**Supplemental Experimental Procedures:**

# A push-pull treatment for strengthening the "lazy eye" in amblyopia

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## Supplemental Experimental Procedures

### *Observers*

Three adults (ages: S1=38; S2=26; S3=24 years old) with amblyopia participated in the study. Their right eye (RE) and left eye (LE) visual acuities were: S1: RE=20/20, LE=$20/50^{-2}$; S2: RE=$20/25^{-1}$, LE=$20/16^{-2}$; S3: RE=$20/16^{-2}$, LE=$20/63^{-2}$. S1 (male) and S3 (female) had hyperopic astigmatism and S2 (female) had myopic astigmatism. None of them had latent or manifest nystagmus. All had previously been diagnosed and treated by their Optometrists with the standard patching therapy but were no longer undergoing treatment at the time of the study (last treated: S1 > 1 year; S2 >17 years and S3 >10 years). The origins of their amblyopia (based on self-reported history) were strabismus for S2 and anisometropia combined with strabismus for S1 and S3. All observers were able to achieve binocular eye alignment at the time of testing. Clinical tests with Worth-4-dot and Bagolini lenses reveal, respectively, that all observers had binocular fusion and normal retinal correspondence. Their optometric examination records indicated that they had good ocular health and stable amblyopia before the start of the push-pull training.

### *Apparatus*

A Macintosh computer running Matlab and Psychophysics Toolbox [1, 2] generated the stimuli on a flat 21" CRT monitor (2048 × 1536 pixels at 75 Hz). The observers viewed the monitor through a mirror haploscope attached to a head-and-chin-rest from a distance of 100 cm.

The experiments performed conformed with the regulatory standards of the Institutional Review Board of Salus University. We ran the following tests and training protocols on the three observers.

*General Procedures*

Relative SED (figure S1A), monocular contrast threshold (figure S1B) and stereopsis threshold (figures S1C and S1D) tests were conducted in the pre- and post-training phases. They were also performed when the observers returned for retention testing. While the same tests were applied, we had to optimize the specific stimulus variables for each observer. This is because amblyopia affects each individual differently resulting in different severity of amblyopic deficits, particularly on SED and stereo ability. For example, it has been found clinically that some amblyopes can perceive depth with contour stereogram but not with random-dot stereogram [3]. Thus, whereas S2 was tested with random-dot stereogram (figure S1D), S1 and S3 were tested with contour stereogram (figure S1C).

*Visual Stimuli*

*1. SED test*

We measured foveal SED using a pair of dichoptic orthogonal gratings (vertical vs. horizontal). The gratings were $1.5^{o}$ (angular diameter) discs presented to the fovea (figure S1A). The contrast of the vertical grating was variable while the contrast of the horizontal grating was fixed. During the trial, the gratings were presented for 400 msec, followed by a 200 msec mask that terminated the trial. The observer responded to his/her percept, horizontal or vertical, by pressing the appropriate key. If he/she saw a mixture of the two gratings, he/she would respond to the predominant orientation perceived. A QUEST [4] procedure adjusted the vertical grating contrast according to the observer's response, while the horizontal grating contrast remained the same (reference contrast). By appropriately adjusting the grating contrast after each trial, the point of equality, where the observer obtained an equal chance of seeing the two gratings (equal predominance), was reached. The contrast obtained defines the balance contrast for the eye that viewed the vertical grating. We then switched the grating orientations between the two eyes, to obtain the balance contrast for the fellow eye (the fixed contrast value of the horizontal grating was individually specified as detailed in the next paragraph). Four to five such blocks of trials were run to obtain the mean balance contrast for each eye. The difference between the LE and RE mean balance contrast values defines the relative SED.

The SED test above was performed on observer S2 with the horizontal grating having a fixed reference contrast of 1.3 log unit. But for observers S1 and S3, due to their excessive SED and the luminance limitation of the computer monitor, we could not obtain their balance contrast values using the same fixed horizontal grating contrast for the two eyes. Accordingly, we reduced the contrast of the reference horizontal grating when presenting it to the strong eye, and increased the contrast of the reference horizontal grating when presenting it to the weak eye. With this way of obtaining the balance contrast of the RE and LE, we derive a measure of *relative SED*. Specifically, for observer S3, the reference contrast of the horizontal grating to the amblyopic LE was fixed at 1.95 log unit when the RE was tested with the variable vertical grating. Then when the LE was tested with the variable vertical grating, the horizontal grating reference contrast in the RE was fixed at 0.4 log unit. Also, observer S3's strong eye viewed its half-images with reduced luminance that was equivalent to viewing through a neutral density filter with 20% transmission. Doing so further reduces the stimulus strength of the sensory dominant eye [5]. For S1, the reference contrast of the horizontal grating to the amblyopic LE was fixed at 1.8 log unit when the RE was tested with the variable vertical grating. Then when the LE was tested with the variable vertical grating, the horizontal grating reference contrast in the RE was fixed at 0.8 log unit. We used the same individualized horizontal grating contrast values (and mean luminance for S3) established for measuring relative SED to test observers S1 and S3 in the pre- and post-training phases. This ensures that changes in relative SED after the training provide a valid measure of the learning effect.

