Eye Movements, Strabismus, Amblyopia and Neuro-Ophthalmology

Rapid Alternate Flicker Modulates Binocular Interaction in Adults With Abnormal Binocular Vision

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Received: November 8, 2022
Accepted: January 24, 2023
Published: February 14, 2023

Citation: Lu Y, Zou L, Chen Y, et al. Rapid alternate flicker modulates binocular interaction in adults with abnormal binocular vision. *Invest Ophthalmol Vis Sci.* 2023;64(2):15. https://doi.org/10.1167/iovs.64.2.15

Purpose. The current understanding of binocular processing is primarily derived from static spatial visual perception: this leaves the role of temporal information unclear. In this study, we addressed this gap by testing the effect of alternating flicker on binocular information processing in adults with abnormal binocular vision. Our goal was to determine which temporal frequency optimally balanced input from both eyes.

Methods. We took measurements in four groups of human adults: 10 normal adults with the individual's nondominant eye covered by a 2% neutral density filter (aged 25.60 ± 1.43 years, experiment 1), 9 nonamblyopic anisometropes (aged 24.33 ± 1.66 years, experiment 2), 7 amblyopes (aged 26.5 ± 1.64 years, experiment 3), and 7 treated amblyopes (aged 24 ± 3.21 years, experiment 4). The balance point (BP), where participants' two eyes are equally effective, was measured using a binocular orientation combination task at four spatial frequencies (SFs; 0.5–4 c/d) and five temporal frequencies (TFs; baseline and 4, 7, 10, and 15 Hz). Its log transformation $|\mathrm{logBP}|$ was taken into further analysis.

Results. We observed clear U-shaped temporal tuning of the $|\mathrm{logBP}|$ for the entire range of TFs (that we measured: trough occurred at 7 Hz). This pattern occurred and was significant in all four groups ($P < 0.001$). In addition, the effect of SFs on $|\mathrm{logBP}|$ was significant in normal, amblyopic, and treated amblyopic groups (all $P < 0.001$) and was marginally significant in the nonamblyopic anisometropic group ($P = 0.086$).

Conclusions. Alternating flicker around 7 Hz may be the optimal temporal frequency for balancing eyes in human adults with binocular imbalance.

Keywords: amblyopia, binocular balance, spatial frequency, temporal frequency, interocular suppression

Amblyopia is an ophthalmic disorder in which the best-corrected visual acuity (BCVA) in one or both eyes is below the normal age-related value. It affects about 1.3% to 3.6% of the population and is often caused by abnormal visual experiences early in life.[1] Besides reduced visual acuity, other symptoms of amblyopia include impairment of contrast sensitivity at medium to high spatial frequencies,[2–4] reduced stereopsis, and other monocular and binocular visual deficits.[5–8] Due to the mismatch of visual information between the two eyes, the amblyopic eye is subject to more interocular suppression than the nonamblyopic eye.[9,10] Binocular masking studies have continuously provided theoretical support for the existence of interocular imbalance in amblyopia.[11–15]

Our current understanding of binocular information processing is primarily limited to static visual perception. Most studies have focused on how spatial parameters, such as spatial frequency, size, and orientation, affect binocular interaction.[16,17] A range of research has shown that visual deficits in amblyopia, including increased binocular suppression, are spatial frequency dependent.[12,13,18,19] Even in so-called treated amblyopia, binocular imbalance still exists, and such imbalance increases with the spatial frequency.[1,20] In contrast, relatively fewer studies have been conducted on how temporal processing affects binocular imbalance.[21–23] Studies based on simultaneous binocular flicker have revealed that the interocular balance points were mainly spatial frequency dependent,[1,19] and temporal frequency has a minor effect on interocular contrast ratios in amblyopes, only limited to the low-to-mid temporal frequencies.[22,23] However, how dichoptic alternative flicker affects binocular imbalance is unclear. The study by Schor et al.[21] showed that the amblyopic perception was greatly enhanced for specific dichoptic flicker stimuli (e.g., 7 Hz). Additional amblyopic studies have also revealed that visual acuity and stereopsis can be improved at 7 Hz.[24,25] Therefore, we hypothesized that dichoptic alternative flicker stimuli could modify binocular interaction by the time difference between interocular excitatory and inhibitory responses, which in turn affects sensory eye balance in binocular processing.

To verify this, we measured the balance point (BP) at different spatiotemporal frequencies in four kinds of groups with abnormal binocular vision using a dichoptic alternating flicker intervention. Specifically, we used the binocular orientation combination paradigm to quantify the BP.[16,26] It reflects the binocular imbalance by calculating the contribution of both eyes to binocular integration during the measurement process. Since participants with abnormal vision have significant binocular imbalance, amblyopes and anisometropes are the optimal candidates for inclusion. As a comparison, two additional groups of participants with varying degrees of binocular balance were recruited. One group consisted of patients with treated amblyopia, which has been shown in previous studies to possess spatial imbalances at moderate to high spatial frequencies,[20] while the other group consisted of normal participants who wearing a 2% neutral density (ND) filter in front of the nondominant eye to simulate binocular imbalance.[27,28] If the dichoptic alternative flicker was valid, we predicted that the interocular imbalance in the four groups would improve to varying degrees at specific temporal frequencies.

MATERIALS AND METHODS

Participants

Four groups of participants were recruited from the Eye Hospital of Wenzhou Medical University. The normal group had ten participants (aged 25.60 ± 1.43 years) wearing a 2% ND filter in front of the nondominant eye. The anisometropic group had nine nonamblyopic adults (aged 24.33 ± 1.66 years). The amblyopic group had seven amblyopes (aged 26.5 ± 1.64 years), and the treated amblyopic group had seven participants (24 ± 3.21 years; see Supplementary Tables S1–S4 for more characteristic details). The included criteria were as follows: (1) BCVA $\leq$0.0 logMAR in both eyes in normal and anisometropic groups, BCVA $\leq$0.1 logMAR in both eyes in the treated amblyopic group, and BCVA >0.1 logMAR in the amblyopic eye in the amblyopic group; (2) spherical equivalent refraction difference between eyes of $\leq$1.25 D in the normal group and $\geq$1.50 D in the anisometropic group; and (3) no other ocular diseases, epilepsy, or other psychiatric diseases. The sample size was calculated using G-power 3.1 (multivariate analysis of variance): assume a medium partial $\eta^2 = 0.04$, $\alpha = 0.05$, power = 0.8, number of measurements = 5 (i.e., five temporal frequencies). Each group had a sample size of 6.4 (approximately equal to 7 per group).

Before the experiment, all participants were optimally refracted and optically corrected. Except for the first author (YL), all participants were naive to the purpose of the experiment. Informed consent was obtained prior to the study, which was approved by the Institutional Review Board of Wenzhou Medical University in China.

