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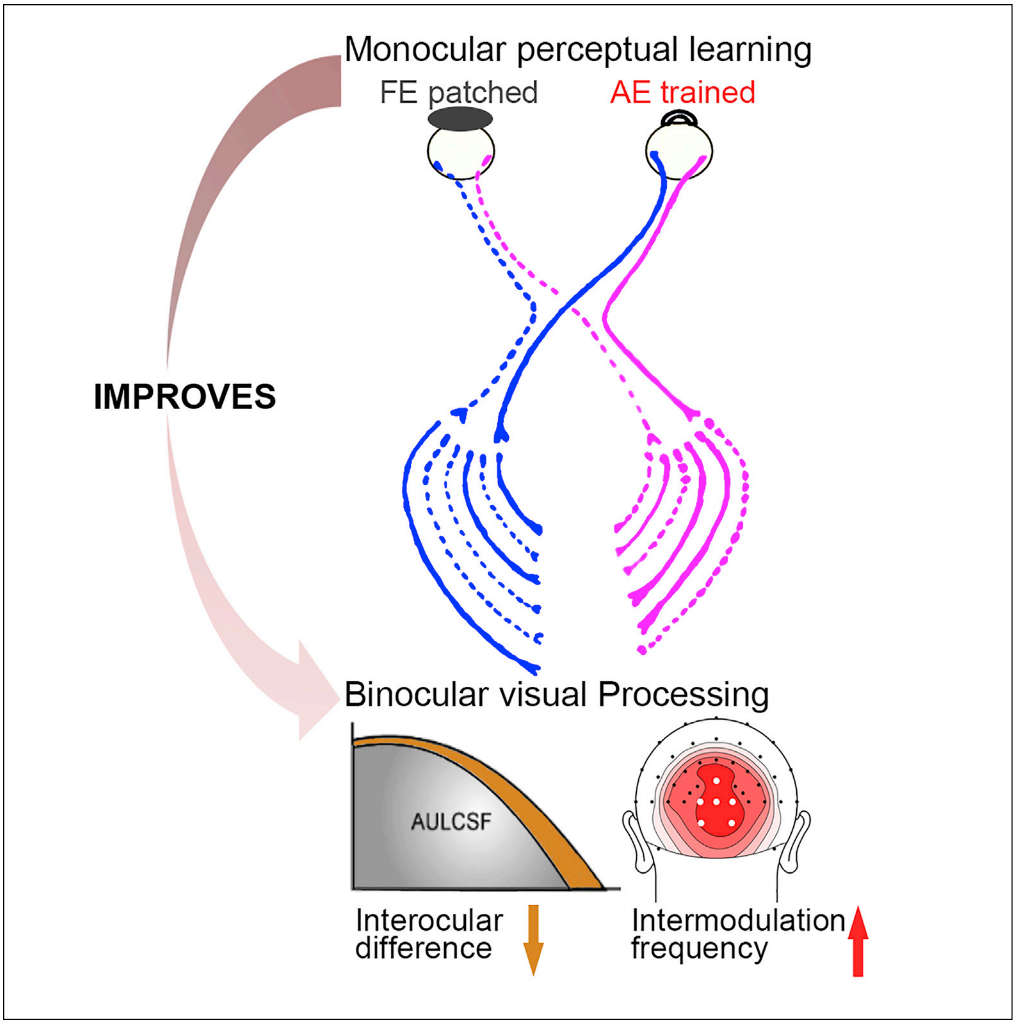

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HIGHLIGHTS

PL reduced the interocular difference

PL increased the amplitude of a binocular SSVEP component

Training in the amblyopic eye improves binocular visual processing

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Effects of Monocular Perceptual Learning on Binocular Visual Processing in Adolescent and Adult Amblyopia

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SUMMARY

Re-establishing normal binocular visual processing is the key to amblyopia recovery beyond the critical period of visual development. Here, by combining perceptual learning, behavioral testing, and steady-state visually evoked potentials (SSVEPs), we examined how monocular perceptual learning in the amblyopic eye could change binocular visual processing in the adolescent and adult amblyopic visual system. We found that training reduced the interocular difference between amblyopic and fellow eyes and increased the amplitude of a binocular SSVEP component, with a significant negative correlation between the two measures. Our results demonstrate that training in the amblyopic eye primarily improves binocular rather than monocular visual processing in the amblyopic visual system, suggesting that behavioral training could potentially address key neural deficits in adolescent and adult amblyopia.

INTRODUCTION

Amblyopia is the most common developmental neuro-visual condition and affects approximately 2%–5% of the world population (Holmes and Clarke, 2006). It is mostly a cortical disorder resulting from the formation of abnormal binocular visual inputs during early postnatal development due to strabismus, large refractive errors, or form-deprivation (Holmes and Clarke, 2006; Hubel and Wiesel, 1964). In animal models, amblyopia is often associated with the abnormal development of ocular dominance columns (Hubel and Wiesel, 1964). In vision clinics, patients with amblyopia exhibit impaired spatial and binocular vision (Holmes and Clarke, 2006). Studies have shown that both monocular and binocular deficits are important predictors of amblyopic visual functions (Hess and Thompson, 2015; Kiorpes, 2006, 2016; McKee et al., 2003). Re-establishing normal binocular visual processing in the amblyopic visual system is the key to amblyopia recovery (Hess and Thompson, 2015; Hubel and Wiesel, 1964; McKee et al., 2003).

In current clinical practice, children with amblyopia are treated by monocularly patching or penalizing the non-amblyopic eye, whereas adolescents and adults with amblyopia are not treated (Holmes and Clarke, 2006). However, a large number of recent studies have shown that monocular perceptual learning in the amblyopic eye could improve visual functions in adolescents and adults with amblyopia (Dosher and Lu, 2017; Hess and Thompson, 2015; Huang et al., 2008; Levi and Li, 2009; Lu et al., 2005; Polat, 2009; Polat et al., 2004; Sagi, 2011; Sasaki et al., 2010; Watanabe and Sasaki, 2015; Zhou et al., 2006). In this study, we ask the following question: How does monocular perceptual learning in the amblyopic eye change binocular visual processing in the amblyopic visual system? We combined perceptual learning, behavioral testing, and steady-state visually evoked potentials (SSVEPs) to address this question.