*2. Monocular contrast threshold test*

The monocular vertical sinusoidal grating (35 cd/m$^2$, 3 cpd, 1.5$^o$) was presented to the test eye, while a homogeneous gray (blank) field with the same mean luminance level was presented to the fellow eye (figure S1B). Each test was conducted using the 2IFC method, whose temporal sequence was: fixation, interval-1 (400 msec), blank (400 msec), interval-2 (400 msec), blank (200 msec), and mask (200 msec). A grating disc was presented at only one interval while the other interval had a blank field. The observer responded whether he/she saw the grating in either interval-1 or -2 by a key press. The grating contrast was adjusted after each trial (by QUEST) to obtain threshold. This contrast threshold test was repeated four times in each eye.

*3. Stereo threshold test*

Owing to differences in the amblyopic observers' stereo ability, S2 was tested with random-dot stereogram while S1 and S3 with contour stereogram. The random-dot stereogram (8$^o$ x 8$^o$, dot size = 0.025$^o$, 35 cd/m$^2$, contrast = 1.0 log%) had a variable crossed-disparity disc target (1.5$^o$) (Figure S1D). The contour stereogram comprised of two dots (0.2$^o$, separated by

1.2$^o$ vertically, 2.3 cd/m$^2$) with variable crossed-disparity, one above and the other below the fixation (0.8$^o$ x 0.8$^o$), against a 35 cd/m$^2$ background (figure S1C).

For both types of stereograms, the standard 2IFC method in combination with the staircase procedure was used to measure the stereo disparity threshold. The temporal sequence of the stimulus presentation was interval-1, blank (400 msec), interval-2, blank (200 msec), and random-dot mask (200 msec). The durations of interval-1 and interval-2 were individually specified (1.6 sec for S1, 200 msec for S2 and S3). After each trial, the observer indicated whether the crossed-disparity target (disc or dots) was perceived at interval-1 or -2. Each test block comprised 10 reversals, and the last 6 reversals were taken as the average threshold. Each block was repeated five times.

*4. Push-pull training protocol*

The key to producing the learning effect (reduction in SED) in the push-pull protocol is the suppression of the strong eye during the training [6, 7] using a binocular rivalry stimulus. Our previous works revealed that the binocular rivalry stimulus could take either the form of a binocular boundary contour (BBC) or monocular boundary contour (MBC) design. An example of an MBC rivalry stimulus is shown in figure S2(B) and a BBC rivalry stimulus is shown in figure S2(C). Notably, the BBC stimulus, which resembles most binocular rivalry stimuli used by other laboratories, has well-defined discs with boundary contours (BC) with orthogonal gratings in the two half-images. Because each half-image has a BC, we refer to the stimulus as a BBC (binocular boundary contour) stimulus. A monocular boundary contour (MBC) rivalry stimulus, on the other hand, does not have a boundary contour in one half-image. Our laboratory has shown that both MBC and BBC rivalry stimuli induce a similar extent of interocular suppression [8, 9] and push-pull learning effect [7].

Observers S1 and S2 were trained on a push-pull protocol with the BBC rivalry stimulus comprising a pair of dichoptic orthogonal gratings (306.7 msec, 1.5$^o$, 3 cpd, 35 cd/m$^2$) (Figure 1 in the main text and Figure S2C). Meanwhile S3 was trained with the MBC rivalry stimulus comprising an MBC disc (306.7 msec, 1.5$^o$, 3 cpd, 35 cd/m$^2$) viewed by the weak amblyopic eye and a background grating (17.2$^o$ x 11.5$^o$ in each half-image, 3 cpd, 35 cd/m$^2$) viewed by both eyes (Figures S2A and S2B).

To begin a 2IFC training trial for either the stimulation in Figure 1 (main text) or Figure S2A, the observer aligned his/her eyes at the fixation target (0.8$^o$ x 0.8$^o$, line width = 0.15$^o$, 15 cd/m$^2$), and then pressed the spacebar on the computer keyboard. This led to the removal of the fixation target, which was replaced by the presentation of a monocular square frame (2.3 cd/m$^2$, 1.5$^o$ x 1.5$^o$ dash outline with frame width of 0.15$^o$ for S1, 1.85$^o$ x 1.85$^o$ dash outline with frame width of 0.12$^o$ for other observers) in the weak amblyopic eye. The interval between the removal of the fixation and the onset of the square frame was 146.7 msec. The square frame acted as a transient attention cue to attract attention to the vicinity of the cue in the weak amblyopic eye [10]. After a 306.7 msec presentation of the cue (cue-lead-time), the BBC stimulus (for S1 and S2, Figure 1 main text) or MBC stimulus (for S3, Figure S2A) was presented for 306.7 msec, followed by a random-dot noise mask (end of interval-1 of the 2AFC trial). The same 306.7 msec cue was presented again 200-400 msec later, followed by the presentation of a second pair of BBC or MBC stimulus (306.7 msec), and a 200 msec mask to terminate the trial. The grating shown to the weak amblyopic eye in this second presentation had a slightly different orientation from the grating shown in the first presentation. The observer's (orientation discrimination) task was to report by a key press whether the first or second grating had a slight counterclockwise orientation. This ended a training trial. Orientation discrimination threshold was obtained by appropriately adjusting the orientation of the subsequent trials using either the QUEST or staircase method.