Apparatus

The binocular balance tests were conducted on a MacBook Pro (13-in., 2017; Apple, Inc., Cupertino, CA, USA) equipped with MATLAB R2016b (The MathWorks, Inc., Natick, MA, USA) and PsychToolBox 3.0.14.[29] Visual stimuli were dichoptically presented via Gamma-corrected head-mount goggles (GOOVIS Pro, AMOLED display; NED Optics, Shenzhen, China). In each eye, the display's refresh rate was 60 Hz, the resolution was 1920 × 1080 pixels, the pixels per degree of the screen was 41.6, and the maximal luminance was 150 cd/m^2. During the experiment, the participant wore Eyetronix Flicker Glasses (Eyetronix Inc Silicon Valley, California, USA), a spectacle frame with liquid crystal lenses that provide alternative occlusion to the two eyes with a 1:1 duration ratio at various temporal frequencies (TFs). In this study, we set the EFG (Eyetronix Flicker Glasses) at 4 Hz, 7 Hz, 10 Hz, and 15 Hz.

Experimental Design

In this study, we measured the BP with a binocular orientation combination task at five temporal frequencies (baseline, 4 Hz, 7 Hz, 10 Hz, and 15 Hz) and four spatial frequencies (0.5 c/d, 1 c/d, 2 c/d, and 4 c/d) using the method of constant stimuli. The distinct temporal and spatial frequencies have all been prerandomized in terms of order by using a random list generated by MATLAB R2016b. Before the experiment started, each participant's sighting dominant eye was determined with the hole-in-the-hand test.[30] Moreover, proper demonstrations were provided with practice trials to ensure participants understood the task. Based on psychophysical performance from practice trials, we established a distinct set of seven interocular contrast ratios (between 0 and 2) for each participant. We performed 10 repetitions for each condition (one orientation configuration and one interocular contrast ratio). Thus, there were a total of 140 trials in each block (2 orientation configurations × 7 interocular contrast ratios × 10 repeats). The interocular contrast ratios and configurations were randomized across different trials. For each participant, the entire experiment was carried out in four consecutive visits, scheduled at the same time each day. During each visit, BP was measured for five different temporal frequencies under one randomly chosen spatial frequency. A participant was allowed to take a 5-minute break after each block (see Fig. 1A).

Stimuli

BP was defined as the interocular contrast ratio in which the two eyes were balanced in binocular combination (i.e., have equal contribution; Fig. 1C). Two horizontally tilted sinusoidal gratings with different orientations were used as stimuli in each trial. The grating presented to each eye had two different configurations. In the first configuration, the grating seen to the dominant eye or fellow eye is oriented counterclockwise (+7.1°) with respect to the horizontal position, while the grating shown to the nondominant eye (NDE) or amblyopic eye (AE) is oriented clockwise (−7.1°). The orientation of the corresponding grating exhibited to each eye in the second arrangement is exactly the opposite of the first configuration. The overall variation in orientation between the eyes in both configurations was 14.2° and the base contrast of the gratings shown to the NDE (or AE) was fixed at 50% (see Fig. 1B). The size of the grating was varied at different spatial frequencies to maintain 2 cycles.

Procedures

There was an alignment phase and a test phase in a typical trial of the binocular orientation combination task. During the alignment phase, the fixation targets appeared first. Participants moved the coordinates of stimuli with crosses and dots to ensure that the images seen by the two eyes were precisely merged throughout the alignment task (see Fig. 1D). It was followed by a 500-ms blank screen made up of a square frame (edge length is three times that of the grating size) around it and diagonal bars in each eye to aid with fusion. Then, the actual test stimuli appeared. The

