

The Flash-lag Effect in Amblyopia

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PURPOSE. Amblyopes suffer a defect in temporal processing, presumably because of a neural delay in their visual processing. By measuring flash-lag effect (FLE), we investigate whether the amblyopic visual system could compensate for the intrinsic neural delay due to visual information transmissions from the retina to the cortex.

METHODS. Eleven adults with amblyopia and 11 controls with normal vision participated in this study. We assessed the monocular FLE magnitude for each subject by using a typical FLE paradigm: a bar moved horizontally, while a flashed bar briefly appeared above or below it. Three luminance contrasts of the flashed bar were tested: 0.2, 0.6, and 1.

RESULTS. All participants, controls and those with amblyopia, showed a typical FLE. However, the FLE magnitude of participants with amblyopia was significantly shorter than that of the control participants, for both their amblyopic eye (AE) and fellow eye (FE). A nonsignificant difference was found in FLE magnitude between the AE and the FE.

CONCLUSIONS. We demonstrate a reduced FLE both in the AE as well as the FE of patients with amblyopia, suggesting a global visual processing deficit. We suggest it may be attributed to a more limited spatiotemporal extent of facilitatory anticipatory activity within the amblyopic primary visual cortex.

Keywords: amblyopia, temporal vision deficits, flash-lag effect

Amblyopia is a neuro-developmental disorder arising from abnormal visual experience during the sensitive period of brain development in the early childhood. Typically, it shows spatial deficits as reflected by reduced acuity,¹ reduced contrast sensitivity,² and spatial inaccuracy.^{3,4} Many studies have demonstrated that amblyopia is not only limited to spatial vision deficits, but also associated with impaired temporal visual processing. Spang and Fahle⁵ reported a poorer temporal resolution by patients' amblyopic eyes (AEs). Tao et al.⁶ found that the AE has a higher temporal synchrony threshold. These deficits might be the consequences of a delay in the processing of information by the AE.⁷⁻¹¹ In particular, by using magnetoencephalography, Chadnova et al. reported an interocular processing delay for the information processed by the AE of approximately 20 ms.¹² However, the fellow eye (FE; non-AE) may also show impaired temporal processing in perception of temporal order.¹³ Indeed, Huang et al.¹⁴ found that patients with amblyopia had deficits in synchrony processing in both eyes, although slightly worse in their AEs. Furthermore, it has been shown that for patients with mild amblyopia, an interocular delay can be detected in the processing of the FE.^{11,15} This evidence of temporal processing deficits indicates that there is a delay in processing visual information in the amblyopic visual system.

In the human brain, there is an intrinsic neural delay in visual information transmission through several processing stages (from the retina to the visual cortex).¹⁶ Consequently,

our processing of visual events lags behind their actual occurrence. To dismiss inaccurate interactions between visual events in real time, our brains could compensate for the neural delay through extrapolation.¹⁷ However, whether the delayed visual system of patients with amblyopia could compensate for the internal neural delay remains unknown.

Because of their temporal processing deficits, patients with amblyopia could experience difficulties compensating for such delay. Such inaccurate compensation would be revealed by motion processing tasks¹⁸⁻²⁰ and visuospatial attention,²¹ in which patients with amblyopia have shown deficits. Another way to investigate this abnormal processing would be by using visual illusions, which trick the brain to anticipate an event. The flash-lag effect (FLE), a well-established illusion in which a flashed object is perceived to lag behind a moving object when their two positions are physically aligned, has been widely used to study the spatiotemporal interactions in visual processing. This effect has mostly been attributed to latency differences in processing: the processing latency of the flashed object would be longer than the latency of the moving one,²²⁻²⁵ extrapolation mechanisms,^{17,26-28} or other mechanisms.²⁹ Transcranial magnetic stimulation (TMS) applied to area MT+, which is involved in visual motion processing and temporal integration, has been shown to significantly reduce the FLE over a long period of time (100 ms before flash to 200 ms after flash).³⁰ Therefore, the FLE provides a valuable tool to investigate temporal processing in amblyopia and

investigate whether the amblyopic visual system can compensate for neural delays.

