Eye Movements, Strabismus, Amblyopia and Neuro-Ophthalmology

Effects of Monocular Flicker on Binocular Imbalance in Amblyopic and Nonamblyopic Adults

Yiqiu Lu,[1–3] Liying Zou,[1–3] Wenjing Wang,[1–3] Ruyin Chen,[1–3] Jia Qu,[1–4] and Jiawei Zhou[1–3]

[1]School of Ophthalmology & Optometry and Eye Hospital, Wenzhou Medical University, Wenzhou, China
[2]State Key Laboratory of Ophthalmology, Optometry and Visual Science, Wenzhou, China
[3]National Clinical Research Center for Ocular Disease, Wenzhou, Zhejiang, China
[4]Oujiang Laboratory (Zhejiang Lab for Regenerative Medicine, Vision, and Brain Health), Eye Hospital, Wenzhou Medical University, Wenzhou, Zhejiang, China

Correspondence: Jiawei Zhou, School of Ophthalmology & Optometry and Eye Hospital, Wenzhou Medical University, 270 Xueyuan West Road, Wenzhou 325027, China; zhoujw@mail.eye.ac.cn.
Jia Qu, School of Ophthalmology & Optometry and Eye Hospital, Wenzhou Medical University, 270 Xueyuan West Road, Wenzhou 325027, China; jqu@wmu.edu.cn.

Received: July 15, 2023
Accepted: March 2, 2024
Published: March 26, 2024

Citation: Lu Y, Zou L, Wang W, Chen R, Qu J, Zhou J. Effects of monocular flicker on binocular imbalance in amblyopic and nonamblyopic adults. *Invest Ophthalmol Vis Sci.* 2024;65(3):33. https://doi.org/10.1167/iovs.65.3.33

Purpose. This study aimed to evaluate the effects of monocular flicker stimulation on binocular imbalance in both amblyopic and nonamblyopic adults.

Methods. Seven amblyopic patients (28.3 ± 3.3 years; four females) and seven normally sighted participants (27.3 ± 4.1 years; five females) participated in the study. We used liquid crystal spectacles to create externally-generated monocular flicker (4, 7, 10, 15, or 20 Hz) and used the metric of log balance point (logBP) to determine whether imposed flicker could change the eyes' equilibrium interocular contrast ratio. Flicker was applied to either the fellow eye vs. the amblyopic eye or dominant eye (DE) vs. non-DE (non-DE) of amblyopic and nonamblyopic participants, respectively. We defined a logBP of 0 to indicate complete binocular balance and an increase in logBP relative to baseline to indicate a relative strengthening of the non-DE or amblyopic eye.

Results. Monocular flicker applied to the DE or fellow eye increased logBP, whereas when applied to the non-DE or amblyopic eye, reduced the logBP. These effects were more pronounced at low temporal frequencies than that at high temporal frequencies. The interaction between eye and temporal frequency was significant in both normals, $F(4, 24) = 58.082$, $P < 0.001$, $\eta^2 = 0.906$, and amblyopes, $F(1.923, 11.538) = 60.555$, $P < 0.001$, $\eta^2 = 0.91$.

Conclusions. Monocular flicker diminishes the contribution of the flickered eye in binocular combination, resulting in a relative dominance of the nonflickered eye in interocular interactions. Furthermore, a more pronounced temporally modulated effect was observed at lower temporal frequencies.

Keywords: monocular flicker, temporal frequency, interocular interaction

Amblyopia, characterized by a unilateral or, less commonly, bilateral decrease in best-corrected visual acuity (BCVA), is a prevalent visual disorder affecting both children and adults,[1] with a prevalence as high as 3%.[2] If left untreated, it can lead to a range of visual impairments and impact psychological development, substantially impacting an individual's quality of life.[3–6] Amblyopes commonly present with binocular visual deficits, including reduced stereopsis[7] and binocular imbalance (i.e., the fellow eye [FE] plays a more dominant role in binocular perception than the amblyopic eye [AE])[8–12] caused by interocular suppression.[13–18] Even in treated amblyopes with fully recovered BCVA, a certain amount of binocular imbalance remains, especially at mid-to-high spatial frequencies.[19]

Recent investigations have shown that this remaining untreated binocular imbalance can potentially be modulated by flicker.[20–23] This can be used for therapeutic benefit. First, for instance, Schor et al.[24] demonstrated that alternately presenting targets to the two eyes at 2 and 7 Hz in amblyopia potentially enables better visual acuity under binocular viewing compared with other temporal frequencies. Next, our preliminary research[25] indicated that binocular alternating flicker at 7 Hz (where flicker stimuli are presented alternately in front of each eye with a 1:1 ratio of light to dark through liquid crystal spectacles) enables better binocular balance in amblyopia. Modulation of binocular imbalance via flicker can also reveal further insights regarding the neural underpinnings of amblyopia: several studies using synchronous binocular flicker (where stimuli are flickered simultaneously in front of both eyes) have observed a slightly larger interocular imbalance at low temporal frequencies in amblyopia.[26]

Both treatment- and etiology-oriented investigations have primarily focused on the effects of binocular flickering. This leaves the relationship between monocular flickering and binocular imbalance relatively unexplored. We posit that the impact of monocular vs. binocular flicker on interocular balance may differ because, in binocular flicker, the flickering stimulus appears either synchronously or alternately in front of both eyes; thus, each eye receives equal visual input. In contrast, with monocular flicker, the flickered eye receives approximately one-half of the visual input because it is only viewing for one-half of the time. Therefore, it potentially decreases the flickered eye's weight in binocular integration, thereby affecting interocular interaction. This effect could be comparable with studies that decrease the stimuli contrast,[27–32] or brightness,[33–37] of the FE, which weakens its contribution in binocular combination and improves binocular balance. However, monocular flicker might affect interocular interaction differently than decreasing stimuli contrast or brightness, not only owing to its temporal rather than spatial nature, but also because flicker itself could play a critical role in binocular viewing.[26] In fact, a recent study by Abuleil et al.[38] further supports this possibility, indicating that, in binocular rivalry tasks, the use of a 9-Hz monocular flicker reduces the relative dominance of the flickered eye, favoring the nonstimulated eye. On the basis of this evidence, we hypothesized that monocular flicker may disrupt the continuous visual stimulation of the flickered eye. The asymmetric binocular visual input that is created may cause the dominant eye (DE) to lose its weight in the integration of visual information, weakening its inhibition of the contralateral (non-DE/AE) eye and thus improving binocular balance.