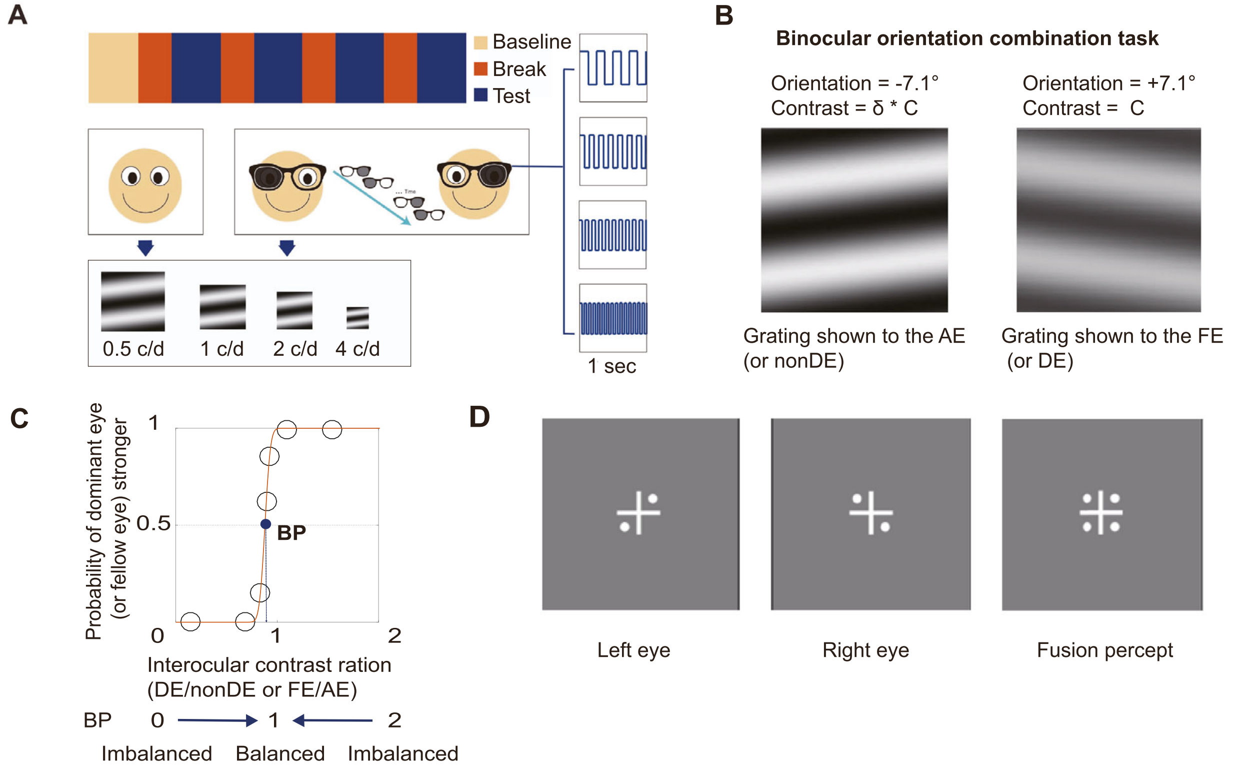

Figure 1. (**A**) The experimental design. Experiments were conducted in four groups (normals, nonamblyopic anisometropes, amblyopes, and treated amblyopes). In the normal group, we added a 2% ND filter on the participant's nondominant eye to simulate binocular imbalance.[27,28] While baseline binocular imbalance was measured without wearing EFG, all other TFs were measured with participants wearing EFG. (**B**) Diagrammatic illustration of the binocular orientation combination paradigm. Two oriented sinusoidal gratings (±7.1°) were dichotically presented to each eye in the binocular orientation combination task. The contrast of the gratings in the non-DE (or AE) was fixed at 50%, while the contrast of the gratings in the DE (or FE) was varied between 0% and 100% with the distinct set of seven interocular contrast ratios (between 0 and 2) for each participant. Participants were required to respond according to the orientation of grating by pressing a left or right key of the keyboard. (**C**) A representative psychometric function. The proportion of trials in which the participants reported that DE (or FE) dominated was plotted as a function of the interocular contrast ratio (DE/non-DE or FE/AE). Cumulative Gaussian distribution function was used to fit this psychometric curve. The BP corresponding to the 50% point of the best-fitting Gaussian function was derived from the fitting, which indicates the point at which the two eyes were balanced in binocular combination. (**D**) The alignment task. Participants were asked to align four dots so that the distance between the neighboring dots was equal. DE, dominant eye; FE, fellow eye.

participants were required to indicate whether the perceived cyclopean grating was oriented in a clockwise or counterclockwise direction by pressing a corresponding keyboard. The visual stimulus remained on the screen until the participants made a choice.

In different trials, the coordinates of the stimuli in the two eyes to which the participant needed to align were recorded. A detailed comparison of the alignment results at low temporal frequencies (i.e., 4 Hz) and high temporal frequencies (i.e., 15 Hz) confirmed that participants were also able to fuse the left and right eye images at low temporal frequencies (for details, see Supplementary Fig. S5).

Data Analysis

After an entire block was completed, the probability in which the orientation of the fused percept tilted toward the grating shown to the dominant (or fellow) eye was computed and plotted against the interocular contrast ratios. A function of cumulative Gaussian distribution was used to fit the psychometric function[12] (see Fig. 1C). The BP was computed by estimating the interocular contrast balance ratio in which the orientation of the fused percept tilted toward the grating shown to the dominant (or fellow) eye 50% of the time. We converted the BP into the absolute value of BP on a log scale (|logBP|) to better illustrate binocular imbalance: a |logBP| value closer to 0 means less binocular imbalance and vice versa.

The |logBP| values measured from each block were plotted against TFs and fitted with a log Gaussian model[31,32]:

$$\phi = \phi 0 + A * exp[-\left(2 * \sigma^{2}\right)^{(-1)} * log\left(TF/TFp\right)^{2} \qquad (1)$$

The function has four free parameters. $\phi 0$ represents a general amplitude, A is the peak amplitude, σ determines the (logarithmic) tuning width, and *TFp* represents the preferred temporal frequency.

Statistical Analysis

We used SPSS software version 20.0 (IBM Corporation, Armonk, NY, USA) to analyze statistics. The difference of |logBP| across various temporal frequencies and spatial frequencies was analyzed using a two-way repeated-measures ANOVA, with their effect size calculated as partial η^2. In addition, we conducted post hoc pairwise *t*-tests (with Bonferroni correction) to compare the |logBP| in five temporal frequencies and four spatial frequencies. The level of significance was set at $P < 0.05$.

RESULTS

Experiment 1. The Effect of Different Temporal Frequencies on Binocular Balance Point in Normal Participants With a 2% ND Filter

Ten normal participants wore a 2% ND filter, and the balance point was examined in five temporal frequencies and four spatial frequencies. The raw mean |logBP| as a function of temporal frequency at spatial frequencies is shown in Figure 2A. At each spatial frequency, a U-shaped trend was observed. |logBP| was the lowest at 7 Hz and increased as temporal frequency became higher or lower. While |logBP| at 7 Hz was lower (more balance) than baseline, values at other temporal frequencies were similar or greater (more imbalance) than baseline. Such data, also fitted into a log-Gaussian model, are presented in Figure 2B. The individual temporal tuning function can be found in Supplementary Figure S1.

A two-way repeated-measures ANOVA revealed a significant difference in |logBP| among different temporal frequencies (F(1.567, 14.107) = 16.850, $P < 0.001$, partial $\eta^2 = 0.652$) and different spatial frequencies (F(3, 27) = 57.321, $P < 0.001$, partial $\eta^2 = 0.864$). No significant interaction was found between temporal frequency and spatial frequency (F(3.548, 31.930) = 2.328, $P = 0.084$, partial $\eta^2 = 0.205$). Post hoc pairwise *t*-test (with Bonferroni correction) showed a significant difference between baseline vs. 7 Hz ($P = 0.001$), 4 vs. 7 Hz ($P = 0.002$), 4 vs. 15 Hz ($P = 0.002$), 7 vs. 10 Hz ($P = 0.032$), 7 vs. 15 Hz ($P = 0.003$), and 10 vs. 15 Hz ($P = 0.003$) in temporal frequencies. There was also a significant difference in 0.5 vs. 1 c/d ($P = 0.03$), 0.5 vs. 2 c/d ($P = 0.001$), 0.5 c/d vs. 4 c/d ($P = 0.001$), 1 c/d vs. 4 c/d ($P = 0.001$), and 2 vs. 4 c/d ($P = 0.001$) in spatial frequencies. This result indicates that the effect of alternating flicker deprivation stimuli on |logBP| was affected by different temporal and spatial frequencies in normal people with a 2% ND filter.

