Intact Perceptual Interactions of Interocular Temporal Phase and Contrast Disparities in Amblyopia

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Purpose. Interocular temporal and contrast differences have been applied separately to improve binocular integration and treat amblyopia. However, the extent to which binocular integration in amblyopia can be improved by combining interocular differences in timing and contrast has yet to be tested. We evaluated how these parameters interact in individuals with amblyopia and in normally sighted controls.

Methods. We developed an interocular flicker integration task in which a pair of dichoptic gratings flickered sinusoidally in counterphase (2 hertz [Hz], 90-degree spatial and temporal offset), producing the appearance of motion. We determined the interocular delay required for optimal integration by adding phase delays to the 90-degree phase offset (left or right eye leading, −80 degrees to +80 degrees). Delays were tested across different interocular contrast conditions: 50%-50%, 70%-30%, or 85%-15%. Amblyopic (n = 12) and control (n = 12) participants reported the perceived motion direction.

Results. Both groups showed broad temporal tuning of flicker integration. Accuracy in the contrast-balanced condition was highest with no added phase delay, and Gaussian fits to the data showed peak performance at a negligible delay (−0.3 degrees). Across both groups, an 85%-15% contrast disparity reduced overall accuracy and shifted peak accuracy to a delay of 28.6 degrees in the higher contrast eye.

Conclusions. Our data demonstrate that interocular temporal and contrast disparities interact similarly in normally sighted individuals and those with amblyopia. In this task, temporal delays and contrast imbalance manipulations interact predictably, such that the effects of imbalanced contrast are counteracted by a leading temporal offset for the eye with the lower contrast stimulus.

Keywords: amblyopia, binocular vision, psychophysics, flicker integration

Amblyopia is a neurodevelopmental disorder of vision, affecting 1%-4% of the population,[1–3] and is characterized by optically uncorrectable vision loss in one or both eyes. Amblyopia results from disruption of balanced binocular vision during visual development, for example, due to eye misalignment, unequal refractive error, or stimulus deprivation. Amblyopia is characterized by a reduction of visual acuity in addition to several other deficits in spatial vision, contrast sensitivity, and binocular integration (e.g., stereopsis; see Refs. 4–6 for reviews).

Although the spatial processing deficits associated with amblyopia are well-characterized, less is known about impairments in temporal processing. Notably, temporal deficits may be linked to spatial impairments, as neural response latencies tend to increase with reduced stimulus intensity.[7,8] Consistent with this prediction, in amblyopic observers, interocular timing differences have been consistently observed across a number of studies, demonstrating delays in visual evoked potentials,[9–11] reaction times,[12,13] and saccadic latencies[14,15] in the amblyopic eye relative to the fellow eye. At a physiological level, these interocular delays could be a consequence of either increased integration time at cortical sites or delays in retinocortical transmission.[9,12,16,17] These could reflect long-term physiological changes in the amblyopic eye pathway associated with the shift in ocular dominance defined by attenuated or delayed cortical evoked responses to monocular stimulation.

Although interocular delays in amblyopia have been shown to correlate with visual acuity,[15,18,19] some studies have demonstrated delays that cannot be explained by interocular sensitivity differences alone. For example, delays in saccadic and manual reaction time are larger than those accounted for by contrast sensitivity.[15,18] Further, methods to assess interocular delays have demonstrated large variability in their magnitude and direction. For example, perceptual measurements of interocular delays with the spontaneous Pulfrich effect have shown varied directionality of delays across participants, with some showing delays in the fellow eye and others in the amblyopic eye.[20,21] Moreover, other work also points to deficits in temporal processing

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more broadly, including deficits in temporal synchrony and temporal order perception[22–24] and changes in the strength of the flash-grab effect[25] and flash-lag effect.[26] These deficits in temporal processing also align with the literature on motion perception in amblyopia, which also shows stimulus-dependent effects. For example, motion perception is often impaired in amblyopia,[27,28] particularly in global motion perception[29] and motion defined form,[30] but may be spared in other contexts.[31–33] Together, the variability and direction of these effects support the idea that temporal deficits may exist beyond those produced by spatial deficits alone, and may be stimulus- and context-dependent.

Understanding both the spatial and temporal deficits in amblyopia may have implications for the development of new treatments. Whereas traditional amblyopia therapies involve occluding or penalizing the fellow eye, recently developed dichoptic treatments aim to promote binocular function (see Refs. 34–36 for reviews). These dichoptic treatments often use rebalanced spatial contrast, in which higher-contrast images are presented to the amblyopic eye compared to the fellow eye to promote binocular integration. More recent dichoptic studies have focused on modulation of relative interocular temporal phase to facilitate binocular integration[37] and to treat amblyopia,[38] based on the principle of spike-timing dependent synaptic plasticity,[39] which dictates that a leading temporal input can facilitate synaptic strengthening. However, how these spatial and temporal parameters interact in the context of fundamental studies remains poorly understood. Specifically, when integrating binocular stimuli, it is unclear if contrast rebalancing alone is sufficient to compensate for temporal delays (and vice versa). Moreover, the potential for particular combinations of temporal and contrast interocular differences to more effectively promote binocular integration than either alone remains unexplored.

