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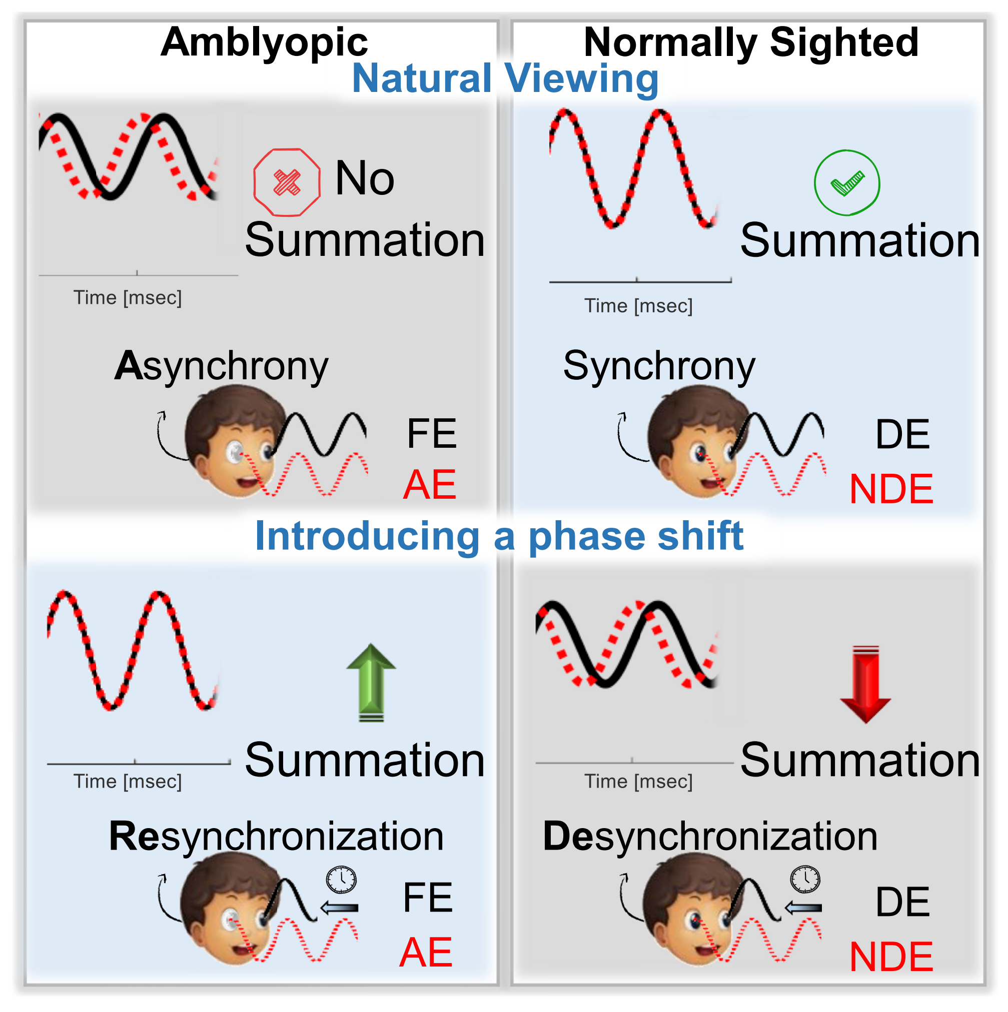

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Highlights

Binocular asynchrony in amblyopic subjects was measured and compensated for

Binocular synchronization improved visual functions in amblyopes

Binocular desynchronization decreased visual functions in neurotypical subjects

Improvement was evident in psychophysical and neurophysiological measures

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Temporal synchronization elicits enhancement of binocular vision functions

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SUMMARY

Integration of information over the CNS is an important neural process that affects our ability to perceive and react to the environment. The visual system is required to continuously integrate information arriving from two different sources (the eyes) to create a coherent percept with high spatiotemporal precision. Although this neural integration of information is assumed to be critical for visual performance, it can be impaired under some pathological or developmental conditions. Here we took advantage of a unique developmental condition, amblyopia ("lazy eye"), which is characterized by an impaired temporal synchronization between the two eyes, to meticulously study the effect of synchronization on the integration of binocular visual information. We measured the eyes' asynchrony and compensated for it (with millisecond temporal resolution) by providing time-shifted stimuli to the eyes. We found that the re-synchronization of the ocular input elicited a significant improvement in visual functions, and binocular functions, such as binocular summation and stereopsis, were regained. This phenomenon was also evident in neurophysiological measures. Our results can shed light on other neural processing aspects and might also have translational relevance for the field of training, rehabilitation, and perceptual learning.

INTRODUCTION

Integration of information from various channels and from body-sensing organs is a critical function of the human perceptual system; deficient integration of inputs entering the CNS can adversely affect daily behavior. One such important example of information integration is the binocular combination of the information arriving from the left and the right eyes.[1,2] Several potential mechanisms combine binocular information: binocular summation (BS),[3] probability summation,[4] rivalry,[5] and inter-ocular suppression, which differ from each other by their neural basis and magnitude. In the current research, we used the term binocular summation (BS) to describe the integration process of information arising from the two eyes, which leads to higher binocular visual function, compared with monocular function. To study the effect of information integration in the visual system on perception, here we investigated how interrupting normative synchronization hampers performance and how correcting deficient integration (in amblyopes) improves performance.

Since the synchronization of information is expected to be critical for integrating rapidly changing stimuli, we focused on evaluating temporal resolution, the ability to discern changes in the stimulus properties over time,[6,7] rather than spatial[8–12] visual resolution. Numerous reports have shown that the BS in the spatial domain is significantly reduced by monocular imbalance.[2,13–15] An important example of ocular imbalance is amblyopia, a developmental condition resulting from the abnormal development of the visual system in the early stages of life,[16] characterized by a unilateral decrease in various spatial resolution dysfunctions,[17–24] the presence of spatial crowding,[25] and reduced temporal functions.[24,26–37] Indeed, various studies have shown that binocular imbalance in the spatial or stimulus energy domain is associated with lower binocular summation,[13,15,38–40] which is restored by adjusting the contrast or stimulus energy of the two eyes.[39,41]

Previous studies have shown a strong association between the temporal and spatial functions in normal and amblyopic subjects[26,34,42]; we, therefore, hypothesized that similar to the spatial domain, the ocular imbalance in amblyopic subjects would be associated with a decrease in BS. We further hypothesized that the slower processing of information arriving from the amblyopic eye[43] elicits desynchronization, which

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interrupts the normal integration of the information, thus resynchronizing and balancing the binocular input, by providing the amblyopic eye with earlier phase-shifted stimuli or brighter stimuli, would increase information integration and BS in the temporal domain. Furthermore, we hypothesized that the desynchronization of information in normally sighted subjects would hamper temporal vision performance.

We studied the temporal function using the critical flicker fusion frequency (CFF) paradigm, which is known to be reduced in amblyopic subjects[44,45] using a customized dichoptic system.[45] We also performed spatiotemporal perception tests using computerized contrast sensitivity tests. In addition, subjects performed a flickering stereopsis test and electrophysiological tests of ssVEP were recorded in response to flickering stimuli. Our results indicated that in contrast to normally sighted subjects, in amblyopic subjects, the binocular CFF was equal to the fellow eye's CFF, suggesting disturbed information integration with no evident BS of flickering stimuli. Interestingly, we found that synchronizing the visual input in amblyopic subjects by providing the amblyopic eye with earlier stimuli or by balancing the input using brighter stimuli resulted in a significant binocular combination, up to the magnitude of normally sighted subjects. In line with this observation, interrupting the synchronization in a normally sighted subject by introducing an inter-ocular phase delay elicited a decrease in BS. A similar effect of binocular synchronization or desynchronization was manifested by the enhancement of binocular function in a flickering stereopsis paradigm as well as in electrophysiological studies. The results suggest that the synchronization of information arriving from various channels is critical for integration. More important, similar to contrast information processing,[41] the mechanism underlying a binocular combination of temporal information remains intact in amblyopic subjects and can be restored by synchronization and balancing the information arriving from the two eyes.

RESULTS

Amblyopic subjects have reduced critical flicker fusion frequency with no apparent binocular temporal summation

To investigate the BS of visual temporal function, 15 normally sighted subjects (8 females, 7 males) and 13 amblyopic subjects (11 females, 2 males) were tested for CFF thresholds at five different luminance levels, for both monocular and binocular viewing conditions. Measurements were carried out by a dichoptic flicker stimulation system using the staircase paradigm, similar to our recent publications[45,46] (see Figure 8 and the STAR Methods for more details). Binocular combination was evaluated by comparing the binocular to the monocular results.

