

# Binocular Imbalance in Amblyopia Depends on Spatial Frequency in Binocular Combination

Yu Mao,<sup>1</sup> Seung Hyun Min,<sup>2</sup> Shijia Chen,<sup>1</sup> Ling Gong,<sup>1</sup> Hao Chen,<sup>1</sup> Robert F. Hess,<sup>2</sup> and Jiawei Zhou<sup>1</sup>

<sup>1</sup>School of Ophthalmology and Optometry and Eye Hospital, and State Key Laboratory of Ophthalmology, Optometry and Vision Science, Wenzhou Medical University, Wenzhou, Zhejiang, China

<sup>2</sup>McGill Vision Research, Department of Ophthalmology and Visual Sciences, McGill University, Montreal, QC, Canada

Correspondence: Jiawei Zhou, School of Ophthalmology and Optometry, Affiliated Eye Hospital, State Key Laboratory of Ophthalmology, Optometry, and Vision Science, Wenzhou Medical University, 270 Xueyuan Xi Rd, Wenzhou, 325035 Zhejiang, P. R. China;

[zhoujw@mail.eye.ac.cn](mailto:zhoujw@mail.eye.ac.cn).

Hao Chen, School of Ophthalmology and Optometry, Affiliated Eye Hospital, State Key Laboratory of Ophthalmology, Optometry, and Vision Science, Wenzhou Medical University, 270 Xueyuan Xi Rd, Wenzhou, 325035 Zhejiang, P. R. China;

[chenhao@mail.eye.ac.cn](mailto:chenhao@mail.eye.ac.cn).

YM and SHM contributed equally.

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**PURPOSE.** To assess the role of spatial frequency on binocular imbalance in binocular combination in adults with amblyopia.

**METHODS.** Ten amblyopes ( $23 \pm 4.9$  [SD] years old; one deprivation, two mixed, seven anisometropia patients) and 10 age-matched normal adults ( $23 \pm 2.3$  years old) participated. The interocular contrast ratio (fellow eye/amblyopic eye, i.e., the balance point [BP]) that resulted in an equal contribution of both eyes in binocular combination was measured using a binocular orientation combination task at 0.5, 1, 2, and 4 cycles per degree (c/d). The extent of binocular imbalance was quantified as the absolute value of the BP on log scale (i.e.,  $|\log BP|$ ).

**RESULTS.** When the base contrast of the amblyopic eye was set at 100% (Experiment 1), the  $|\log BP|$  was found to be significantly affected by stimulus spatial frequency ( $F(1.44, 26.01) = 51.6, P < 0.001, \eta_g^2 = 0.40$ ) and group ( $F(1, 18) = 66.97, P < 0.001, \eta_g^2 = 0.74$ ), the interaction between spatial frequency and group was also significant ( $F(1.44, 26.01) = 38.12, P < 0.001, \eta_g^2 = 0.33$ ). Such spatial frequency-dependent binocular imbalance remained present, even when the base contrast of the amblyopic eye was set at equal suprathreshold contrast levels across spatial frequencies (Experiment 2).

**CONCLUSIONS.** Binocular balance was more disrupted at higher spatial frequencies in binocular combination in amblyopia. This imbalance might not originate solely from the amblyopic eye's deficit in contrast sensitivity but is likely to be related to the difference in contrast sensitivity between the eyes.

**Keywords:** amblyopia, binocular vision, attenuation, interocular suppression, spatial frequency

Previous studies highlight the prevalence of abnormal binocular interactions in amblyopia. For instance, in the amblyopic visual system, excitatory connections that drive binocular summation can be normal<sup>1,2</sup>; however, stereopsis can be defective, as are the inhibitory interactions that underlie interocular suppression.<sup>3</sup> In particular, there seems to be an asymmetry in the interocular inhibitory connections between the amblyopic and fellow eyes in the amblyopic visual system; the suppression of the amblyopic eye by the fellow eye is larger in magnitude than that of the fellow eye by the amblyopic eye. Numerous dichoptic masking studies have corroborated this asymmetry of interocular suppression.<sup>4-8</sup> The role of suppression could be pivotal in that there is evidence that the loss of binocularity (i.e., suppression) is the primary defect with the loss of monocular acuity being a secondary consequence.<sup>9,10</sup> Consequently, researchers have designed a training protocol that aims to reduce the magni-

tude of suppression as a first step in the treatment of amblyopia. These studies report an improvement in both binocular and monocular functions in patients with strabismic, as well as anisometropic, amblyopia after therapy designed to reduce suppression.<sup>11-15</sup> Along the same lines, Thompson et al.,<sup>16</sup> Clavagnier et al.,<sup>17</sup> and Moret et al.<sup>18</sup> showed that contrast sensitivity improvements for the amblyopic could be made in adults with amblyopia using repetitive transcranial magnetic stimulation (rTMS) and high-frequency transcranial electrical stimulation. In an animal study, Castano-Castano et al.<sup>19</sup> found that amblyopic rats can produced an almost full recovery in visual acuity after eight sessions of anodal transcranial direct current stimulation on the visual cortex. Although the mechanism of action here is unclear, rTMS and Continuous theta burst stimulation (cTBS) change the balance between excitation and inhibition in the brain and are likely to be also targeting interocular suppression.

The binocular imbalance of amblyopic observers is thought to depend on spatial frequency.<sup>20–22</sup> Ding et al.<sup>22</sup> measured binocular imbalance in binocular combination using suprathreshold sine wave gratings at three frequencies (0.68, 1.36, and 2.72 cycles per degree [c/d]) in both normal and amblyopic observers. They observed that the amblyopic eye needed a higher contrast than that of the fellow eye to achieve binocular balance, and that this difference peaked at high spatial frequencies.<sup>22</sup> However, their demonstration of a spatial frequency dependence is not well-founded because of the narrow range of spatial frequencies investigated. Kwon et al.<sup>20</sup> later showed that binocular imbalance in amblyopic observers is also dependent on spatial frequency using a band-pass filtered letter chart through a binocular rivalry paradigm at frequencies 0.5 to 5 c/d. They reported that amblyopic binocular imbalance is more noticeable at intermediate and high spatial frequencies.<sup>20</sup> Finally, Reynaud and Hess<sup>21</sup> used a suprathreshold contrast matching task to investigate binocular balance and reported a larger imbalance of binocular vision at high spatial frequencies in amblyopes. In summary, recent evidence supports the view that the binocular imbalance in amblyopia is greater the higher the spatial frequency.

We were interested in examining binocular imbalance using a binocular combination task at a suprathreshold level, which pertains to an everyday visual experience, and furthermore, whether this is simply a consequence of the monocular contrast threshold elevation for the amblyopic eye or whether it reflects a loss of function at a binocular site. To answer this, we examined binocular imbalance using a binocular combination approach for suprathreshold stimuli. To do so, we measured binocular imbalance as a function of spatial frequency in adults with amblyopia using a binocular orientation combination task that we had previously developed and validated.<sup>23</sup> In this task, observers were viewed with suprathreshold sinewave gratings at four different spatial frequencies (0.5, 1, 2, and 4 c/d). One grating shown to one eye was tilted clockwise, and another to the other eye, counter-clockwise. Subjects were asked to report the orientation of the fused grating percept. Our findings are in line with studies of binocular combination in amblyopia in that they show that the interocular stimulus contrast can be used to achieve an equal weight for the contributions from the two eyes,<sup>9,22,24,25,37,45,46</sup> which in this case would be the perception of a fused horizontal grating. Using this approach, one can demonstrate that the weight associated with the amblyopic eye signal is much weaker than that associated with the fellow eye signal when it comes to binocular combination; this agrees with numerous studies that use binocular combination of suprathreshold sinewave gratings.<sup>22,24,25</sup> This form of asymmetric weighting was more marked at higher spatial frequencies, consistent with recent studies.<sup>20,22</sup> Importantly, we also matched the visibility of stimuli based on the contrast threshold at each spatial frequency for the amblyopic eye, and still observed a more severe binocular imbalance at higher spatial frequencies. These results indicate that the binocular imbalance is not just a consequence of the contrast sensitivity deficit at high spatial frequencies in the amblyopic eye.<sup>26</sup> In other words, monocular deficits in contrast sensitivity are not the sole basis of binocular imbalances as assessed by binocular combination at high spatial frequencies in amblyopia. However, our assessment of the role of interocular contrast threshold difference in amblyopia indicates that the difference in contrast threshold between

eyes may be strongly related to the binocular imbalance in amblyopia.