SSVEPs are often used to tag neural responses to visual stimuli at specific temporal frequencies (Norcia et al., 2015). The technique has been widely used to investigate neural responses during binocular rivalry (Katyal et al., 2016; Regan and Regan, 1988; Zhang et al., 2011). In response to dichoptically presented visual stimuli flickering at two different temporal frequencies (f_1, f_2), SSVEP components presented at fundamental (f_1, f_2) and harmonic frequencies (mf_1, nf_2) are associated with monocular visual processing and SSVEP components at the intermodulation frequencies $m\,f_1 \pm n\,f_2$ are associated with binocular visual processing (Baitch and Levi, 1988; Regan and Regan, 1989; Zhang et al., 2011). Here, we used the amplitudes of

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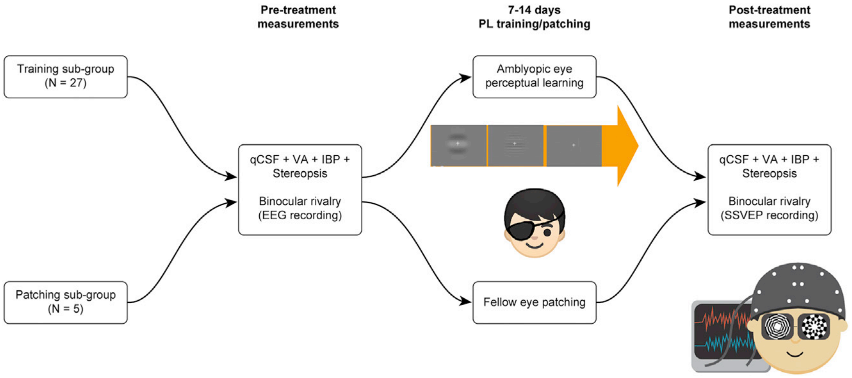

Figure 1. Experimental Procedure

Subjects in the treatment groups were either trained in a monocular 2AFC identification task in the amblyopic eye or received patching treatment in the fellow eye. Before and after treatment, we measured monocular visual acuity, monocular contrast sensitivity function (Hou et al., 2010), interocular balance point in binocular phase combination (Ding and Sperling, 2006), stereopsis, and SSVEPs while the subjects viewed flickering binocular rivalry stimuli. See also Table S1 for clinical details.

the intermodulation components of SSVEP to evaluate changes in binocular visual processing following perceptual learning in anisometropic amblyopia. We hypothesized that perceptual learning would reduce the interocular difference between amblyopic and fellow eyes and that this reduction would be associated with higher amplitudes of intermodulation SSVEP components.

RESULTS

A total of forty-six patients with anisometropic amblyopia and twelve subjects with normal vision participated in this study. Twenty-seven of the amblyopic subjects were trained in a monocular two-alternative-forced-choice (2AFC) identification task at the cutoff spatial frequency in the amblyopic eye (Huang et al., 2008), and five of these subjects received patching treatment (see Supplemental Information for details). We recorded SSVEPs while the subjects viewed binocular rivalry stimuli consisting of a pair of incompatible circular checkerboard patterns flickering at two different temporal frequencies. The SSVEPs were recorded for all subjects at baseline and for those in the treatment groups after treatment (Figure 1). To gauge the impact of perceptual learning on amblyopic vision, a number of visual functions, including monocular visual acuity (VA), monocular contrast sensitivity function (CSF), interocular balance point (IBP) in binocular phase combination, and stereopsis (Hou et al., 2010; Huang et al., 2008; McKee et al., 2003), were also assessed before and after treatment (Figure 1).

SSVEPs and Behavioral Measurements at Baseline

We first evaluated SSVEPs in all the subjects at baseline (see Supplemental Information for details). The SSVEPs exhibited robust monocular responses at the two fundamental (f1, f2) and second harmonic flicker frequencies (2f1, 2f2) ($M_{f1} = 5.122 \pm 0.417$, $t_{57} = 9.889$, $p < 0.001$; $M_{f2} = 6.535 \pm 0.573$, $t_{57} = 9.661$, $p < 0.001$; $M_{2*f1} = 4.538 \pm 0.424$, $t_{57} = 3.659$, $p = 0.001$; $M_{2*f2} = 5.367 \pm 0.450$, $t_{57} = 5.301$, $p < 0.001$) (Figure 2A). The SSVEPs also exhibited significant binocular responses at a series of intermodulation frequencies (Cunningham et al., 2017; Liu-Shuang et al., 2014; Rossion et al., 2012), with the clearest response recorded at f1+f2 ($M_{f1+f2} = 2.1589 \pm 0.133$, $t_{57} = 8.711$, $p < 0.001$) (Figure 2B). We further assessed the correlation between SSVEP responses and behavioral measures of monocular and binocular visual functions. For the amblyopic subjects, only the amplitude of the 2*f2 component was negatively correlated with the cutoff spatial frequency of the amblyopic eye, cutoff_{AE} ($r = -0.276$, $p = 0.036$); none of the other correlations between monocular behavioral measures in amblyopic and fellow eyes (VA, AULCSF [Area Under the Log CSF, see Figure 2C for diagram]) and the amplitudes of f1, 2*f1, f2, or 2*f2 was significant (all $p > 0.064$). Across all the subjects at baseline, the amplitude of the f1+f2 component was negatively correlated with the interocular AULCSF difference ($r = -0.312$, $p = 0.017$; Figure 2D). None of the other correlations between binocular behavioral measures (interocular visual acuity difference, IBP, stereopsis) and amplitudes of SSVEP intermodulation components was significant (all $p > 0.067$). We therefore focused on the amplitude of the f1+f2 component in subsequent analyses.