Under both monocular and binocular conditions and for both amblyopic subjects and normally sighted subjects, increasing stimulus luminance levels were associated with higher CFF values (Figures 1A and 1B Mann-Kendall test p for trend = 0.027). In the amblyopic subjects, the amblyopic eyes showed significantly lower CFF, compared with the fellow eye; the mean difference was 25% (paired two-tailed t-test: t(4) = 12.36, $p<<0.001$) (Figure 1B). Importantly, BS of up to a factor of 1.11 of the CFF was found in normally sighted subjects (Figure 1A and 1C), as is evident by the higher CFF value in the binocular compared with the monocular condition; however, the difference was statistically significant only for the lower luminance level (paired two-tailed t-test: 24.92 vs. 27.56Hz, t(14) = -5.2, $p < 0.001$; 26.53 vs. 28.77 t(14) = -6.35, $p < 0.001$; 29.13 vs. 30.02 t(14) = -2.68, $p = 0.018$ for a luminance of 2.5, 5, and 10 cd/m^2, respectively). Interestingly, in contrast with normally sighted subjects, no BS was found in amblyopic subjects; for all luminance levels, the binocular CFF values were similar to the CFF values of the fellow (normal) eye (paired two-tailed t-test $p > 0.1$ for all luminance levels) (Figures 1B and 1D), suggesting that the binocular information integration in the temporal domain malfunctions in amblyopes.

Both normally sighted subjects and amblyopic subjects show a binocular summation in the spatial visual function

In contrast to the lack of BS in the temporal domain in our amblyopic subjects, previous studies reported various magnitudes of BS in spatial and spatiotemporal tasks in amblyopic subjects.[15,47] In order to study the magnitude of BS in the spatial domain in our subjects and set-up, normally sighted and amblyopic subjects performed a computerized contrast sensitivity task at spatial frequencies of 3, 6, and 12 cpd. In addition, to evaluate the spatiotemporal function, a backward masking task[48–50] was performed (see the STAR Methods). All tests were performed using a "PSY—psychophysical tool" similar to our previous reports[42,51–54] for both eyes monocularly and binocularly (see the star methods).

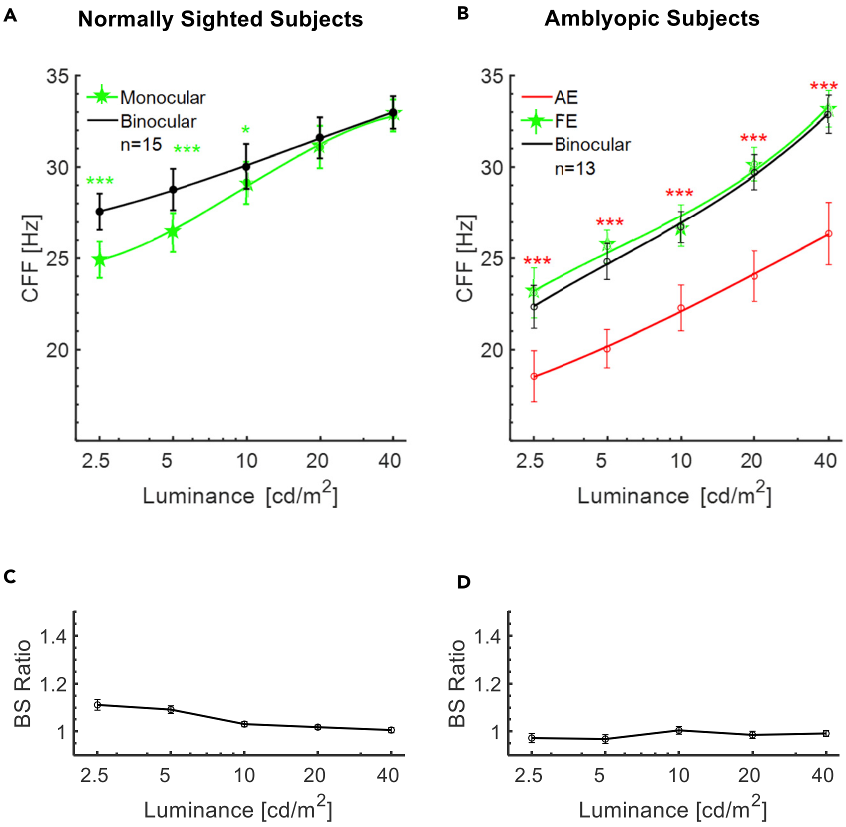

Figure 1. The effect of the Luminance level on CFF and Binocular summation (BS)

(A) CFF as a function of luminance level in normally sighted subjects. The black line represents binocular values and the green line represents monocular values.

(B) CFF as a function of the luminance level in normally sighted subjects. The black line represents binocular values, the green line represents FE values, and the red line represents AE values; the amblyopic eye showed a significant decrease of 5.52Hz in the CFF ($p \ll 0.001$).

(C) Binocular summation in normally sighted subjects as a function of the luminance level. BS Ratio = binocular values/monocular values.

(D) Binocular summation as a function of the luminance level in amblyopic subjects. BS Ratio = binocular values/FE values. Error bars refer to the SEM. Statistical significance was indicated as:*$p<=0.05$**$p \ll 0.01$ ***$p \ll 0.001$.

In accordance with previous reports, for both normally sighted (Figures 2A, 2C, S1A, and S1C) and amblyopic (Figure 2B, 2D, S1B, and S1D) subjects, the results show that a binocular summation of contrast sensitivity is evident. In normally sighted subjects, a significantly higher contrast sensitivity was obtained in binocular, compared with monocular conditions for all spatial frequencies (paired two-tailed t-test: $t(8) = 12$, $t(8) = 17.6$, $t(8) = 27.6$, of 3,6 and 12 cpd, respectively, $p \ll 0.001$ for all SFs); the difference was larger at lower spatial frequencies (Figure 2A, Mann-Kendall test p for trend = 0.003). Similarly, in amblyopic subjects, for 6 and 12 cpd, the binocular contrast sensitivity was higher compared with the fellow (normal) eye (Figure 2B, paired two-tailed t-test: $t(7) = -2.27$, $p = 0.018$, $t(7) = -4.88$, $p = 0.004$ for an SF of 6, 12 cpd, respectively).

Binocular summation was also found for both amblyopic and normally sighted subjects in the backward masking task, as is evident with significantly higher thresholds found for the binocular compared with the monocular condition (Figure S2). In amblyopes, the BS ratio was increased only for the longer ISI, compared with the normal (Figure S2).

Thus, the results show that in contrast with the "pure" temporal function paradigm task (CFF), information integration leading to BS was found in amblyopic subjects in the spatial domain, though it is slightly lower compared with normally sighted subjects. Furthermore, the spatiotemporal backward masking paradigm

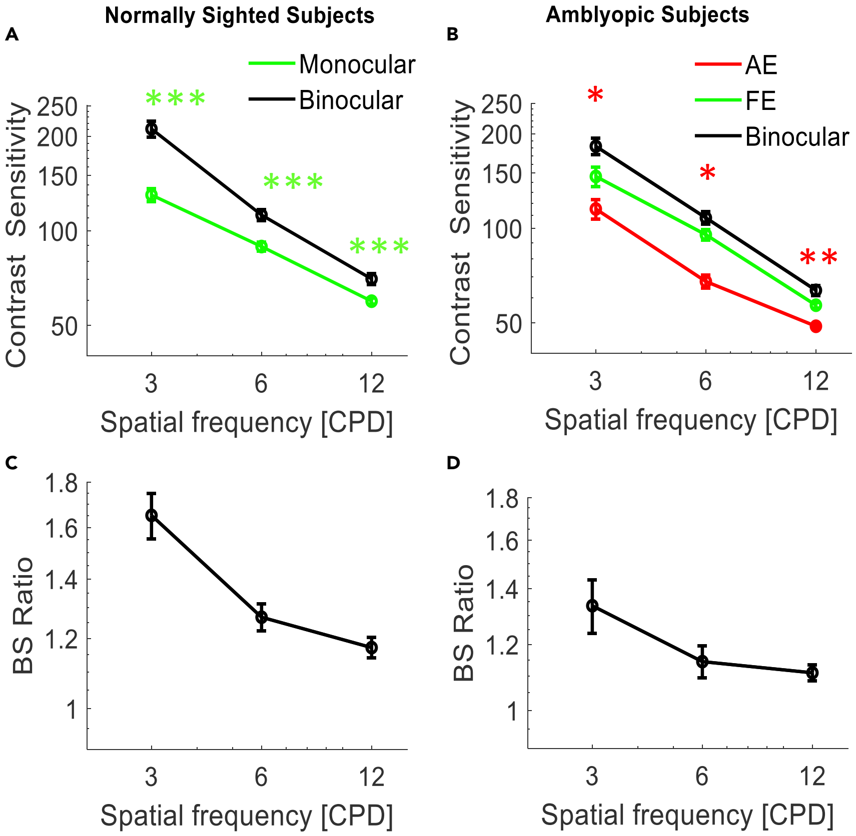

Figure 2. Contrast sensitivity as a function of SF

(A) CS tests in normally sighted subjects. Black dots represent values under binocular conditions and blue dots represent values under monocular conditions. Monocular tests showed lower contrast sensitivity than binocular tests. ***p<=0.001 (mean difference of 22.3%).

