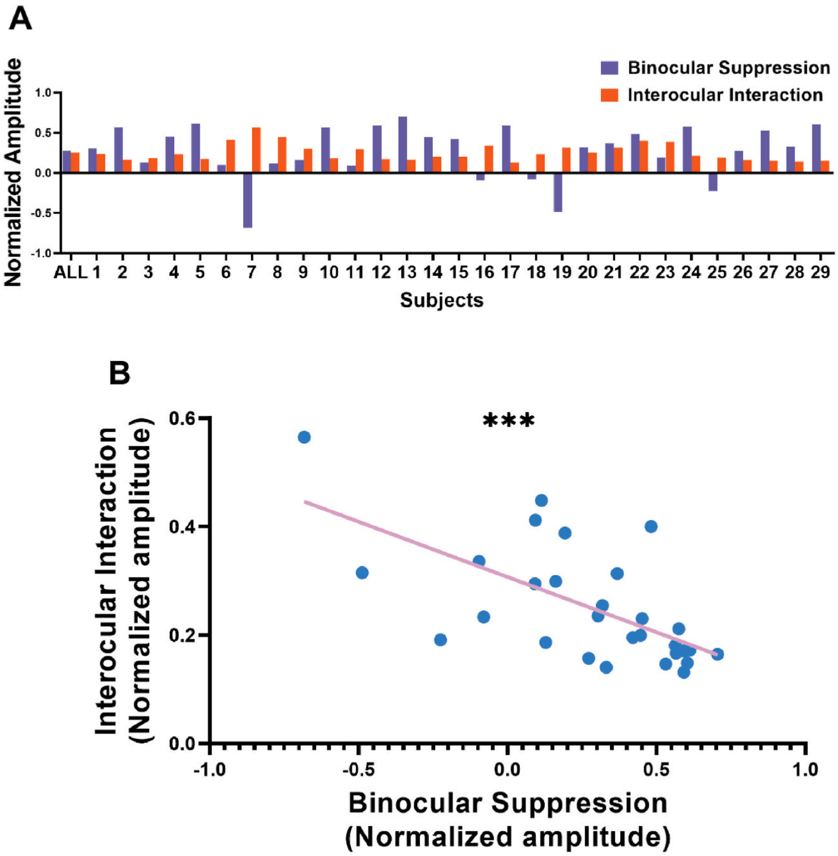

Fig. 3. Correlations of Binocular Interactions in the Anisometropic Amblyopic Visual Cortex. (A) This panel displays the mean normalized amplitude across participants and the normalized amplitude for each individual, measured for binocular suppression (purple bar) and interocular interaction (orange bar). **(B)** The graph illustrates the negative correlation between binocular suppression and interocular interaction in all participants (pink line). Data for all participants are depicted as the mean; asterisks indicate statistical significance; ***$p < 0.001$. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

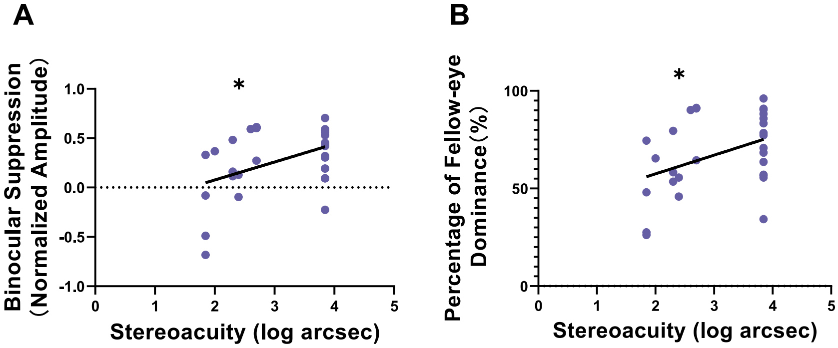

Fig. 4. Correlations of Stereoacuity in the Anisometropic Amblyopic Visual Cortex. (A) The graph depicts a positive correlation between binocular suppression and stereoacuity, measured in log arcsec, for all participants (black line). **(B)** The graph shows a positive relationship between the percentage of fellow-eye dominance and stereoacuity, expressed in log arcsec, across all participants (black line). Data for all participants are depicted as the mean; asterisks indicate statistical significance; $^*p < 0.05$.