The goal of the present study is to assess interocular delays and evaluate how they are impacted by interocular contrast differences in normally sighted participants and in patients with amblyopia. To test these relationships, we used an interocular flicker integration paradigm,[40,41] in which participants viewed a dichoptic pair of flickering gratings with a spatial and temporal offset between the eyes, resulting in the combined appearance of smooth motion. The underlying logic is that any interocular delays that are observed either at, or prior to, the time of this integration would alter the physical timing delays needed to perceive the motion. Specifically, our hypotheses were: (1) the interocular temporal phase offset optimal for binocular integration would be shifted in the amblyopic eye-leading direction as compared with normally sighted subjects; and (2) temporal phase and contrast would interact in both groups to shift the optimal temporal offset for binocular integration. Specifically, we predicted that optimal integration would occur when the eye with the lower-contrast stimuli had the leading offset.

Methods

Participants

We recruited a total of 29 participants through the Department of Ophthalmology at Boston Children's Hospital. Procedures were approved by the Institutional Review Board at Boston Children's Hospital, and participants gave written informed assent and consent before participating in the study. Following the exclusions listed below, the final sample consisted of 24 participants, who were assigned to the control and amblyopic groups based on the following criteria: patients with amblyopia ($N = 12$, ages = 8–43 years old, median = 13, interquartile range [IQR] = 10.5−19.5) had at least a one-line difference in interocular best-corrected visual acuity, with visual acuity corrected to 20/25 or better in their fellow eye, and a confirmed history of an amblyogenic factor. We additionally excluded participants lacking motor fusion and/or with manifested strabismus with best correction at near. Normally sighted controls ($N = 12$, ages = 6–27 years old, median = 11.5, IQR = 10–17) had a corrected visual acuity of 20/25 or better in each eye, with no manifest strabismus (see Procedure for screening details, and see the Table for a summary of the clinical characteristics and Supplementary Table S1 for individual participant data).

Apparatus and Stimuli

In our study, participants completed an interocular flicker integration task in which a pair of dichoptic horizontal gratings flickered sinusoidally in counterphase. Previous work has shown that participants with normal binocular vision can integrate flickering images shown separately to each eye to produce an illusion of motion seen binocularly.[40,41] This can be produced by showing participants a dichoptic pair of gratings that flicker with a 90-degree spatial and temporal phase offset in the images between the eyes, resulting in the combined appearance of smooth motion. We used this cyclopean motion illusion as a tool to evaluate binocular function in amblyopia and to determine whether binocular integration in amblyopia can be improved by varying differences in timing and contrast between the two eyes.

The experiment was programmed with the Psychophysics Toolbox version 3[42–44] in MATLAB. Stimuli were presented on a gamma-corrected LG 42LM6200-UE passive 3D monitor (resolution of 1920 × 1080 and 60 hertz [Hz] refresh rate). Participants were positioned 95 cm from the display and viewed the stimulus through circularly polarized glasses (worn over their corrective lenses). At this distance, the display subtended 43.4 degrees horizontally and 25.2 degrees vertically. Display crosstalk was reduced to approximately 2% using a subtractive cancellation procedure, which was implemented with the *StereoCrosstalkReduction* routine in Psychtoolbox.

On each trial, participants viewed a pair of screen-centered dichoptic Gabors presented on a grey background (65.6 cd/m^2). The Gabors were shown for 1000 ms, flickering in counterphase at 2 Hz with a sinusoidal temporal profile. To produce the appearance of upward or downward motion, as shown in Figure 1A, the Gabors were oriented horizontally (spatial frequency of 0.7 cpd; contrast envelope of σ = 0.86 degrees) and were always offset 90 degrees in spatial phase between the two eyes ($\varphi = -45$ degrees in the left eye, and $\varphi = +45$ degrees in the right eye). These settings were selected from previous work showing that these parameters reliably produce the appearance of continuous motion across the two eyes,[41] which was confirmed through pilot experiments. In addition, we chose to use a spatial frequency easily perceivable for participants with amblyopia, and we used horizontal rather than vertical Gabors to minimize any possible attenuation of the motion illusion produced by small horizontal vergence errors or common ocular misalignments.

We additionally varied two parameters between trials: the relative temporal phase between the two eyes, and the rela-

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Table. Summary of Clinical Data From Participants in Each Group

Group	Total	Median Age, Y	Median Interocular Acuity Difference, LogMAR	Median Acuity - Worse Eye, LogMAR	Median SER (D) - AE or OD	Median SER (D) - FE or OS	Median Stereoacuity, Arcsec
Amblyopia	12 (7 M, 5 F)	13 (10.5 to 19.5)	0.23 (0.12 to 0.46)	0.20 (0.10 to 0.37)	1.25 (0.13 to 4.47)	0.00 (0.00 to 0.97)	60 (45 to 100)
Control	12 (7 M, 5 F)	11.5 (10 to 17)	0.00 (0.00 to 0.00)	0.00 (0.00 to 0.00)	−1.50 (−3.09 to −0.44)	−1.75 (−3.03 to −0.38)	40 (40 to 50)

AE, amblyopic eye; D, diopters; F, female; FE, fellow eye; M, male; OD, right eye; OS, left eye; SER, spherical equivalent refractive error (signed values).
Values in parentheses represent interquartile ranges.