## METHODS

### Participants

Ten patients with amblyopia (age:  $23 \pm 4.9$  [SD] years; seven male patients; two patients with mixed amblyopia, one patient with deprivation amblyopia, seven patients with anisometropia) and 10 age-matched adults ( $Z = -0.077$ ,  $P = 0.939$ ) with normal or corrected-to-normal vision (age:  $23 \pm 2.3$  years; four male normal adults; mean binocular visual acuity  $\leq 0.0$  logMAR) participated in the first experiment of our study. A subset of the patients (A2, A5, A6, A9, and A10) participated in the second experiment. The Table outlines the clinical characteristics of the 10 patients. If necessary, we corrected the refractive errors of all subjects before collecting data. All subjects were naive to the purpose of the study and provided written informed consent. The study was approved by the institutional review boards at Wenzhou Medical University and is in line with the Declaration of Helsinki.

### Apparatus

Using MATLAB R2016b (The Mathworks, Inc., Natick, MA, USA) with PsychToolBox 3.0.14<sup>27</sup> and Psykinematics (v2.0.1 GPU edition; KyberVision, Sendai, Miyagi, Japan) on a MacBook Pro (13-in., 2017; Apple, Inc., Cupertino, CA, USA), we conducted two experiments. For the binocular imbalance measurement, we dichoptically presented stimuli via head-mount goggles that had been Gamma-corrected (GOOVIS, AMOLED display; NED Optics, Shenzhen, China). They had a refresh rate of 60 Hz, a resolution of  $2560 \times 1600$  pixels, and a maximal luminance of  $150 \text{ cd/m}^2$ . For the contrast threshold measurement, using an ASUS monitor (ASUS PG279Q; AsusTek Computer Inc., Taipei, Taiwan) with a refresh rate of 60 Hz and a resolution of  $2560 \times 1440$ , we monocularly presented stimuli at a viewing distance of 60 cm to measure the contrast threshold of each of the eyes of our participants.

### Stimuli and Design

**Experiment 1: Binocular imbalance when the base contrast of the gratings shown to the amblyopic eye was set at 100%.** In our first experiment, we measured the balance point (BP) with a binocular orientation combination task at four spatial frequencies (0.5, 1, 2, and 4 c/d) using the method of constant stimuli. BP was defined as the interocular contrast ratio in which the two eyes were balanced in binocular combination (i.e., have equal contribution). We included two orientations of horizontally tilted sinusoidal gratings, one for each eye, at two different configurations. In the first configuration, the grating shown to the dominant (or fellow) eye had an orientation of  $+7.1^\circ$  counter-clockwise relative to the horizontal position, whereas a counterpart grating shown to the nondominant (or amblyopic) eye had that of  $-7.1^\circ$  clockwise relative to the horizontal position. In the second configuration, the grating shown to the nondominant (or amblyopic) eye had an orientation of  $+7.1^\circ$  counter-clockwise relative to the horizontal position, whereas a counterpart grating shown to the dominant (or fellow) eye had that of  $-7.1^\circ$  clockwise relative to the horizontal position. As a result, the total differ-

TABLE. Clinical Details of the Patients

Subject	Sex/Age	Type	Refraction OD/OS	VA (logMAR) OD/OS	Strabismus (pd)	RDS (Arc Seconds)	History
A1	M/12	depr	+3.00/-1.00*180 +0.50	0.70 0	∅	400	Detected capsular cataract at 11 years old, then received surgery and patched until now and has received vision therapy for 3 months
A2	F/22	mix	-6.00/-3.00*75 -6.00	0.22 -0.08	XP 10	100	Detected at 13 years old, patched occasionally for 1 year
A3	F/19	mix	-1.50/-4.50*180 +1.00/-6.50*175	0.10 0.22	X(T) 15	100	Detected at 12 years old, then received strabismus surgery for X(T) and patched occasionally for 6 months
A4	M/22	anis	-3.25/-0.50*11 +2.50/-1.50*10	-0.08 0.22	∅	400	Detected at 6 years old, patched for 1 year
A5	M/24	anis	-14.25/-1.00*180 -7.75/-0.75*10	0.70 0	∅	400	Detected at 18 years old, no treatment
A6	M/30	anis	-13.25/-2.00*40 -1.50/-1.75*7	0.22 0	∅	800	Detected at 10 years old then wore glasses until now and no patching treatment
A7	M/25	anis	-1.25/-0.50*55 +4.00/-2.00*125	0 0.5	∅	400	Detected at 18 years old, no treatment
A8	M/27	anis	+0.50 +2.50	0 0.22	∅	100	Detected at 9 years old, patched for 1 month
A9	M/25	anis	-0.50 +5.00/-3.00*178	0 0.70	∅	400	Detected at 18 years old, no treatment
A10	F/24	anis	plano +2.25	0 0.6	∅	400	Detected at 10 years old, no treatment

anis, anisometropia; depr, deprivation; mix, strabismic + refractive; ∅, Without strabismus OD, right eye; OS, left eye; plano, emmetropia pd, prism diopters; RDS, randot stereoacuity; VA, vision acuity; XP, exophoria; X(T), intermittent exotropia.

ence of orientation between the eyes was 14.2°. For patients with amblyopia, the base contrast of the grating shown to the amblyopic eye was fixed at 100% (Fig. 1C). However, for adults with normal vision, the base contrast of the grating shown to the dominant eye was fixed at 50%. The sign of eye dominance of normal adults was determined by a hole-in-the-hand test<sup>28</sup> before the start of data collection; proper demonstrations were provided with practice trials to ensure observers had understood the task. Based on psychophysical performance from practice trials, we established a distinct set of seven interocular contrast ratios (between 0 and 1) for each subject. We repeated each condition (one orientation configuration and one interocular contrast ratio) 20 times. Thus there were 280 trials (2 orientation configurations × 7 interocular contrast ratios × 20 repetitions) total in each block at each spatial frequency. We randomized the interocular contrast ratios and configurations throughout each trial. The test of BPs at different spatial frequencies were randomized in different observers.

**Experiment 2. Binocular imbalance when the base contrast of the gratings shown to the amblyopic eye was matched for suprathreshold contrast across spatial frequencies.** During the second experiment, we measured the BP of amblyopes with stimuli that were matched in their suprathreshold contrast for the amblyopic eyes across spatial frequencies (Fig. 1C). First, we measured the contrast threshold of the amblyopic eyes from five amblyopes (A2, A5, A6, A9, and A10) at four spatial frequencies (0.5, 1, 2, and 4 c/d). The subjects were asked to discriminate the orientation of the presented gratings (either 7.1° counter-clockwise or 7.1° clockwise relative to the horizontal position) with the untested eye occluded. The threshold contrast for the discrimination was obtained using a two-down/one-up staircase procedure and ended at the sixth

reversal point. The contrast of the stimuli was decreased proportionally by 50% before the first reversal and 12.5% thereafter when subjects correctly performed two consecutive trials and was increased by proportionally 25% when subjects incorrectly performed one trial. We repeated each staircase procedure three times. The last five reversal points of each repetition were averaged to obtain the threshold.