In order to characterize the temporal visual processing deficits in human amblyopia, in the present study, we explored the FLE in adults with unilateral strabismic and anisometropic amblyopia. Given the already known temporal processing deficits,^{5,6,13,14} we expected a larger FLE in amblyopia. We used a common FLE paradigm: a vertical bar moved horizontally while a flashed bar briefly appeared above or below the moving bar. Then, participants were asked to judge whether the flashed bar was presented ahead or behind the moving bar. Previous studies have found contrast of stimuli could influence the FLE perception.^{31–35} As patients with amblyopia present a reduced contrast sensitivity^{2,36} which is known to affect the processing time of visual stimuli,⁹ we also sought to explore whether the reduced contrast sensitivity of patients with amblyopia would have an influence on the FLE at different contrasts. We assessed the FLE in the amblyopic population by monocularly testing both the AE and the FE at three different contrasts and compared the results with a control group.

METHODS

Apparatus

Stimuli were presented on a Mac computer (OSX, 10.10.5) running Matlab R2018a (the MathWorks) with PsychTool-Box version 3.0.9 extension.^{37,38} They were displayed on a gamma-corrected CRT monitor (Iiyama MA203DTD: 45.1 cm × 36.1 cm viewing area), with a maximum luminance of 82 cd/m², resolution of 1280 × 1024 pixels, and a refresh rate of 100 Hz. Observers viewed the screen monocularly, wearing a dark opaque patch over the untested eye, at a viewing distance of 57 cm in a dark room.

Subjects

Eleven amblyopic adult subjects (average age: 33.5 years old, range: 20–66 years old) and 11 control subjects (average age: 27.8 years old, range: 21–38 years old) with normal or corrected-to-normal vision participated in this study. The clinical details of subjects with amblyopia are presented in Table 1. The dominant eyes of the control subjects were determined with the Porta test. This study followed the tenets of the Declaration of Helsinki and was approved by the Ethics Review Board of the McGill University Health Center. Informed consent was obtained from all participants before data collection.

Stimuli and Procedure

The FLE was measured with a standard procedure: one bar in the left hemifield moved rightward toward the vertical meridian. During its motion, another bar was flashed at a fixed position, but with a variable timing relative to the stimulus initiation in each trial. This foveopetal stimulus presented in the left hemifield has been reported to drive the largest FLE.³¹ There were two possible configurations of the bars in this study. In the first configuration, the moving bar was shown in the lower visual field, and the flashed bar was shown in the upper visual field (Fig. 1A). In the second configuration, the moving bar appeared in the upper visual field, and the flashed bar appeared in the lower visual field.

An orange fixation point was presented at the center of the screen throughout the experiment.

Moving and flashed bars were the same size (5 degrees × 1 degree). The vertical distance between the nearest edges of the bars (bottom edge of the top bar and the top edge of the bottom bar) was 1 degree. The speed of the moving bar was constant at 18 degrees/s. The moving bar moved for 1000 ms and disappeared at the constant position of 4 degrees horizontal distance from the vertical meridian. The flashed bar was presented at a fixed position 8 degrees from the vertical meridian for 1 frame (~10 ms). The timing of the flash bar was varied within 11 timepoints (–100, –80, –60, –50, –40, –30, –20, –10, 20, 40, and 60) ms (i.e. –10, –8, –6, –5, –4, –3, –2, –1, 2, 4, and 6 frames) relative to the time when the moving bar reached the azimuth (x coordinates) of the flash. Therefore, the flashed bar could be presented for up to 100 ms before the moving bar was physically aligned with the flashed bar, or up to 60 ms after the bar passed the flash position. In each trial, the subject was asked to judge whether the flashed bar was presented at the left (behind) or the right side (ahead) of the moving bar when the “flash” occurred by using a keyboard. The timepoints distribution was skewed toward negative values in order to ensure that the subjects would report an equivalent amount of types of judgements (e.g. “left” and “right”) over probes in the experiment. In one block, each timepoint was tested with 10 repetitions, yielding a total of 110 (11 × 10) trials/block. Three luminance contrasts of the flashed bar were tested: 0.2, 0.6, and 1. The contrast of the bar was defined as the luminance increment of the bar relative to the constant mean grey background (41 cd/m²). Subjects were tested with the left and right eyes separately. Thus, there were a total of 12 conditions (2 stimuli configurations × 3 contrast ratios × 2 eyes). One condition was tested per block, and repeated two times. The order of the conditions was randomized.