Suppression + 2.38; $F = 6.35$, $p < 0.01$; $R^2 = 0.33$). To assess the distribution of the regression coefficients empirically, we refitted the model on 5000 bootstrapped samples. The bootstrap estimate of bias was minimal and did not affect the outcome of the linear model. Additionally, we employed Bayesian linear regression to compare different models. The combined AE and suppression model showed a higher BF_{10} when compared to the Null model ($BF_{10} = 8.80$), the Suppression model ($BF_{10} = 3.81$), and the AE model ($BF_{10} = 3.81$), respectively. Overall, the AE and suppression model demonstrated the best fit. These findings highlight the critical role of binocular suppression and visual acuity of the amblyopic eye as significant predictors of stereoacuity.

3.4. Neural responses and dominance in controls

In this study, ten control participants were included. The analysis revealed that the mean response to the left eye stimulus did not significantly differ from the mean response to the right eye stimulus (left eye: 0.51 ± 0.10 μV; right eye: 0.52 ± 0.11 μV; Wilcoxon signed-rank test: $z = -0.26$, $P = 0.85$). Additionally, there was no significant discrepancy in the mean response between the dominant and non-dominant eyes (dominant eye: 0.53 ± 0.11 μV; non-dominant eye: 0.51 ± 0.10 μV; Wilcoxon signed-rank test: $z = -0.87$, $P = 0.43$). These findings indicate that neural responses in normal participants demonstrated equivalent responses from both eyes. The balanced binocular responses in normal adults suggest that the stimulus conditions between the two eyes in our binocular rivalry paradigm did not exhibit significant differences.

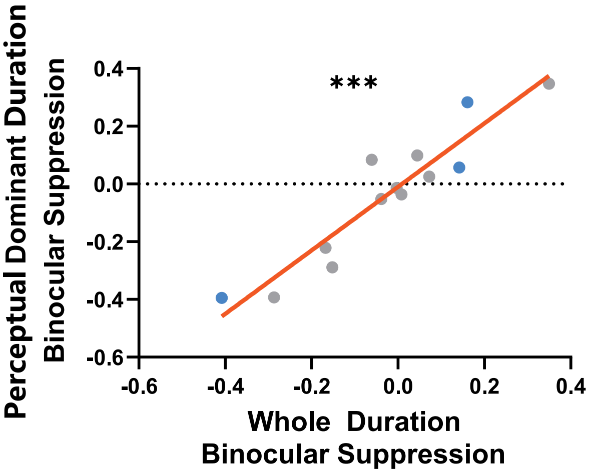

Fig. 5. Correlations of Binocular Suppressions during Perceptual Dominant Duration and Whole Duration in the Visual Cortex. A positive correlation (orange line) is observed between perceptual dominant and whole duration binocular suppressions in controls ($N = 10$, grey circle) and amblyopic participants ($N = 3$, blue circle) who provided perceptual reports. Data for all participants are presented as the mean; Asterisks indicate statistical significance; $^{***}p < 0.001$. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.5. *Perceptual dominant duration correlated with whole dominant duration*

To account for the potential impact of dominant time during binocular rivalry on amplitude, we categorized each eye's amplitude based on the reported dominant eye in all participants who provided perceptual reports. For instance, the Amplitude of the left eye for perceptual reports was obtained when participants reported the left eye's perceptual phase. This allowed us to calculate the binocular suppression during the duration of perceptual dominance. A significant correlation was observed between the binocular suppression across the entire duration and the binocular suppression during the perceptual dominant duration (Perceptual Dominant Binocular Suppression: 0.04 ± 0.06; Whole Duration Binocular Suppression: 0.03 ± 0.06; Spearman rank correlation: Rho = 0.88, $p < 0.001$), shown in Fig. 5.