(B) CS tests in amblyopic subjects. The black dots represent values under binocular conditions, the green dots represent values for FE, and the red dots represent values for AE. The amblyopic eye showed a significant decrease in contrast sensitivity compared with the normal eye: a mean difference of 31.3% from binocular and 19.17% from the normal eye.

(C) BS Ratio in normally sighted = binocular values/monocular values.

(D) BS Ratio in Amblyopic subjects = binocular values/FE values.

Error bars refer to the SEM. Statistical significance was indicated:*p<=0.05**p≪0.01 ***p≪0.001.

demonstrated the dependence of BS on the temporal aspects of the stimuli; amblyopic subjects showed BS only at longer ISIs.

Synchronizing and balancing binocular input elicited a significant temporal binocular summation in amblyopic subjects

We hypothesized that the lack of inter-ocular information integration in the temporal domain (Figures 1B and 1D), in contrast with the integration found in the spatial domain (Figures 2B and 2D), is caused by a lack of information synchronization during binocular cortical processing. We also hypothesized that binocular imbalance further contributes to the lack of integration; it is caused by inter-ocular differences in contrast gain, as the amblyopic eye is "weaker" than the normal eye. This loss of balance can dramatically decrease the binocular summation, as is modeled and reported by the gain control and other theories.[13,36,55–57]

In an attempt to understand the importance of inter-ocular synchronization in the integration of rapidly changing or flickering stimuli arriving from the two eyes, we first studied how interrupting synchronization in normally sighted subjects hampers performance and then studied whether correcting deficient integration in amblyopic subjects improves performance. To this end, we measured the binocular combination in the CFF paradigm in both amblyopes and normally sighted subjects while introducing a phase shift of various magnitudes (0.1π or 0.5π). In a second set of experiments, we adjusted the imbalance by increasing the luminance provided to the amblyopic eye.

Effect of de-synchronizing and synchronizing ocular input

CFF tests were performed on 13 amblyopic and 15 normally sighted subjects at five different luminance levels under monocular and binocular viewing conditions, similar to the experiments described above. A phase shift (of $0.1\ \pi$ or $0.5\ \pi$) was presented to the amblyopic eye in amblyopic subjects and to the non-dominant eye in normally sighted subjects.

In normally sighted subjects (Figures 3A and 3C), interrupting the normative integration of the information by adding a phase of 0.5π significantly affected the binocular summation and reduced the binocular CFF by an average of 2.2 Hz (mean ± STD 27.56 Hz ± 3.8 vs. 25.39 Hz ± 4.6, paired two-tailed t-test: $t(14) = -4.029$, $p = 0.0012$), to levels that were now not different from the monocular CFF (paired two-tailed t-test: $t(14) = 0.6$, $p = 0.56$), thus further suggesting the disruption of summation. The addition of a smaller phase of 0.1π caused a non-statistically significant smaller change in the CFF (paired two-tailed t-test: $t(14) = -1.6$, $p = 0.12$); the CFF in this condition was also not statistically different, compared with the monocular condition.

In contrast to normally sighted subjects, synchronizing the binocular visual input in amblyopic subjects by introducing a phase advance to the flickering stimuli elicited a significant binocular summation at low luminance stimuli, which was evident from the higher binocular CFF, compared with the CFF measured in the fellow eye and compared with the CFF measured binocularly with no phase advance (Figure 3B). This effect was statistically significant for 2.5 cd/m^2 (mean ± STD, 27Hz ± 0.67 vs. 22.33Hz ± 1.16, paired two-tailed t-test, between introducing a phase advance and CFF measured binocularly with no phase advance, $t(12) = 4.66$, $p = 0.006$), and 5 cd/m^2 (mean ± STD 27.35Hz ± 0.6 vs. 24.83Hz ± 0.98, $t(12) = 2.9$, $p = 0.0125$). The calculated BS ratio (Figure 3D) was highest for a phase advance of 0.1π (equivalent to ~2ms at 23Hz) at 2.5 cd/m^2, reaching an average value of 1.21, and decreased when the stimuli luminance increased. Importantly, when synchronization was induced, the binocular CFF in amblyopic subjects reached the level of the binocular CFF in normally sighted subjects for 2.5 cd/m^2 and 5 cd/m^2 (ANOVA analysis $p = 0.204$, $F(1) = 1.94$). (Figure S3).

A control condition experiment, where amblyopic subjects were introduced with an opposite phase (the fellow eye was introduced with an earlier stimulus, compared with the amblyopic eye), showed no significant increase in the binocular CFF above the fellow (normal) eye condition (Figure S4).

Balancing ocular input in the gain domain

Since the CFF increases with increasing luminance, we further hypothesized that increasing the stimulus luminance presented to the amblyopic eye would also balance the stimuli provided to the two eyes and would enable the binocular summation, similar to the effect of introducing a phase shift. To this end, CFF tests were performed on amblyopic subjects under a binocular viewing condition, where the luminance to the amblyopic eye was increased by a factor of 4 (Figure 4A). Indeed, introducing an inter-ocular luminance difference elicited a significant binocular summation, which was manifested by an increase in the binocular CFF (FE in 2.5 cd/m^2 and AE in 10 cd/m^2), compared with the binocular CFF, with no luminance difference (27.19Hz vs. 22.33 Hz, paired two-tailed t-test: $t(12) = 6.25$, $p \ll 0.001$) or compared to the amblyopic eye monocularly measured at the increased luminance (27.19 Hz vs. 22.27 Hz, paired two-tailed t-test: $t(12) = 7.6$, $p < 0.001$). Similar results were found for FE in 5 cd/m^2 and AE in 20 cd/m^2, compared to the amblyopic eye monocularly measured at the increased luminance (30.33Hz vs. 24.03 Hz, paired two-tailed t-test: $t(12) = 6.8$, $p \ll 0.001$). The binocular CFF measured under these conditions in amblyopic subjects was similar to that of normally sighted subjects (Figure 4A, two-sample t-test: $p = 0.29$ $p = 0.32$). The calculated BS ratios in these experiments were 1.204 and 1.16 for FE at 2.5 cd/m^2 + AE at 10 cd/m^2 and FE at 5 cd/m^2 + AE at 20 cd/m^2 (Figure 4B).

Synchronizing inter-ocular input enhanced the binocular function in a flickering stereopsis paradigm in amblyopic subjects

Following the demonstration of the effect of inter-ocular synchronization on the binocular summation in the CFF task, we further hypothesized that the synchronization of the binocular input, thus enabling the integration of information arriving from the two eyes, can significantly enhance binocular function in more