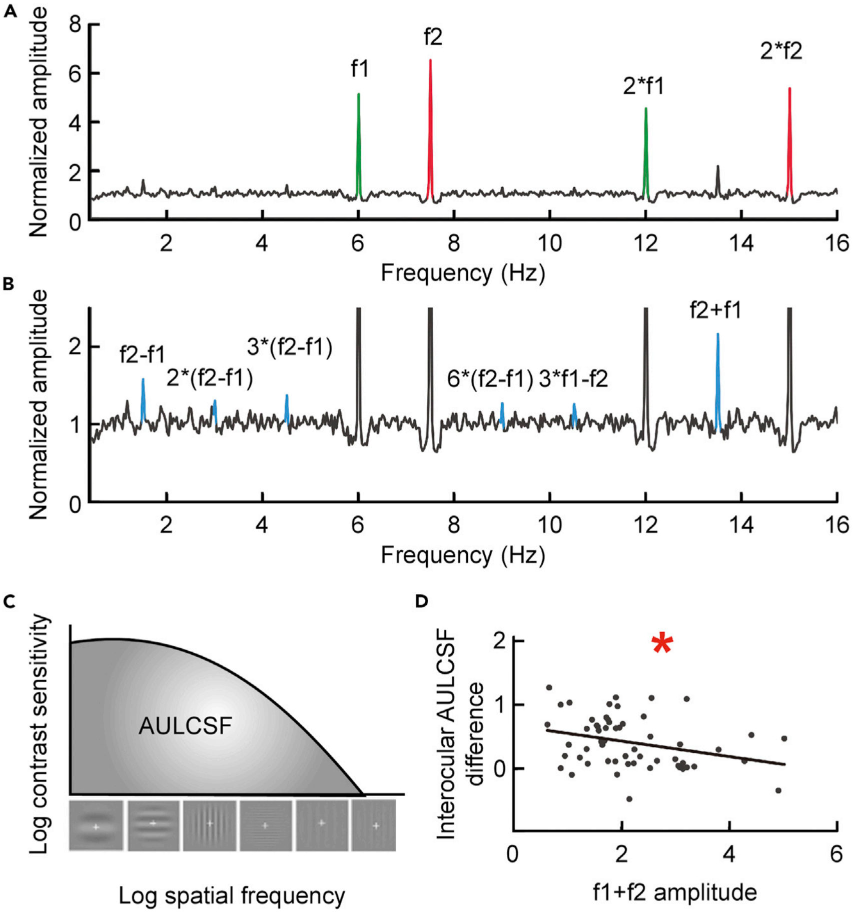

Figure 2. Illustration of the SSVEP Components, AULCSF, and the Correlation between the Amplitude of the f1+f2 Component and the Interocular AULCSF Difference at Baseline

(A) The average baseline SSVEP spectrum across all 58 subjects. The fundamental and second harmonic components are highlighted (Red: f2 and 2*f2 components are associated with the amblyopic eye; Green: f1 and 2*f1 components are associated with the fellow eye).

(B) An enlarged version of (A) with blue-highlighted SSVEP intermodulation components (f2-f1, 2*f2-2*f1, 3*f2-3*f1, 6*f2-6*f1, 3f1-f2, and f1+f2).

(C) A schematic diagram of AULCSF.

(D) A scatterplot of the interocular AULCSF difference versus the amplitude of the f1+f2 SSVEP component across all subjects at baseline and result of correlation analysis. An asterisk * indicates a significance level of $p < 0.05$.

See also Figure S1 for scalp topography.

Effects of Perceptual Learning

We then examined the effects of perceptual learning. A two-way ANOVA with eye (fellow eye and amblyopic eye) and training (pre-training and post-training) factors showed a significant main effect of eye ($F_{1,26}$ = 76.332, $p < 0.001$, partial η^2 = .746), a significant main effect of training ($F_{1,26}$ = 17.455, $p < 0.001$, partial η^2 = 0.402), and a significant interaction between the two factors ($F_{1,26}$ = 5.271, p = 0.030, partial η^2 = .169). Consistent with previous findings (Hess and Thompson, 2015; Huang et al., 2008; Levi and Li, 2009; Levi and Polat, 1996; Zhou et al., 2006), perceptual learning significantly improved the AULCSF of the amblyopic eye (M_{diff} = 0.130 ± 0.023, t_{26} = 5.713, $p < 0.001$), reduced the interocular AULCSF difference (M_{diff} = -0.074 ± 0.033, t_{26} = -2.292, p = 0.030; Figure 3A), but had no significant effect on the AULCSF of the fellow eye (M_{diff} = 0.056 ± 0.032, t_{26} = 1.772, p = 0.088). It also improved the cutoff spatial frequency and visual acuity of the amblyopic eye as well as stereopsis (Table 1).

Perceptual learning had no significant effect on the SSVEP components associated with monocular processing in the amblyopic (f2, 2f2) and fellow (f1, 2f1) (all $p > 0.08$) eyes. However, it did increase the