Data Analysis

The data were analyzed with Matlab R2018b (the MathWorks). We fitted the psychometric function describing the proportion of “left” responses at each timepoint by a logistic function using least squares estimation (Matlab’s `nlinfit`). The estimated midpoint of the logistic function defines the point of subjective equality (PSE), where participants would yield 50% left response and 50% right response when indicating the perceived alignment between the moving bar and the flashed bar (Fig. 1B). Significant PSE shift from zero would then characterize the FLE magnitude.

RESULTS

The PSE shift from 0 was significantly different in all 12 conditions for both control and amblyopic groups (for all, $P < 0.001$). All participants, controls and those with amblyopia, showed a typical FLE, that is, they perceived the flashed bar to be at the left of the moving bar when the two bars were physically aligned (i.e. the flashed bar was lagging behind the rightward moving bar). The FLE magnitude for each eye in different luminance contrast conditions is reported in Table 2 and Figure 2 with the data averaged between the 2 bars spatial configurations (see Supplementary Fig. S1 for the scatterplots of the FLE magnitude between the two stimuli configurations for the 11 subjects with amblyopia).

TABLE 1. Clinical Details of Subjects With Amblyopia

Subject	Age/Aex	Type	Eye	Refraction	VA	Squint	Suppression	Randot	Patching	Surgery
A1	66/F	Mixed	FE (OS) AE (OD)	+4.25/-2.00 × 96 degrees +5.00	20/20 20/400	Non measured	Strong	NA	Around 4 y	Strabismic surgery at 16 y
A2	56/M	Mixed	FE (OD) AE (OS)	-1.25/-0.50 × 30 degrees +2.50/-1.50 × 75 degrees	20/20 20/50	L exo 6 degrees	No	NA	No	No
A3	28/F	Strab	FE (OS) AE (OD)	Plano -0.75/-0.50 × 60 degrees	20/20 20/63	R exo 15 degrees	Strong	NA	Around 2y to 7y	2 strabismic surgeries at 2y
A4	27/M	Mixed	FE (OD) AE (OS)	-0.50 +2.00	20/16 20/63	L exo 5 degrees	Central	NA	No	No
A5	22/F	Aniso	FE (OD) AE (OS)	+0.25/-0.25 × 70 degrees +1.50/-1.50 × 10 degrees	20/20 20/50	∅	No	200	No	No
A6	30/F	Aniso	FE (OS) AE (OD)	Plano +2.50	20/12.5 20/16	∅	Weak central	100	No	No
A7	24/F	Aniso	FE (OD) AE (OS)	+10.25/-0.75 × 155 degrees +10.25/-0.75 × 90 degrees	20/25 20/40	∅	No	NA	From 3 y to 5 y, and 8 y to 9 y	No
A8	40/M	Strab	FE (OS) AE (OD)	-1.25/-0.25 × 103 degrees +0.50/-1.25 × 90 degrees	20/12.5 20/40	R exo 10 degrees	Central	NA	From 10 y to 12 y	No
A9	20/F	Mixed	FE (OS) AE (OD)	-0.50 -3.25	20/25 20/100	R exo 10 degrees	Weak	800	No	No
A10	34/M	Mixed	FE (OD) AE (OS)	-1.00 +1.50	20/10 20/50	L eso 10 degrees	Central	400	No	No
A11	22/M	Strab	FE (OD) AE (OS)	-2.75 -1.25	20/25 20/80	L exo 6 degrees	Central	100	No	No

VA, visual acuity; FE, fellow eye; AE, amblyopic eye; Strab, strabismus; Aniso, anisometropia; exo, exotropia; eso, esotropia.