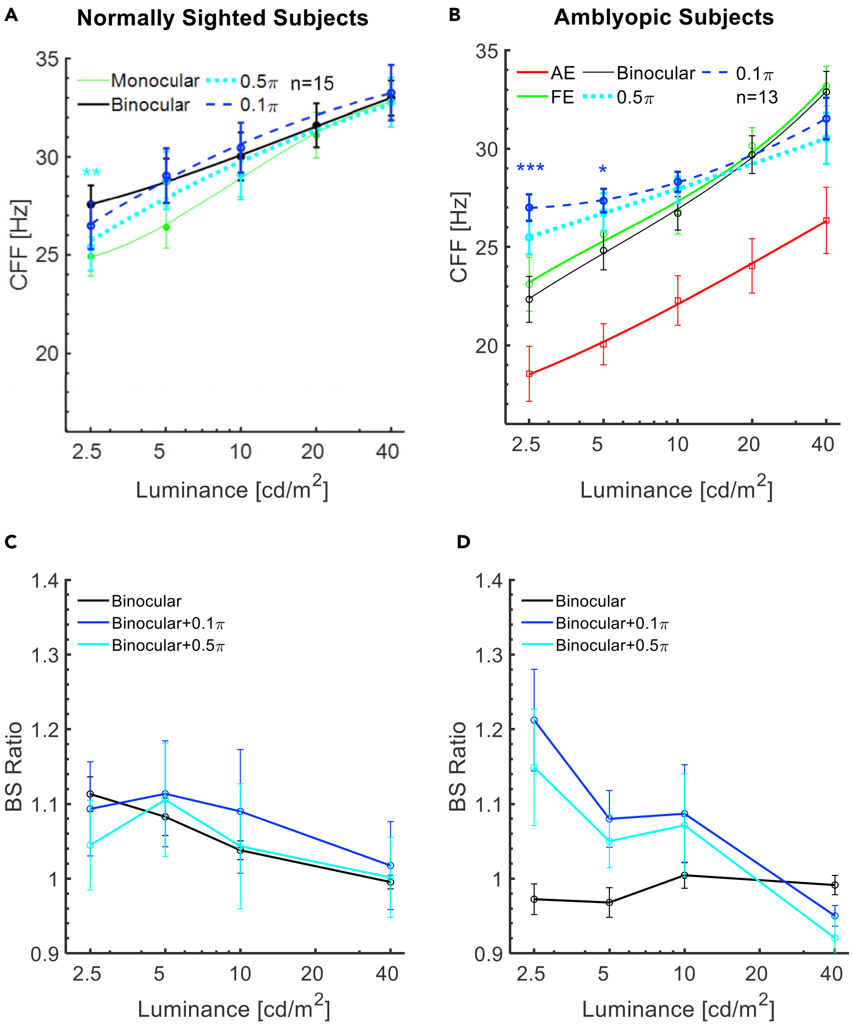

Figure 3. The effect of Inter-ocular phase delays on binocular summation and CFF

(A) CFF as a function of the luminance level for various phase values in normally sighted subjects. The black line represents values under binocular conditions and the green line represents values under monocular conditions. The blue line represents 0.1π phase and the cyan line represents 0.5π phase in binocular vision.

(B) CFF values as a function of the luminance level in amblyopic subjects at intraocular delays of 0.1π phase and 0.5π phase. The black line represents binocular values, the green line represents values for FE, and the red line represents values for AE. The blue line represents 0.1π phase and the cyan line represents 0.5π phase in binocular vision. CFF values significantly increased with the presentation of phase to AE- 4.67Hz ($p = 0.0006$) and 2.61HZ ($p = 0.012$).

(C) Binocular summation as a function of the luminance level for normally sighted subjects. The solid black line represents binocular values as measured with no intraocular phase, the solid cyan line represents binocular values as measured with an intraocular phase of 0.5π, and the solid blue line represents binocular values as measured with an intraocular phase of 0.1π. BS Ratio = binocular values/monocular values.

(D) Binocular summation as a function of the luminance level for amblyopic subjects. The solid black line represents binocular values as measured, the solid cyan line represents binocular values as measured with an intraocular phase of 0.5π, and the solid blue line represents binocular values as measured with an intraocular phase of 0.1π. BS Ratio = binocular values/FE values.

Error bars refer to the SEM. Statistical significance was indicated as:*$p<=0.05$**$p\ll0.01$ ***$p\ll0.001$.

complex tasks. We opted to test stereopsis, an important daily life function,[58–61] which is highly dependent on the proper function of each eye separately and on the proper integration of the information at the spatiotemporal domains.[62] To test the temporal aspects of stereopsis, we developed a flickering stereopsis test, where flickering stereo images are introduced to the eyes. The stereo threshold (disparity angle) is

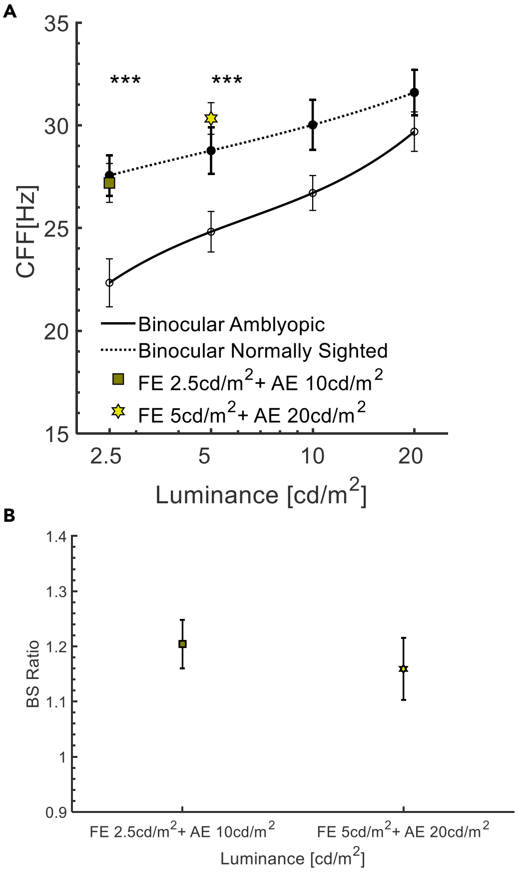

Figure 4. The effect of Inter-ocular luminance difference on CFF values

(A) The effect of the inter-ocular luminance level difference on CFF values for amblyopic subjects. The solid black line represents binocular values as measured in normally sighted subjects, the black dashed lines represent binocular values as measured in amblyopic subjects.

(B) Binocular summation improvement. The dark yellow square represents binocular values as measured in amblyopic subjects where the FE stimulus was at 2.5 cd/m^2 and the AE stimuli were at 10 cd/m^2. The bright yellow star represents binocular values as measured in amblyopic subjects where the FE stimulus was at 5 cd/m^2 and the AE stimulus was at 20 cd/m^2.

Error bars refer to the SEM. Statistical significance was indicated: ***p≪0.001.

evaluated using a staircase under the 2TAFC paradigm (see Figure 8 and the STAR Methods); lower thresholds suggest a better stereopsis function. We hypothesized that in the amblyopic subjects, where there is an inter-ocular asynchrony, introducing an earlier image to the amblyopic eye would enhance the stereo function under a flickering condition. The test was performed on 6 amblyopic and 10 normally sighted subjects.

Indeed, synchronization of the inter-ocular input by presenting the amblyopic eye with an earlier image (Δt = 4ms) significantly improved the stereopsis function, as manifested by a significantly lower disparity threshold (Figures 5C and 5D, 557″ vs. 385″, paired two-tailed t-test: t(9) = -2.5, p = 0.0002). Further increasing the timing difference (16, 66ms) caused the stereopsis function to deteriorate.

In line with our hypothesis and in contrast with amblyopic subjects, interrupting information integration by the desynchronization of the stereo images in normally sighted subjects significantly deteriorated stereopsis (Figures 5A and 5C); all asynchrony times showed higher thresholds (147″ vs. 212″, 226″, 661″ for a phase delay of Δt = 4, 16, and 66ms, respectively); the difference was in a dose-response manner (Mann Kendall *p* for trend ≪0.001). It should be mentioned that as expected,[63,64] for both amblyopes and normally sighted subjects the flickering stereopsis threshold (with no phase shift) was significantly lower compared with the

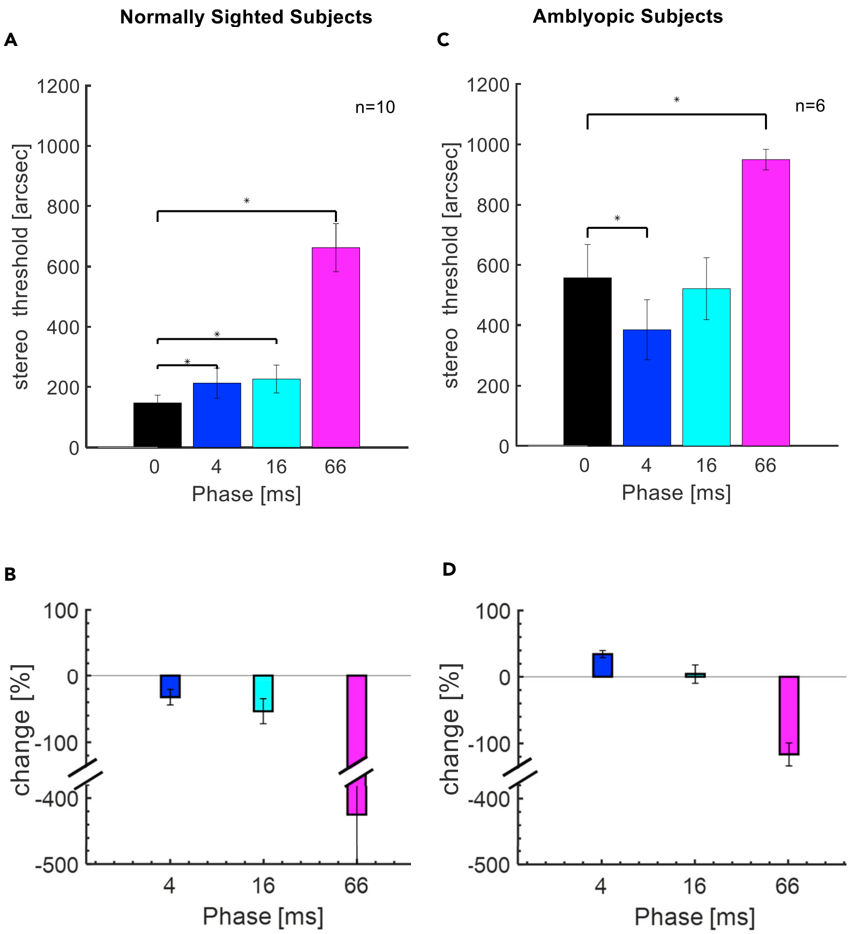

Figure 5. The effect of phase on temporal stereopsis

(A) The effect of phase on stereopsis values for normally sighted subjects. Introducing a phase shift between the eyes significantly increased the threshold of stereopsis (p = 0.035), where a large time difference of 66 ms between eyes almost completely abolished binocular vision and the ability to perceive stereopsis ($p \ll 0.001$).