The contrast threshold of the amblyopic eye was always higher at 4 c/d than at the other spatial frequencies. Therefore to match the visibility of the amblyopic eye across spatial frequencies, we established the base contrast of the grating shown to the amblyopic eye at:

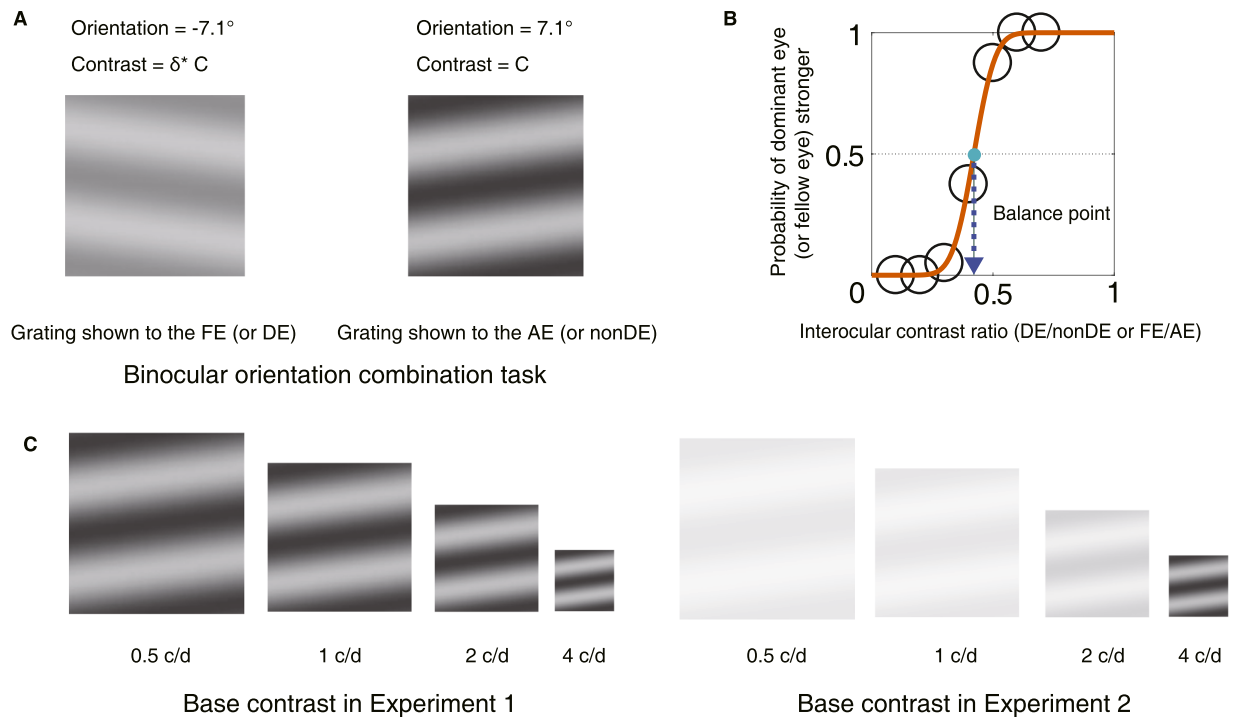
$$\text{base contrast}_{x\text{ c/d}} = \frac{\text{contrast threshold}_{x\text{ c/d}}}{\text{contrast threshold}_{4\text{ c/d}}} \quad (1)$$

where  $x\text{ c/d}$  refers to any of the 0.5, 1, and 2 c/d, and contrast threshold is the one from the amblyopic eye. *Contrast threshold*<sub>4 c/d</sub> refers to the contrast threshold of the amblyopic eye at 4 c/d. Therefore the base contrasts at 0.5, 1, 2, and 4 c/d in the amblyopic eye were matched to an identical suprathreshold level, that is,  $1/\text{Contrast threshold}_{4\text{ c/d}}$  times of the contrast threshold of the amblyopic eye (Equation 1).

By using the same procedure as that in Experiment 1, the patients subsequently performed the binocular orientation combination task to remeasure their BPs at 0.5, 1, and 2 c/d. The test order of these three spatial frequencies were randomized in different observers.

## Procedure

As was the case in our previous study,<sup>23</sup> there was an alignment phase and a test phase in a typical trial of the binocular orientation combination task. During the alignment phase,



**FIGURE 1.** An illustration of the binocular orientation combination task. **(A)** The stimuli in the binocular orientation combination task. In this task, two horizontal sinusoidal gratings with equal and opposite tilts ( $\pm 7.1^\circ$ ) were displayed to each eye. FE = fellow eye, DE = dominant eye, AE = amblyopic eye, nonDE = nondominant eye. **(B)** A psychometric function of one subject. The  $y$ -axis represents the probability in which the orientation of the fused percept tilted toward the grating shown to the dominant (or fellow) eye. The  $x$ -axis represents interocular contrast ratio (fellow eye/amblyopic eye or dominant eye/nondominant eye). The psychometric function was fitted via a cumulative Gaussian function of distribution. The BP is the interocular contrast ratio in which the orientation of the fused percept tilted toward the grating shown to the dominant (or fellow) eye 50% of the time. **(C)** In the first experiment, the base contrast of the grating shown to the amblyopic eye was set at 100%. In the second experiment, it was different for each spatial frequency (0.5, 1, 2, and 4 c/d). We matched the visibility of the grating (suprathreshold level) at 0.5, 1, and 2 c/d to that at 4 c/d for the amblyopic eye. The gratings are presented here for illustration and do not represent their actual sizes on the screen display.

subjects were asked to align the dichoptic presented crosses with fusion surrounding frame and diagonal bars in the two eyes. The alignment phase ensured all subjects were correctly aligned throughout the block. The coordinates of the two crosses were then used to present the stimuli in the followed test phase. Next a blank screen (comprised of a surrounding frame and diagonal bars in each eye to facilitate fusion) was displayed for 500 ms. This followed the test phase, during which a horizontal sinusoidal grating with a differing tilt was shown to each eye. Subjects were asked to report whether the fused grating appeared to be tilted in either a clockwise or counter-clockwise direction by pressing a keyboard. The gratings were shown until subjects completed the task. The next trial then started automatically after subjects' response.

In a typical trial of the contrast threshold measurement, a horizontally oriented ( $\pm 7.1^\circ$ ) Gabor, with a  $\sigma$  size of  $2^\circ$ , was shown to the tested eye for 117 ms. Subjects were asked to report whether the Gabor appeared to be tilted in either a clockwise or counter-clockwise direction by pressing a keyboard. Auditory feedback was provided to indicate the correctness of the response. The next trial started 500 ms after subjects' response.

### Data Analyses

We used a function of cumulative Gaussian distribution to fit the psychometric function, that is, the probability in which

the orientation of the fused percept tilted toward the grating shown to the dominant (or fellow) eye as a function of the interocular contrast ratio (dominant/nondominant or fellow eye/amblyopic eye). Then we estimated the BP, which is the interocular contrast balance ratio in which the orientation of the fused percept tilted toward the grating shown to the dominant (or fellow) eye 50% of the time. The BP of amblyopic adults is typically less than 1 due to their binocular imbalance. The BP of normal adults is usually approximately 1 with some deviation. We transformed the BP into the absolute value of BP in log scale ( $|\log BP|$ ) to capture the distance of the BP from the value of 1 (i.e.,  $|\log BP|$  shows the binocular imbalance). In an ideal observer with balanced eyes,  $|\log BP|$  would always be 0. However, an observer with imbalanced eyes might have a larger  $|\log BP|$ . In the following test, binocular imbalance means  $|\log BP|$ , whereas balance point means BP.