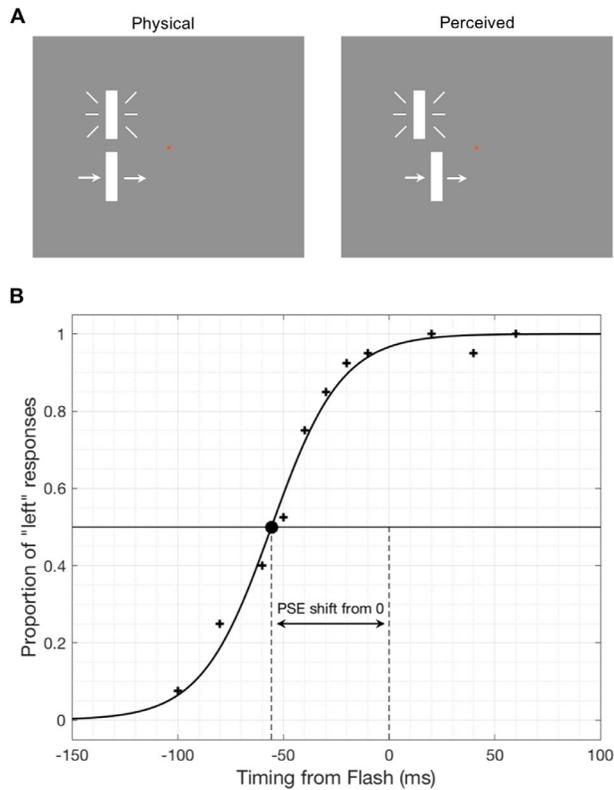


FIGURE 1. (A) The left panel illustrates the physical stimuli. In this example, one bar moved horizontally rightward toward the vertical meridian in the left hemifield. The flashed bar is presented at the time when the moving and flashed bar are physically aligned. The right panel illustrates the typical perception: the moving bar is perceived to be ahead of the flashed bar. (B) Psychometric function of one representative participant in the experiment: Proportion of “left” response as a function of the relative timing of the appearance of the flashed bar. Negative value means the flashed bar was presented before the moving bar physically aligned with the flashed bar. Datapoints are fitted with a logistic function. PSE, point of subjective equality.

We performed between-subject repeated measures ANOVA tests with contrast (3 levels) as a within-subject factor to compare FLE magnitude between groups. We found that the mean FLE magnitude of the AE was significantly smaller than that of the non-dominant eye (NDE) of controls ($F[1, 20] = 7.75, P = 0.011$). To investigate whether the FE processing of patients with amblyopia was comparable to control eyes, we compared the mean FLE magnitude of FE to that of dominant eye (DE) of controls. The results showed that the mean FLE magnitude of FE in the amblyopic group

TABLE 2. Mean FLE Magnitude of the Control and Amblyopic Groups Under Different Contrast Conditions

Contrast Conditions	Controls		Amblyopes	
	DE	NDE	FE	AE
0.2	100.3 ± 10.7	93.3 ± 10.5	52 ± 5.8	53 ± 7.8
0.6	74 ± 7	73.8 ± 8.2	43.3 ± 6	42.4 ± 8.4
1	68.9 ± 7.8	64.1 ± 7.7	41.2 ± 2.1	41.8 ± 7.7

Data are expressed as the mean ± standard error. FLE, flash-lag effect; DE, dominant eye; NDE, non-dominant eye; FE, fellow eye; AE, amblyopic eye.

was significantly smaller than that of DE in controls ($F[1, 20] = 14.15, P = 0.001$). In addition, the effects of contrast were significant (AE versus NDE: $F[2, 40] = 19.08, P < 0.001$; FE versus DE: $F[1.56, 31.2] = 23.19, P < 0.001$), and the interactions between group and contrast were significant as well (AE versus NDE: $F[2, 40] = 3.47, P = 0.041$; FE versus DE: $F[1.56, 31.2] = 6.53, P = 0.007$). We also conducted within-subject repeated measures ANOVA tests with eyes (2 levels) and contrast (3 levels) as within-subject factors. The results show that the FLE magnitude was not significantly different between NDE and DE in the control group ($F[1, 10] = 2.88, P = 0.12$), or between AE and FE in the amblyopic group ($F[1, 10] = 0.002, P = 0.965$). Figure 2 clearly shows that the FLE magnitude is comparable between the eyes in both the control and amblyopic groups. Altogether, our results indicate that the amplitude of the FLE is similar in the AE and FE of patients with amblyopia, although it is smaller than what is observed in controls.

Figure 3 shows the FLE differences between different contrast conditions in the two groups. We calculated the differences between FLE of contrast ratio 0.2 minus FLE of contrast 1, and between FLE of contrast 0.6 minus FLE of contrast 1. Zero means no difference compared with FLE of contrast 1. In the control group, there was a 34.4 ms longer FLE of contrast 0.2 over FLE of contrast 1 with DE ($P = 0.003$), and a 29.2 ms longer FLE with NDE ($P = 0.003$; see Fig. 3A; two-sided Wilcoxon signed rank test). The mean differences between FLE of contrast 0.6 minus FLE of contrast 1 with NDE was significantly different from zero ($P = 0.041$, two-sided Wilcoxon signed rank test). However, these differences were not significant in the amblyopic group for either the AEs or FEs (Fig. 3B).