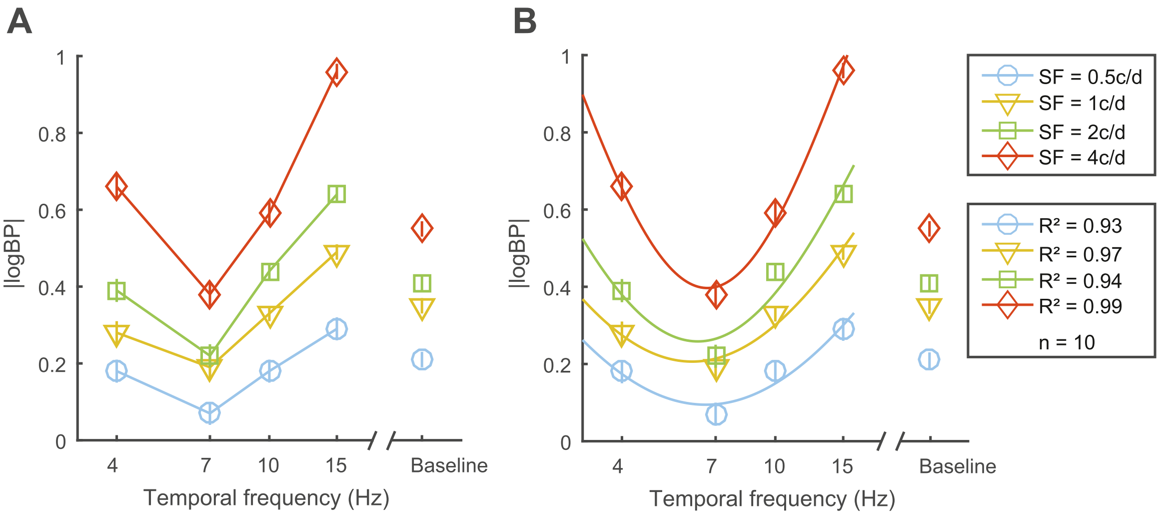

FIGURE 2. Mean balance point (|logBP|) as a function of the temporal frequency in normal participants. (**A**) Raw data. (**B**) Fitted function. *Error bars*: standard errors across the 10 participants. Participants' nondominant eye wore a 2% ND filter.

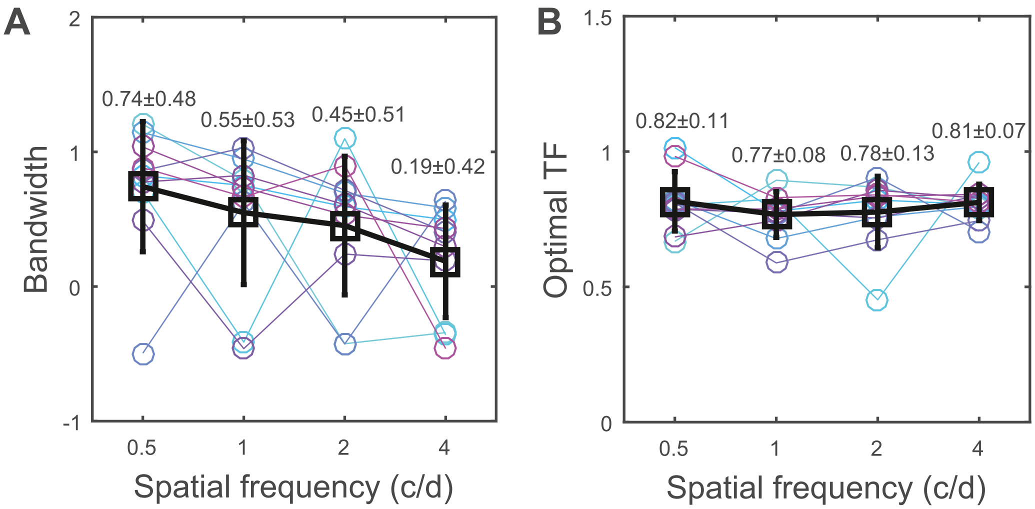

FIGURE 3. Parameters of the fitted tuning curve as a function of spatial frequency in normal participants with a 2% ND filter. (**A**) Bandwidth. (**B**) Optimal TF. Each color of dot represents one participant's result. Rectangle represents the mean value at different spatial frequencies. *Error bars*: standards errors across the 10 participants.

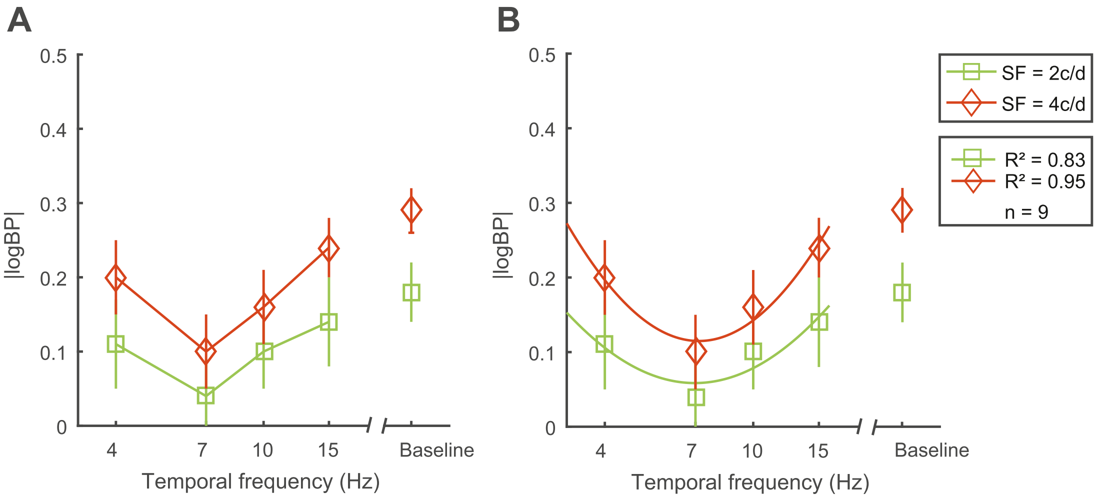

Figure 4. Mean balance point (|logBP|) as a function of the temporal frequency in the nonamblyopic anisometropes. (**A**) Raw data. (**B**) Fitted function. *Error bars*: standard errors across the nine participants.

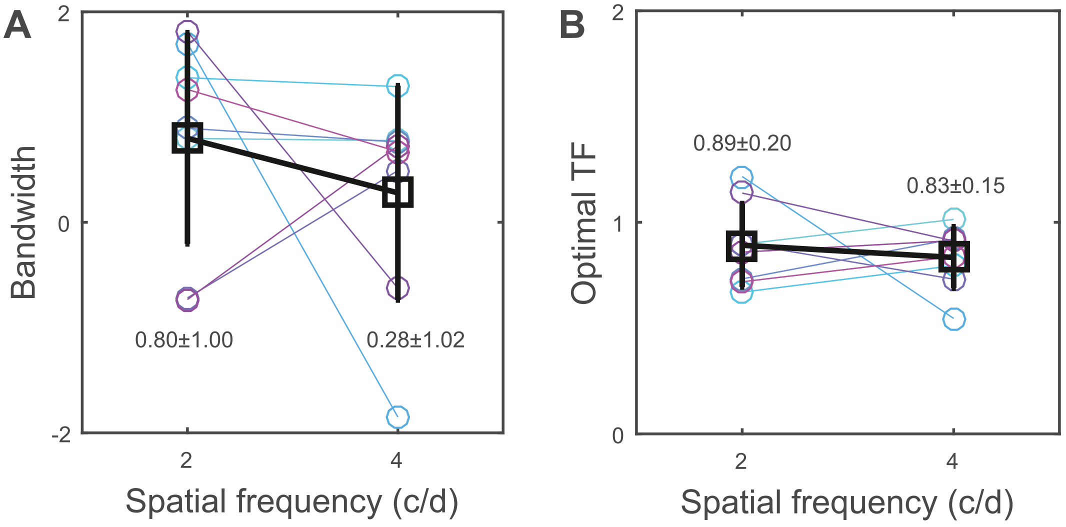

Figure 5. Parameters of the fitted tuning curve as a function of spatial frequency in nonamblyopic anisometropes. (**A**) Bandwidth. (**B**) Optimal TF. Each color of dot represents one participant's result. *Rectangle* represents the mean value at different spatial frequencies. *Error bars*: standard errors across the nine participants.