To verify this finding, we conducted a series of monocular flicker interventions. Specifically, we administered externally generated monocular flicker stimuli using liquid crystal spectacles at five temporal frequencies to both the DE/FE and the non-DE/amblyopic eye (AE) of both nonamblyopic and amblyopic participants. We used a binocular orientation combination task,[39,40] which assesses interocular balance by quantifying each eye's contribution to binocular combination, i.e., the binocular balance point (BP), during measurement. If our hypothesis is correct, then applying flicker to either eye of a nonamblyopic participant (whether it be the dominant or non-DE) would disrupt their nearly balanced binocular state, leading to an imbalance. Similarly, when flicker is applied to the DE of amblyopes, the existing binocular imbalance could be improved, potentially resulting in a shift of ocular dominance to the contralateral (amblyopic) eye.

Methods

Participants

The study included fourteen participants, comprising seven normally sighted participants (28.3 ± 3.3 years; four females) and seven amblyopic participants (27.3 ± 4.1 years; five females). All subjects were naive to the study's purpose. The inclusion criteria were as follows: (1) BCVA of $\leq$0.0 logMAR in both eyes in the nonamblyopic group; BCVA of >0.1 logMAR in the AE and an interocular BCVA difference of >0.2 logMAR in the amblyopic group; and (2) no other ocular diseases, epilepsy, or other psychiatric diseases. Detailed clinical information about amblyopic patients can be found in Supplementary Tables S1. Before the study, written informed consent was obtained from all participants. The study protocol was reviewed and approved by the institutional review boards of the Affiliated Eye hospital of Wenzhou Medical University, and it adhered to the principles outlined in the Declaration of Helsinki.

Apparatus

The experiment was conducted using a MacBook Pro (13-inch, 2017; Apple, Inc., Cupertino, CA, USA) running MATLAB R2016b (The MathWorks, Inc., Natick, MA, USA) and PsychToolBox 3.0.14.[41,42] Visual stimuli were presented dichoptically using gamma-corrected head-mount goggles (GOOVIS Pro, AMOLED display; NED Optics, Shenzhen, China). The goggles had a refresh rate of 60 Hz, a resolution of 1600 × 900 pixels, a pixel density of 34.6 pixels per degree of visual angle, and a maximum luminance of 150 cd/m^2. The Eyetronix Flicker Glasses (EFG; Eyetronix Inc., Santa Clara, CA, USA), a spectacle frame with liquid crystal lenses, were used to generate monocular flicker stimulus. In particular, these glasses produce a light–dark flicker stimulus with a 1:1 duration ratio for one eye. We referenced pertinent literature[24,43–45] and considered the frequency limitations of flicker glasses (limitation not exceeding 20 Hz), selecting five temporal frequencies (4, 7, 10, 15, and 20 Hz) for our study. Participants were required to wear the EFG during the experiment.

Experimental Design

For each participant, the effects of monocular flicker on their left and right eyes were assessed in two separate sessions conducted on two consecutive days. During each visit, the BP was measured with and without (i.e., baseline) monocular flicker. The EFG was used to administer monocular flickering to participants' DE/FE and non-DE/AE. The flicker had temporal frequencies of 4, 7, 10, 15, and 20 Hz (Fig. 1A). A participant was allowed to take a 5-minute break after each frequency test.

The BP was defined as the interocular contrast ratio at which the two eyes had an equal contribution in a binocular orientation combination task (Fig. 1B). Before the experiment, the DE of nonamblyopic participants was determined using a hole-in-the-hand test.[46] Appropriate demonstrations and practice trials were provided to ensure participants' understanding of the tasks. Based on the results from the practice trials, seven interocular contrast ratios (FE/AE; DE/non-DE) ranging from 0 to 2 were selected for each subject to test their BPs using the method of constant stimuli. Each orientation combination configuration and contrast ratio were tested 10 times, resulting in a total of 140 trials (2 orientation configurations × 7 contrast ratios × 10 repetitions) in each block for a temporal frequency. The order of the configuration and contrast ratios were randomized across trials.

Stimuli

Each experimental trial used two horizontally titled sinusoidal gratings as stimuli. The size of the grating was two cycles with a spatial frequency of 0.42 c/d. The grating presented to each eye had two configurations. In the first configuration, the grating presented to the DE (or FE) was oriented clockwise (−7.1°) from the horizontal, while the grating presented to the non-DE (or AE) was counterclockwise (+7.1°) from the horizontal. The second configuration featured reversed orientations compared with the first configuration. The base contrast of the gratings shown to the non-DE (or AE) remained constant at 50%. The contrast of the grating shown to the DE (or FE) varied from 0% to 100%,