Finally, in order to investigate whether the difference between the amblyopic and control groups could be attributable to a greater expertise of the control participants (most of them being experienced laboratory members), we examined whether there is a performance asymmetry in FLE between experienced and nonexperienced subjects. According to their total amount of participation in any psychophysical experiment in our laboratory before, controls and subjects with amblyopia were respectively divided into two subgroups: experienced (who participated more than 5 times) and nonexperienced (≤ 5 times) groups. We assessed the difference of FLE magnitude between the two subgroups in the control (experienced [$n = 6$] vs. nonexperienced [$n = 5$]) and amblyopic group (experienced [$n = 6$] vs. nonexperienced [$n = 5$]). The average FLE magnitude in the two subgroups for controls and subjects with amblyopia is plotted in Figure 4, with all conditions pooled together. We can clearly see on these bar graphs that the FLE magnitude is equivalent between the experienced and nonexperienced participants in each of the control group and amblyopic group. Hence, the observed difference cannot be attributable to the experience of the observers. These differences were not significant ($P = 0.67$ and $P = 0.094$ for controls and subjects with amblyopia, respectively, two-sided Wilcoxon rank sum test; see Fig. 4).

DISCUSSION

By using the FLE phenomenon, we examined whether the amblyopic visual system could compensate for the neural processing delay in perceiving moving objects. We found that: (1) subjects with amblyopia show an FLE, that is, they perceive the flashed bar to lag behind the moving bar when

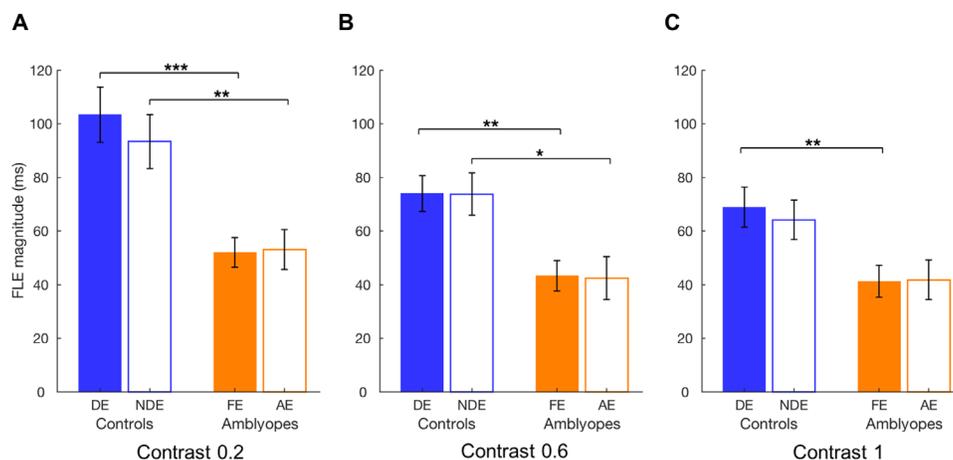


FIGURE 2. Mean FLE magnitude of the two groups under different contrast conditions (A–C). Results are compared between the eyes (filled versus open bars) of healthy control group and amblyopic group (blue and orange, respectively). FLE, flash-lag effect; DE, dominant eye; NDE, non-dominant eye; FE, fellow eye; AE, amblyopic eye. Error bars represent the standard errors. *** $P \leq 0.001$; ** $P < 0.01$; * $P < 0.05$.

