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Supplemental Information

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

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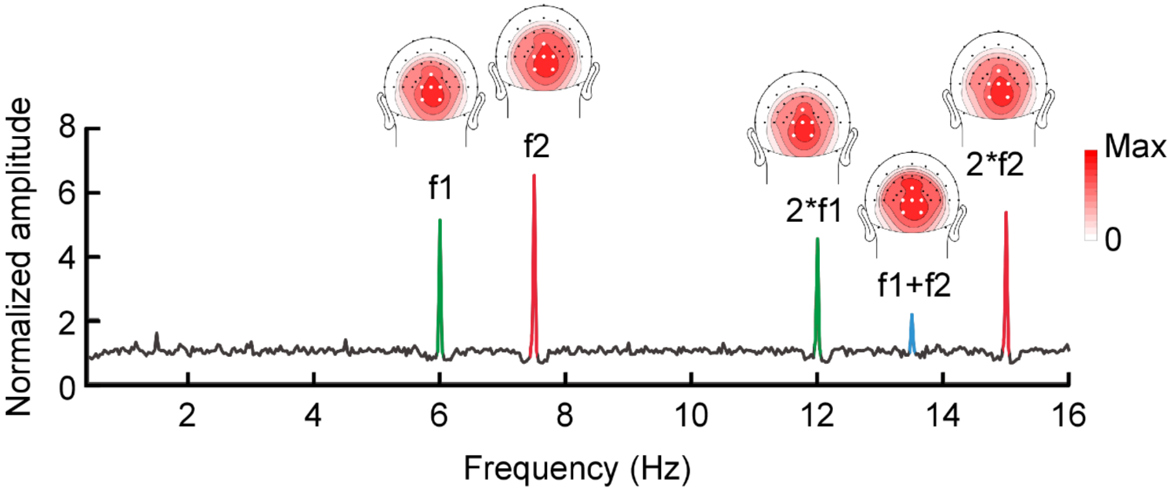
Supplementary materials



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):

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

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).

Supplementary references

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