### Statistical Analysis

We used SPSS software version 23.0 (IBM Corporation, Armonk, NY, USA) and R software<sup>29</sup> to analyze statistics. Data were visualized using MATLAB. The normal distribution of data were assessed via the Shapiro-Wilk test. Homogeneity of variance assumption was examined via the Levene test. When a respective dataset was normally distributed, we used parametric measures, such as analysis of variance (ANOVA). When it was not normally distributed, we used



nonparametric procedures, such as the Friedman test, the Wilcoxon test, and a nonparametric (rank-based) ANOVA-like test of longitudinal data using the package 'nparLD' designed for R software.<sup>30</sup>

The nonparametric ANOVA-like test from the R package 'nparLD' is essentially a rank-based sample approximation from F-distribution's quantiles. Post hoc tests were performed when there was a significant main effect. *P* values were corrected based on the Bonferroni method. We report ANOVA-type statistics<sup>31</sup> throughout the article. As for the effect size, relative treatment effect (RTE) is reported. An RTE is a probability in which a randomly drawn observation from a particular subgroup (i.e., one of the four tested spatial frequencies) has a larger value than that from the combined mean distribution (the subdataset from the pertinent spatial frequency). To illustrate, an RTE of 0.2 for a subgroup means that there is a probability of 20% for the value of a randomly chosen observation to be higher than that from the rest of the data in the subgroup. Because RTE is a probability value, its range is from 0 to 1. When there is no effect, RTE is 0.5.

We also computed the slope of the binocular imbalance as a function of spatial frequency (i.e., |logBP| vs. spatial frequency). Subsequently, we compared the slope between the amblyopes and the adults with normal vision using the Welch *t*-test. Effect size is reported as *d*. The Pearson correlation test was used for data that were normally distributed, and nonparametric Spearman correlation test for data that were not normally distributed. The level of significance was established at *P* < 0.05.

## RESULTS

### Experiment 1. Binocular imbalance when the base contrast of the gratings shown to the amblyopic eye was set at 100%

We were interested in seeing whether spatial frequency would influence the binocular imbalance of adults with amblyopia or normal vision. In Figure 2, we plotted individuals' psychometric functions for the binocular orientation task. It is clear that the fitted BP decreased as spatial frequency increased for all amblyopes and some normal controls. To visualize the magnitude of binocular imbalance for both adults with normal vision and amblyopia, we plotted the averaged |logBP| (i.e., the absolute value of the BP on log scale) as a function of spatial frequency as shown in Figure 3. We found that spatial frequency dependence of |logBP| was quite different between the two groups. First, we checked whether our dataset per each group (control and amblyopes) was normally distributed by performing the Shapiro-Wilk test and found that both datasets had normal distributions (*P* > 0.05). Then we checked for homogeneity of variance assumption by performing the Levene test. Although the variance was not homogeneous across the two populations (*P* < 0.05), we realized that ANOVA would still be apt because we had an equal number of subjects in both groups (*n* = 10 in each group). Therefore a 2-way mixed ANOVA was performed (within-subject factor: spatial frequency; between-subject factor: subject group). We found a significant main effect of subject group ( $F(1, 18) = 66.97, P < 0.001, \eta_g^2 = 0.74$ ), and a significant main effect of spatial frequency ( $F(1.44, 26.01) = 51.6, P < 0.001, \eta_g^2 = 0.40$ ). Moreover, a significant interaction between subject

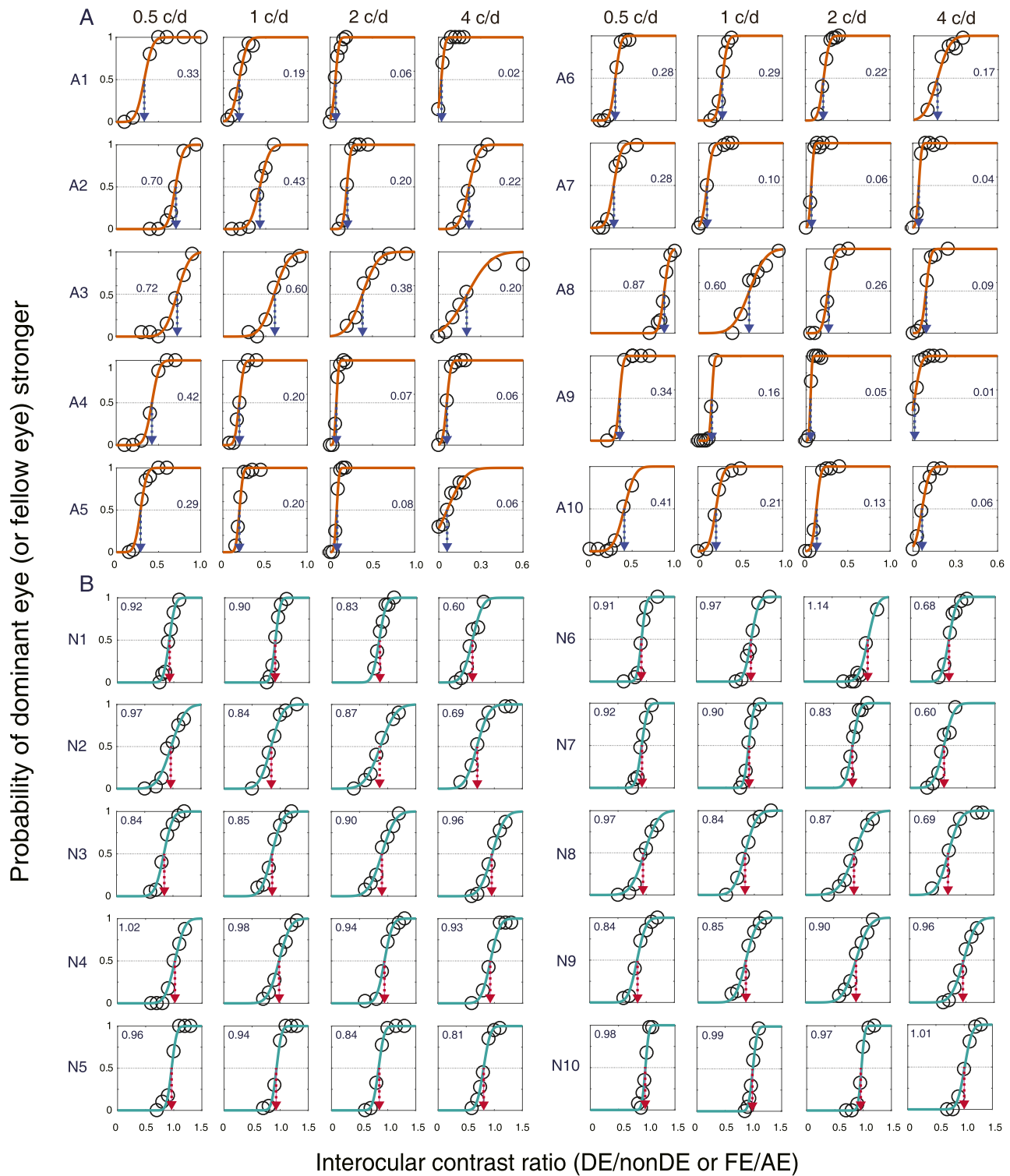
group and spatial frequency was found ( $F(1.44, 26.01) = 38.12, P < 0.001, \eta_g^2 = 0.33$ ). Because we found a main effect of subject group, we investigated the effect of the subject group at every spatial frequency via post hoc analysis, and adjusted *P* values according to the Bonferroni method. We found that on all spatial frequencies, the main effect of subject group was significant (adjusted *P* < 0.001); in other words, the |logBP|s were significantly different between groups at all spatial frequencies. Moreover, because we found a main effect of spatial frequency, we investigated the effect of spatial frequency at each group via post hoc analysis. We found that on both groups, the main effect of spatial frequency was significant (control group, *P* = 0.048; amblyopia group, *P* < 0.001); in other words, the |logBP|s were significantly different between all spatial frequencies at both groups, but more so in the amblyopic group.