tive spatial contrast. As shown in Figure 1B, a 90-degree temporal offset between the 2 eyes produces the appearance of smooth continuous motion in one direction (e.g., upward). The opposite direction of motion can be produced by reversing the 90-degree temporal offset between the 2 eyes. On each trial, we added one of seven relative temporal delay conditions to this standard (90-degree) temporal delay: −80 degrees, −40 degrees, −20 degrees, 0 degrees, +20 degrees, +40 degrees, and +80 degrees (Fig. 1C). Positive values correspond to a temporally delayed stimulus in the amblyopic eye, whereas negative values correspond to a delayed stimulus in the fellow eye. At 2 Hz, these correspond to −111, −56, −28, 0, +28, +56, and +111 ms, respectively. For control participants, one eye was randomly assigned to receive the same contrast manipulation as the amblyopic eye (higher contrast); for these participants, positive values indicate that the higher-contrast stimulus was temporally delayed relative to the other eye. In addition to varying the relative phase difference between the two eyes, the absolute phase was randomly selected on each trial (from 0 degrees to 360 degrees in 12-degree steps). For example, the standard 90-degree offset condition (i.e. 0-degree delay added) could be produced by many possible temporal phase pairings between the left and right eyes: 0 degrees and 90 degrees, 12 degrees and 102 degrees, 24 degrees and 114 degrees, and so forth.

The relative contrast level (i.e. contrast imbalance) between the two eyes also varied between trials in one of 2 conditions: 50%-50% and 85%-15%. A subset of 13 participants (4 amblyopic and 9 control) additionally completed a 70%-30% contrast imbalance condition. To minimize the contribution of ceiling or floor effects to participants' performance, we first determined the summed peak contrast level to be tested across the two eyes using an initial adjustment task (see Procedure), which was determined for each participant and each contrast imbalance condition. To facilitate stable ocular vergence, each Gabor was centered within a rectangular frame for the duration of the trial, which served as a fusion lock box (and was either black or white on each trial depending on feedback; see Procedure), with a height of 6.84 degrees and a stroke width of 0.20 degrees.

Procedure

Vision Screening. Participants underwent a screening performed by an ophthalmologist, optometrist, certified orthoptist, or ophthalmic technician, as part of a routine clinic visit on the same day as testing. This included measurements of Snellen visual acuity, stereoacuity (via the Titmus or Randot stereo test), and ocular alignment using a cover test. Group measurements from the screening are reported in the Table, and individual participant data are reported in Supplementary Table S1.

Contrast Adjustment Procedure. To measure temporal tuning in the flicker integration task, the summed contrast level across the two eyes needed to be close to, or slightly above, the participants' contrast threshold to avoid ceiling or floor effects in performance. This summed contrast level was determined individually for each participant and each contrast imbalance condition using a baseline contrast adjustment task prior to the main experiment. For example, a participant might be tested at a summed peak contrast level of 10% contrast. This would mean that in the 50%-50% balance condition, the peak contrast would be 5% in each eye. A summed contrast level of 20% for the