The bandwidth (Fig. 3A) and the optimal TF (Fig. 3B) were derived from each participant's temporal frequency tuning curve and plotted against spatial frequency. A one-way repeated-measure ANOVA showed that there was no significant difference among four spatial frequencies for either bandwidth ($F(3, 27) = 2.425$, $P = 0.087$, partial $\eta^2 = 0.212$) or optimal TF ($F(3, 27) = 0.568$, $P = 0.641$, partial $\eta^2 = 0.059$). These results indicated that different spatial frequencies did not make a significant change to the shape of the temporal tuning function.

Experiment 2. The Effect of Different Temporal Frequencies on Binocular Balance Point in Nonamblyopic Anisometropes

Similar experiments were performed in nonamblyopic anisometropic participants. Since baseline binocular imbalance only existed at relatively higher spatial frequencies (2 c/d: 0.20 ± 0.08, 4 c/d: 0.32 ± 0.14) and two eyes were relatively balanced in low to medium spatial frequencies (baseline of 0.5 c/d: 0.04 ± 0.04, baseline of 1 c/d: 0.06 ± 0.03), the effect of EFG on binocular balance at different temporal frequencies was tested only at spatial frequencies of 2 and 4 c/d. The raw mean |logBP| as a function of temporal frequencies at different spatial frequencies is shown in Figure 4A. At each spatial frequency, a U-shaped trend was observed. |logBP| was lowest at 7 Hz and increased as temporal frequency became higher or lower. While |logBP| at 7 Hz was lower than baseline, values at other temporal frequencies were similar to or greater than baseline. Such data, also fitted into a log-Gaussian model, are presented in Figure 4B. The individual temporal tuning function can be found in Supplementary Figure S2.

A two-way repeated-measures ANOVA showed that the |logBP| of the anisometropes were significantly different

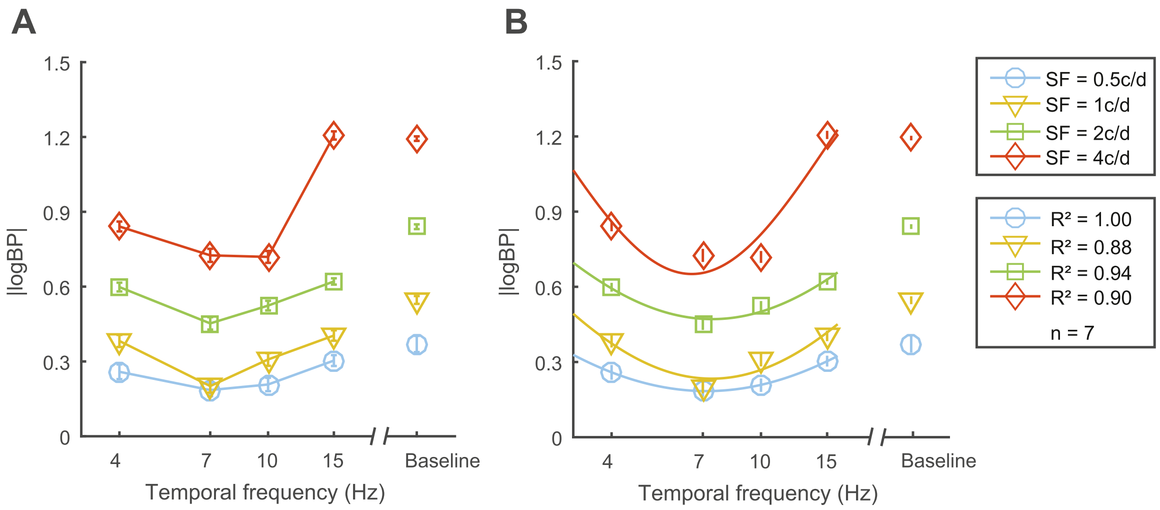

FIGURE 6. Mean balance point (|logBP|) as a function of the temporal frequency in the amblyopes. (**A**) Raw data. (**B**) Fitted function. *Error bars*: standard errors across the seven participants.

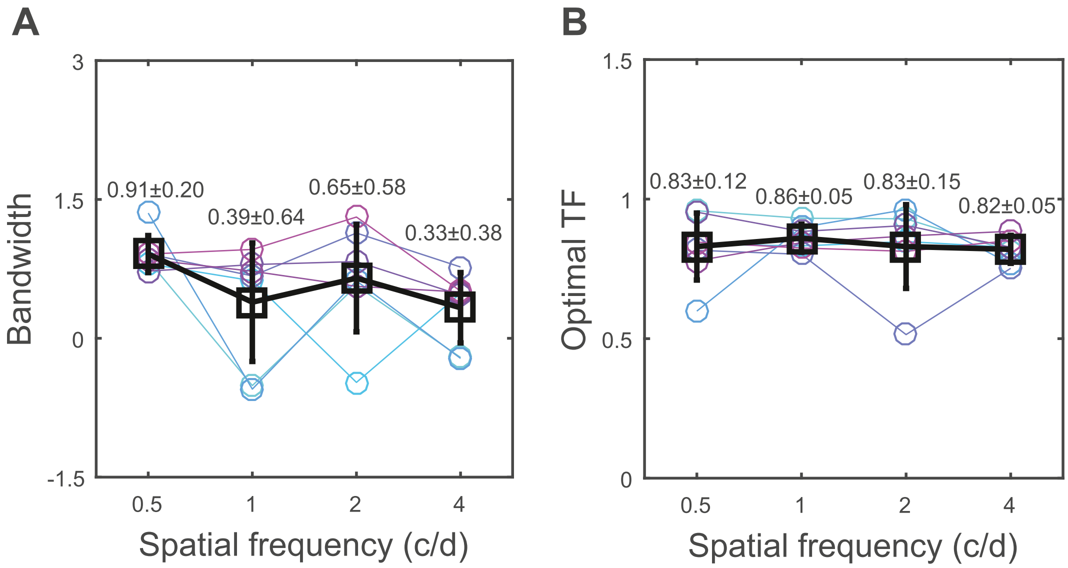

FIGURE 7. Parameters of the fitted tuning curve as a function of spatial frequency in amblyopes. (**A**) Bandwidth. (**B**) Optimal TF. Each color of dot represents one participant's result. *Rectangle* represents the mean value at different spatial frequencies. *Error bars*: standards errors across the seven participants.