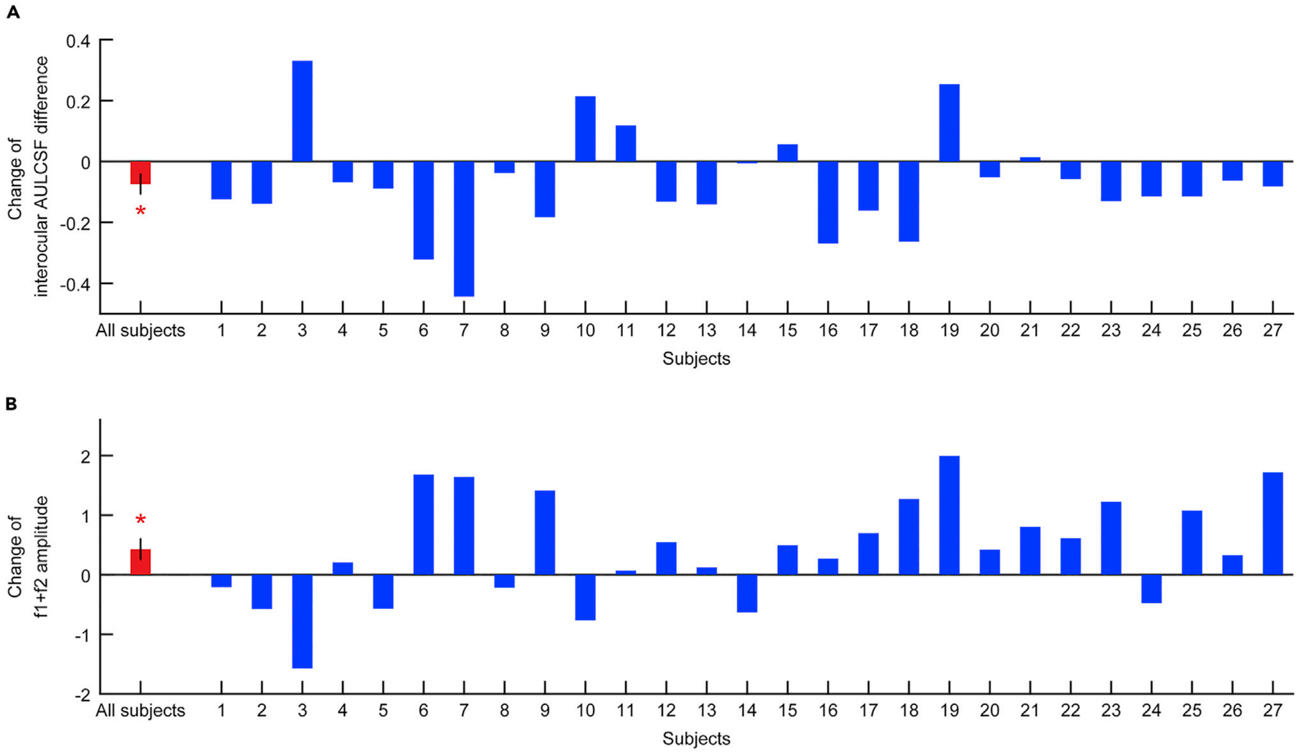

Figure 3. Effects of Perceptual Learning

(A) Effects of perceptual learning on the interocular AULCSF difference.

(B) Effects of perceptual learning on the amplitude of the f1+f2 SSVEP component. One-sample t test for the change of interocular AULCSF difference or the amplitude of the f1+f2 SSVEP component. Data are represented as mean ± SEM. An asterisk * indicates a significance level of $p < 0.05$.

amplitude of the f1+f2 SSVEP component in 19 of the 27 amblyopic subjects, producing a significant effect across all subjects ($M_{pre} = 2.025 \pm 0.153$, $M_{post}=2.453 \pm 0.181$, $M_{diff}=0.428 \pm 0.171$, $t_{26}= 2.495$, $p = 0.019$) (Figure 3B). Most interestingly, we found that there was a significant negative correlation between reductions in the interocular AULCSF differences and increases in the amplitude of the f1+f2 SSVEP component following perceptual learning ($r = -0.436$, $p = 0.023$; Figure 4). This significant correlation held true even after we controlled for changes in SSVEP components at the fundamental and second harmonic frequencies (f1, f2, 2*f1, 2*f2) in a multivariable regression analysis ($\beta = -0.481$, $p = 0.024$). In addition, we also found that there was a significant correlation between changes in the stereopsis and increases in the amplitude of the f1+f2 SSVEP component ($r = 0.387$, $p = 0.046$; $\beta = 0.430$, $p = 0.046$ in the multivariable regression analysis controlling for f1, f2, 2*f1, 2*f2). There was no significant correlation between changes in any monocular behavioral measure and changes in SSVEP components associated with monocular processing (f1, 2*f1, f2, and 2*f2; all $p > 0.050$).

In addition to the pre-/post-training assessments, subjects also performed a monocular 2AFC identification task during the training period. Focusing on the first and last days of training, we found that perceptual learning significantly improved the contrast threshold ($M_{start} = 2.208 \pm 0.494$, $M_{end}=3.183 \pm 1.032$, $M_{diff} = 0.967 \pm 0.995$, $t_{26} = 4.287$, $p < 0.001$), and the improvement was significantly correlated with the increase of f2 amplitude ($r = 0.415$, $p = 0.031$). However, the correlation became only marginally significant when we used multi-variate regression to control for other SSVEP components (f1, 2*f1, 2*f2, f1+f2) ($\beta = 0.364$, $p = 0.096$).

Control for the Influence of Patching

To control for the influence of patching during the training procedure, five additional patients with anisometropic amblyopia completed 10–13 days of patching treatment. The only difference between the patching and perceptual learning groups was that patching was applied instead of training. A two-way ANOVA with group (training and patching) and treatment (pre-treatment and post-treatment) factors showed a significant interaction effect for AULCSF of AE ($F_{1,30} = 4.875$, $p = 0.035$, partial $\eta^2 = 0.140$) (main effect of group