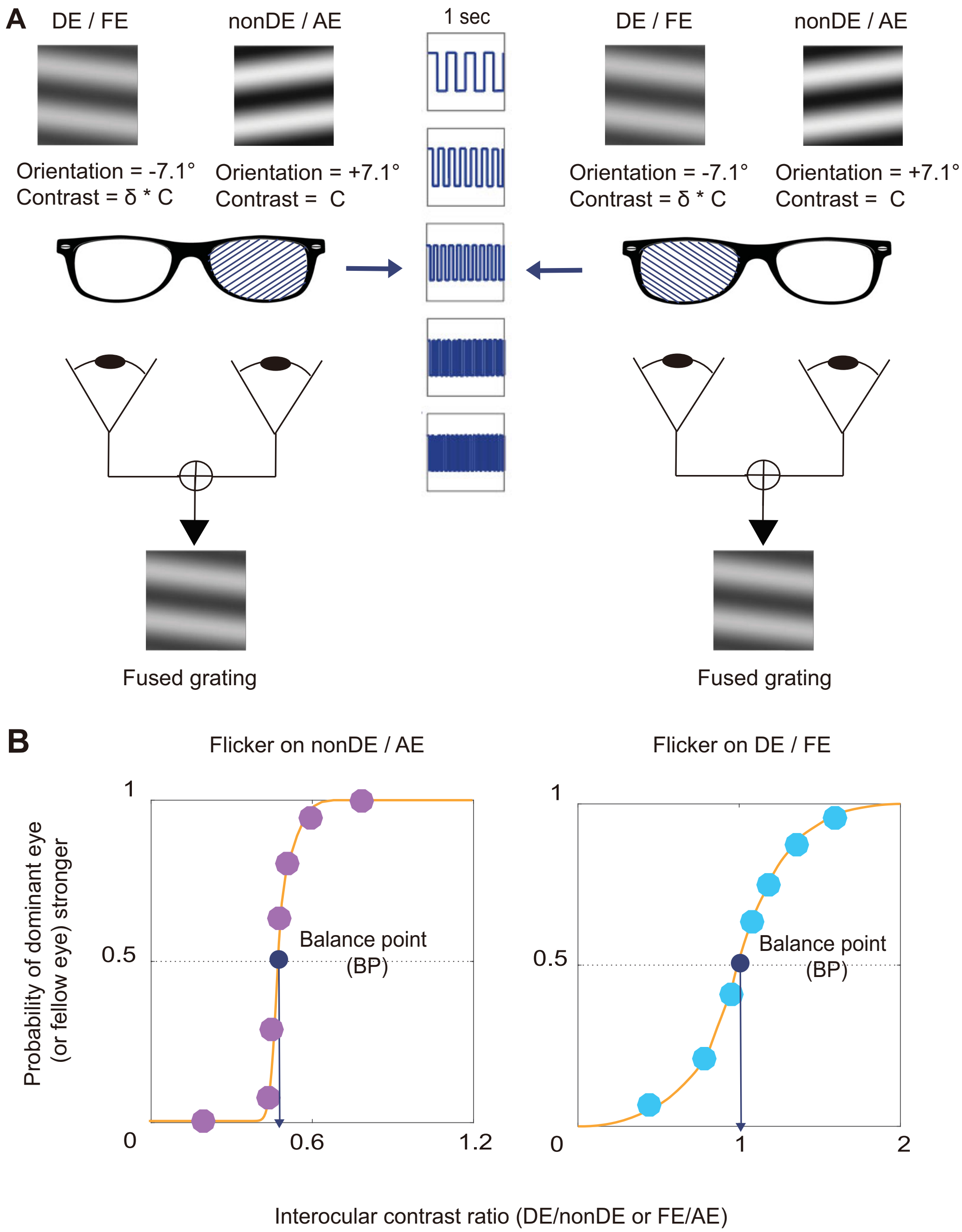

Figure 1. Experimental setup and psychometric function. (**A**) In the binocular orientation combination task, two sinusoidal gratings oriented at ±7.1° were dichoptically presented to each eye. Participants wore monocular flicker glasses across five temporal frequencies (i.e., 4, 7, 10, 15, and 20 Hz) and were instructed to respond to the orientation of the fused grating by pressing either the left or right key on the keyboard. (**B**) The psychometric function was established by plotting the percentage of trials where participants indicated the dominance of DE (or FE) as a function of the interocular contrast ratio (DE/non-DE or FE/AE). A cumulative Gaussian distribution function was used to fit this curve. The BP, corresponding with the 50% point on the optimally fitted Gaussian function, was derived from this fit, indicating the equilibrium of the two eyes in binocular combination.

corresponding to an interocular contrast ratio (DE/non-DE or FE/AE) ranging from 0 to 2 (Fig. 1A).

Procedures

A typical trial in the binocular orientation combination task comprised an alignment and a test phase. During the alignment phase, participants were instructed to align the dichoptic targets, which included crosses and dots, to ensure the correct fusion of the images. Subsequent to the alignment phase, a blank screen was presented for 500 ms, during which a square frame was displayed in each eye to facilitate fusion. The test phase commenced thereafter. In this phase, two horizontally oriented gratings were dichoptically presented to the two eyes for 1 second. After the disappearance of the stimuli, participants were required to judge whether the perceived combined gratings were oriented in a clockwise or counterclockwise direction and provide their responses via keyboard.

Quantification

Upon completion of a block, we used a cumulative Gaussian distribution to fit the psychometric function (Fig. 1B). We computed the probability of the fused percept's orientation tilted toward the dominant (or fellow) eye's grating and plotted this against the interocular contrast ratios (DE/non-DE or FE/AE). The BP was determined as the interocular contrast ratio where the fused percept's orientation tilted toward the dominant (or fellow) eye's grating 50% of the time. To quantify binocular imbalance, we converted the BP values into logarithmic values (log BP [logBP]): When the logBP is 0, it indicates a state of binocular balance. Furthermore, to better illustrate the impact of monocular flicker stimuli on eye dominance, we calculated the logBP difference relative to baseline (LDRB) at different temporal frequencies:

$$LDRB_{\ TF} = logBP_{\ TF} - logBP_{\ Baseline} \tag{1}$$

When the LDRB is >0, it indicates a strengthening of the non-DE or AE.

Statistical Analysis

The statistical analysis involved the following steps: Firstly, a two-way repeated-measures ANOVA was conducted with within-subject factors of eye (e.g., flicker on non-DE/AE and flicker on DE/FE) and temporal frequency (e.g., 4, 7, 10, 15, and 20 Hz), post hoc pairwise paired *t* tests with Bonferroni correction were performed to compare the LDRB values at different temporal frequencies and between eyes in each group. Subsequently, a mixed repeated-measures ANOVA was conducted with a between-subjects factor of group (e.g., nonamblyopic and amblyopic group) and within-subjects factors of eye and temporal frequency. Pairwise post hoc comparisons were used to examine whether the LDRB or slopes (from linear regression of LDRB vs. temporal frequency curve) values were similar between nonamblyopic participants and amblyopes.

Statistical analyses and data visualization were conducted using R and SPSS 20.0 (IBM Corporation, Armonk, NY, USA). All tests were performed using a two-tailed significance level (α) of 0.05.

Results

logBP vs. Flicker Temporal Frequency

Application of monocular flicker shifted binocular balance away from the flickered eye in both nonamblyopic (Fig. 2) and amblyopic (Fig. 3) participants. In nonamblyopic participants, the average baseline logBP was −0.01 ± 0.01, which is close to the ideal binocular balance level (i.e., logBP of 0). Monocular flicker applied to the DE dramatically increased the logBP, whereas when applied to the non-DE, it decreased the logBP. The effect of monocular flicker on logBP was more evident at low temporal frequencies than that at high temporal frequencies in both cases. For example, when flicker was applied to the DE, the average logBP increased to 0.26 ± 0.02, 0.25 ± 0.01, 0.20 ± 0.02, 0.17 ± 0.02, and 0.12 ± 0.01 at 4 Hz, 7 Hz, 10 Hz, 15 Hz, and 20 Hz, respectively (Fig. 2B). Then, when flicker stimulation was applied to the non-DE, this reduced the logBP to −0.44 ± 0.03, −0.43 ± 0.02, −0.36 ± 0.02, −0.26 ± 0.02, and −0.22 ± 0.02 at 4 Hz, 7 Hz, 10 Hz, 15 Hz, and 20 Hz, respectively.