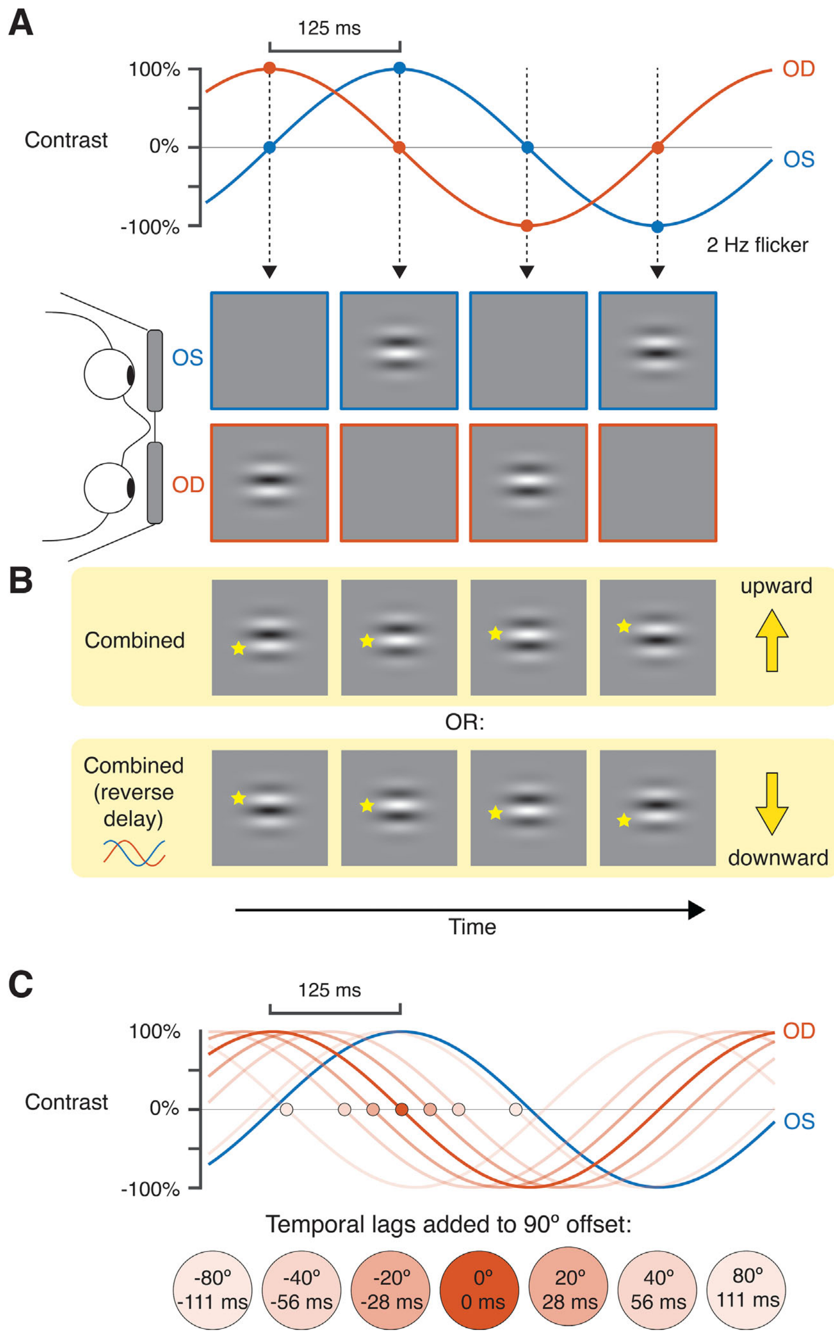

Figure 1. Motion integration task design and manipulation of dichoptic temporal phase. (**A**) Sample temporal profile of the stimulus, which consisted of a pair of dichoptic flickering Gabors viewed through polarized glasses that were spatially and temporally offset from each other by 90 degrees (OD = right eye, shown in *red*; and OS = left eye, shown in *blue*). Note that the left and right eye stimuli had complete spatial overlap at the center of the display and are shown here with a vertical spatial offset for illustrative purposes. (**B**) The combined binocular percept (*upper yellow panel*) produces the appearance of continuous upward motion across the two eyes. A visible landmark (a high luminance region) is marked with a star (⋆) to illustrate its position change over time in the combined stimulus (not shown in the experiment). The percept of motion in the opposite direction (downward motion; *lower yellow panel*) can be produced by reversing the temporal phase offset between the two eyes. (**C**) One of seven possible temporal delays (shown in *different shades of red*) were added to the standard 90-degree temporal offset to assess the temporal tuning of binocular flicker integration.

70%-30% condition would mean that the contrast would be 14% in one eye and 6% in the other, etc.

The adjustment procedure consisted of three phases. First, participants completed a temporal adjustment in which the dichoptic Gabor stimulus was visible continuously at full contrast. Participants moved a slider underneath the stimulus (controlled using the left/right arrow keys) to adjust the interocular temporal delay. Participants were instructed to select the delay that produced the smoothest appearance of motion (choosing from 1 of the 7 delays tested in the main experiment). This procedure was completed once for each of the contrast imbalance conditions. The chosen delay from this first phase was then used for the contrast adjustment procedure.

Next, participants viewed the stimulus continuously while adjusting a slider underneath the stimulus to manipulate its contrast. The initial summed contrast level was set to 100% (on the right side of the slider), and participants were instructed to adjust the contrast downward by moving the slider leftward via keypresses until the motion was no longer visible. The contrast level was adjustable downward to 5% summed contrast on a continuous logarithmic scale. The third and final phase was identical, except that participants adjusted the slider upward from 5% contrast until the motion began to be visible. Motion direction (upward or downward) was randomly selected on each adjustment screen, and each adjustment screen was completed once for each contrast imbalance condition before moving on to the next phase. The contrast levels from the upward and downward adjustment phases were averaged within each imbalance condition. To ensure that the stimulus was above threshold, participants completed the main task with the summed contrast level set to 1.25 times the contrast level calculated from the adjustment screen.

The mean adjusted contrast level was 14.6% for the amblyope group and 12.4% for the controls, which were not significantly different from one another ($F(1, 21) = 1.18$, $P = 0.29$; after excluding one outlier setting for the controls more than 3 SDs above the mean). The similar settings between the two groups are likely due to the low spatial frequency of the stimulus (0.7 cpd), consistent with previous work showing minimal interocular contrast sensitivity differences in amblyopic observers at low spatial frequencies.[45] Contrast settings were also not significantly different between the 85%-15% and 50%-50% imbalance conditions $F(1, 21) = 0.30$, $P = 0.59$, possibly due to difficulty in reliably observing motion with the contrast-imbalanced stimulus for some participants.

We used an adjustment procedure, rather than a forced-choice task, to minimize the testing time because the sample consisted primarily of pediatric participants. To verify that our results are robust to the method used to select the contrast level, we additionally tested a group of 12 normally sighted adults with the same task, all of whom completed three contrast imbalance conditions (50%-50%, 70%-30%, and 85%-15%). In this experiment, contrast levels were selected using a forced-choice motion discrimination task, in which contrast varied based on a staircase procedure. These results are comparable to the amblyopic and control participants in the main experiment, and are reported in the Supplementary Materials (see Supplementary Note).

Flicker Integration Task. Following the adjustment procedure, participants completed the main task, in which they viewed the dichoptic flickering stimulus for 1000 ms on each trial. Following the stimulus, participants reported its motion direction as upward or downward using the up and down arrow keys, respectively, and were given unlimited time to respond. After each response, participants were given feedback regarding their accuracy in the form of a beep (600 Hz for a correct response, or sum of 140, 280, and 560 Hz tones for an incorrect response; 250 ms each). Additionally, feedback was provided on the following trial, the color of the frame surrounding the grating was white following a correct response, and black following an incorrect response. Trials were separated by an intertrial interval of 500 ms, during which only the Nonius box was visible.