	Pre-training (Mean ± SE)	Post-training (Mean ± SE)	PL Change (Mean ± SE)	t-Value	p Value
SSVEP-Normalized Value					
Fellow eye					
f1	4.875 ± 0.665	4.459 ± 0.710	−0.416 ± 0.380	−1.096	0.283
2*f1	4.869 ± 0.704	4.117 ± 0.578	−0.753 ± 0.422	−1.782	0.086
Amblyopic eye					
f2	5.495 ± 0.820	6.139 ± 0.781	0.644 ± 0.656	0.982	0.335
2*f2	5.223 ± 0.653	4.724 ± 0.585	−0.499 ± 0.642	−0.777	0.444
Interocular					
f2-f1	1.397 ± 0.139	1.090 ± 0.101	−0.307 ± 0.172	−1.786	0.086
2*f2-2*f1	1.236 ± 0.106	1.040 ± 0.115	−0.197 ± 0.159	−1.236	0.227
3*f2-3*f1	1.296 ± 0.145	1.243 ± 0.103	−0.054 ± 0.185	−0.292	0.773
6*f2-*6*f1	1.327 ± 0.147	1.206 ± 0.099	−0.120 ± 0.138	−0.874	0.390
3*f1-f2	1.148 ± 0.097	1.162 ± 0.138	0.014 ± 0.140	0.098	0.923
f1+f2	2.025 ± 0.153	2.453 ± 0.181	0.428 ± 0.171	2.495	0.019*
Behavioral Measurements					
Fellow eye					
$AULCSF_{FE}$	1.442 ± 0.047	1.498 ± 0.035	0.056 ± 0.032	1.772	0.088
$Cutoff_{FE}$	1.391 ± 0.026	1.384 ± 0.025	−0.007 ± 0.016	−0.453	0.654
VA_{FE}	−0.049 ± 0.019	−0.073 ± 0.020	−0.024 ± 0.008	−3.008	0.006*
Amblyopic eye					
$AULCSF_{AE}$	0.814 ± 0.072	0.944 ± 0.065	0.130 ± 0.023	5.713	<0.001**
$Cutoff_{AE}$	0.954 ± 0.048	1.020 ± 0.046	0.066 ± 0.019	3.530	0.002*
VA_{AE}	0.473 ± 0.062	0.341 ± 0.048	−0.132 ± 0.021	−6.311	<0.001**
Interocular and binocular metrics					
Interocular AULCSF difference	0.628 ± 0.072	0.554 ± 0.070	−0.074 ± 0.033	−2.292	0.030*
Interocular cutoff difference	0.437 ± 0.054	0.364 ± 0.049	−0.073 ± 0.026	−2.836	0.009*
VA interocular difference	−0.522 ± 0.061	−0.414 ± 0.046	0.108 ± 0.024	4.628	<0.001**
Interocular balance point	0.445 ± 0.060	0.471 ± 0.067	0.026 ± 0.045	−0.598	0.557
Stereopsis	0.003 ± 0.001	0.005 ± 0.001	0.002 ± 0.001	2.239	0.034*

Table 1. Mean Values of SSVEPs at Target Frequencies and Behavioral Measures in Amblyopic Subjects before and after Training

Note: f1 = 6 Hz, f2 = 7.5 Hz. Only 20 subjects completed the binocular phase combination task. A single asterisk indicates a significance level of $p < 0.05$. Two asterisks indicate a significance level of $p < 0.001$.

factor: $F_{1,30} = 1.092$, $p = 0.304$, partial $\eta^2 = 0.035$; main effect of treatment factor: $F_{1,30} = 4.501$, $p = 0.042$, partial $\eta^2 = 0.130$). Further analysis showed a significant AULCSF treatment effect in the training group ($F_{1,30} = 29.99$, $p < 0.001$) but no significant AULCSF changes before and after patching in the control group ($F_{1,30} < 0.005$, $p = 0.963$). No significant interaction was found for other electrophysiological or behavioral assessments.

DISCUSSION

As a neuro-visual condition resulting from abnormal binocular visual experience during development, amblyopia can only be successfully treated by restoring normal binocular visual processing. In this study,

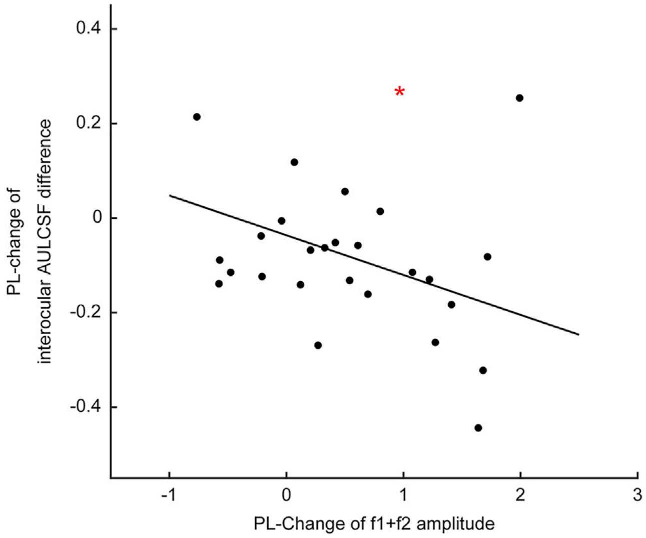

Figure 4. A Scatterplot of Changes in the Interocular AULCSF Difference and the Amplitude of the f1+f2 SSVEP Component and Result of Correlation Analysis

Asterisk indicates a significance level of $p < 0.05$.

we show that monocular perceptual learning in the amblyopic eye reduced the interocular difference between the amblyopic and fellow eyes and increased the amplitude of a binocular SSVEP component in adults with anisometropic amblyopia; furthermore, there was a significant negative correlation between the two. These results suggest that monocular perceptual learning in the amblyopic eye could improve binocular visual processing in the amblyopic visual system.

A large number of recent studies have shown that extensive perceptual learning in the amblyopic eye can improve monocular and binocular visual functions (Hess and Thompson, 2015; Huang et al., 2008; Levi and Li, 2009; Li et al., 2013; Polat, 2009; Polat et al., 2004; Zhou et al., 2006). The current study is the first to demonstrate the effects of monocular perceptual learning on amblyopic binocular visual processing using SSVEPs. By measuring the intermodulation f1+f2 component of SSVEP before and after perceptual learning, we were able to demonstrate that the change in the amplitude of the component was correlated with behavioral improvements that have been reported in many previous psychophysical studies (Huang et al., 2008; Levi and Polat, 1996; Li et al., 2013; Lu et al., 2005; Zhou et al., 2006). We also did not observe reliable correlation between the behavioral improvements that followed perceptual learning and the changes in the amplitudes of monocular SSVEP components. Collectively, our results suggest that monocular perceptual learning in the amblyopic eye to a large extent improved binocular rather than monocular visual processing in the amblyopic visual system. This is consistent with previous reports showing that monocular perceptual learning in the amblyopic eye led to improved vision in both amblyopic and fellow eyes (Hess and Thompson, 2015; Huang et al., 2008; Levi and Li, 2009; Polat, 2009).