We also computed the slope of binocular imbalance as a function of spatial frequency (i.e., |logBP| vs. spatial frequency), and subsequently compared the slopes between the groups. First, we examined whether there were any outliers in the dataset in each subject group and found none. Next we found that our dataset in each group was normally distributed by performing the Shapiro-Wilk test (*P* > 0.05). Finally, by performing the Levene test, we checked for homogeneity of variance assumption and found that the dataset in each group had heterogeneous variances (*P* = 0.020). Therefore because the variance was heterogeneous, we performed the Welch *t*-test, which reported a significant difference between the groups ( $t(9.94) = -6.82, P < 0.001, d = 3.05$ ).

### Experiment 2. Binocular imbalance when the base contrast of the gratings shown to the amblyopic eye was matched for suprathreshold contrast across spatial frequencies

Previous studies show that monocular contrast sensitivity of the amblyopic eye is reduced for high spatial frequencies.<sup>32,33</sup> In Experiment 1, a fixed contrast of 100% was used as the base contrast for the amblyopic eye. However, this methodology raises the question of whether the spatial frequency-dependent binocular imbalance that we observed in Experiment 1 was simply because of the higher monocular contrast threshold of the amblyopic eye at high spatial frequency. To answer this question, we conducted Experiment 2, in which binocular imbalance was measured when the base contrast of the gratings shown to the amblyopic eye was set as the same suprathreshold contrast (i.e., visibility-matched) across spatial frequencies. Because amblyopes display "contrast constancy," namely they see contrast veridical at or just above threshold,<sup>34,35</sup> our suprathreshold contrast criteria would have also ensured that all the stimuli were seen to be of the same perceptual contrast across spatial frequencies.

Five amblyopes (A2, A5, A6, A9, and A10) participated in Experiment 2. First, we measured the contrast sensitivity at four spatial frequencies (0, 1, 2, 4 c/d) for both the amblyopic and fellow eye. As shown in previous studies, the contrast sensitivity of the amblyopic eye was impaired increasingly at high spatial frequency. A Friedman test indicated that contrast sensitivity was significantly different across spatial frequency (*P* = 0.0036, Kendall's W [effect size] = 0.90). Because a significant main effect of spatial frequency was

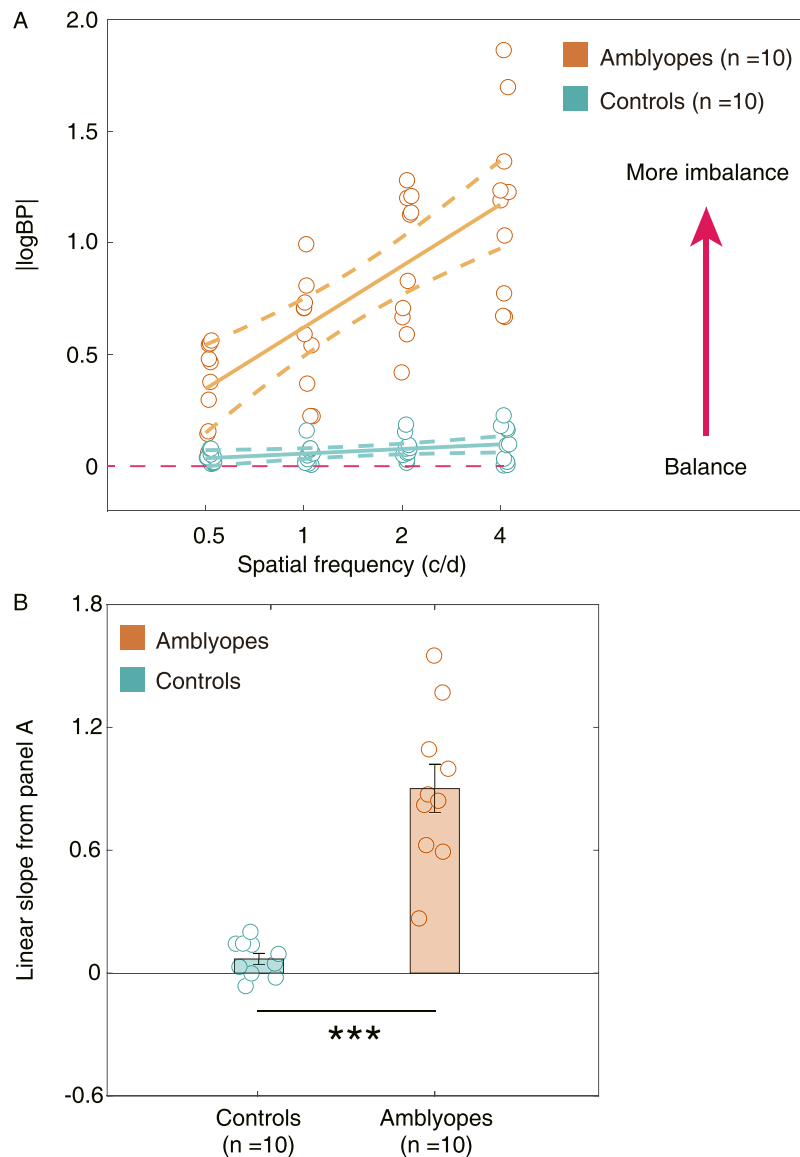


**FIGURE 2.** Psychometric functions of all subjects from the first experiment. **(A)** The orange psychometric fits are from 10 amblyopic patients (A1-10). The dotted arrow lines (navy) denote the estimated BPs. **(B)** The cyan psychometric fits are from 10 normal observers (N1-10). The dotted arrow lines (red) denote the estimated BPs.

found, multiple pairwise comparisons were performed via the Wilcoxon signed-rank tests as post hoc analysis to identify at which spatial frequency did the contrast sensitivity differ significantly; *P* values were adjusted according to the Bonferroni method. The contrast sensitivity of these five

amblyopes was significantly different between 0.5 and 4 c/d, and 1 and 4 c/d (adjusted *P* = 0.048).