(B) The temporal stereopsis change from static stereopsis for normally sighted subjects.

(C) The effect of phase on stereopsis values for amblyopic subjects. Introducing a phase shift between the eyes with the AE being presented with the earlier stimuli at a small-time shift (4msec) resulted in a significantly improved threshold ($p \ll 0.001$), indicating improved stereopsis.

(D) The temporal stereopsis change from static stereopsis for amblyopic subjects. The black bars represent binocular values in the flickering condition. The blue bars represent the 4msec phase, the cyan bars represent the 16msec phase, and the magenta bars represent the 66 msec phase in binocular vision.

Error bars refer to the SEM. Statistical significance was indicated:*p<=0.05.

static presentation of the stimuli (data not shown, 73″vs. 147″ in normally sighted subjects, 301″ vs. 557″ in amblyopes).

Electrophysiological evidence for the synchronization-induced enhancement of binocular function

Following the psychophysical investigation of ocular synchronization and binocular summation, we proceeded with an electrophysiological evaluation using visual evoked potential (VEP). Steady-state VEP was recorded in 9 amblyopic and 7 normally sighted subjects in response to flicker stimuli of 15 Hz at two different luminance levels (5 and 40 cd/m^2) under both monocular and binocular viewing conditions. We evaluated the effect of introducing inter-ocular phase shifts of various durations on the recorded VEP amplitude.

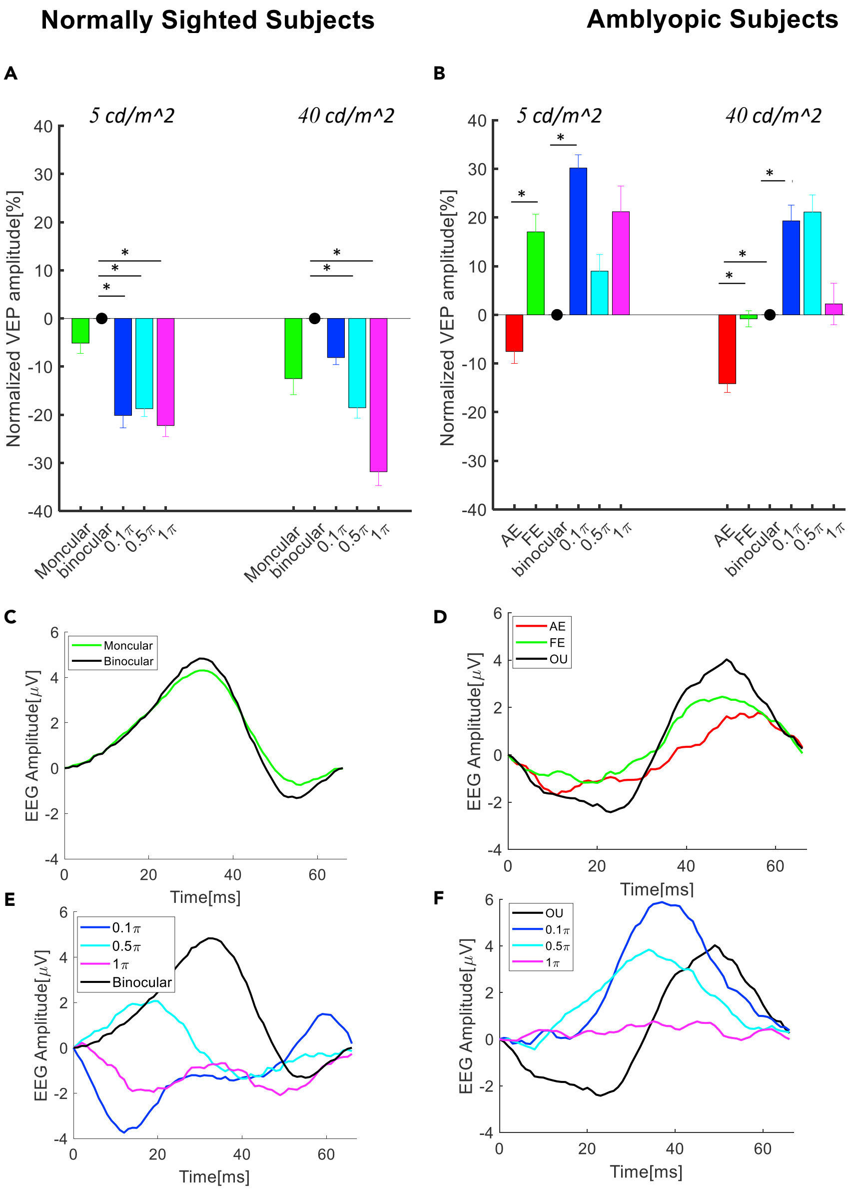

Figure 6. The effect of inter-ocular phase delays on temporal VEP amplitude

(A) Normalized VEP amplitude for normally sighted subjects. All results are normalized to the results of binocular amplitude; the result was subtracted from 1 to calculate the difference.

(B) Normalized VEP amplitude for amblyopic subjects. All results are normalized to the results of binocular amplitude and the result was subtracted from 1 to calculate the difference.

(C) Representative VEP signals of normally sighted subjects; the black line represents waves under binocular viewing conditions; the green line represents waves under monocular viewing conditions.

(D) Representative VEP signals of amblyopic subjects. The black line represents typical waves under binocular viewing conditions, the green line represents typical waves under FE viewing conditions, and the red line represents typical waves under AE viewing conditions.

(E) Representative VEP signals of normally sighted subjects with the addition of the intraocular phase. The black line represents binocular values, the blue line represents the 0.1π phase, the cyan line represents 0.5π phase, and the magenta line represents 1π phase in binocular vision.

(F) Representative VEP signals of amblyopic subjects with the addition of the intraocular phase. The black line represents binocular values, the blue line represents 0.1π phase, the cyan line represents 0.5π phase, and the magenta line represents 1π phase under binocular viewing conditions.

Error bars refer to the SEM. Statistical significance was indicated: $^{*}p<=0.05$.

For normally sighted subjects, desynchronization of the inter-ocular input by introducing a 0.1π phase shift decreased the VEP amplitude by up to 20.2% for 5 cd/m^2 and 8.11% for 40 cd/m^2 (Figure 6A), compared with no phase shift; the difference was statistically significant only for the 5 cd/m^2 (paired two-tailed t-test: t(5) = 3.3, p = 0.02; t(5) = 2.46, p = 0.057, for 5 cd/m^2 and 40 cd/m^2, respectively), in agreement with both the CFF and the flickering stereopsis paradigms. A larger phase (0.5π and 1π) resulted in a statistically significant decrease in VEP amplitude at both luminance levels; the decrease in VEP amplitude for 40 cd/m^2 showed a dose-response. In contrast, in amblyopic subjects, synchronizing the inter-ocular input by adding a 0.1π phase shift (earlier stimuli to the amblyopic eye by ∼3.3ms at 15Hz), significantly increased the binocular VEP amplitude, compared with binocular viewing with no phase shift, up to an increase of 28.2% (Figure 6B) for both luminance levels (paired two-tailed t-test: t(8) = -3.68, p = 0.008, 5 cd/m^2; t(8) = -2.6, p = 0.03, 40 cd/m^2). Further increasing the phase up to 1π gradually reduced the amplitude to the baseline binocular levels. These results are in agreement with our results obtained in the psychophysical tests for both the CFF and flickering stereo paradigm, further exploring the effect of inter-ocular synchronization on binocular temporal performance in amblyopic subjects.

Characteristic averaged VEP responses of one subject to the various stimuli conditions are shown in Figures 6C-6F. In contrast to the perfectly aligned responses to monocular and binocular stimuli in normally sighted subjects (Figure 6C), the response of the amblyopic eye is delayed, compared with the fellow eye or the binocular response. Synchronizing the ocular input in amblyopic subjects by a phase shift of 0.1π elicited a significantly larger and earlier response: the larger phase shift caused a significantly reduced or abolished response (Figure 6F).