We adopted four behavioral measures in this study: monocular visual acuity, monocular contrast sensitivity function, interocular balance point, and stereopsis. Visual acuity measures the limit of spatial resolution in high contrast, whereas the contrast sensitivity function is a more comprehensive assessment of spatial vision (Pelli and Bex, 2013). The interocular balance point in phase combination is largely an assessment of interocular inhibition in supra-threshold contrast (Huang et al., 2009). Stereopsis is a popular clinical measure of binocular function in amblyopia. Here, we found that the interocular difference in AULCSF and stereopsis but not the interocular balance point and interocular visual acuity difference was most correlated with the SSVEP intermodulation components. We speculate that interocular phase combination and visual acuity may reflect both inhibitory and excitatory processes in binocular processing (Hess and Jenkins, 1980; Hess and Malin, 2003) and could not be evaluated with the SSVEP measures used in this study.

SSVEP studies using binocular rivalry paradigms have shown a non-linear relationship between the intermodulation frequencies and binocular visual processing (Baitch and Levi, 1988; Regan and Regan, 1989;

Zhang et al., 2011), although it remains unclear whether the relationship reflects binocular competition or integration (Gordon et al., 2019; Tong et al., 2006). In this study, we found that increased f1+f2 amplitude was correlated with decreased interocular AULCSF difference and increased stereopsis. Note that the decrease of interocular AULCSF difference and the increase of stereopsis both indicated improvement of binocular balance. The results suggest that the observed increase of f1+f2 amplitude in the binocular conflict paradigm might be related to improved binocular integration. On the other hand, perceptual learning improved binocular balance in the amblyopic visual system and may lead to better inter-ocular conflict resolution. Additional studies are necessary to evaluate this.

Huang et al. (2008) and Hou et al. (2011) showed that, for adults with amblyopia, perceptual learning in contrast detection at the cutoff spatial frequency can transfer to a wide range of spatial frequencies and to motion detection and discrimination in a wide range of temporal frequencies. These results suggest that the amblyopic visual system may possess more plasticity than the normal visual system. Our results are in line with those previous results. Using the same cutoff spatial frequency training paradigm, Huang et al. (2009) found that perceptual learning improved contrast sensitivity and visual acuity in the amblyopic visual system via a combination of internal additive noise reduction and external noise exclusion. Xu et al. (2006) and Huang et al. (2007) found that both increased additive noise and mismatched perceptual template underlay performance deficits in the amblyopic visual system, although the degree of perceptual template mismatch increased with the spatial frequency of the test stimuli. That perceptual learning reduced internal noise and improved external noise exclusion suggests that the training scheme can address both mechanisms underlying amblyopic deficits. Performance improvements in high external noise conditions are potentially related to improved forward and backward masking, whereas improved performance in all the external noise conditions may be related to improved temporal integration in the amblyopic visual system.

Limitations of the Study

Our control experiment with patching only showed that mere repetition of the pre-/post-training assessments did not produce improved behavioral performance or improved f1+f2 amplitude. We note that the control group had only five subjects, which may limit our statistical power in observing patching effects. In addition, it also is possible that the observed training effects in the current study were due to the influences of both training and patching. Therefore, the effects of patching were not entirely ruled out in this study. Further investigations with more subject and only training (no patching) are necessary.

Conclusions

In summary, by combining perceptual learning, behavioral testing, and SSVEP, we found that monocular perceptual learning in the amblyopic eye improved binocular visual processing in the amblyopic visual system. These results suggest that it is possible to use behavioral training to address a key issue in amblyopia treatment, that is, the recovery of binocular processing.

METHODS

All methods can be found in the accompanying Transparent Methods supplemental file.

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.isci.2020.100875.

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AUTHOR CONTRIBUTIONS

J.L. and X.W. designed the research; L.G., S.D., L.F., and Z.C. performed the research; L.G. analyzed the data; and Z.-L.L., J.L., and X.W. wrote the manuscript.

DECLARATION OF INTERESTS

Zhong-Lin Lu: Commercial Relationship(s), Adaptive Sensory Technology: Code I (Personal Financial Interest), Adaptive Sensory Technology: Code P (Patent). All remaining authors declare no conflicting interests.

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Supplemental Information

Effects of Monocular Perceptual Learning on Binocular Visual Processing in Adolescent and Adult Amblyopia

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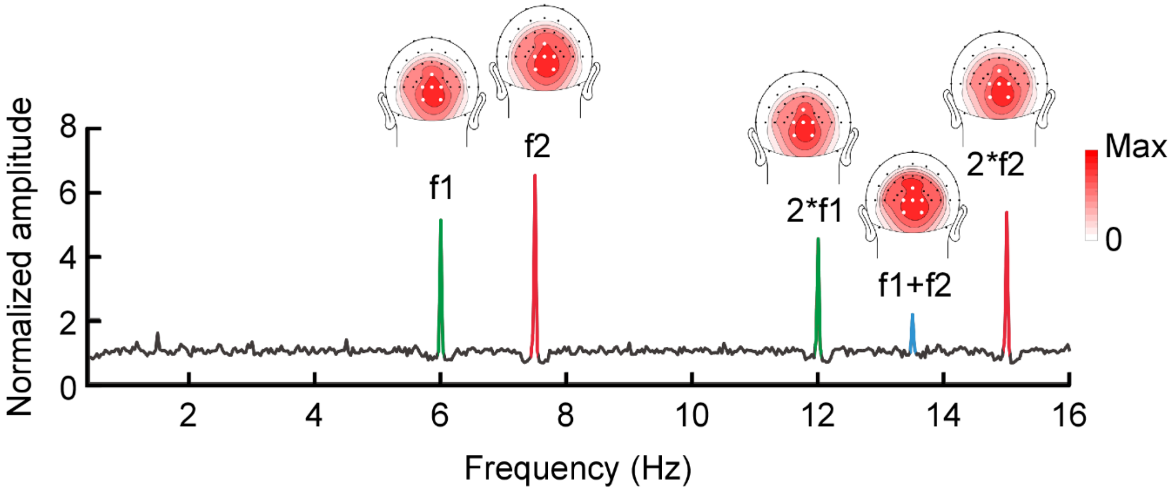
Supplementary materials