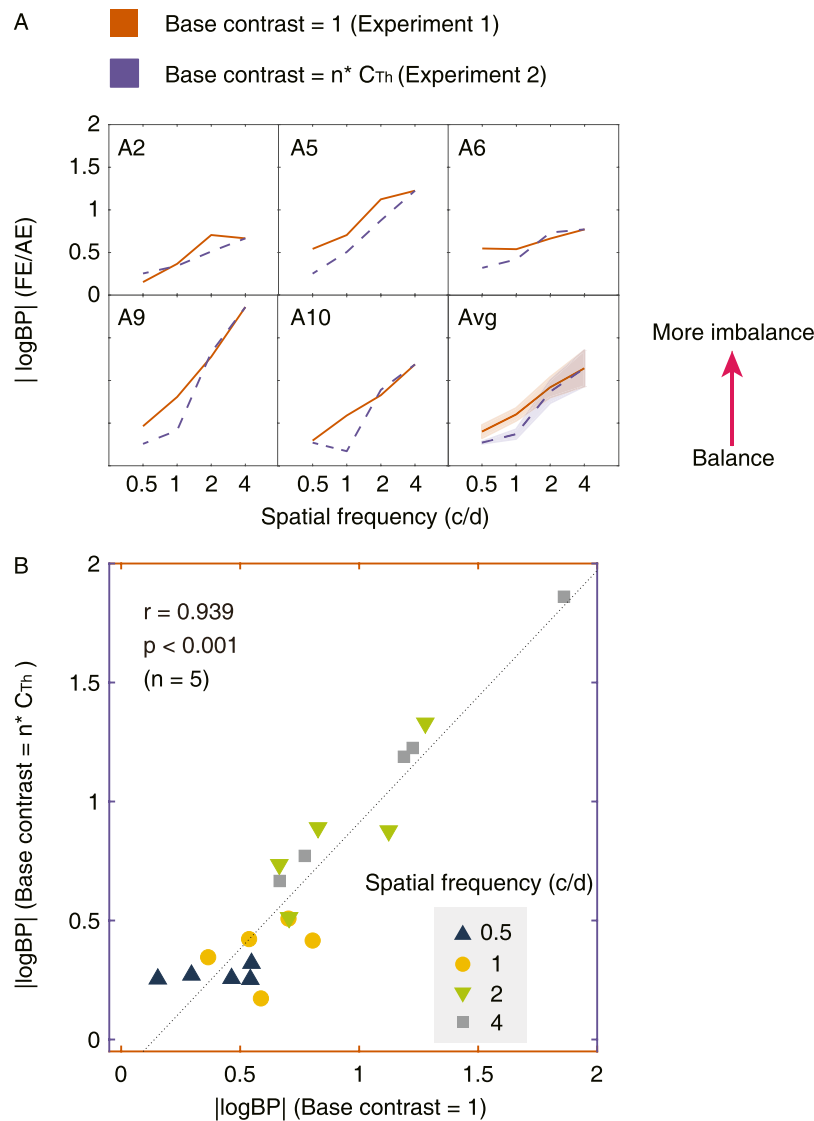
In Figure 4A, we illustrate the magnitude of binocular imbalance by plotting the visibility-matched  $|\log BP|$  as a function of spatial frequency for five amblyopes



**FIGURE 3.** Comparison of the results from Experiment 1 between the normal ( $n = 10$ ) and amblyopic ( $n = 10$ ) observers. **(A)** The binocular imbalance, that is, the absolute value of the BP in log scale ( $|\log BP|$ ), as a function of spatial frequency. *Orange points* denote amblyopic observers; *cyan points* denote normal observers. The *solid lines* and surrounded *dashed lines* represent the best linear fit with 95% confidence interval. The horizontal *red dashed line* represents ideal binocular balanced eyes. **(B)** Averaged slopes of the linear regression from panel A. The linear regression slope was calculated for each subject, and the values were averaged across each subject group. *Cyan bar* represents the averaged slope of the normal observers, *orange* the averaged slope of the amblyopic counterparts. *Error bars* show  $\pm$  SEM. \*\*\*Represent  $P < 0.001$  in a two-tailed paired samples  $t$ -test.

(purple plots in Fig. 4A). To quantify whether there was a significant main effect of spatial frequency, we performed several statistical tests. First, we checked whether the data as grouped by each spatial frequency were normally distributed via the Shapiro-Wilk test, which reported a non-normal distribution ( $P < 0.05$ ). Because our datasets were not normally distributed, we performed a nonparametric procedure, namely, the Friedman test (within-subject factor = spatial frequency) to examine whether the averaged visibility-matched  $|\log BP|$  increased significantly as spatial frequency increased. Similar to that in the first experiment (see the orange lines in Fig. 4A), the visibility-matched  $|\log BP|$  (i.e., when the suprathreshold contrast of stimuli shown to the amblyopic eye was matched across spatial

frequencies) significantly increased as spatial frequency increased:  $P = 0.0029$ , Kendall's  $W$  (effect size) = 0.94. Because a significant main effect of spatial frequency was found, multiple pairwise comparisons were performed via the Wilcoxon signed-rank test as post hoc analysis to identify at which spatial frequency did the visibility matched  $|\log BP|$  differ significantly;  $P$  values were adjusted according to the Bonferroni method. Visibility-matched  $|\log BP|$  of these five amblyopes were significantly different between 0.5 and 2 c/d, 0.5 and 4 c/d, 1 and 2 c/d, and 1 and 4 c/d (adjusted  $P = 0.048$ ). Therefore, whether the stimuli shown to the amblyopic eye is of a fixed absolute contrast (e.g., Experiment 1) or of the same suprathreshold contrast (e.g., Experiment 2) across spatial frequen-



**FIGURE 4.** Comparison of the results from amblyopic observers ( $n = 5$ ) between Experiments 1 and 2. **(A)** The  $|\log BP|$  is plotted as a function of spatial frequency for five patients and their average in different subpanels. In each subpanel, *solid orange curves* represent results from Experiment 1 in which the base contrast of the amblyopic eye was fixed at 100% and that of the fellow eye modulated at seven contrast levels in the measure. *Dashed purple curves* represent results from Experiment 2 in which the base contrast of the gratings shown to the amblyopic eye was set as equal suprathreshold contrast level (i.e.,  $1/Contrast\ threshold_{4\ c/d}$  times of the contrast threshold of the amblyopic eye) across spatial frequencies. The *shaded areas* in the last subpanel indicate the range of  $\pm$  SEM between subjects. **(B)** Each point represents the result of one amblyopic observer. The correlation was found to be significant,  $r = 0.939$ ,  $P < 0.001$ .

cies, the binocular imbalance increased as spatial frequency increased.

Figure 4B shows the relationship between the binocular imbalance values from both experiments ( $x$ -axis: Experiment 1,  $y$ -axis: Experiment 2). A two-tailed Pearson correlation test revealed a strong correlation ( $r = 0.939$ ,  $P < 0.001$ ). It should be noted that for 4 c/d, the criteria of setting the base contrast in Experiment 1 (fixed at 100%) and that in Experiment 2 ( $1/Contrast\ threshold_{4\ c/d}$  times of the contrast threshold of the amblyopic eye) are the same. Therefore we used patients' results at 4 c/d from Experiment 1 instead of remeasuring them in Experiment 2. Including the binocular imbalance values when the gratings were shown at 4 c/d would make the correlation in Figure 4B seem more robust. We thus reassessed the correlation between both experiments after excluding the

condition when the gratings were shown at 4 c/d. Nonetheless, we still observed a significant correlation ( $\rho = 0.754$ ,  $P = 0.001$ ). We were interested in whether the amblyopes exhibited different levels of binocular imbalance across spatial frequency depending on the experiment (first and second). The binocular imbalance from Experiment 1 was obtained when the base contrast shown to the amblyopic eye was set at 100%, whereas in Experiment 2 the base contrast of the amblyopic eye was visibility matched (see details earlier). First, we performed the Shapiro-Wilk test and found that our dataset as grouped by different spatial frequency and experiment type was not normally distributed ( $P < 0.05$ ). For this reason, nonparametric (rank-based) ANOVA-like analyses of longitudinal data were performed (two within-subject factors: experiment type, spatial frequency). The main effect of spatial frequency was significant ( $F(1.27,$



$\infty$ ) = 45.68,  $P < 0.001$ , RTEs = 0.19, 0.35, 0.69, and 0.77 for 0.5, 1, 2, and 4 c/d), so was the main effect of experiment type ( $F(1, \infty) = 19.10$ ,  $P < 0.001$ , RTEs = 0.55 and 0.45 for Experiments 1 and 2). Post hoc analysis tests for a main effect of spatial frequency showed a significant difference in  $|\log BP|$  between all pairs of the four spatial frequencies ( $P < 0.001$ ). However, the interaction was nearly significant ( $F(2.05, \infty) = 2.80$ ,  $P = 0.060$ ). The near-significant interaction effect indicates that the plots of  $|\log BP|$  are not parallel in the function of spatial frequency between the two experiment types. Nevertheless, the significant main effect of spatial frequency indicates that even if the visibility of the grating shown to the amblyopic eye is matched in terms of suprathreshold across spatial frequencies, binocular imbalance is increasingly impaired at high spatial frequencies. This suggests that an increased monocular threshold solely does not explain the existence of binocular imbalance in amblyopia.