The inter-ocular synchronization between the eyes was further quantified using the cross-correlation analysis (see the STAR Methods). The inter-ocular synchronization in normally sighted subjects was high, as is suggested by the short inter-ocular phase of 1.5ms (Figure 7A). In contrast, the inter-ocular phase in amblyopic subjects was, on average, 6ms, significantly longer compared with the normally sighted subjects (Figure 7B) (two-sample t-test: t(11) = 6.07, p<<0.001, for both luminance levels), suggesting significantly lower synchronization in amblyopic subjects.

We further evaluated the similarity between recorded VEP in the various stimulus conditions (monocular, binocular, and binocular with an added phase) to a 15Hz sinusoid, in both normal and amblyopes by calculating a correlation between these two waves. This analysis provides a simple measure of how much the VEP response is similar to a sinusoid. In normally sighted subjects (Figure 7C), the correlation increased in a binocular condition, compared with a monocular condition (paired two-tailed t-test: t(5) = -3.15, p = 0.025, at 40 cd/m^2), and significantly degraded when a phase shift was added to the stimulus presented to one of the eyes (Mann-Kendall p for trend = 0.0037). In contrast, increasing the inter-ocular synchronization in the amblyopic subjects (Figure 7D) by adding a phase shift caused an increase in the correlation to sinusoid (Mann-Kendall test p for trend = 0.027). These results suggest that inter-ocular synchronization is evident with more synchronized and coherent VEP signals.

DISCUSSION

Integration of multisensory information or sensory information arriving from two body sides (e.g. vision, hearing) is important for normal development and sensory function.[55] A unique example is the ability of the auditory system to detect microsecond's order of the asynchronic information arising from the two cochleae for resolving sound source localization.[56] In contrast, in the visual system, the binocular information synchronization is important for normal perception and stereopsis, whereas delayed binocular information is thought[57,65] to elicit the so-called Pulfrich Phenomenon. The Pulfrich phenomenon is a neuro-ophthalmic miss-perception in which two-dimensional moving objects are perceived as three-dimensional. This phenomenon is often observed in neurological diseases that affect the eyes non-symmetrically[66] or it can be experimentally induced by a monocular optical filter in normally sighted subjects.[67–69]

Studying the visual temporal function in amblyopic subjects can serve as a unique model where temporally imbalanced input is provided by two sensing organs sides, since the information arriving from the amblyopic eye is both attenuated and delayed,[34,43] compared with the information arriving from the fellow eye. Indeed, a spontaneous Pulfrich phenomenon can also be experienced in some amblyopic subjects,[70,71] probably because of a delayed processing time or an increase in retino-cortical transmission. An important aspect of the binocular integration of

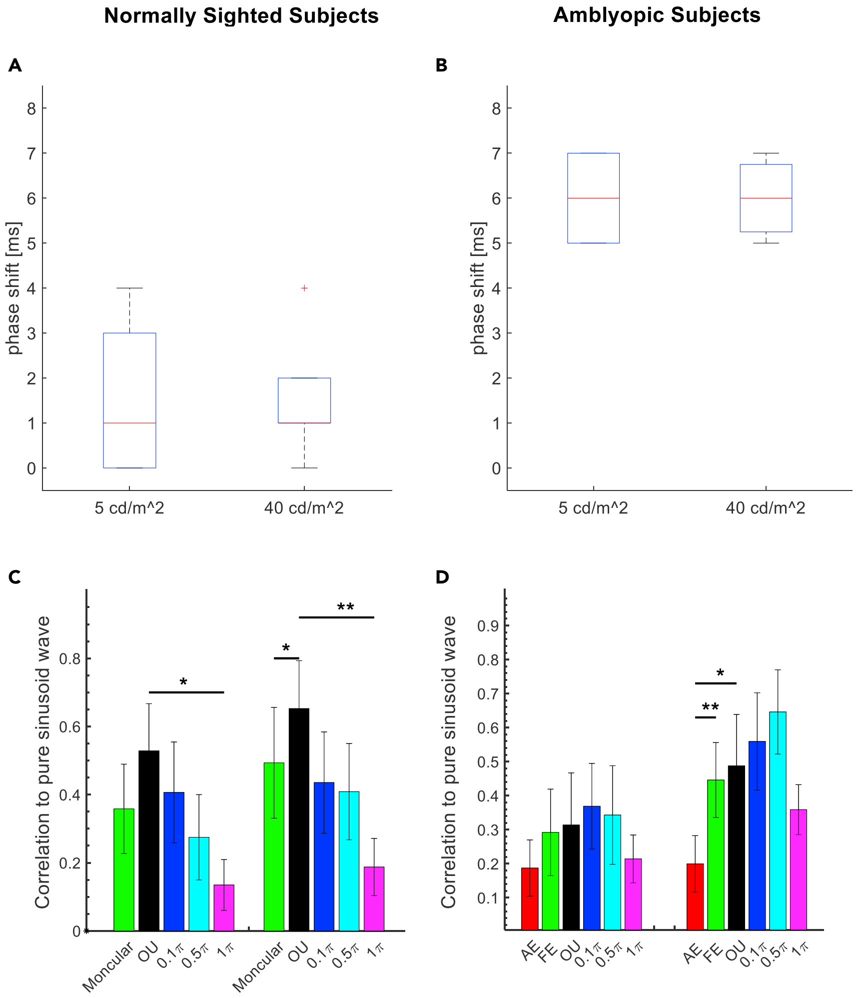

Figure 7. Phase shift evaluation by the cross-correlation of VEP recordings

Temporal delays between eyes were estimated by the peak of the cross-correlation of the VEP signals arising from each eye.

(A) Normally sighted. (B) Amblyopic subjects. The cross-correlation peak of VEP signals for 5 and 40 cd/m^2 stimuli presented at 15 Hz for 9 amblyopic subjects (8 females 1 male) and 7 normally sighted subjects under monocular viewing conditions. (C) Correlation with a sinusoid wave for normally sighted subjects.

(D) Correlation with a sinusoid wave of amblyopic subjects.

Error bars refer to the SEM. Statistical significance was indicated: *$p<=0.05$, **$p \ll 0.01$.

information is the binocular summation (BS), which is an increase in binocular performance, compared with the monocular condition. Numerous reports showed that the BS in the spatial domain is significantly reduced by a monocular imbalance.[15,72] In the case of amblyopic subjects, where a large monocular imbalance is evident, BS is indeed usually reduced[35,47,73–75]; however, it can be regained by adjusting the contrast of the amblyopic eye.[39,41] A possible model showing the effect of ocular imbalance on the BS was suggested by Pardhan[47] and Ding,[13,38,76–78] with maximum inter-ocular summation and minimum inter-ocular inhibition occurring when the two eyes have equal contrast sensitivities and the same spatial phase. Others suggested[39] that the reduced function of the amblyopic eye itself is a direct reason for the lower BS. Giving an advantage to the amblyopic eye can balance the information, therefore reducing the inhibition exerted on the amblyopic eye and increasing the binocular summation.[41,79]

Although the temporal component of visual input was not included in the models described above, we found that the same principles of ocular balance apply to visual processing in the temporal domain. Here we studied inter-ocular temporal balance in both normally sighted and amblyopic subjects, where the information arriving from the fellow eye is both of a higher energy level and is also processed earlier in the visual cortex; thus, here the binocular balance is further reduced; therefore, maximal inhibition is exerted on the amblyopic eye, leading to poor or a missing BS.

In normally sighted subjects, we found that the disruption of temporal information integration by desynchronization, obtained by the addition of a phase delay, canceled the BS, which was readily evident when the eyes were synchronized (with no phase shift). It should be mentioned, that in contrast with the significant desynchronization-related loss of function in the flickering stereo task (see later in discussion), the loss of function in the CFF task was modest and was found mainly with a large phase shift (0.5π). These results suggest preserved temporal compensatory and adaptation processes in the normally sighted visual system, in agreement with Richard et al.[80]

In contrast with the normally sighted subjects, we found that in amblyopes, where the information is inherently not balanced and synchronized, there was no evident BS in the temporal domain. We then found that synchronizing the inter-ocular information by a phase shift, while keeping the stimulus energy intact, had a significant effect on binocular summation in both the CFF and the flickering stereo tasks. It is worth noting that synchronizing binocular input for increasing binocular CFF was only effective at low stimulus luminance levels. One possible explanation is the fact that latency is increased in response to low luminance stimuli,[81] leading to greater inter-ocular time differences; therefore, the effect of inter-ocular synchronization is increased. Another potential explanation is the fact that we used a fixed phase shift that implies different relative delays at different luminance levels.