Supplementary Figure 1. Grand average amplitude topography, Related to Figure 2. Amplitude topographies are shown for the fundamental (6 Hz, 7.5 Hz) and second harmonic (12 Hz, 15 Hz) frequencies and the intermodulation frequency (f1+f2=13.5 Hz). Maximal amplitudes were at electrodes around Oz (highlighted in white). The topography of each SSVEP component is displayed with its individual colour scale and ranged from 0 to the maximal amplitude value of the SSVEP component (Rossion and Boremanse, 2011; Rossion et al., 2012).

Transparent Methods

Subjects

Forty-six patients with anisometropic amblyopia (16 females, 12 to 25 years old with a mean age of 15.9 ± 4.0 years; see Supplementary Table 1 for clinical details) and twelve subjects with normal vision (all males, 21 to 30 years old with a mean age of 24.4±3.2 years) were recruited to participate in the acquisition of baseline measurements. Twenty-seven of the amblyopic subjects (13 females, mean age 15.8 ± 4.0 years old) participated in training, while five (all males, mean age 19.6 ± 4.0 years old) received patching treatment. Other amblyopic subjects did not participate in training for personal reasons (e.g., residential address far from hospital, low motivation for receiving treatment).

During training, the subjects performed a 2AFC orientation identification task near their individual cut-off spatial frequency in their amblyopic eye for 7 to 15 days (Huang et al., 2008). They were also instructed to patch their fellow eye for two hours per day during the same period. Subjects receiving patching treatment were instructed to patch their fellow eye for two hours per day for 10 to 13 days. Before and after training or patching, we assessed monocular visual acuity (VA), monocular contrast sensitivity function (CSF), interocular balance point (IBP) in binocular phase combination(Hou et al., 2010), stereopsis, and SSVEP in binocular rivalry(Norcia et al., 2015; Zhang et al., 2011).

The CSF was measured with the qCSF method (Hou et al., 2010), and the Area Under the Log CSF (AULCSF) and cut-off acuity were derived as summary CSF metrics (Hou et al., 2010). Because seven subjects did not complete the binocular phase combination test, the interocular balance point data obtained from the remaining twenty subjects were used in subsequent analyses.

This study followed the tenets of the Declaration of Helsinki and was approved by the Zhongshan Ophthalmic Center Ethics Committee. Informed consent was obtained from all subjects prior to data collection.

Experimental procedure

Psychophysics measurements

Contrast sensitivity function

The qCSF method was applied to assess the contrast sensitivity function (Hou et al., 2010). Stimuli were digits presented on a gamma-corrected 46-inch LCD monitor (Model: NEC LCD P463) with a resolution of 1920×1080 pixels, a mean luminance of 50 cd/m^2 and a 60 Hz vertical refresh rate. Subjects first viewed the display from a distance of 4.5 m in a dark room. They were instructed to read out the Arabic number that appeared on the center of screen. The spatial frequency and contrast of the stimulus in each trial were controlled by the qCSF algorithm, and the digits were resized according to the corresponding spatial frequency (Zheng et al., 2019). The experimenter, who had access to the ground truth, coded the subjects' reports as numbers. If subjects gave an “I don’t know” response, the response was marked as “incorrect”. No feedback was provided. A new trial started 500 ms after the response. Each eye was separately examined in 35 trials with three digit stimuli in each trial. The entire examination took approximately 25 minutes.

Visual acuity

VA was measured using a tumbling E EDTRS chart viewed from a 4-m distance at a luminance of 500 cd/m^2 and is expressed in logMAR units. The chart followed EDTRS standards and consisted of 5 optotypes per line for a total of 12 lines with optotype size decreasing from 1.0 logMAR to -0.3 logMAR in steps of 0.1 logMAR. A forced-choice testing method was used. VA was scored using the standard technique of subtracting 0.02 logMAR for each correctly identified optotype.

Stereopsis measurements

The stereoscopic depth perception was assessed using the Randot Preschool Test viewed from a distance of 40 cm (Levi et al., 2015).

Interocular balance point (IBP) in binocular phase combination

The binocular phase combination task (Ding and Sperling, 2006) was performed with two horizontal sinusoidal gratings viewed at a distance of 68 cm, subtending 3 × 3 degree^2. Two gratings were identical spatial frequencies that were oriented with a 45° phase difference to measure the interocular balance point. The contrast of the grating in the amblyopic eye was fixed at 100%, while the contrast of the grating in the fellow eye was varied. The gratings contained two complete cycles at a spatial frequency of 0.293 cpd. The program measured phase differences with interocular contrast ratios at 0, 0.1, 0.2, 0.4, 0.8 and 1.0. The subjects could adjust the position of a line at a step size of 4° to indicate the perceived phase. Two grating configurations (with either +22.5°or −22.5° of phase) were used to cancel potential bias reflecting an upward or downward preference. The perceived phase was defined as the difference between the phases measured in the two configurations and used to calculate the effective contrast ratio in this task. Each pair of interocular contrast ratios was repeatedly measured in four blocks. The data obtained from the binocular phase combination were fitted using a modified interocular gain-control model (Huang et al., 2009):

$$\varphi = 2\tan^{-1}\left[\frac{\eta^{1+\gamma}-\delta^{1+\gamma}}{\eta^{1+\gamma}+\delta^{1+\gamma}}\tan\left(\frac{\theta}{2}\right)\right] \tag{1}$$

The interocular balance point (IBP) was determined as the interocular contrast ratio at which the two eyes were balanced in the binocular phase combination. In this model, the perceived phase of the cyclopean grating φ is determined by only one parameter, γ , and the interocular contrast ratio (balance ratio, BR) δ at the interocular balance point (i.e., when φ =0) would therefore be at η for amblyopic vision (Ding and Sperling, 2006).