A similar pattern was found in amblyopes, in which flicker applied to the FE increased the logBP, while flicker applied to the AE reduced the logBP (Fig. 3A). Again, the effect was more pronounced at low temporal frequencies than at high temporal frequencies. Stimulation of the FE increased the average logBP from −0.37 ± 0.02 at baseline to 0.14 ± 0.05, 0.14 ± 0.05, −0.01 ± 0.04, −0.09 ± 0.03, and −0.09 ± 0.05 at 4 Hz, 7 Hz, 10 Hz, 15 Hz, and 20 Hz flicker, respectively. On the contrary, stimulation of the AE reduced the logBP to −0.77 ± 0.05, −0.78 ± 0.06, −0.66 ± 0.06, −0.57 ± 0.04, and −0.53 ± 0.03 at 4 Hz, 7 Hz, 10 Hz, 15 Hz, and 20 Hz flicker, respectively (Fig. 3B).

LDRB vs. Flicker Temporal Frequency

Considering the interobserver variability in baseline logBP values, we calculated the LDRB at different temporal

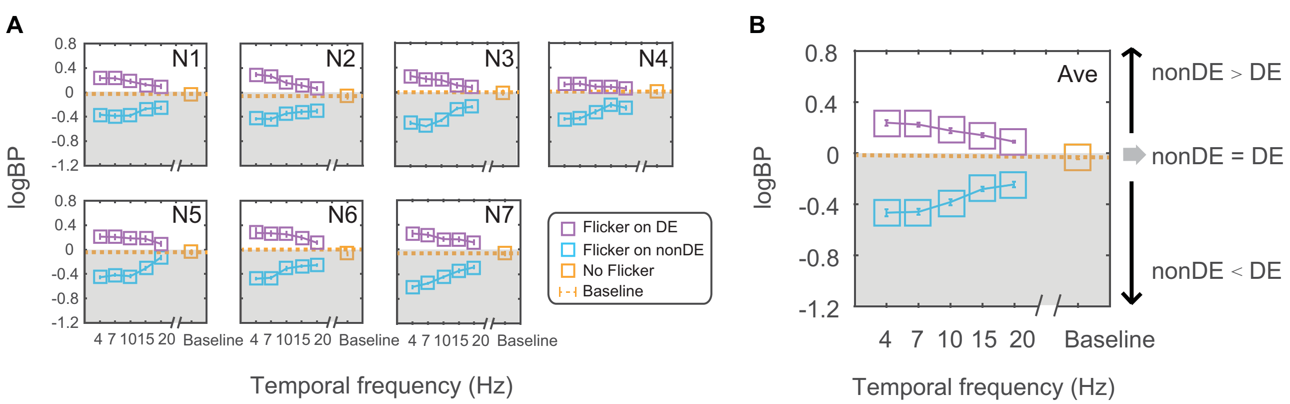

Figure 2. logBP as a function of flicker temporal frequencies for each nonamblyopic participant and their average. (**A**) The logBP of each nonamblyopic participant across five temporal frequencies (i.e., 4, 7, 10, 15, and 20 Hz). (**B**) The average logBP of nonamblyopic participants (n = 7). *Error bars* in the average plots represent standard errors across the seven participants. The *blue squares* and *purple squares* represent the logBP measured with flicker applied to the non-DE and DE, respectively. The *yellow square* represents the baseline level of binocular balance measured with no flicker applied. Date points in the grey area (i.e., logBP < 0) indicate that the DE is stronger in binocular combination.

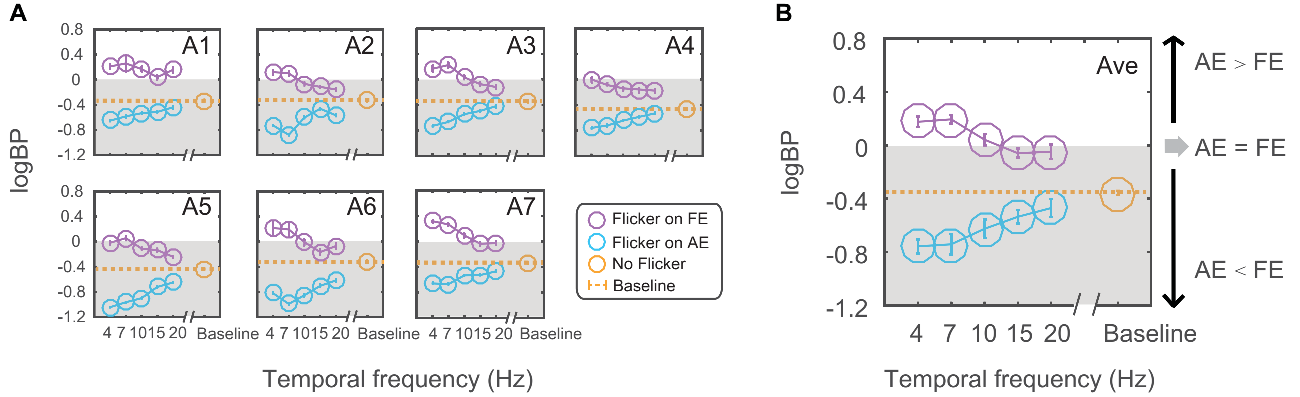

Figure 3. logBP as a function of flicker temporal frequencies for each amblyope and their average. (**A**) The logBP of each amblyopic participant across five temporal frequencies (i.e., 4, 7, 10, 15, and 20 Hz). (**B**) The average logBP of amblyopic participants (n = 7). *Error bars* in the average plots represent standard errors across the seven participants. The *blue circles* and *purple circles* represent the logBP measured with flicker applied to the AE and FE. The *yellow circle* represents the baseline level of binocular imbalance measured without the adding of flicker. Data points in the grey area (i.e., logBP < 0) indicate that the FE is stronger in binocular combination.

frequencies to better illustrate the effects of monocular flicker. Figure 4A shows the LDRB of each nonamblyopic participant and their average LDRB as a function of the temporal frequency of monocular flicker. It's evident that monocular flicker produced opposite effects when applied to the two eyes. This observation is supported by the results of a two-way repeated measures ANOVA, which revealed significant effects of eye, F(1,6) = 470.917, $P < 0.001$, η^2 = 0.987, and temporal frequency, F(4,24) = 3.866, $P = 0.015$, η^2 = 0.392, on the LDRB. Moreover, there was a significant interaction between eye and temporal frequency, F(4, 24) = 58.082, $P < 0.001$, η^2 = 0.906. The latter results suggest that the temporal frequency tuning curves were significantly different when monocular flicker was applied to different eyes.