The results in Experiment 2 indicate that the spatial frequency dependence of binocular imbalance that we found in Experiment 1 might not be explained by the difference of amblyopic eye's contrast threshold across different spatial frequencies. One interesting issue is whether the binocular imbalance is linked to the interocular contrast threshold ratio across spatial frequencies. To answer this question, we conducted a correlation analysis between the measured BP in Experiment 1 and the interocular contrast threshold ratio of the five patients who participated in Experiment 2. A significant correlation was found between them:  $r = -0.64$ ,  $P = 0.002$ .

In our measurement, the binocular imbalance (i.e., BP) was defined as the interocular contrast ratio when resulted in an equal contribution of both eyes in binocular combination (Equation 2):

$$\text{Balance point} = \frac{\text{Contrast}_{FE}}{\text{Contrast}_{AE}}, \quad (2)$$

which could be rewritten based on the contrast thresholds of the two eyes (Equation 3):

$$\text{Balance point} = \frac{\text{Contrast}_{FE}}{\text{Contrast}_{AE}} = \frac{N_{FE} \times \text{Contrast threshold}_{FE}}{N_{AE} \times \text{Contrast threshold}_{AE}}. \quad (3)$$

Therefore, to further illustrate the relationship between the binocular imbalance in binocular orientation combination and interocular contrast threshold ratio, we normalized the measured BPs in Experiments 1 and 2 to individuals' interocular contrast thresholds (Equation 4):

$$\begin{aligned} \text{Normalized balance point} \\ = \frac{N_{FE}}{N_{AE}} = \text{Balance point} \times \frac{\text{Contrast threshold}_{AE}}{\text{Contrast threshold}_{FE}} \end{aligned} \quad (4)$$

The normalized BP thus indicates the difference between the measured BP and the interocular contrast threshold ratio. If the normalized BP is close to 1, it shows that interocular difference in contrast threshold might be relevant to binocular imbalance in amblyopia. However, if it deviates significantly from 1, interocular threshold difference might not be relevant to the binocular imbalance.

Figure 5A illustrates the normalized BPs from both experiments as a function of spatial frequency. Patients' BPs

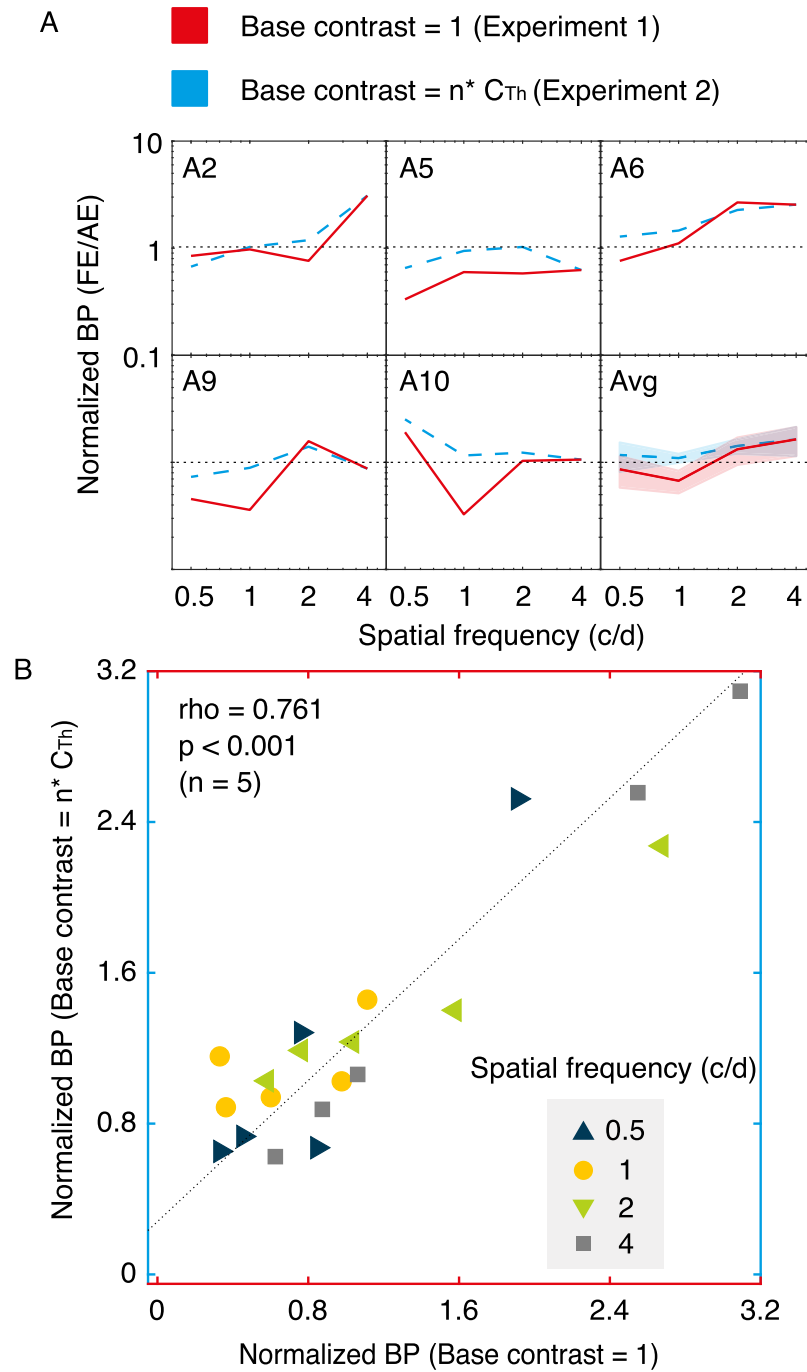
were not that different across spatial frequencies after being normalized to their interocular contrast threshold ratios. This is true for both Experiments 1 and 2. Statistical tests were performed to verify this qualitative observation. First, we separated the entire dataset by the experiment type (1 or 2) and spatial frequency, and then checked whether each of the subdataset assumed a normal distribution using the Shapiro-Wilk test. Because we found that some of the datasets had non-normal distribution, we opted for a nonparametric procedure to verify the qualitative observation from Figure 5A. To do so, nonparametric (rank-based) ANOVA-like analyses of longitudinal data were performed (two within-subject factors: experiment type, spatial frequency). The main effect of spatial frequency was not significant ( $F(2.35, \infty) = 1.75$ ,  $P = 0.16$ , RTEs = 0.39, 0.41, 0.61, and 0.60 for 0.5, 1, 2, and 4 c/d). However, the main effect of experiment type was significant ( $F(1, \infty) = 37.48$ ,  $P < 0.001$ , RTEs = 0.43 and 0.57 for Experiments 1 and 2). The interaction between the two within-subject factors was not significant ( $F(2.06, \infty) = 1.71$ ,  $P = 0.18$ ). These findings indicate that the normalized BP was different between the two experiments. Moreover, one-sample Wilcoxon test revealed no significant difference between the normalized BPs (at each spatial frequency and experiment) and 1 ( $P > 0.063$ , effect size  $r > 0.06$ ).

In addition, we plotted correlation between the normalized BPs from both experiments (Fig. 5B). A Spearman correlation test revealed a significant correlation between the normalized BPs from both experiments ( $\rho = 0.761$ ,  $P < 0.001$ ). We also checked the correlation after excluding the condition in which the gratings had a spatial frequency of 4 c/d. A significant correlation was found ( $r = 0.865$ ,  $P < 0.001$ ).