Each participant completed 20 trials for every unique combination of temporal delay (7 conditions), and contrast imbalance (either 2 or 3 levels), for a total of either 280 or 420 trials (7 × [2 or 3] × 20). Phase delays, contrast imbalance conditions, and motion directions (up or down) were all randomly interleaved throughout the experiment. Participants were given the opportunity to take frequent breaks, at least once every 50 trials. Completion times ranged from 8 to 20 minutes, with slightly longer average completion times for participants who completed 3 balance conditions compared to 2 (approximately 16 vs. 11 minutes, respectively).

Analysis

To determine how the temporal phase delay and contrast imbalance conditions affected participants' accuracy, for each participant, we calculated the proportion of correct responses for each unique combination of temporal delay and contrast imbalance condition. The resulting values from the full set of 24 participants were compared with a 2 (group) × 2 (contrast imbalance: 50%-50% and 85%-15%) × 7 (phase delay) mixed-model ANOVA. We also separately analyzed the performance of the subset of 13 participants who completed 3 contrast imbalance conditions with a 2 (group) × 3 (contrast imbalance) × 7 (phase delay) mixed-model ANOVA. Post hoc tests were corrected for multiple comparisons using Holm's procedure. Statistical analyses were completed in JASP (version 0.19.1).

In addition, to determine the phase delay that produced the highest accuracy, we fit a Gaussian function to each participants' accuracy across the phase delay conditions. Mean (μ), standard deviation (σ), and height (α) were free parameters, and floor performance was fixed at 50% (chance performance). Functions were fit separately for each contrast imbalance condition using a least-squares fitting procedure. To remove unreliable fits, we excluded conditions in which participants did not perform significantly above chance, based on a binomial test (calculated across all phase delays for a given imbalance condition). In addition, we excluded fits where the σ parameter was greater than 80 degrees (i.e. for these fits, ± 1 standard deviation exceeded the range of phase values tested). The resulting mean (μ) corresponded to the phase delay required for the highest accuracy for that condition. These values were compared between the different imbalance conditions with a paired samples *t*-test.

Results

Accuracy

All Participants. Figures 2A and 2B show the mean accuracy for all 24 participants in the 50%-50% and 85%-15% contrast imbalance conditions. A 2 (group) × 2 (contrast imbalance: 50%-50% and 85%-15%) × 7 (phase delay) mixed-model ANOVA showed significantly higher overall accuracy in the 50%-50% condition compared to the 85-15% condition (79.6% vs. 65.4%), $F(1, 22) = 73.10$, $P < 0.001$, $\eta^2_p = 0.77$. We also observed a significant main effect of phase delay $F(1, 3.45) = 23.62$, $P < 0.001$, $\eta^2_p = 0.52$, after adjusting the degrees of freedom using the Greenhouse-Geisser correction for sphericity ($\varepsilon = 0.58$; Mauchly's test: $\chi^2(20) = 43.15$, $P = 0.002$). As expected, across contrast imbalance conditions and groups, accuracy was highest with no phase delay