4. Discussion

We employed frequency-tagged SSVEPs to assess neural responses to each eye within a standard binocular rivalry paradigm involving 29 anisometropic amblyopes and 10 normal controls. Among the control group, SSVEP amplitudes in response to the left and right eyes exhibited no significant difference. In contrast, SSVEP amplitudes in response to the fellow eye were notably larger than those evoked by the amblyopic eye in amblyopic individuals. This discrepancy highlights the utility of SSVEP measurements in quantifying the neural imbalance between the two eyes in anisometropic amblyopia. Moreover, our findings revealed that the degree of binocular suppression, as measured by SSVEPs, correlated with the stereoacuity of individual amblyopic patients. These results underscore the potential of SSVEP measurements during binocular rivalry as effective neural indices for assessing visual deficiencies in amblyopia.

4.1. *Neural measurement during binocular rivalry*

Binocular rivalry occurs when bistable perceptions are presented to the two eyes, resulting in alternating neural activities that can be quantified through SSVEP measurements, providing direct evidence for visual studies [32]. In our study, normal controls underwent SSVEP testing during binocular rivalry, revealing that the mean amplitude induced in the right and left eyes did not exhibit significant differences. Furthermore, the mean amplitude induced by binocular rivalry stimuli showed no significant difference between the dominant and non-dominant eyes. These results align with a prior study indicating that sensory eye dominance in binocular rivalry does not strongly correlate with subjective dominance as assessed through clinical evaluation [33]. The positive correlation between perceptual dominance and whole-duration binocular suppression also indicated a consistent ocular dominance across different dominant durations, suggesting that binocular rivalry stimuli tagged with different frequencies remain relatively stable. The findings from normal observers helped rule out potential confounding factors. Differences in stimulation frequencies and dominant phases had minimal impact on our results. None of these factors yielded significant differences in controls, which implies that the results obtained in amblyopic individuals cannot be attributed to these factors. Monocular amblyopia, characterized by binocular imbalance and a reduction in monocular visual acuity and stereoacuity, is expected to manifest as imbalanced neural responses and abnormal binocular interactions, as measured by SSVEP during binocular rivalry stimuli.

4.2. *Neural measurement of binocular imbalance in amblyopia*

Disruptions in binocular balance arise when visual percepts in both eyes are inequivalent [23]. Our study has corroborated that neural dominance and neural amplitude from the fellow eye were significantly higher than those from the amblyopic eye in anisometropic amblyopia. This difference suggests a reduced amplitude in the amblyopic eye with strong inhibition originating from the fellow eye towards the amblyopic eye. These findings are consistent with prior literature, which has shown that SSVEP responses and fMRI activation elicited by the amblyopic eye are markedly diminished in the visual cortex [22,34,35]. These unequal neural responses in the visual cortex signify suppressive interactions between the eyes, manifesting as binocular imbalance in amblyopes [5,8].

Binocular interactions in the visual cortices include both excitatory and inhibitory interactions between two eyes [36–38]. Notably, in amblyopia, binocular suppression is enhanced, leading to diminished neuronal activity associated with the amblyopic eye compared to the fellow eye [39,40]. Conversely, excitatory interactions—assessed through the intermodulation term of steady-state visual evoked potentials (SSVEPs) when each eye is exposed to distinct flickering frequencies—are attenuated in strabismic amblyopia and are linked to impaired stereopsis [41]. Our research indicates a potential connection between binocular suppression and interocular interaction, which may reflect an imbalance between excitation and inhibition among neurons. The dysregulation of the excitatory-inhibitory balance has garnered significant attention in recent research related to visual deficits and their recovery [42–44]. Excessive inhibition in the visual cortex leads to impaired visual functions in amblyopia [45,46]. Our results have demonstrated a robust negative correlation between binocular suppression and interocular interaction, underscoring the imbalanced relationship between inhibitory and excitatory influences in the amblyopic visual cortex [47]. Binocular interactions including both suppression and interaction serve as objective outcome measurements in SSVEPs, providing empirical evidence of the imbalance between neural inhibition and excitation in amblyopia. These indices offer a potent foundation for further research and innovative investigations in amblyopia.

Another potential explanation for the correlation between binocular suppression and interocular interaction warrants consideration. Given that the fellow eye tends to be highly dominant in amblyopia, individuals may experience less mixed perception during binocular rivalry. The reduced duration of mixed perception could lead to lower intermodulation responses in SSVEPs. This could, in part, explain the observed correlation between binocular suppression and interocular interaction.