To further assess the difference, pairwise post hoc comparison between specific temporal frequencies were conducted, revealing significant differences including: 20 Hz vs. 4 Hz (flicker on non-DE: P = 0.005; flicker on DE: P = 0.003), 20 Hz vs. 7 Hz (flicker on non-DE: P = 0.003; flicker on DE: P = 0.001), 15 Hz vs. 4 Hz (flicker on non-DE: P = 0.003; flicker on DE: P = 0.03), 15 HZ vs. 7 Hz (flicker on non-DE: P = 0.004; flicker on DE: P = 0.015), 10 Hz vs. 15 Hz (flicker on non-DE: P = 0.028), 20 Hz vs. 10 Hz (flicker on DE: P = 0.008), and 20 Hz vs. 15 Hz (flicker on DE: P = 0.016). These results suggest a more prominent flicker effect at low temporal frequencies than that at high temporal frequencies in nonamblyopic participants.

The patterns of temporal frequency tuning curves in amblyopic participants, as illustrated in Figure 4B, closely resemble those observed in nonamblyopic participants (Fig. 4A). A two-way repeated measures ANOVA revealed significant effect of eye, F(1,6) = 320.036, $P < 0.001$, η^2 = 0.982, but not of temporal frequency, F(4,24) = 0.46, P = 0.765, η^2 = 0.071. The interaction between eye and temporal frequency was significant, F(1.923, 11.538) = 60.555, $P < 0.001$, η^2 = 0.91, highlighting that the distinct temporal frequency tuning curves when monocular flicker was applied to different eyes in amblyopes.

The observed tuning curves suggest that the effects of monocular flicker on binocular vision are not uniform across all temporal frequencies, emphasizing the importance of considering both the eye of stimulation and the temporal frequency in interventions targeting binocular vision in amblyopia. Further pairwise post hoc comparisons indicated significant differences between specific temporal frequencies in the following conditions: 20 Hz vs. 4 Hz (flicker on AE: P = 0.002; flicker on FE: P = 0.007), 20 Hz vs. 7 Hz (flicker on AE: P = 0.002; flicker on FE: P = 0.008), 15 Hz vs. 4 Hz (flicker on AE: P = 0.005; flicker on FE: P = 0.008), 15 Hz vs. 7 Hz (flicker on AE: P = 0.03; flicker on FE: P = 0.006), 10 Hz vs. 4 Hz (flicker on FE: P = 0.011), 10 Hz vs. 7 Hz (flicker on FE: P = 0.04). These results also suggest a more prominent flicker effect at low temporal frequencies than that at high temporal frequencies in amblyopes.

To compare the LDRB between nonamblyopic controls and amblyopes, a mixed repeated-measure ANOVA was conducted. The analysis revealed significant effect of eye, F(1,12) = 720.727, $P < 0.001$, η^2 = 0.984; group, F(1,12) = 9.408, P = 0.01, η^2 = 0.439; and an interaction between eye and group, F(1,12) = 9.641, P = 0.009, η^2 = 0.446. Pairwise post hoc comparisons were conducted to further explore the differences. The results showed that the LDRB was similar when flicker was applied to the AE in amblyopes compared with when flicker was applied to the non-DE in nonamblyopic individuals (P = 0.468). However, when flicker was applied to the FE in amblyopes, the LDRB was higher than that observed when flicker was applied to the DE in controls ($P < 0.001$).

To evaluate the variability in the trend of binocular imbalance with respect to temporal frequency, the LDRB vs. temporal frequency curves were fitted with linear regression and the resultant slopes were compared between nonamblyopic and amblyopic participants. A mixed repeated-measure ANOVA was performed, with a within-subjects factor for eyes and a between-subjects factor for groups. The analysis revealed a significant effect of the eye, F(1,12) = 236.487, $P < 0.001$, η^2 = 0.952, an interaction between eye and group, F(1,12) = 5.987, P = 0.031, η^2 = 0.333, but not of group, F(1,12) = 2.544, P = 0.137, η^2 = 0.175. A pairwise post hoc comparison showed that there were differences in the slope between the DE and FE (P = 0.015) and no differences between the non-DE and AE (P = 0.516).