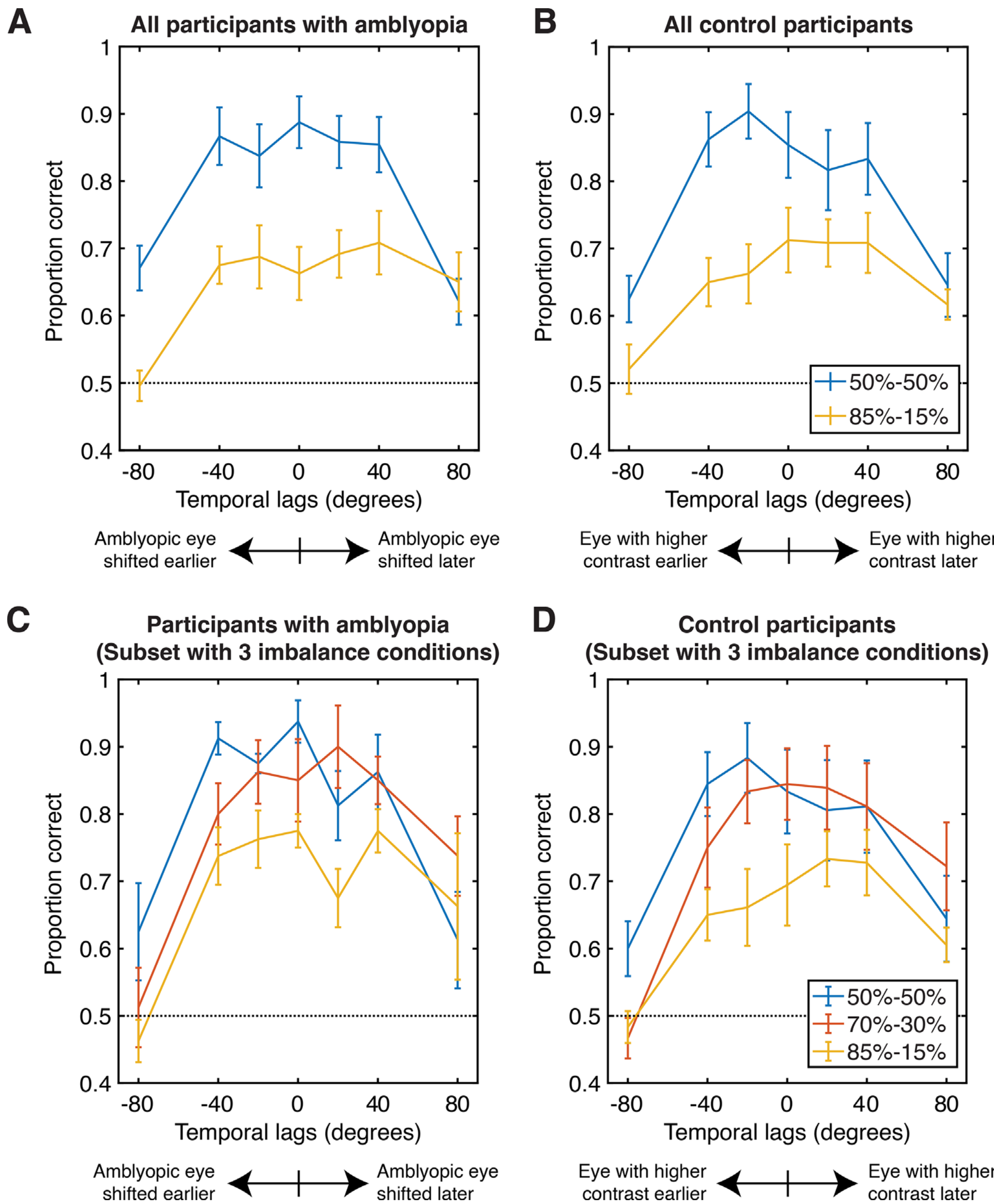

FIGURE 2. Task performance for amblyopic and control subjects as a function of temporal phase and contrast conditions. Proportion of correct responses for each phase lag (added to the standard 90-degree offset) for all participants in the amblyopic ($N = 12$; **A**) and control ($N = 12$; **B**) groups. Results are also shown separately for a subset of participants in the amblyopic ($N = 4$; **C**) and control ($N = 9$; **D**) groups who completed three contrast imbalance conditions. *Blue*, *red*, and *yellow lines* indicate the contrast balance conditions (50%-50%, 70%-30%, and 85%-15%, respectively). In the 70%-30% and 85%-15% conditions, contrast was higher in the amblyopic eye (selected at random for controls). The *dashed line* at 0.50 represents chance performance, and error bars represent ± 1 standard error of the mean (SEM).

(added to the 90-degree offset), and accuracy was reduced at extreme delays (i.e. the ±80-degree conditions).

These effects were qualified by a significant contrast imbalance × phase delay interaction, $F(6, 132) = 6.21$, $P < 0.001$, $\eta_p^2 = 0.22$. As expected, the contrast imbalance conditions shifted the overall tuning function of performance across phase delays, which was supported by the results of pairwise post hoc tests. In the even balance (50%-50%) condition, performance was symmetric around the condition with no phase delay, indicated by significantly worse in the −80-degree and +80-degree conditions relative to the other 5 phase delay conditions (0, ±20 degrees, ±40 degrees, all $p_{holm} < 0.001$) with no difference between the −80 and +80-degree conditions ($p_{holm} = 1.0$) However, this symmetry was not observed in the 85%-15% imbalance condition. Participants were significantly less accurate in the −80-degree condition compared with the other 6 phase delay conditions, including the +80-degree condition (all $p_{holm} \leq 0.011$). In addition, participants were more accurate in the 50%-50% condition compared to the 85%-15% condition at all phase delays (all $p_{holm} < 0.001$), except for the +80-degree delay ($p_{holm} = 1.0$).