SSVEP in binocular rivalry

Stimuli

Binocular rivalry stimuli were presented on a 27-inch LCD monitor (ASUS) using an active shutter stereo-goggle (NVIDIA 3D Vision 2) at a mean luminance of 150 cd/m^2. The monitor was gamma-calibrated at a refresh rate of 120 Hz to ensure a 60 Hz presentation in each eye. A chinrest was used to minimize the subjects' head movements.

A pair of incompatible circular checkerboard patterns adopted from a previous SSVEP binocular rivalry study (Zhang et al., 2011) was presented simultaneously to each eye through the goggles, with an annular window with a 10° visual angle. The two patterns reversed their contrast at 6 Hz and 7.5 Hz, respectively. Subjects viewed the display in a dark room at a distance of 1.0 m. Successive frames were seen by only one eye with no perceptible flicker at the high alternation rate. Subjects fixated on a central dark mark that remained visible throughout the experiment and actively monitored the parafoveal rivalrous stimuli. Each trial lasted 30 s, and each subject completed six trials with 10 s of rest between them.

EEG data acquisition

The subjects were seated in a shielded room. The EEG signals were amplified and digitized using a SynAmps 2 64-channel Amplifier with the 64-channel Quick-Cap in accordance with the international 10–20 system (Compumedics, USA), which allows fast and simple electrode placement. Signals were recorded from 21 posterior electrodes with a focus on covering the occipital scalp region, and the impedance of each electrode was kept below 10 kV. Horizontal and vertical electrooculograms (HEOG and VEOG) were also recorded to monitor eye movements. A reference electrode was placed between Cz and CPz. The data were sampled at 1000 Hz and filtered with a 0.05–100 Hz bandpass filter.

By stimulating the two eyes using stimuli flickering at two different frequencies, f_1 and f_2, we were able to tag the activities of monocular neurons according to EEG signals at the fundamental frequencies and their harmonics, $m*f_1$ and $n*f_2$, where m and n are integers. The activities of binocular neurons, which combine inputs from the two eyes and possess binocular nonlinearities, such as rectification, squaring, and/or divisive normalization, were tagged by EEG signals at the nonlinear intermodulation frequencies $m*f_1 \pm n*f_2$ (Regan and Regan, 1988; Sutoyo and Srinivasan, 2009; Tsai et al., 2012; Victor and Conte, 2000).

Perceptual learning

Subjects were trained with gratings at their individual cut-off spatial frequencies. A 2AFC orientation identification task with a three-down one-up staircase procedure was used for training. Each trial started with a 259-ms fixation cross placed in the centre of the display. The stimuli were sinusoidal luminance gratings generated by a psychophysical software Psykinematix43 installed on a MacBook Pro laptop. The stimuli were presented on a gamma-calibrated Dell 17-inch color CRT monitor (refresh rate = 85 Hz) at a 10.8 bits monochromatic mode to ensure high grayscale resolution. The mean luminance was 50cd/m^2. The untrained eye was patched during training. The stimuli were viewed monocularly at a 120 cm, with its diameter subtending 2 degrees of visual angle. The edge of the stimulus was blurred by a half-Gaussian 0.5° ramp. Each stimulus was oriented either horizontally or vertically and presented at an interval of 120 ms, and the subjects were asked to judge its orientation using the computer keyboard. During training, a brief tone followed each correct response. This response also initiated the next trial. Each subject performed ten training sessions a day, with each session consisting of 70 ~ 100 trials. Training began from the day CSF was tested and lasted for seven to fourteen days. Overall, each subject completed approximately 5,000-10,000 trials or eight hours of training (Huang et al., 2008).

Data analysis

Behavioural data

For the qCSF data, the cut-off acuity and AULCSF (log CSF) and with the CSF at 1, 1.5, 3, 6, 12, and 18 cpd were calculated using the trapezoid method. Both the spatial frequency and the contrast sensitivity in the logarithmic value were generated. We computed the area under the log CSF (AULCSF) for spatial frequencies ranging from 1.5 cpd to 18 cpd. We also computed the cut-off spatial frequency, which was defined as the spatial frequency at which the contrast sensitivity was 2.0 (threshold: 0.5).

EEG data

EEG was analysed using a customized toolbox (mfeeg: http://sourceforge.net/p/mfeeg) programmed with MATLAB (Mathworks, Natick, MA, USA). The topographic maps were generated with a customized MATLAB function based on EEGLAB (Delorme and Makeig, 2004; Li et al., 2018). Continuous EEG recordings were bandpass-filtered from 1 to 30 Hz and cut into six epochs (30 s each). SSVEP responses were obtained by applying the Fast Fourier transform (FFT) on the averaged epochs. In addition, the signal-to-noise-ratio (SNR) at each frequency was computed by taking the value at each frequency and dividing it by the average value of the 5 neighbouring frequencies on either side to normalize the differences in the spectrum values across different frequencies, different conditions and different subjects (Boremanse et al., 2013; Rossion and Boremanse, 2011). A one-sample t-test was conducted to test whether the SNR at each target frequency was significantly above background noise (SNR = 1) (Cunningham et al., 2017; Liu-Shuang et al., 2014; Rossion et al., 2012). EEG signals from 21 channels were located in the occipital scalp region. Since scalp topography showed that maximal IM responses were obtained at the electrodes surrounding Oz (Supplementary Figure 1), the signals from six electrodes (Oz, POz, O1, O2, CB1, CB2) were averaged for further analysis (additional analysis on Oz showed consistent results).

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