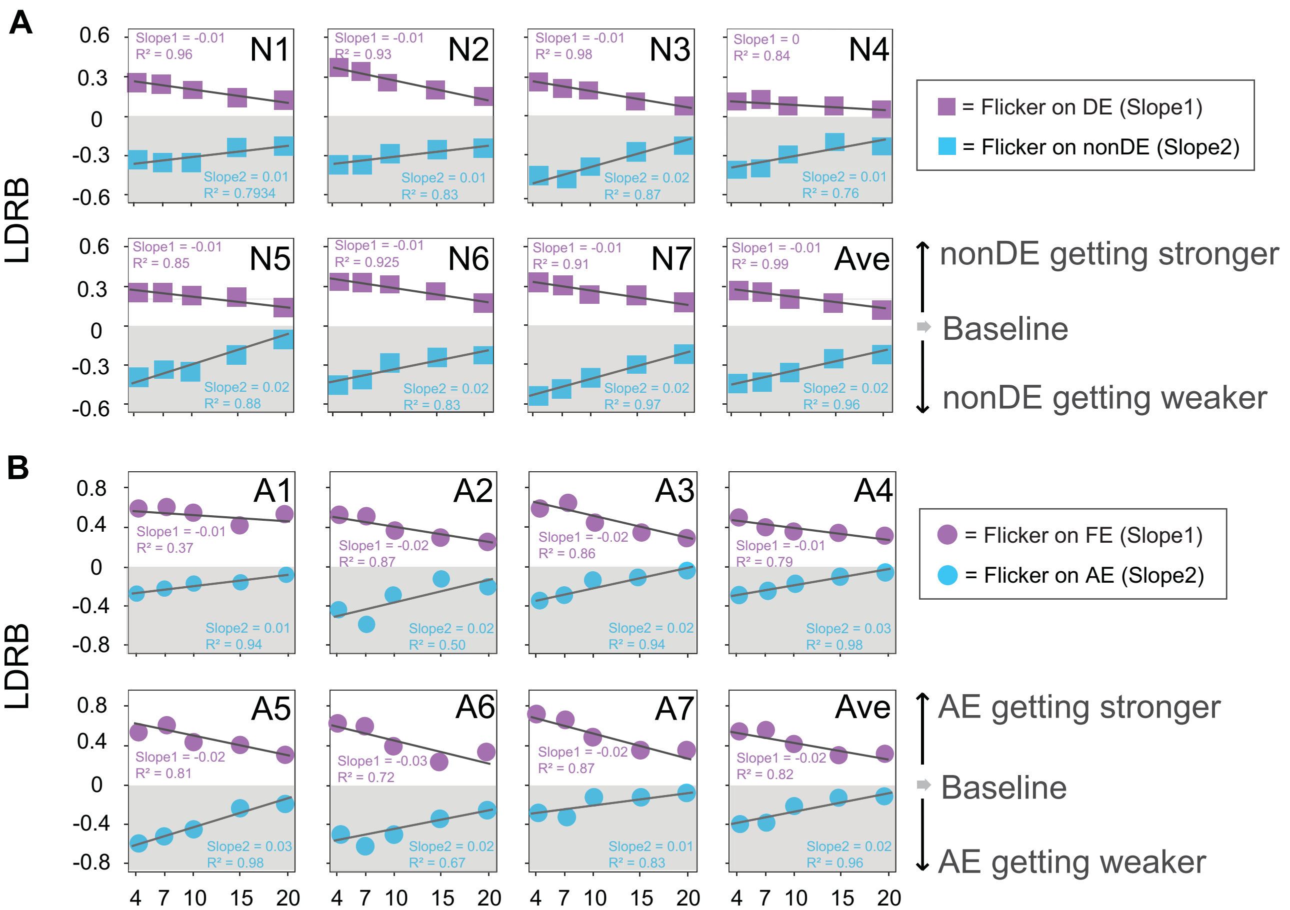

Figure 4. The individual and average LDRB in nonamblyopic participants and amblyopes. (**A**) The individual and average LDRB of nonamblyopic participant ($n = 7$) across five temporal frequencies (i.e., 4, 7, 10, 15, and 20 Hz). The *blue squares* and *purple squares* represent the LDRB when flicker is applied to the non-DE and DE, respectively. (**B**) The individual and average LDRB of amblyopes ($n = 7$) across five temporal frequencies. The *blue* and *purple circles* represent the LDRB when flicker is applied to the AE and FE, respectively. *Error bars* in the average plots represent standard errors across the seven participants. Data points in the grey area (i.e., LDRB < 0) indicate that the non-DE/AE is getting weaker in binocular combination.

Discussion

This study aimed to investigate the effects of externally generated monocular flicker stimuli on binocular interactions. This was done by quantifying the impact of flicker stimuli at various temporal frequencies (e.g., 4, 7, 10, 15, and 20 Hz) on binocular imbalance in both nonamblyopic and amblyopic participants. Our findings reveal the following key insights. (1) Monocular flicker significantly decreases the contribution of the flickered eye to binocular combination, resulting in a relative dominance of the nonflickered eye in interocular interactions. (2) The temporally modulated effect is more pronounced at lower temporal frequencies than at higher ones.

In this study, the externally generated monocular flicker operated with a 1:1 light-to-dark ratio, resulting in the flickered eye receiving only one-half of the visual input compared with the nonflickered eye. Under fixed conditions of spatial frequency (0.42 c/d) and visual grating presentation duration (1 second), this halved presentation time consequently decreases the weight of the flickered eye in binocular integration, thus diminishing its potential suppressive effect on the contralateral eye. This observation aligns with the contrast-gain model proposed by Ding et al.[40] However, a pertinent question arises: Why is this suppression effect most pronounced at low temporal frequencies?

Previous research on flicker stimuli[25] indicated that flickering could alter luminance transmission, leading to a decrease in the average luminance in front of the flickered eye. Although luminance reduction in one eye could lessen its contribution in binocular integration,[35–37] could the flickering-induced luminance transmission alternation explain the temporal frequency dependency pattern found in the current study? To verify this hypothesis, we measured the luminance transmission across five various temporal frequencies of flicker stimuli (refer to Supplementary Table S2, Supplementary Fig. S1, and Supplementary Fig. S2 for details). The results showed that luminance transmission decreases as temporal frequency increases, with average luminance at higher frequencies (e.g., 20 Hz) being lower than that at lower frequencies (e.g., 4 Hz). However, this trend does not align with the temporally modulated effects we observed, suggesting that the luminance differences between eyes caused by flickering do not fully explain this phenomenon.

Another plausible explanation is that the external flicker stimuli may act as a patching or blurring effect, subsequently weakening the flickered eye in binocular combination. Unlike previous studies[26,38] that generate flicker through software programming, displaying targets (such as numbers, letters, or gratings) at various durations to create target flickered at different temporal frequencies, in our study, monocular flicker is produced with liquid crystal lenses. These lenses generate specific temporal frequency flicker stimuli in front of the eye with a 1:1 light:dark ratio through electronic control. This externally generated flicker stimulus may act as temporal noise, disrupting the target (grating) and reducing the signal-to-noise ratio of visual input, thereby diminishing the weighted contribution of the flickered eye in binocular integration.[47] To preliminarily validate this conjecture, we had seven participants with normal vision to measure their monocular contrast threshold under single-eye flicker conditions at various temporal frequencies. Four of these participants were from the normal group (N1, N4, N6, and N7), and the remaining three were recruited additionally. The results showed that the monocular contrast threshold gradually decreases as the temporal frequency increases (see Supplementary Fig. S3 for details). Notably, this temporal frequency dependency pattern aligns with the binocular measures conducted in the current study.

Our study proposes an effective method for regulating binocular balance. Existing research has shown that amblyopic patients demonstrate improved performance in stereoscopic assessments when both eyes achieve greater balance.[48–50] Various training methods, such as binocular contrast modulation,[27,51] push–pull training,[52–54] or synchronization of binocular latencies,[55] have demonstrated the potential for yielding long-term gains in visual acuity and stereopsis among amblyopic patients. These observations align with the perspective of binocular combination modeling, suggesting correlations between different types of binocular combination processing,[10] which may share a common early contrast gain control mechanism.[14] Therefore, based on the binocular balance optimization protocol we proposed in this study, it is worthwhile to further explore its short- and long-term effects on other binocular functions in amblyopic patients in the future.