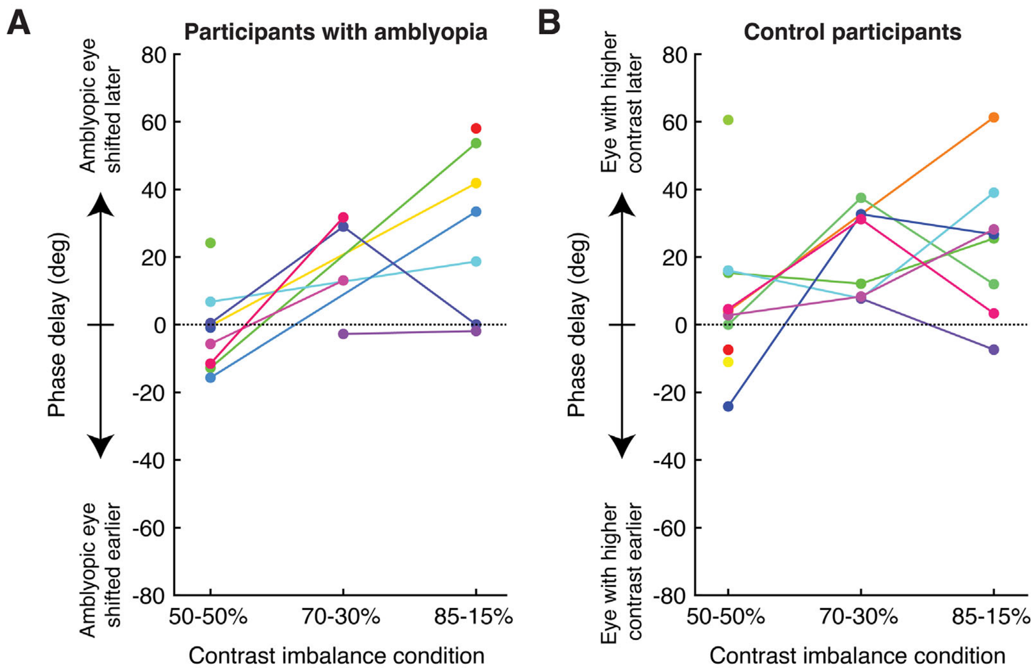

Figure 3. Contrast imbalance shifts the temporal offset for peak performance. Individual participant data showing the phase delay corresponding to peak accuracy (μ parameter based on a Gaussian fit) for participants in the amblyopic (**A**) and control (**B**) groups across all contrast imbalance conditions (50%-50%, 70%-30%, and 85%-15%). *Connected lines* represent individual participants, and data points are omitted in cases where the inclusion criteria were not met (see Analysis), or where the participant did not complete the 70% to 30% condition.

There was no difference in overall accuracy between the amblyopic and control groups, $F(1, 22) = 0.007$, $P = 0.93$, and none of the interactions involving group were significant ($F < 1.50$, $P > 0.18$), indicating that each group responded similarly to the contrast and phase delay manipulations.

Subset With Three Imbalance Conditions. As shown in Figures 2C and 2D similar results were observed for the subset of 13 participants who completed 3 contrast imbalance conditions (50%-50%, 70%-30%, and 85%-15%). As before, we observed a main effect of contrast imbalance, $F(2, 22) = 18.35$, $P < 0.001$, $\eta_p^2 = 0.63$, with significantly lower accuracy in the 85%-15% condition compared to the 70%-30% and 50%-50% conditions ($p_{bolm} = 0.003$, and 0.002, respectively). The main effect of phase delay was significant ($F(6, 66) = 22.97$, $P < 0.001$, $\eta_p^2 = 0.68$), as was the interaction between phase delay and contrast imbalance, $F(12,32) = 2.30$, $P = 0.01$, $\eta_p^2 = 0.17$, indicating the effect of phase delay depended on contrast level. Participants performed significantly better in the 50%-50% condition compared to the 85%-15% condition at all lags 0 degrees or lower (all $p_{bolm} < 0.04$). Participants also performed better in the 50%-50% condition compared to the 70%-30% condition at a lag of −40 degrees ($p_{bolm} = 0.009$), and better in the 70%-30% condition compared to the 85%-15% condition at lags of 0 degrees and −20 degrees ($p_{bolm} = 0.014$ and 0.02, respectively).

As before, the amblyopic and control groups did not differ in overall accuracy $F(1, 11) = 0.39$, $P = 0.55$, and none of the interactions involving group were significant ($F < 1$, $P > 0.86$), with the 2 groups responding similarly to the stimulus manipulations.

Phase Delay Corresponding to Peak Accuracy

The accuracy data indicate that the contrast imbalance manipulation shifted performance across phase delays. To further evaluate this, we fit a Gaussian to each participant's accuracy as a function of phase delay, to estimate the phase delay that produced the highest accuracy (given by the μ parameter). Individual participant fits are shown in Supplementary Figures S1 and S2, and the delays corresponding to the fitted peaks are shown in Figure 3. Five participants with amblyopia and 7 participants in the control group had reliable fits in both the 50%-50% and 85%-15% conditions, meeting the inclusion criteria for this analysis (see Analysis). Across groups, the phase delay that produced the highest accuracy was significantly higher in the 85%-15% condition compared to the 50%-50% condition (28.6 degrees vs. −0.3 degrees, $t(11) = 4.28$, $P = 0.001$). In addition, with a subset of 3 amblyopes and 6 controls that met the inclusion criteria in each condition, the phase delay corresponding to peak accuracy was higher in the 70%-30% condition compared to the 50%-50% condition (22.6 degrees vs. −0.2 degrees, $t(8) = 3.15$, $P = 0.014$).

Finally, to confirm that these results were robust across analysis methods, we fit a sinusoid to each participants' proportion of responses for a given motion direction as a function of temporal lag in each condition (which captured each inflection or null point in addition to the peaks of the function). The phase lags from these fits correlated strongly with the delay (μ) parameter from the Gaussian fits shown in Figure 3 ($r(43) = 0.95$, $P < 0.001$), and we observed similar results (see Supplementary Figs. S3–S5).

Discussion

Together, our results demonstrate that both participants with amblyopia and normally sighted controls respond similarly to temporal delays and contrast imbalance manipulations in a binocular integration flicker task. Specifically, both groups showed broad temporal tuning of flicker integration, with the highest accuracy for temporal offsets within ±40 degrees of the standard (90-degree) offset and decreasing at extreme temporal offsets (±80 degrees). Notably, contrast imbalances (70%-30% or 85%-15%) decreased performance in both groups and shifted the optimal temporal delay for integration—in the contrast imbalance conditions, performance was highest when the image in the eye with higher contrast was delayed.