## DISCUSSION

Accumulating evidence suggests that the clinical characteristics of amblyopia depend on spatial frequency. Monocular contrast sensitivity for the amblyopic eye is reduced more for high spatial frequencies.<sup>32,33</sup> Perceived spatial distortions<sup>47</sup> and phase discrimination are intact at low but not at high frequencies.<sup>36</sup> More importantly, it has been suggested that the binocular imbalance is also more marked at high spatial frequencies.<sup>20,22</sup> The question is, "Is there a direct causal relationship between the monocular contrast sensitivity deficit and the binocular balance deficit in the high spatial frequency range in binocular combination?" Therefore we investigated the extent of the binocular imbalance across four spatial frequencies (0.5, 1, 2, and 4 c/d) using a binocular orientation combination task, which allowed precise measurements of binocular balance even at high frequencies.<sup>23</sup>

We performed two experiments. For the first experiment, we set the base contrast of the amblyopic eye to 100%, whereas modulating the contrast of the fellow eye to measure binocular balance (Fig. 1C). Our findings agree with recent studies<sup>22,25,37</sup>; namely, that a stimulus of fixed and equal contrast in the amblyopic eye was weighted much less than that of the fellow eye across a wide range of frequencies but more so at high frequencies (Fig. 3). For the second experiment, we measured the contrast threshold of each amblyope at each spatial frequency. This was done at a much shorter presentation duration of 117 ms compared with the binocular combination measure (unlimited, but in practice



**FIGURE 5.** Normalized BPs under the two conditions. **(A)** The *solid red curve* represents normalized BP ( $N_{BP}$ ) from Experiment 1 in which the base contrast of the amblyopic eye was fixed at 100%; the *dashed blue curves* from Experiment 2 in which the perceived visibility of the stimuli was matched across spatial frequencies. The center horizontal *dotted line* (i.e., a value of 1) indicates the prediction that the BPs are affected totally by interocular contrast threshold difference across spatial frequencies.  $N_{BP}$  is close to 1 in all spatial frequencies under the two conditions (all  $P > 0.063$ , effect size  $r > 0.06$ ; one-sample Wilcoxon test). The averaged  $N_{BP}$  is plotted in the last subpanel for both conditions. The *shaded areas* indicate the range of  $\pm$  SEM between subjects. **(B)** Each point represents the result of one amblyopic observer. A significant correlation was found between the two normalized BPs:  $\rho = 0.761$ ,  $P < 0.001$ ; Spearman correlation test.

typically 1–2 seconds). However, we would not expect this short duration to have affected our measure of the contrast sensitivity deficit in amblyopia because it does not exhibit a stimulus duration dependency.<sup>38</sup> The base contrast of the amblyopic eye was subsequently set so that, as a function of spatial frequency, it was at a fixed suprathreshold value (Fig. 1C). If the contrast sensitivity dependence on spatial

frequency in the amblyopic eye was the sole factor in determining how the binocular imbalance changed with higher frequencies, this experiment would have corrected for this and as a result the effect of spatial frequency on binocular imbalance that was observed in Experiment 1 would be much reduced or eliminated. However, for base contrast stimuli of equal suprathreshold contrast shown to the ambly-

opic eye, we still observed a more noticeable binocular imbalance at high spatial frequencies (Fig. 4). These findings thus suggest that the monocular contrast sensitivity dependence on high frequencies in the amblyopic eye (i.e., signal attenuation in the amblyopic eye) is inadequate to explain why binocular imbalance increases with spatial frequency in amblyopia.

Contrast sensitivity, which is assessed at threshold level, and binocular balance, which has been often measured with suprathreshold stimuli,<sup>20–22</sup> might not have a direct relationship. Monocular deficits on contrast sensitivity at intermediate and high frequencies have been repeatedly reported.<sup>32,33</sup> Conversely, several studies have reported normal suprathreshold contrast perception in amblyopia.<sup>26,34,35,39</sup> These conflicting reports may suggest that measurements from contrast sensitivity (measured with stimuli shown at threshold level) and binocular imbalance (often assessed with suprathreshold stimuli) might not be directly related. At low spatial frequency (e.g., 0.68 c/d), Huang et al.<sup>25</sup> reported that the effective contrast of the amblyopic eye in binocular combination is approximately 11% to 28% of the same contrast presented of the fellow eye. This proportion is much less than the ratio of contrast sensitivity (0.73–1.42), suggesting that the phenomena of contrast sensitivity deficit and binocular imbalance could depend on separate mechanisms. Ding et al.<sup>22</sup> also found a larger difference in the contrast between the amblyopic and fellow eyes than an interocular difference in contrast sensitivity to attain effective binocular imbalance at up to 2.72 c/d. However, both studies do not clarify the relationship between binocular imbalance and interocular difference in contrast threshold. For the spatial frequency range that we assessed in the current study (i.e., 0.5–4 c/d), to quantify the relationship between binocular imbalance and interocular difference in contrast threshold, we normalized the BP of each amblyope (see Methods) at each spatial frequency. The normalized BP did not deviate significantly from 1 (Fig. 5), suggesting that interocular difference in contrast threshold is strongly related to the extent of the binocular imbalances at high spatial frequencies. Our results indicate that although monocular attenuation, as reflected by poor contrast sensitivity at high frequencies in the amblyopic eye, is not solely responsible for the spatial frequency dependent binocular imbalance in binocular combination, the contrast threshold difference between the eyes is likely to be a contributing factor. This same explanation has been advanced by Baker et al.<sup>2</sup> to account for interocular masking results in amblyopes. If this is the case, it would be interesting to see whether treatments that target reducing the interocular contrast threshold difference<sup>13,40–42</sup> would also recover the binocular balance at high spatial frequencies. Nevertheless, because the observations from our Experiment 2 were from only five patients, future studies with larger sample size will need to see whether this is the case in different types of amblyopia. Actually, not all studies involving interocular masking in amblyopia would agree with this view. A number of recent studies have demonstrated that there is greater masking of the amblyopic eye by the fellow eye and vice versa for stimuli of equal-suprathreshold contrast.<sup>8,21</sup> These results argue that to explain the unequal interocular masking in amblyopia one needs to consider models involving changes in the contrast-gain of interocular connections and not just the interocular contrast sensitivity. This is also supported by Huang et al.<sup>43</sup> who measured binocular phase and contrast combination of suprathreshold sinewave grat-

ings in anisometric amblyopia and fitted the data to a multichannel model of contrast gain control.<sup>44</sup> Their results suggest that signals in the amblyopic eye are highly attenuated and that the contrast gain control from the fellow eye is stronger than that of the amblyopic eye, resulting in strengthened suppression of the signal and gain control of the amblyopic eye (direct and indirect interocular inhibition). Furthermore, Ding et al.<sup>22</sup> also found that the sensitivity deficit alone is not responsible for binocular imbalance in amblyopia. Thus at the present time there is some controversy on the exact nature of the interocular masking that characterizes amblyopia. However, what one can say is that binocular balance and interocular inhibition (as reflected by interocular masking) may not be one and the same thing.

## CONCLUSIONS

There are three possible mechanisms that could underlie the binocular imbalance that characterizes amblyopia: attenuation at a purely monocular site (e.g., poor contrast sensitivity) in the amblyopic eye, imbalanced interocular contrast sensitivity (binocular site), or an imbalance in the binocular inhibitory interaction between the amblyopic and fellow eyes. Our results show that amblyopia exhibits a more noticeable binocular imbalance at higher spatial frequencies in binocular combination; the difference in contrast threshold between eyes is likely to be strongly related to the binocular imbalance in amblyopia. There may be a functional distinction between binocular balance and interocular suppression, the latter being reflected by masking behavior.

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