However, we must acknowledge certain limitations in our study. First, the sample size was relatively small, consisting of only seven adult amblyopes, all of whom had late-onset amblyopia that been diagnosed in the teen years. Second, all the patients included in this study were anisometropic amblyopes. Therefore, further research with larger sample sizes and diverse clinical profiles could provide additional insights into the generalizability of these findings and their potential applications in the clinical management of amblyopia. It is also interesting to explore whether our strategy in modulating binocular imbalance extends to other binocular disorders, such as strabismus.[56]

Conclusions

Our study delves into the impact of externally generated monocular flicker on binocular balance, encompassing both nonamblyopic and amblyopic participants. The findings illuminate the fact that externally generated monocular flicker diminishes the contribution of the flickered eye in binocular combination, resulting in a relative dominance of the non-flickered eye in interocular interactions. Furthermore, a more pronounced temporal effect was observed at lower temporal frequencies. These results contribute significantly to our understanding of the role played by various flicker stimuli in binocular information processing and hold the potential to usher in the development of new patient-friendly treatment approaches.

Acknowledgments

The authors thank Ziyu Xu for his dedication to measure the luminance transmission under different temporal frequency conditions. Thanks to Kevin Willeford for proofreading this paper. Thanks to the two anonymous reviewers for their insightful comments on the interpretation of the results of the paper.

Supported by the National Key Research and Development Program of China Grant (2023YFC3604104), the Natural Science Foundation for Distinguished Young Scholars of Zhejiang Province, China (LR22H120001), the Non-profit Central Research Institute Fund of Chinese Academy of Medical Sciences (2023-PT320-04), and the Project of State Key Laboratory of Ophthalmology, Optometry and Vision Science, Wenzhou Medical University (J02-20210203) to JZ., the Key Research and Development Plan in Zhejiang Province (2021C03102) to JQ., and the National Natural Science Foundation of China Grant NSFC 12205221 to RC.

Disclosure: **Y. Lu**, None; **L. Zou**, None; **W. Wang**, None; **R. Chen**, None; **J. Qu**, None; **J. Zhou**, None

References

1. Cruz OA, Repka MX, Hercinovic A, et al. Amblyopia preferred practice pattern. *Ophthalmology*. 2023;130(3):136–178.
2. Multi-ethnic Pediatric Eye Disease Study Group. Prevalence of amblyopia and strabismus in African American and Hispanic children ages 6 to 72 months the multi-ethnic pediatric eye disease study. *Ophthalmology*. 2008;115(7):1229–1236.
3. Holmes JM, Clarke MP. Amblyopia. *Lancet*. 2006;367(9519):1343–1351.
4. Carlton J, Kaltenthaler E. Health-related quality of life measures (HRQoL) in patients with amblyopia and strabismus: a systematic review. *Br J Ophthalmol*. 2011;95(3):325–330.
5. Davidson S, Quinn GE. The impact of pediatric vision disorders in adulthood. *Pediatrics*. 2011;127(2):334–339.
6. Felius J, Chandler DL, Holmes JM, et al. Evaluating the burden of amblyopia treatment from the parent and child's perspective. *J AAPOS*. 2010;14(5):389–395.
7. Walraven J, Janzen P. TNO stereopsis test as an aid to the prevention of amblyopia. *Ophthalmic Physiol Opt*. 1993;13(4):350–356.
8. Mao Y, Min SH, Chen S, et al. Binocular imbalance in amblyopia depends on spatial frequency in binocular combination. *Invest Ophthalmol Vis Sci*. 2020;61(8):7.
9. Zhou J, Liu R, Feng L, Zhou Y, Hess RF. Deficient binocular combination of second-order stimuli in amblyopia. *Invest Ophthalmol Vis Sci*. 2016;57(4):1635–1642.
10. Zhou J, Huang PC, Hess RF. Interocular suppression in amblyopia for global orientation processing. *J Vis*. 2013;13(5):19.
11. Kwon M, Wiecek E, Dakin SC, Bex PJ. Spatial-frequency dependent binocular imbalance in amblyopia. *Sci Rep*. 2015;5:17181.
12. Li J, Thompson B, Lam CSY, et al. The role of suppression in amblyopia. *Invest Ophthalmol Vis Sci*. 2011;52(7):4169–4176.
13. Meese TS, Georgeson MA, Baker DH. Binocular contrast vision at and above threshold. *J Vis*. 2006;6(11):1224–1243.