The reported magnitude of BS found for contrast tasks varies for different studies (see the meta-analysis by Baker[15]), probably because of different stimulus parameters and potential inter-ocular performance differences and interaction between these two components. In this review, the magnitude of binocular summation was, on average, between 1.4 and 2, in agreement with our results in normally sighted subjects for the low spatial frequencies contrast stimuli. In the same task, the BS found for amblyopes was lower, compared with normally sighted subjects, in alignment with the notion that balanced input from the eyes is important for BS.[15,76,82] In both normally sighted subjects and amblyopes, the BS found in our subjects significantly decreased with higher SFs, consistent with the trend found by Baker.[15] This could arise from the fact that in higher SFs, the imbalance between the eyes is increased (see also Figure S1 in the Supp. Results).

The magnitude of the BS in the temporal domain (the CFF task) in normally sighted subjects was 1.1, significantly lower than with the contrast task. Our results are in agreement with previous studies, which showed that higher temporal frequencies[83] or the so-called stimuli-speed[15] were associated with lower BS. This can also be supported by our results showing the significant dependence of the BS on the inter-stimuli interval (ISI) found in the backward masking test, where BS was highest in longer ISIs, and decreased significantly with shorter ISIs. Moreover, in amblyopic subjects, BS was evident in this task only at an ISI of 120ms and longer, further suggesting the importance of long integration times on BS and the differences between normal and amblyopes.

In addition to synchronizing the binocular input by a temporal shift, we showed that binocular summation up to the level of normally sighted subjects could be elicited in amblyopic subjects by increasing the stimulus luminance level presented to the AE (Figures 4A and 4B), thus balancing the binocular input; these results are in line with previous reports on binocular spatial vision.[76,84]

We further found that the desynchronization of the binocular information in normally sighted subjects elicited a significant decrease in the flicker stereo function. Importantly, synchronizing the binocular information in amblyopes by introducing a phase shift (an earlier image by 4ms) to the amblyopic eye elicited a significant improvement in the flicker stereopsis task; the effect deteriorated when the shift was increased to 16ms and above, probably as it exceeded the inter-ocular time delay. These results further stress the importance of inter-ocular synchronization in the process of binocular information integration.

It should be mentioned that stereopsis is a unique visual function requiring the use of both eyes and is dependent upon spatial functions, normal oculomotor control, and the development of binocular brain mechanisms.[85] Thus, measuring the stereo acuity is considered the gold standard test for studying binocular visual functions.[86] The detectable rate of change in disparity information (important for stereopsis processing) is much slower

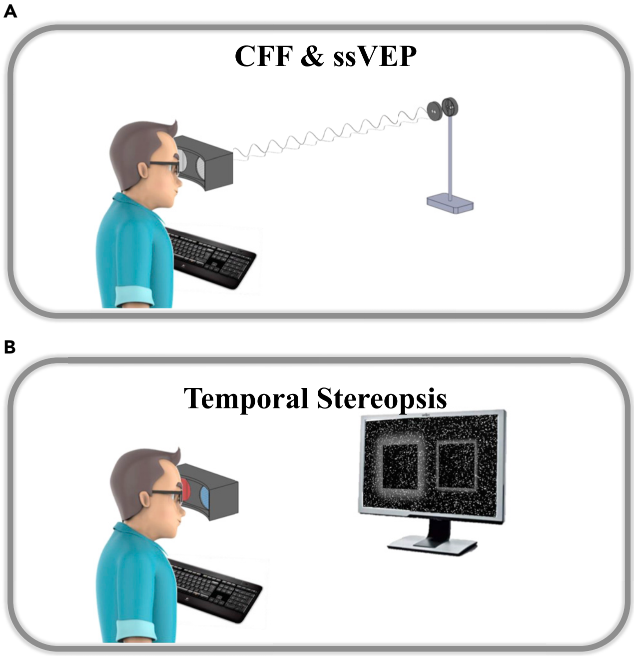

Figure 8. Experimental setup

(A) Illustration of the Dichoptic CFF measurement system.

(B) Illustration of the temporal stereopsis investigation system.

compared with luminance information[87–89]; therefore, stereopsis significantly deteriorates under a flickering condition,[64] which was also shown in our (both normally sighted and amblyopic) subjects. Furthermore, we suggest that the dependency on precise timing further reduces the flickering stereopsis in amblyopic subjects, since the amblyopic eye input is significantly delayed compared with normal eye input.

Hubel and Wiesel laid the foundations for a better understanding of the physiological basis of binocularity[90]; visual striate cortex (V1) recordings in cats showed cells that responded to stimuli presented to both retinas. Single cortical cells in the striate cortex show various degrees of binocular interactions such as summation,[90,91] facilitation, and inhibition.[92,93] More recent work that recorded VEP signals in normally sighted subjects, in response to contrast stimuli, showed binocular interactions starting from V1 with a good fit to gain control models.[94] In line with this, VEP studies have shown that amblyopic subjects' VEP signals not only exhibit low amplitudes and low signal-to-noise ratios—they also exhibit longer latencies and phase-misalignment, compared with normally sighted subjects.[43,95] We, therefore, wanted to investigate the effect of binocular information synchronization on the VEP signals in both normally sighted subjects and amblyopes. Our results showed that de-synchronizing the ocular information in normally sighted subjects elicited a significant decrease in VEP amplitude. In contrast, synchronizing the binocular information in amblyopes by introducing a phase shift (an earlier stimulus to the amblyopic eye) elicited a significant increase in VEP amplitude. Both results are in strong agreement with the psychophysical measurement of binocular CFF and flickering stereopsis.

In the current study, we also presented a novel analysis method for evaluating the inter-ocular asynchrony by calculating the cross-correlation of the VEP recorded in response to stimuli presented to each eye separately. Our results (average (SD) of 6ms (1ms)) are comparable to those of other studies reporting a few milliseconds delay between AE and FE, varying from 0.5 to 20 ms (under different stimulus conditions and different types of amblyopia).[43,95–97]

Our findings, showing a significant increase in binocular CFF and in the flickering stereopsis task (which by definition requires binocularity, and is highly dependent on the inter-ocular synchronization of the information) support the hypothesis suggested by Baker[41] that the neural circuits responsible for the binocular functions function in patients with amblyopic, and can be activated by synchronizing the binocular information.

Our results might have implications for amblyopia training-based treatments, since balancing the binocular input during treatment can significantly enhance improvement, which was shown in the spatial or luminance domain.[98,99] These studies have shown that balancing the visual input provided to the two eyes during training can improve both the vision in the AE and the binocular function. We suggest that in addition to balancing the spatial and luminance information, temporal synchronization of the eyes during treatment can further enhance the learning effect and increase the resulting binocular function.

In conclusion, we presented here evidence that balancing the binocular input can elicit binocular temporal performance up to the level of normally sighted subjects. These results were further supported by a significant increase in a flickering stereopsis task and electrophysiological VEP recordings in normally sighted and amblyopic subjects. Further research will explore the implications of these findings for developing an effective vision training tool based on temporal characteristics.

Limitations of the study

This study had a few limitations. First, due to the small number of subjects, we did not divide the results into amblyopia sub-groups (e.g., strabismic and anisometropic), which might have different effects on binocular vision. Second, in the ssVEP experiments we used a frequency of 15Hz rather than the threshold frequency (i.e., CFF) because the VEP amplitude was small (probably because of the target stimulus size). Future studies should try to address the effect of synchronization on the VEP amplitude at the fusion frequency. Finally, the screen refresh rate used in the flickering stereopsis experiment limited the phase shift to multiples of 4ms. Future studies using higher refresh rates should measure the optimal phase shift more accurately.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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- RESOURCE AVAILABILITY
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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2023.105960.

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AUTHOR CONTRIBUTIONS

A.E.E., N.F., U.P., and Y.M. designed the study, as well as wrote and edited the article. A.E.E. conducted the experiment as well as collected and analyzed the data. All authors reviewed the article. Y.M. supervised the study and provided funding.

DECLARATION OF INTERESTS

The authors declare no conflict of interest.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RSOURCE	SOURCE	IDENTIFIER
Software and algorithms		
Matlab 2018b	Mathworks	www.mathworks.com
Other		
laptop-based CFF measuring system	Eisen-Enosh et al.[45]	https://doi.org/10.1016/j.exer.2020.108290

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Yossi Mandel (yossi.mandel@biu.ac.il).

Materials availability

This study did not generate new unique reagents or other materials.

Data and code availability

- All data reported in this paper will be shared by the lead contact upon request.
- This paper did not report the original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Participants

The CFF measurements were performed on a total of 28 subjects: 15 normally sighted subjects (8 females, 7 males aged 26.3 ± 2.4 years old, mean ± STD) with no known neurological conditions and with normal corrected vision, and on 13 amblyopic subjects (11 females, 2 males, aged 30.4 ± 11 years old, mean ± STD).