across different temporal frequencies ($F(2.196, 17.569) = 10.184$, $P = 0.001$, partial $\eta^2 = 0.560$), with no significant differences in spatial frequencies ($F(1, 8) = 3.839$, $P = 0.086$, partial $\eta^2 = 0.324$). Also, no significant interaction was found between temporal frequencies and spatial frequencies ($F(4, 32) = 0.382$, $P = 0.820$, partial $\eta^2 = 0.046$). Post hoc pairwise *t*-test (with Bonferroni correction) showed significant difference in baseline vs. 7 Hz, 4 vs. 7 Hz, and 7 vs. 15 Hz ($P = 0.01, 0.04, 0.032$).

To further evaluate the effect of spatial frequency on the shape of the tuning function, we analyzed how the bandwidth and the optimal TF changed with different spatial frequencies (Fig. 5). One of the participant's data was an outlier and eliminated. The remain eight participants' data were included in the analysis. Paired-samples *t*-test showed that there was no significant difference in 2 c/d and 4 c/d for bandwidth ($P = 0.419$) or optimal TF ($P = 0.591$).

Experiment 3. The Effect of Different Temporal Frequencies on Binocular Balance Point in Amblyopes

For experiment 3, seven amblyopes were recruited and tested. The raw data and fitted function of mean balance point (|logBP|) in amblyopes are shown in Figure 6, and the individual temporal tuning function separately can be found in Supplementary Figure S3.

A two-way repeated-measures ANOVA indicated that there was a significant difference in |logBP| among different temporal frequencies ($F(4, 24) = 20.069$, $P < 0.001$,

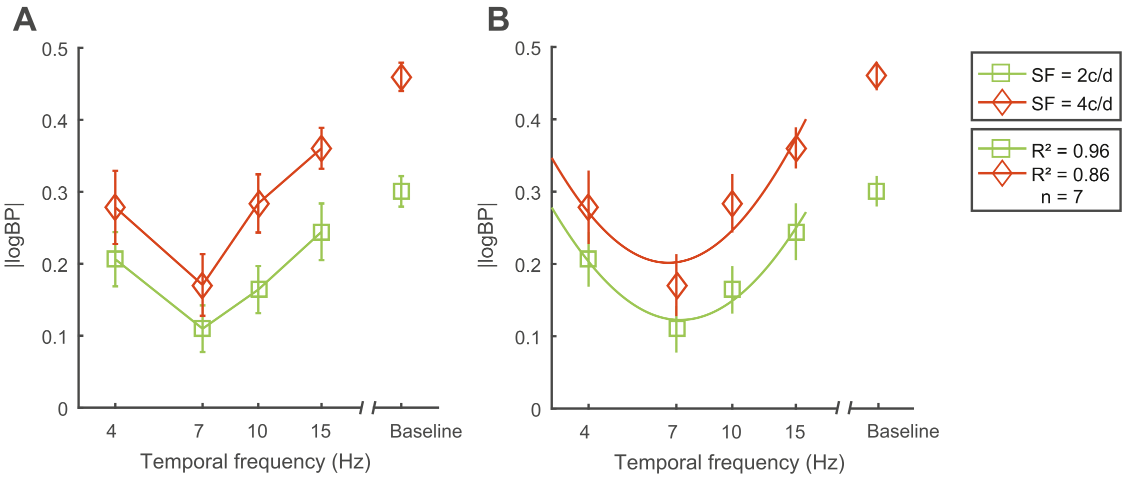

Figure 8. Mean balance point (|logBP|) as a function of the temporal frequency in the treated amblyopes. (**A**) Raw data. (**B**) Fitted function. *Error bars*: standard errors across the seven participants.

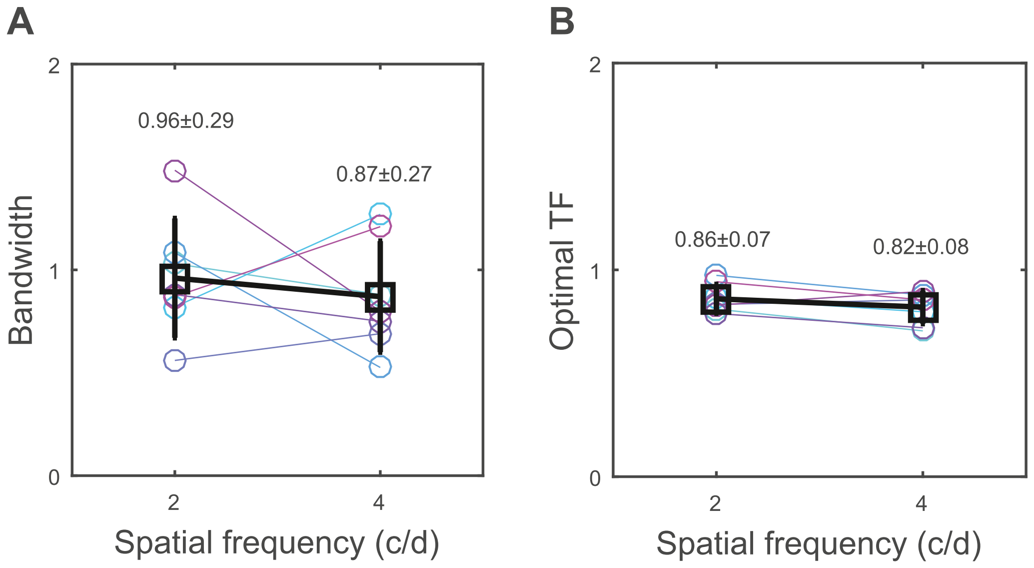

Figure 9. Parameters of the fitted tuning curve as a function of spatial frequency in treated amblyopes. (**A**) Bandwidth. (**B**) Optimal TF. Each color of dot represents one participant's result. *Rectangle* represents the mean value at different spatial frequencies. *Error bars*: standards errors across the seven participants.

partial $\eta^2 = 0.770$) and different spatial frequencies ($F(1.336, 8.019) = 31.643$, $P < 0.001$, partial $\eta^2 = 0.841$). No significant interaction was found between temporal frequency and spatial frequency ($F(2.127, 12.760) = 3.196$, $P = 0.073$, partial $\eta^2 = 0.348$). Post hoc pairwise *t*-test (with Bonferroni correction) showed a significant difference in baseline vs. 7 Hz ($P = 0.012$), baseline vs. 10 Hz ($P = 0.025$), 4 vs. 7 Hz ($P = 0.025$), 4 vs. 15 Hz ($P = 0.043$), 7 vs. 15 Hz ($P = 0.007$), and 10 vs. 15 Hz ($P = 0.036$) in temporal frequencies, and there was a significant difference among all spatial frequencies ($P < 0.001$).

