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Effects of monocular perceptual learning on binocular visual processing in adolescent and adult amblyopia

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Abstract

Re-establishing normal binocular visual processing is the key to amblyopia recovery beyond the critical period of visual development. Here, by combining perceptual learning, behavioural 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 behavioural 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, while children with amblyopia are treated by monocularly patching or penalizing the non-amblyopic eye, 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, behavioural 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 \( mf_1 \pm nf_2 \) are associated with binocular visual processing (Baitch and Levi, 1988; Regan and Regan, 1989; Zhang et al., 2011). Here, we used the amplitudes of 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 Supplementary Materials 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 Supplementary Materials for details). The SSVEPs exhibited robust monocular responses at the two fundamental (f1, f2) and second harmonic flicker frequencies (2f1, 2f2) ($M_{f1} = 5.122\pm0.417$, $t_{57}=9.889$, $p<0.001$; $M_{f2} = 6.535\pm0.573$, $t_{57}=9.661$, $p<0.001$; $M_{2f1} = 4.538\pm0.424$, $t_{57}=3.659$, $p=0.001$; $M_{2f2} = 5.367\pm0.450$, $t_{57}=5.301$, $p<0.001$) (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 behavioural measures of monocular and binocular visual functions. For the amblyopic subjects, only the amplitude of the 2*f2 component was negatively correlated with the cut-off spatial frequency of the amblyopic eye, cut-off$_{AE}$ ($r = -0.276$, $p = 0.036$); none of the other correlations between monocular behavioural measures in amblyopic and fellow eyes (visual acuity, area under the log CSF) 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 behavioural 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.

**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 $\eta^2 = .746$), a significant main effect of training ($F_{1,26} = 17.455$, $p < 0.001$, partial $\eta^2 = 0.402$), and a significant interaction between the
two factors ($F_{1,26} = 5.271, p = 0.030, \text{partial } \eta^2 = .169$). 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 ($M_{\text{diff}} = 0.130\pm0.023, t_{26} = 5.713, p < 0.001$), reduced the interocular AULCSF difference ($M_{\text{diff}} = -0.074\pm0.033, t_{26} = -2.292, p = 0.030$; Figure 3a), but had no significant effect on the AULCSF of the fellow eye ($M_{\text{diff}} = 0.056\pm0.032, t_{26} = 1.772, p = 0.088$). It also improved the cut-off 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 ($f_2, 2f_2$) and fellow ($f_1, 2f_1$) (all $p > 0.08$) eyes. However, it did increase the amplitude of the $f_1+f_2$ SSVEP component in 19 out of the 27 amblyopic subjects, producing a significant effect across all subjects ($M_{\text{pre}} = 2.025\pm0.153, M_{\text{post}} = 2.453\pm0.181, M_{\text{diff}} = 0.428\pm0.171, t_{26} = 2.495, p = 0.019$) (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 $f_1+f_2$ 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 ($f_1, f_2, 2*f_1, 2*f_2$) 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 $f_1+f_2$ 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 behavioural 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 \( (M_{\text{start}}=2.208\pm0.494, M_{\text{end}}=3.183\pm1.032, M_{\text{diff}} = 0.967\pm0.995, t_{26}=4.287, p < 0.001) \), 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 \( (F_{1,30} = 4.875, p=0.035, \text{partial } \eta^2 = 0.140) \) (main effect of group factor: \( F_{1,30} = 1.092, p=0.304, \text{partial } \eta^2 = 0.035; \) main effect of treatment factor: \( F_{1,30} = 4.501, p=0.042, \text{partial } \eta^2 = \) \)}.
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 behavioural 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, 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 behavioural 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 behavioural 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 behavioural measures in this study: monocular visual acuity, monocular contrast sensitivity function, interocular balance point, and stereopsis. Whereas visual acuity measures the limit of spatial resolution in high contrast, 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 $f_1+f_2$ 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 $f_1+f_2$ 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. Whereas performance improvements in high external noise conditions are potentially related to improved forward and backward masking, improved performance in all the external noise conditions may be related to improved temporal integration in the amblyopic visual system.

**Limitations of study**

Our control experiment with patching only showed that mere repetition of the pre-/post-training assessments did not produce improved behavioral performance, nor 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, behavioural 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 behavioural training to address a key issue in amblyopia treatment, that is, the recovery of binocular processing.

Methods

Any methods and additional references are provided in supplementary materials.

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Author contributions

LJR and WX designed the research; GL, DSY, FL and CZP performed the research; GL
analysed the data; and ZLL, LJR and WX wrote the manuscript.

Competing interests statement

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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Figure legends

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 Supplementary Table 1 for clinical details.

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 scatter plot 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 Supplementary Figure 1 for scalp topography.

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.

Figure 4. A scatter plot of changes in the interocular AULCSF difference and the amplitude of the f1+f2 SSVEP component and result of correlation analysis. An asterisk * indicates a significance level of p < 0.05.

Supplementary Table 1. Clinical details of the subjects with amblyopia at baseline, Related to Figure 1. Note: M: male, F: female, AE: amblyopic eye, FE: fellow eye. OD: right eye, OS: left eye. Aniso: anisometropic amblyopia, Mixed: mix of anisometropic and strabismic amblyopia.
Tables

Table 1. Mean values of SSVEPs at target frequencies and behavioural measures in amblyopic subjects before and after training.

|                      | 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*  |
| **Behavioural measurements** |                      |                           |                       |         |         |
| Fellow Eye           |                          |                           |                       |         |         |
| AULCSF<sub>FE</sub>  | 1.442±0.047              | 1.498±0.035               | 0.056±0.032           | 1.772   | 0.088   |
| Cut-off<sub>FE</sub>  | 1.391±0.026              | 1.384±0.025               | -0.007±0.016          | -0.453  | 0.654   |
| V<sub>AE</sub>       | -0.049±0.019             | -0.073±0.020              | -0.024±0.008          | -3.008  | 0.006*  |
| Amblyopic Eye        |                          |                           |                       |         |         |
| AULCSF<sub>AE</sub>  | 0.814±0.072              | 0.944±0.065               | 0.130±0.023           | 5.713   | <0.001**|
| Cut-off<sub>AE</sub>  | 0.954±0.048              | 1.020±0.046               | 0.066±0.019           | 3.530   | 0.002*  |
| V<sub>AE</sub>       | 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*  |
| Cut-off Difference    | 0.437±0.054              | 0.364±0.049               | -0.073±0.026          | -2.836  | 0.009*  |
| Metric                  | Mean 1 ± SEM 1 | Mean 2 ± SEM 2 | Mean 3 ± SEM 3 | t-score | p-value |
|------------------------|---------------|---------------|---------------|---------|---------|
| 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*  |

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.
Highlights

- PL reduced the interocular difference
- PL increased amplitude of a binocular SSVEP component
- Training in the amblyopic eye improves binocular visual processing