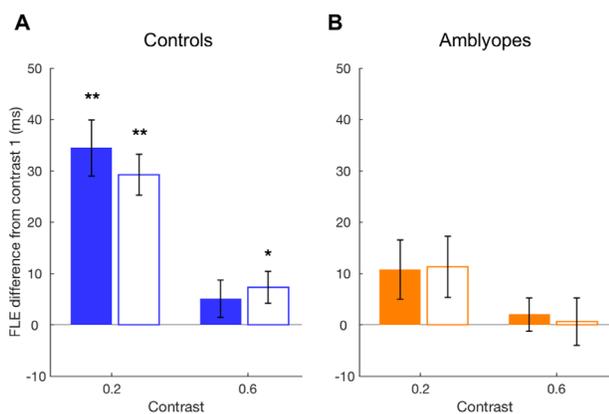


FIGURE 3. FLE amplitude differences between the low contrast conditions and the full contrast condition in the two eyes (filled versus open bars). (A) control group. (B) amblyopic group. FLE, flash-lag effect; Error bars represent the standard errors. ** $P < 0.01$; * $P < 0.05$.

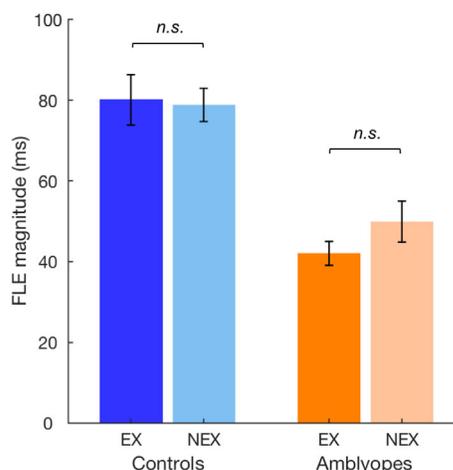


FIGURE 4. Mean magnitude of FLE in experienced (dark color bars) and nonexperienced (light color bars) groups for controls (blue bars, left pair) and amblyopes (orange bars, right pair). FLE, flash-lag effect; EX, experienced group; NEX, nonexperienced group. Error bars represent the standard errors. n.s., not significant.

their two positions are physically aligned. However, contrary to expectations, the magnitude of the FLE in amblyopia is significantly smaller compared with controls. (2) There is no difference in FLE between the AE and the FE at any contrast ratio condition studied. (3) Patients with amblyopia do not show a significant increase in FLE when the flashed bar contrast was reduced, whereas controls did.

The reduced FLE magnitude we report here adds more evidence that amblyopia is associated with not only spatial deficits but also temporal deficits. In addition, the spatiotemporal deficit, as reflected in the FLE, is not limited to the AE. The processing associated with the FE of the amblyopic participants is also affected. Previous studies have reported that different temporal aspects of vision can be altered in the amblyopic visual system. Decreased temporal resolutions⁵ and increased temporal synchrony thresholds^{6,14} have been identified for the amblyopic visual system. Chadnova et al., additionally, reported an interocular processing delay of around 20 ms in patients with amblyopia relative to controls.¹² With those different paradigms, the temporal deficits observed falls in the same range as in our present study (20–40 ms). An interocular delay would cause patients with amblyopia to experience a spontaneous Pulfrich effect (perception of a stimulus moving in depth whereas it is moving in plane, when viewed binocularly).^{8,11,15} Furthermore, it would be interesting to investigate how patients with amblyopia experience the FLE in depth³⁹ as they would likely have perceived the trajectory of the moving stimulus erroneously.

Actually, the spontaneous Pulfrich phenomenon patients with amblyopia experience can, in some cases, be caused by a delay of the FE.^{11,15} Evidence for temporal processing impairments in the fellow eye has been found. Huang et al.¹⁴ noted that patients with monocular amblyopia showed impairments in temporal discrimination involving both eyes, although slightly worse in their AEs. Temporal deficits associated with fellow eye processing in unilateral amblyopia have also been reported in motion perception. Global motion threshold^{19,40,41} and motion-defined form thresholds^{20,42,43} associated with the FE processing are also elevated in patients with amblyopia. In our study, observers with amblyopia behaved similarly in the FLE task whether they used their AE or their FE. Taken together, this body

of evidence indicates that unilateral amblyopia may cause abnormal integration of space and time for visual processing associated with either eye, potentially due to the disruption of binocular development. Furthermore, the reduced FLE we observed cannot be accounted by a constant delay as this would affect equally both the moving and flashed bars.