4.3. Relationship between neural and behavioral measurements in amblyopia

We investigated the relationship between behavioral functions and neural indices of binocular interactions and dominance in amblyopia. Our models aimed to identify predictive factors for both monocular and binocular functions. Results demonstrated that the visual acuity of the amblyopic eye, in combination with binocular suppression measured from SSVEPs, effectively predicted stereoacuity. Notably, this model is the winning model compared with other models. This suggests that assessing both binocular suppression and visual acuity of the amblyopic eye as neural and behavioral indicators of imbalance is a more predictive measurement of deficiencies in binocular vision in amblyopia.

However, in contrast to previous studies involving different types of amblyopic patients [12], our research did not find a correlation between binocular suppression and visual acuity of the amblyopic eye in our anisometropic amblyopic participants. This suggests that binocular suppression, as measured by SSVEPs, may not directly correlate with visual acuity in anisometropic amblyopes. Further investigation is needed to determine whether the inclusion of mixed and strabismus amblyopes would affect the results. Our findings also contribute to a new correlation model that links stereoacuity, binocular suppression, and visual acuity, supporting previous research that emphasized the strong correlation between increased suppression and poor stereoacuity [48].

Suppression in anisometropic amblyopia has been suggested as a crucial factor correlated with binocular combination in psychophysical investigations [41,49]. This highlights the significance of binocular interactions in understanding amblyopia. Our results have provided electrophysiological evidence for correlations between interocular interaction and suppression, as well as between interocular interaction and dominance. These findings indicate that the neural imbalance in amblyopia can be detected through SSVEPs and is relevant to the expression of interocular interaction in the visual cortex.

A prior study has supported the notion that a reduction in intermodulation in SSVEPs disrupts stereoacuity and binocular interactions in strabismic amblyopia [41]. Additionally, changes in intermodulation have been found to correlate with improvements in perceptual learning [50,51]. However, in our anisometropic amblyope group with ages across younger children and adolesence, we did not observe a direct relationship between interocular interaction and behavioral indicators. It is possible that the link between interocular interaction and behavioral indexes is more complex in children than adults.

5. Conclusions

In summary, our study provides evidence that binocular suppression measured by SSVEPs serves as a reliable neural predictor for assessing stereroacuity in amblyopia. Integrating SSVEP measurements with traditional eye tests can result in more objective and precise diagnostic tools for evaluating vision abilities in individuals with amblyopia. Furthermore, SSVEP measurements offer an implicit assessment method, making them particularly advantageous in scenarios where obtaining self-reports from patients may be challenging.

CRediT authorship contribution statement

Jingyi Hu: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation, Conceptualization. **Jing Chen:** Writing – review & editing, Methodology, Formal analysis, Conceptualization, Investigation, Software, Visualization. **Minbin Yu:** Supervision, Funding acquisition, Writing – review & editing, Conceptualization, Investigation, Project administration, Resources. **Yixuan Ku:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Project administration, Investigation, Formal analysis, Conceptualization.

Significance

The study demonstrates significant differences in SSVEP amplitudes between the fellow and amblyopic eyes, indicating the effectiveness of SSVEPs in indexing neural imbalances in anisometropic amblyopia. Moreover, the study reveals a negative correlation between binocular suppression and interocular interaction measured by SSVEP, as well as a significant correlation between binocular suppression and stereoacuity, suggesting that SSVEP-derived indices can serve as effective neural predictors for stereoacuity in amblyopia. These results hold promise for enhancing the precision and objectivity of diagnosing amblyopia, particularly in early detection among children and when patient self-reports may be unreliable.

Funding disclosure

The National Natural Science Foundation of China (Grant No. 32171082), The Health Commission of Guangzhou, China (Grant No. YW2024GZGX03-1), The Health Commission of Guangzhou, China (Grant No. 3030901004049), The Interdepartmental Collaborative Projects, China (Grant No. 3031101185), the Natural Science Foundation of Guangdong Province, China (Grant No. 2015A03031201) provided funding for this work.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We acknowledge the contributions of Daming Deng, Zidong Chen, Zitian Liu, Yiru Huang, Yanyan Wu, QingQing Liu, Yuanzhou Huang, Wenyan Liu, and Yudi Mao in the areas of patient referral and paradigm design as well as their useful recommendations for experiments.

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