14. Huang CB, Zhou J, Lu ZL, Zhou Y. Deficient binocular combination reveals mechanisms of anisometropic amblyopia: signal attenuation and interocular inhibition. *J Vis*. 2011;11(6), doi:10.1167/11.6.44.
15. Ding J, Klein SA, Levi DM. Binocular combination of phase and contrast explained by a gain-control and gain-enhancement model. *J Vis*. 2013;13(2):13.
16. Baker DH, Meese TS, Hess RF. Contrast masking in strabismic amblyopia: attenuation, noise, interocular suppression and binocular summation. *Vision Res*. 2008;48(15):1625–1640.
17. Zhou J, Reynaud A, Yao Z, et al. Amblyopic suppression: passive attenuation, enhanced dichoptic masking by the fellow eye or reduced dichoptic masking by the amblyopic eye? *Invest Ophthalmol Vis Sci*. 2018;59(10):4190–4197.
18. Gong L, Reynaud A, Wang Z, et al. Interocular suppression as revealed by dichoptic masking is orientation-dependent and imbalanced in amblyopia. *Invest Ophthalmol Vis Sci*. 2020;61(14):28.
19. Chen S, Min SH, Cheng Z, et al. Binocular visual deficits at mid to high spatial frequency in treated amblyopes. *iScience*. 2021;24(7):102727.
20. Vera-Diaz F, Moore B, Hussey E, Srinivasan G, Johnson C. A flicker therapy for the treatment of amblyopia. *Vis Dev Rehab*. 2016;2:105–114.
21. Yuan Y, Zhu C, Wang P, et al. Alternative flicker glass: a new anti-suppression approach to the treatment of anisometropic amblyopia. *Ophthalmic Res*. 2021;64(6):967–973.
22. Lin W, Wei J, Wang W, et al. Rapid alternate monocular deprivation does not affect binocular balance and correlation in human adults. *eNeuro*. 2022;9(3):ENEURO.0509–21.2022. 1–8.
23. Min SH, Chen S, Xu J, et al. A randomized clinical trial comparing Eyetronix flicker glass and patching for treatment of amblyopia in children reveals similar improvements in vision. *Front Neurosci*. 2021;15: 622729.
24. Schor C, Terrell M, Peterson D. Contour interaction and temporal masking in strabismus and amblyopia. *Am J Optom Physiol Opt*. 1976;53(5):217–223.
25. 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.
26. Kosovicheva A, Ferreira A, Vera-Diaz FA, Bex PJ. Effects of temporal frequency on binocular deficits in amblyopia. *Vision Res*. 2019;163:52–62.
27. Gao TY, Guo CX, Babu RJ, et al. Effectiveness of a binocular video game vs placebo video game for improving visual functions in older children, teenagers, and adults with amblyopia: a randomized clinical trial. *JAMA Ophthalmol*. 2018;136(2):172–181.
28. Birch EE, Jost RM, De La Cruz A, et al. Binocular amblyopia treatment with contrast-rebalanced movies. *J AAPOS*. 2019;23(3):160.e1–160.e5.
29. Pineles SL, Aakalu VK, Hutchinson AK, et al. Binocular treatment of amblyopia: a report by the American Academy of Ophthalmology. *Ophthalmology*. 2020;127(2):261–272.
30. Manh VM, Holmes JM, Lazar EL, et al. A Randomized trial of a binocular iPad game versus part-time patching in children aged 13 to 16 years with amblyopia. *Am J Ophthalmol*. 2018;186:104–115.
31. Pediatric Eye Disease Investigator Group, Holmes JM, Manny RE, et al. A randomized trial of binocular dig rush game treatment for amblyopia in children aged 7 to 12 years. *Ophthalmology*. 2019;126(3):456–466.
32. Xiao S, Angjeli E, Wu HC, et al. Randomized controlled trial of a dichoptic digital therapeutic for amblyopia. *Ophthalmology*. 2022;129(1):77–85.
33. Ding J, Levi DM. Rebalancing binocular vision in amblyopia. *Ophthalmic Physiol Opt*. 2014;34(2):199–213.
34. Pediatric Eye Disease Investigator Group Writing Committee, Rutstein RP, Quinn GE, et al. A randomized trial comparing Bangerter filters and patching for the treatment of moderate amblyopia in children. *Ophthalmology*. 2010;117(5):998–1004.e6.
35. Min SH, Mao Y, Chen S, Hess RF, Zhou J. Modulation of mean luminance improves binocular balance across spatial frequencies in amblyopia. *iScience*. 2022;25(7): 104598.
36. Zhou J, Hess RF. Neutral-density filters are not a patch on occlusion. *Invest Ophthalmol Vis Sci*. 2016;57(10):4450–4451.
37. Zhou J, Jia W, Huang CB, Hess RF. The effect of unilateral mean luminance on binocular combination in normal and amblyopic vision. *Sci Rep*. 2013;3:2012.
38. Abuleil D, McCulloch DL, Patterson H, Thompson B. Modulation of binocular rivalry with rapid monocular visual stimulation. *Eur J Neurosci*. 2021;53(4):1008–1018.
39. Wang Y, He Z, Liang Y, et al. The binocular balance at high spatial frequencies as revealed by the binocular orientation combination task. *Front Hum Neurosci*. 2019;13: 106.
40. Yehezkel O, Ding J, Sterkin A, Polat U, Levi DM. Binocular combination of stimulus orientation. *R Soc Open Sci*. 2016;3(11):160534.
41. Brainard DH. The Psychophysics toolbox. *Spatial Vis*. 1997;10(4):433–436.
42. Kleiner M, Brainard DH, Pelli D, Ingling A, Murray R, Broussard C. What's new in Psychtoolbox-3. *Perception*. 2007;36:1–16
43. Hammett ST, Larsson J. The effect of contrast on perceived speed and flicker. *J Vis*. 2012;12(12):17.
44. Eren OE, Straube A, Schöberl F, Ruscheweyh R, Eggert T, Schankin CJ. Age- and frequency-dependent changes in dynamic contrast perception in visual snow syndrome. *J Headache Pain*. 2021;22(1):148.
45. Beste C, Wascher E, Güntürkün O, Dinse HR. Improvement and impairment of visually guided behavior through LTP- and LTD-like exposure-based visual learning. *Curr Biol*. 2011;21(10):876–882.
46. Dane A, Dane S. Correlations among handedness, eyedness, monocular shifts from binocular focal point, and nonverbal intelligence in university mathematics students. *Percept Mot Skills*. 2004;99(2):519–524.
47. Ding J, Sperling G. A gain-control theory of binocular combination. *Proc Natl Acad Sci USA*. 2006;103(4):1141–1146.
48. Eisen-Enosh A, Farah N, Polat U, Mandel Y. Temporal synchronization elicits enhancement of binocular vision functions. *iScience*. 2023;26(2):105960.
49. Ooi TL, He ZJ. Sensory eye dominance: relationship between eye and brain. *Eye Brain*. 2020;12:25–31.
50. Wang X, Baldwin AS, Hess RF. Balanced binocular inputs support superior stereopsis. *Invest Ophthalmol Vis Sci*. 2021;62(12):10.
51. Hess RF, Thompson B, Black JM, et al. An iPod treatment of amblyopia: an updated binocular approach. *Optometry*. 2012;83(2):87–94.
52. Ooi TL, Su YR, Natale DM, He ZJ. A push-pull treatment for strengthening the "lazy eye" in amblyopia. *Curr Biol*. 2013;23(8): R309–310.

Investigative Ophthalmology & Visual Science

53. Xu JP, He ZJ, Ooi TL. Effectively reducing sensory eye dominance with a push-pull perceptual learning protocol. *Curr Biol*. 2010;20(20):1864–1868.
54. Xu JP, He ZJ, Ooi TL. A binocular perimetry study of the causes and implications of sensory eye dominance. *Vision Res*. 2011;51(23–24):2386–2397.
55. Huang X, Xia H, Zhang Q, et al. New treatment for amblyopia based on rules of synaptic plasticity: a randomized clinical trial. *Sci China Life Sci*. 2022;65(3):451–465.
56. Zhou J, Wang Y, Feng L, Wang J, Hess RF. Straightening the eyes doesn't rebalance the brain. *Front Hum Neurosci*. 2017;11:453.