Perception of object position involves an accumulation of signals over space and time. The visual system does not have access to the immediate information of a moving object because the transmission of that information from the retina to the cortex takes time.¹⁶ One way the brain could compensate for this neural processing delay is through extrapolation, a general compensation mechanism,⁴⁴ which presumably accounts for the FLE.^{17,27,28,45,46} In this study, we observed that the FLE in amblyopia is reduced. It is known that fixation status could affect perception of FLE.⁴⁷ However, previous studies have reported that fixation is more unstable in AEs than in FEs^{48,49} and also that the characteristics of fixation stability are similar in FEs of patients with amblyopia and healthy controls.^{50–52} Therefore, the reduced FLE we observe in both eyes of patients with amblyopia is unlikely to be a consequence of poorer fixation. Albeit reduced, our results showed that amblyopes did exhibit an FLE, which may suggest that their brains still could compensate for this neural processing delay. However, compared with controls patients with amblyopia had smaller magnitude of FLE, which suggests that their perception was closer to the physical stimulus. It is unlikely that the smaller FLE is attributed to subjects with more experience, because we observed similar performance between experienced and nonexperienced subgroups for both controls and subjects with amblyopia (see Fig. 4). Thus, we speculate that the smaller FLE in amblyopia may be due to singular processing of their visual system.

Most studies have explained the FLE in terms of a faster processing of the moving bar or an extrapolative delay compensation.²⁹ Such faster processing could be due to facilitatory neural activity on the moving bar trajectory path within the V1 retinotopic representation.^{53–56} At the bar onset, a wave of activity would emerge at the bar retinotopic location in V1.⁵⁷ This wave of activity would propagate across the V1 surface through horizontal connections, thus depolarizing neighboring neurons subliminally.⁵⁸ This subliminal depolarization would characterize a pre-activation of these neighboring neurons, which are coding adjacent locations in the visual field. Then, when the bar moves across the visual field, its retinotopic representation moves to a pre-activated cortical location, which would facilitate the response of visual neurons.^{53,59,60} Hence, these neurons would give a faster response than the neurons responding to the stationary flash.

Such an hypothesis would substantiate previous observations that the FLE emerges from relatively high-level cortical processes.^{30,53,61,62} In this context, the smaller FLE we observed for amblyopes – suggesting the difference of latencies between the moving and flashed bars is reduced – could be the consequence of defects in cortical structure and function. Previous studies on animal models have shown that amblyopia is associated with a range of morphological and physiological changes in the visual cortex, patchy modifications in the projection of the local and long-range horizontal connections in V1,⁶³ reduced strength of cellular interaction,⁶⁴ and alteration in the balance of excitatory and inhibitory connections to neurons.^{65,66} This altered balance could be characterized by asymmetries in surround

suppression mediated by horizontal connections in V1.⁶⁷ Such cortical changes may alter the retinotopic representation of V1^{68–70} and therefore disturb the propagation of the facilitatory neural activity, which would affect the neural delay. Consequently, patients with amblyopia may present a longer neural response time for the moving bar, hence causing a reduction in the difference of latencies between the moving and flashed bars, due to a limited or slower extent of propagating facilitatory activity waves across the cortex. Such alterations could be asymmetric between the two hemispheres.^{71,72} However, our results cannot address this question because our stimuli were always presented in the left visual hemifield.

The visual latency is known to vary with the properties of a stimulus, such as its luminance and contrast.^{73,74} Weaker visual inputs generate slower neural responses; the time to perceive the stimuli is longer at a low stimulus contrast. It has been reported that FLE decreases with a weaker motion signal,^{33,35} and increases with a decrease in contrast of moving and flashed object compared to the background³¹ or with only a decrease in contrast of the flashed object.^{22,25} Consistent with previous studies,^{22,25} we observed in controls that the magnitude of the FLE increased as the contrast of the flashed bar decreased (see Figs. 2, 3A). The largest FLE was obtained at the lowest stimulus contrast. However, this pattern was barely observable in subjects with amblyopia. A trend was present but the increased amplitude of FLE at lowest contrast was not significant (see Fig. 3B). So far, we cannot provide a satisfactory explanation for this observation.

In conclusion, we demonstrate an impaired FLE in both the AE and FE in amblyopia, meaning this temporal processing deficit affects their whole visual system. It might indicate that the amblyopic visual system does not accurately compensate for the intrinsic neural delay derived from visual information transmission from the retina to the cortex. This temporal processing deficit may be attributable to a more limited spatiotemporal extent of facilitatory activity waves across the amblyopic primary visual cortex.

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