Contrary to our hypothesis, the pattern of performance across phase delays was comparable for participants with amblyopia and control subjects under both balanced and imbalanced contrast conditions. In participants with amblyopia, motion discrimination was best in the 50%-50% condition when there was no added delay, and overall performance was comparable to normally sighted participants. In conjunction with previous studies,[46–48] the results suggest that the basic mechanism underlying this phenomenon, which requires binocular integration, is intact in amblyopia. Interestingly, the contrast manipulation shows that with no delay added, a contrast imbalance (70%-30% or 85%-15%) reduced performance. This is notable given that amblyopic observers show abnormal binocular interactions, with interocular balance points that are significantly shifted in favor of the fellow eye, even at low spatial frequencies.[49]

Although the data show an effect of contrast manipulation on binocular integration, with an average shift of 28.6 degrees (39.7 ms) in the tuning function (peak performance) for amblyopic and normally sighted observers in the 85%-15% condition, this method may not have been sufficiently sensitive to capture full impact of the temporal manipulation (i.e. phase delay). Our data show broad temporal tuning across interocular phase delays, and small interocular timing shifts may not have been detected. We also note that, given the cyclical nature of the stimulus, a very large delay would also produce similar tuning functions to those observed in the study (i.e. centered on 0 degrees for a 50%-50% delay), but this is much less likely given a 2 Hz flicker rate, as this would require a very large (250 ms) delay.

As we noted previously, interocular delays in amblyopia can be stimulus-specific. For example, one difference from previous work measuring interocular delays in amblyopia in the context of binocular integration (e.g., the spontaneous Pulfrich effect), is that the stimulus used in this study did not rely on stereoscopic information. Moreover, some studies have shown that temporal deficits – observed through either response latencies for monocular input or through binocular interactions – may be more pronounced for higher spatial frequencies, reflective of deficits in spatial processing.[50–53] In contrast, others show interocular delays are larger than those explained by interocular differences in contrast sensitivity alone.[15,18] Further work would be needed to understand how spatial and temporal properties of the stimulus may interact, as it is possible that these interocular delays with balanced contrast (50%-50%) could potentially be seen with higher spatial and temporal frequencies than the ones tested here. The stimulus parameters we used were based on previous work showing optimal performance in this task at lower spatial frequencies.[41] Carney et al.[48] previously showed that amblyopic performance in a motion discrimination task decreased as spatial frequency increased. Similar results were obtained in studies by Agrawal et al.[46] and McKee, Levi, and Movshon,[47] additionally demonstrating impaired performance in strabismic amblyopes compared to anisometropic amblyopes.[46–48] Because our goal was to isolate the temporal properties of the illusion and how they vary across interocular contrast levels, we chose a lower spatial frequency to provide a reliable substrate on which contrast and temporal phase could exert measurable effects. This also may account for the comparable contrast levels between the two groups in the matching procedure prior to the experiment (see Procedure). Whether and how the spatial frequency dependence of motion integration performance in amblyopia may affect the contrast-temporal phase interactions observed in this study is ripe for future study.

Although the results did not support our predictions regarding the temporal manipulation, our results are nonetheless consistent with other work demonstrating relatively spared dichoptic motion perception in amblyopia.[46–48] Identifying the mechanisms underlying this relatively spared performance would require further investigation. For example, one possible explanation for the similar performance between the two groups is that participants may have relied on feature tracking cues to judge motion direction. In other words, Shadlen and Carney argued that the illusion is produced by an early binocular mechanism[41]; however, similar performance can be achieved through higher-order processes including tracking of the stimulus features over time.[54] We note that dichoptic motion perception is still possible when feature-tracking cues are reduced or eliminated, which supports the existence of this mechanism.[40,55] However, this particular stimulus does not allow us to differentiate between these competing explanations. Further work measuring dichoptic motion perception in the absence of feature-tracking cues would be needed to establish the mechanisms supporting performance in amblyopic observers.

Our results are also consistent with some studies showing that the temporal properties of binocular interactions are similar in amblyopia compared with normally sighted observers. For example, a previous study by Huang et al.[56] showed that the temporal profiles of interocular suppression in amblyopic subjects are comparable to dichoptic masking in normally sighted participants. More recently, Zhou et al.[57] demonstrated that, when shown dichoptic stimuli flickering at different rates, amblyopic and control subjects show similarly increased perceptual dominance of stimuli that flicker at intermediate temporal frequencies. In both studies, amblyopic subjects showed more interocular imbalance, but otherwise showed qualitatively similar effects of temporal frequency modulation to control participants. Finally, the large individual differences in the temporal offset required for optimal flicker integration, as well as variable changes in performance in response to the contrast manipulation (see Fig. 3), also align with previous findings across different perceptual tasks.[19,20]

Both contrast imbalance and temporal offsets have both been used to facilitate binocular integration in amblyopia.[37,38] However, the interaction between these parameters has not been previously investigated. Our findings suggest that combined manipulation of these parameters alters perceptual responses predictably in both normally sighted and amblyopic observers. Our results also suggest that, for some stimuli, temporal offsets favoring the amblyopic eye may not always improve performance in amblyopic observers, particularly in the context of contrast-balanced dichoptic stimuli. Importantly, patients with amblyopia required varying degrees of temporal delays to optimize binocular integration, and future studies aimed at answering whether these individual differences are relevant to responses to dichoptic therapy may serve to improve outcomes.

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