The bandwidth (Fig. 7A) and optimal TF (Fig. 7B) for the amblyopes' tuning curves measured were also analyzed. One-way repeated-measures ANOVA revealed that there was no significant difference among four spatial frequencies for either bandwidth ($F(3, 18) = 2.595$, $P = 0.084$, partial $\eta^2 = 0.302$) or optimal TF ($F(3, 18) = 0.256$, $P = 0.856$, partial $\eta^2 = 0.041$).

Experiment 4. The Effect of Different Temporal Frequencies on Binocular Balance Point in Treated Amblyopes

Considering that previous studies have shown that binocular imbalance in treated amblyopes exists mainly at medium to high spatial frequencies,[20] we assessed the effect of different temporal frequencies in experiment 4 only at 2 c/d and 4 c/d. Figure 8 shows the raw data and the fitted function of the mean balance point (|logBP|) for seven treated amblyopes, while the temporal tuning functions for individual participants can be found in Supplementary Figure S4.

A two-way repeated-measures ANOVA indicated that there was a significant difference in |logBP| among different temporal frequencies ($F(4, 32) = 12.240$, $P < 0.001$, partial $\eta^2 = 0.605$) and different spatial frequencies ($F(1, 8) = 11.874$, $P = 0.009$, partial $\eta^2 = 0.597$). No significant interaction was found between temporal frequency and spatial frequency ($F(4, 32) = 2.034$, $P = 0.113$, partial $\eta^2 = 0.203$). Post hoc pairwise *t*-test (with Bonferroni correction) showed a significant difference in 7 Hz vs. baseline ($P = 0.006$), 7 vs. 4 Hz ($P = 0.002$), and 7 vs. 15 Hz ($P = 0.003$) in temporal frequencies, and there was a significant difference between 2 c/d and 4 c/d in spatial frequencies ($P = 0.009$). The paired-samples *t*-test showed no significant difference between the bandwidths of 2 c/d and 4 c/d (Fig. 9A, $P = 0.614$) or the optimal TF (Fig. 9B, $P = 0.103$).

DISCUSSION

Four groups were tested in this study. Three of them had a preexisting binocular imbalance (nonamblyopic anisometropes, amblyopes, and treated amblyopes). In another group, the binocular imbalance was simulated in binocularly balanced participants by placing a 2% ND filter in front of the nondominant eye. The effect of EFG on binocular balance was measured at various temporal frequencies. As we predicted, we did find that binocular imbalance is also modulated by the flicker frequency. There is a universal U-shaped temporal tuning curve for each spatial frequency in participants with abnormal vision. The tuning curves reach the trough, the maximal binocular balance, at a temporal frequency of 7 Hz and rise when the frequency becomes lower or higher. This result shows that the dichoptic alternative flicker does improve the interocular balance of the eyes.

Two Potential Mechanisms for TF Tuning Curves

With EFG, each eye is stimulated alternatively. During the stimulation-on phase, the stimulated eye makes excitatory responses and develops inhibitive signals to the fellow eye.[33] We speculate that such an inhibitive signal could only be initiated when sufficient excitatory responses have been integrated over time. Previous studies provided evidence that is consistent with this speculation. First, in both normal and amblyopic participants, interocular suppression takes around 150 ms to develop, which is much longer than the excitatory response.[34–36] Second, it has been suggested that interocular suppression in amblyopes and dichoptic masking in normal participants are caused by changes in interocular contrast gain control.[35,36] The longer the time course of stimulus, the more effective it is.[37] During the stimulus-off phase, as the stimulus switch to the other eye, the excitatory responses do not immediately disappear but gradually fade away. Such visual persistence provides the neural basis for binocular interaction. Therefore, interocular summation of excitatory and inhibitory signals depends on the flicker frequency (Fig. 10).

To illustrate, at low temporal frequency, each cycle is relatively long. Sufficient excitatory responses thus could be integrated within one stimulation-on phase to initiate inhibitive signals (Fig. 10A, top row). At high temporal frequencies, each cycle becomes much shorter. Although no sufficient excitatory responses could be integrated within one stimulus-on phase, they do not fade much during the stimulus-off phase either. Therefore, enough excitatory responses could be integrated over continuous cycles to develop inhibitive signals to the other eye (Fig. 10A, bottom row). Since the interocular suppression is asymmetrical,[11,13] with the inhibitory effect from the dominant eye to the nondominant eye being much stronger, this leads to greater interocular imbalance. The middle range temporal frequency, around 7 Hz, leads to a peculiar combination. Not enough excitatory responses could be integrated during the stimulus-on phase, and most of the excitatory responses fade away during the stimulus-off phase. Therefore, not many excitatory responses could be integrated over continuous cycles either. Consequently, the asymmetrical binocular suppression is substantially reduced or even eliminated