All subjects underwent a comprehensive eye examination by a qualified optometrist (A.E.E.). Subjects were refracted by dry retinoscopy and tested using a binocular 'Randot stereo' test, a cover-test, and underwent a general eye examination including fundus ophthalmoscopy and a slit lamp examination of the anterior segment. The criteria for inclusion were visual acuity (ETDRS chart) better than 0.1 LogMar with a difference of less than 0.2 LogMar between eyes, stereopsis better than 40″, and no ocular or neurological diseases. The mean stereopsis of the subject groups was better than 40″, and the mean visual acuity (LogMar) was as follows: far monocular: −0.04, far binocular: −0.07, near monocular: −0.01, near binocular: 0. For amblyopic subjects, the criteria for inclusion were a difference between the eyes of at least 0.2 LogMar and an indication of the presence of strabismus or a difference in the refractive error.

The details of the visual parameters of the amblyopic subjects appear in Table S1 in the supplemental information. Temporal Stereopsis measurements were performed on 10 healthy participants (6 females, 4 males aged 28.05 ± 8.1 years old, mean ± STD) with no known neurological conditions and with normal corrected vision, and on 6 amblyopic subjects (5 females, 1 male, aged 23.85 ± 3 years old, mean ± STD). ssVEP measurements (see below) were performed on 7 healthy participants (3 females, 4 males aged 29.9 ± 4.2 years old, mean ± STD) with no known neurological conditions and with normal corrected vision, and on 9 amblyopic subjects (8 females, 1 male, aged 30.6 ± 11.3 years old, mean ± STD).

The objectives of the experiment and its implementation were detailed and clearly explained to all subjects, and they all filled out a medical questionnaire. All participants signed an informed consent form, and the study was approved and conducted according to the IRB Committee by the Bar-Ilan University Ethics Committee.

METHOD DETAILS

CFF measurements

Dichoptic system design considerations

The experiments were performed using our previously described[45,46] method and device for dichoptic measurement of CFF. In short, the subject is seated behind Linear Polarizing glasses mounted on a chin rest (Figure 8A). Both the glasses and the LED filter are orthogonally polarized so that each eye is stimulated by only one LED. In order to create binocular testing conditions, with characteristics similar to the monocular examination, we designed the system apparatus so that the two LEDs were perceived as a single LED.

The experiment begins with a dark adaption period of 3 min (a mean room luminance of 0.001 cd/m^2), which was found to be enough for CFF testing using our approach.[46]

Experimental paradigm

During the adaptation time, the experimental procedure was explained to the subject. Experiments were designed to evaluate the CFF threshold through a psychophysical test, based on a discrimination task using the two temporal alternative forced-choice paradigm (2TAFC) with a stimulus duration of 1 s, similar to our previous publications.[45,46] The target stimulus temporal features were modulated using the staircase method, as described below. Subjects were requested to discriminate between a target stimulus (a flickering light at various frequencies) and a flickering light at a frequency of 120Hz, which is significantly higher than the CFF in humans and is therefore perceived as continuous light. The staircase method was implemented by modifying the stimulus frequency using an adaptive 3:1 method according to the participant's response[46,100] (with fixed-step sizes of 2 Hz) of the 3-up-1-down staircase (3:1).[101] The test was finalized upon the completion of 8 reversals (a change in the direction of the stimulus frequency); the CFF was defined as the mean of the last six reversal values. The CFF threshold calculation is in 79% correct.[101,102] The entire procedure was repeated twice to increase reliability and the CFF threshold was calculated as the mean of the two repetitions. The test can be performed in a combined manner so that during a specific trial both eyes are tested, the stimulus is randomly presented to either eye or to both eyes simultaneously, the phase difference is randomly set. The test duration is 20 min, and the results are saved for further analysis. The interface used to control the experimental paradigm and the psychophysical tests was developed using MATLAB.

Visual spatial tests

Advanced computerized tests to examine the spatial visual perception were tested. The tests were performed using a procedure described by our group.[51–54] The tests are based on a 'PSY – psychophysical tool' software including contrast sensitivity and backward masking (BM). Gabor patches at various spatial frequencies as well as contrast and duration times (as is detailed for each test) were used as the visual stimuli.[103] Stimuli are presented on an 'EIZO FORIS- FG2421 "Turbo 240"' computer screen at a 100 Hz refresh rate. The high-performance 'EIZO' screen was calibrated as follows: Brightness = 100, Contrast = 50, Resolution 1024 x 768. The screen was calibrated, and gamma corrected. The task was either to detect a Gabor with different contrast levels or to detect a Gabor target in the masking experiments.

Flicker stereopsis

The stimuli were presented on a Dell Alienware 25 Monitor AW2521HFL equipped with a GeForce GTX 1650 Graphics Card, NVIDIA. Visual stimuli were generated under the control of MATLAB with the Psychophysics Tool-box[104] in 64-bit Microsoft Windows. The experiment was conducted in a dark room, so that the screen provided the only measurable light input to the eyes. The screen was set to a spatial resolution of 1920 X 1080pixels with a refresh rate of 240 Hz, calibrated, and gamma corrected. At a 100 cm viewing distance, the screen provided ~64 pixels per degree of visual angle, and pixels subtended ~55'' of arc (arcsec). The stimulus consisted of two squares (3 × 3°) and was presented on a black screen with a white random dot background. Each square was composed of a combination of random red and blue dots. The subject wore glasses with red and blue filters so that the red dots were presented to the right eye and the blue dots to the left eye. Stereopsis depth was controlled by manipulating the disparity (between the red and the blue dots) and the subjects' task was to choose which square (left or right) protrudes in a 2AFC paradigm (Figure 8B). The stereopsis depth was modified in a staircase (3 up 1 down) paradigm in order to find the stereopsis threshold.

A validation test of precise stimulus presentation time was performed as described in the supplemental methods, Figure S5. We further excluded inter-ocular cross talk by a cross-talk test (see the supplemental methods Figure S6).

ssVEP

VEP recordings

VEP recordings were performed using the CURRY 8 X (Compumedics Neuroscan) system. Signals were amplified 4,000 times (SynAmps RT 64-channel Amplifier). Electrode impedances were kept below 10 kΩ and the sampling rate was 1.0 kHz, the recording time was 5s. Using a custom-written MATLAB program, we processed the information from the Oz (inion) central electrodes in the visual (the reference electrode was taken as Fz).

The stimulus consisted a 15 Hz flickering light presented for 5 s using our customized device.[45] The stimulus was randomly presented to each eye separately or to both eyes. In addition, an inter-ocular phase shift of 0.1π, 0.5π, or 1π was randomly introduced. The stimulus size was 0.47°.

VEP was segmented using the stimulus markers that specify the start of the flickering. The trend in the segmented data was removed and the data were filtered with a band-pass filter with cut-off frequencies of 0.1–100 Hz. The first second was deleted from the analysis because it is highly affected by the abrupt change from dark to light at the beginning of each trial. The next 4 s were averaged for a wave of 15 Hz.

Cross-correlation analysis

In the current study we used a unique method for the quantitative evaluation of the similarity between the eyes. Cross-correlation is a measurement of similarity between multiple time series and in particular, to find where they best match.[105] Here we used MATLAB to calculate cross-correlation between the right and left eye waves. We then estimated the inter-ocular delay by finding the time difference between the two waves that gives the highest correlation.

Correlation to a sinusoid wave

In the current study we also evaluated the match between the stimulus and the response. In order to evaluate the signal quality of the VEP signal, the cross-correlation function in MATLAB was performed between VEP signals and a 15 Hz sinusoid wave (the wave that constituted the stimulus itself).

QUANTIFICATION AND STATISTICAL ANALYSIS

A paired two-tailed t-test, as planned comparisons, was used to compare two conditions within the same subjects; the significance is reported as t and p values. A two-sample, two-tailed Welch t-test was used to compare the normally sighted and amblyopic subjects; the significance is reported as t and p values. When the p value was smaller than three zero digits, we reported it as $p \ll 0.001$.

Two-way ANOVA was used for assessing the main effects and the interactions. For comparisons with degrees of freedom higher than 1, the post hoc tests of a multiple comparison test were applied (for any ANOVA).

The two-tailed Mann-Kendall test was used with a null hypothesis of trend absence when there were results in multiple consecutive test values, against the alternative of trend in results, *p* for trend value was reported.

Analyses were performed using the MATLAB software (Mathworks, Waltham, MA) Statistics and Machine Learning Toolbox, with significance set at $p < 0.05$. A Quantile-Quantile plot of the data, which was plotted before the statistical analysis, showed a normal distribution of the data. The number of subjects tested (n) is indicated in the figures, and all data are reported as the mean ± SEM(SE).