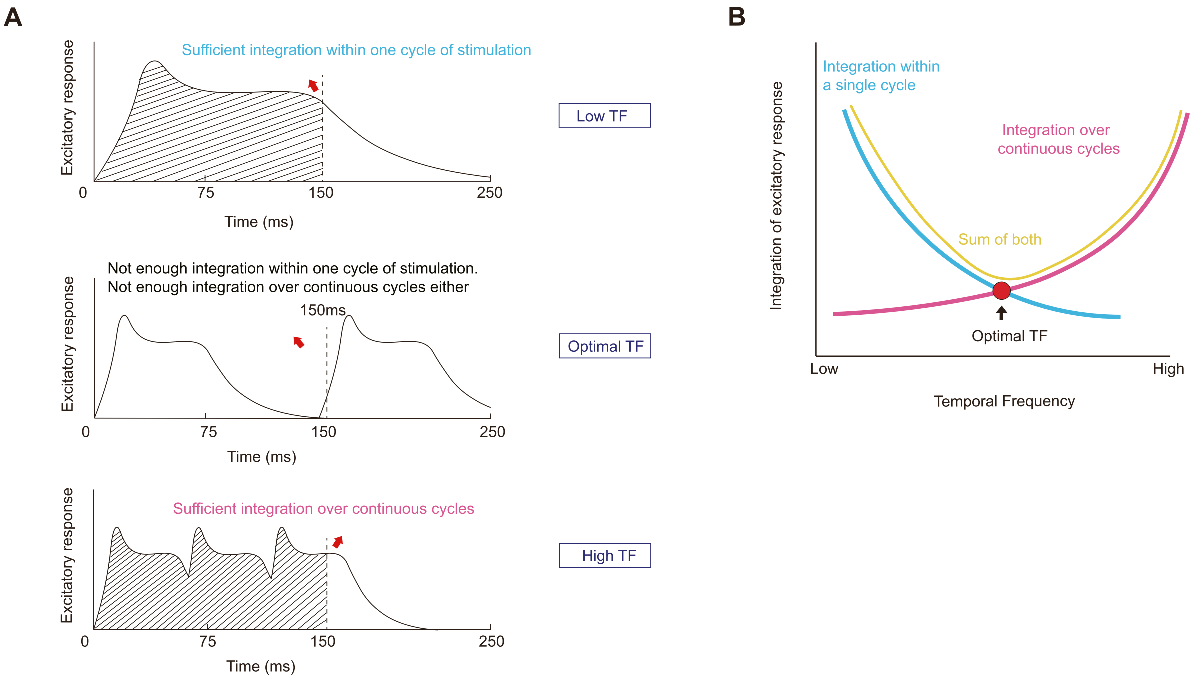

FIGURE 10. Illustration of the potential mechanism of U-shaped TF tuning curves. (**A**) Excitatory response at different temporal frequencies. (**B**) Integration of excitatory response at different temporal frequencies.

(Fig. 10A, middle row). Such a change leads to improved binocular balance and forms the trough of the U-shaped tuning curve (Fig. 10B). The trough at 7 Hz is consistent with the findings from the study by Schor et al.,[21] in which five participants with strabismus and amblyopia were presented with alternating stimuli of different SOAs (Stimulus onset asynchrony). Visual acuities were significantly improved at 7 Hz (SOA = 71 ms).

Previous studies also reported that perceived brightness changes with flickering frequency. A 7-Hz stroboscopic stimulation may lead to an enhancement of perceived brightness and contrast.[38] Solomon and Tyler[39] reported that the estimated brightness and apparent contrast increased when the stimulus flickered at a temporal frequency of 7.5 Hz. This is also consistent with the Brücke–Bartley effect.[40] Related studies by Zhou et al.[27] and Min et al.[28] also showed that changes in the brightness of the amblyopic eye and the fellow eye could correct the binocular imbalance in amblyopia through a modified gain-control model. We tested the luminance transmission of the EFGs and found that it is different at various temporal frequencies. For instance, the transmission rate is 7% at 15 Hz and 30% at 7 Hz. Participants also reported that the experimental gratings seen while wearing the 7-Hz EGF were significantly brighter than other frequencies during testing.

Differences in TF Tuning Curves

There were also subtle differences among the four groups. In normal group, the baseline measures are much lower than the flicker measures. However, the opposite is true in the other groups. One possibility for this difference is that the binocular imbalance that we simulated in the normal controls was achieved by adding an ND filter to their nondominant eye. The temporal effect of EFGs might be different in participants with simulated binocular imbalance and in participants with naturally occurring binocular imbalance. In addition, the ND filter itself causes temporal delays,[11,41,42] which might also affect the temporal tuning of the EFGs to binocular imbalance.

Relationship to Previous Studies

Previous studies have reported no improvement in binocular balance in adult humans after wearing EFG for 1 hour.[43] The improvement in stereopsis is debatable.[24,25,44,45] The findings from this study should not be compared to these findings directly. First, the binocular balance was quantified while EFG was on in this study. Previous studies measured binocular balance or stereopsis with EFG off (i.e., the aftereffects). Second, in Lin et al.,[43] normal participants were tested, who have mostly balanced eyes at low spatial frequencies and only slight interocular imbalance at medium and high spatial frequencies.[12] Therefore, alternating flicker-deprived stimulation may not have significant improvement in such participants. In contrast, participants with large binocular imbalances were used in this study.

The notion that inhibitive binocular interaction could be alleviated with specific flickering frequencies needs future investigation. There are several weaknesses inherit in the current study design. First, for the choice of temporal frequency, four different temporal frequencies (e.g., 4 Hz, 7 Hz, 10 Hz, and 15 Hz) were selected for this study. However, the refresh frequency of the computer display is 60 Hz, and only 7 Hz is not compatible with it. To verify this, we added additional tests at two noncompatible temporal frequencies (i.e., 13 Hz and 17 Hz) in five new normal participants with a 2% ND filter. The results confirm that 7 Hz is still the optimal TF (see Supplementary Fig. S7). If possible, more different temporal frequencies should be explored further to better clarify the temporal effect in binocular interaction. Second, the stimulus duration was not fixed. The participants looked at the stimulus as long as they preferred. To test if temporal integration of excitatory response is key to initiating a suppressive signal to the other eye, the stimulus presentation has to be fixed. Third, combinations of various stimulus durations and flicker frequencies should be tested. Adding delay in front of the dominant eye would also help to illustrate the potential mechanism. More important, such studies may point out a new strategy in amblyopic treatment. Instead of having alternating occlusion between the two eyes, the amblyopic eye should be left open, and flickering frequency should be added only to the dominant eye. This strategy may not only reduce the suppression from the dominant eye to the amblyopic eye, and it still allows the interocular suppression from the amblyopic eye to the dominant eye.

CONCLUSION

Our results suggest that binocular balance of human adults is tuned to a specific temporal frequency. Alternating flicker around 7 Hz may be the optimal temporal frequency to balance the eyes of adults with binocular imbalance. This further explains and supports the previous findings that 7 Hz significantly improves amblyopic vision. There is reason to believe that dichoptic alternative flicker can change the binocular interaction, further affecting abnormal perception and bringing more practical results in treating binocular function amblyopic patients.

Acknowledgments

The authors thank Kevin Willeford for proofreading this paper.

Supported by the National Natural Science Foundation of China Grant NSFC 31970975 (JZ); the Natural Science Foundation for Distinguished Young Scholars of Zhejiang Province, China (LR22H120001) (JZ); and the Project of State Key Laboratory of Ophthalmology, Optometry and Vision Science, Wenzhou Medical University (No. J02-20210203) (JZ); The Key Research and Development Plan in Zhejiang Province (2021C03102) (JQ); and National Natural Science Foundation of China Grant NSFC 12205221 (RC).

Disclosure: **Y. Lu**, None; **L. Zou**, None; **Y. Chen**, None; **Y. Mao**, None; **J. Zhu**, None; **W. Lin**, None; **D. Wu**, None; **R. Chen**, None; **J. Qu**, None; **J. Zhou**, None

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