Each block of trials to obtain threshold comprised about 50 trials. Multiple blocks were run during a training session (S1: 18 blocks; S2: 15 blocks; S3: 9-15 blocks). Each training session lasted about 1.5 hours. The difference in the number of blocks accomplished by the observers per session was due to the length of the mini-break they took in between blocks.

A variant of the task used in the push-pull protocol: To offer variety, we also designed separate push-pull protocols that required observers to perform a contrast discrimination task, instead of the orientation discrimination task. The stimulus variables were the same as those above, except that the dominant gratings in the weak eye varied in contrast rather than orientation (Figure S2C for S1 and S2 and Figure S2B for S3). The observers selected the 2IFC interval with the higher grating contrast.

During each training session, we alternated 3 blocks of orientation discrimination task with 3 blocks of contrast discrimination task.

**Supplemental References**


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**Figure S1.** Stimuli used for measuring visual functions immediately before (pre) and after (post) training, as well as during the retention testing. **(A)** Binocular rivalry stimulus for measuring SED. To obtain the LE balance contrast the vertical grating contrast is varied relative to the fixed contrast of the horizontal grating (RE) until the observer perceives both gratings at equal predominance. The RE balance contrast is obtained in a similar manner when the two orthogonal gratings in the two eyes are switched. The difference in the LE and RE balance contrast values defines the relative SED. **(B)** Stimulus for measuring the monocular contrast threshold. **(C)** Contour stereogram for measuring binocular disparity threshold. With (uncross) fusion, the dots above and below the plus sign are seen in the front depth. **(D)** Random-dot stereogram for measuring binocular disparity threshold. With (uncross) fusion, a disc is seen in the front depth.

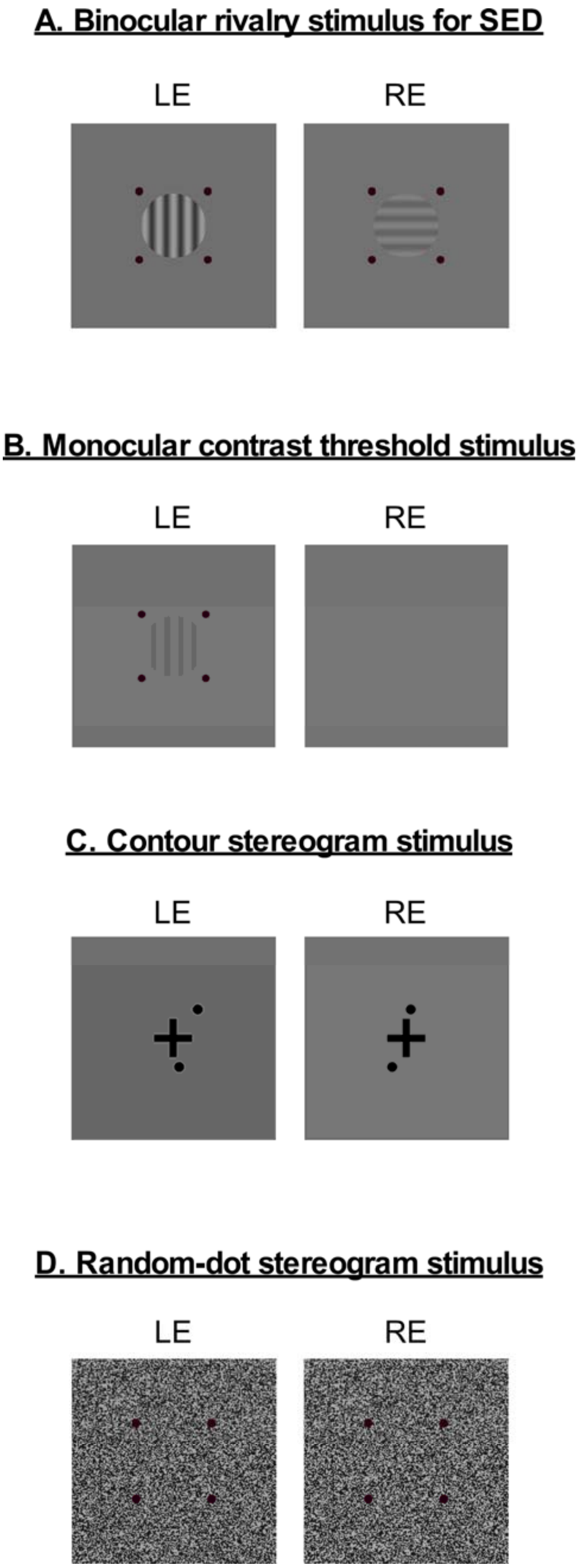
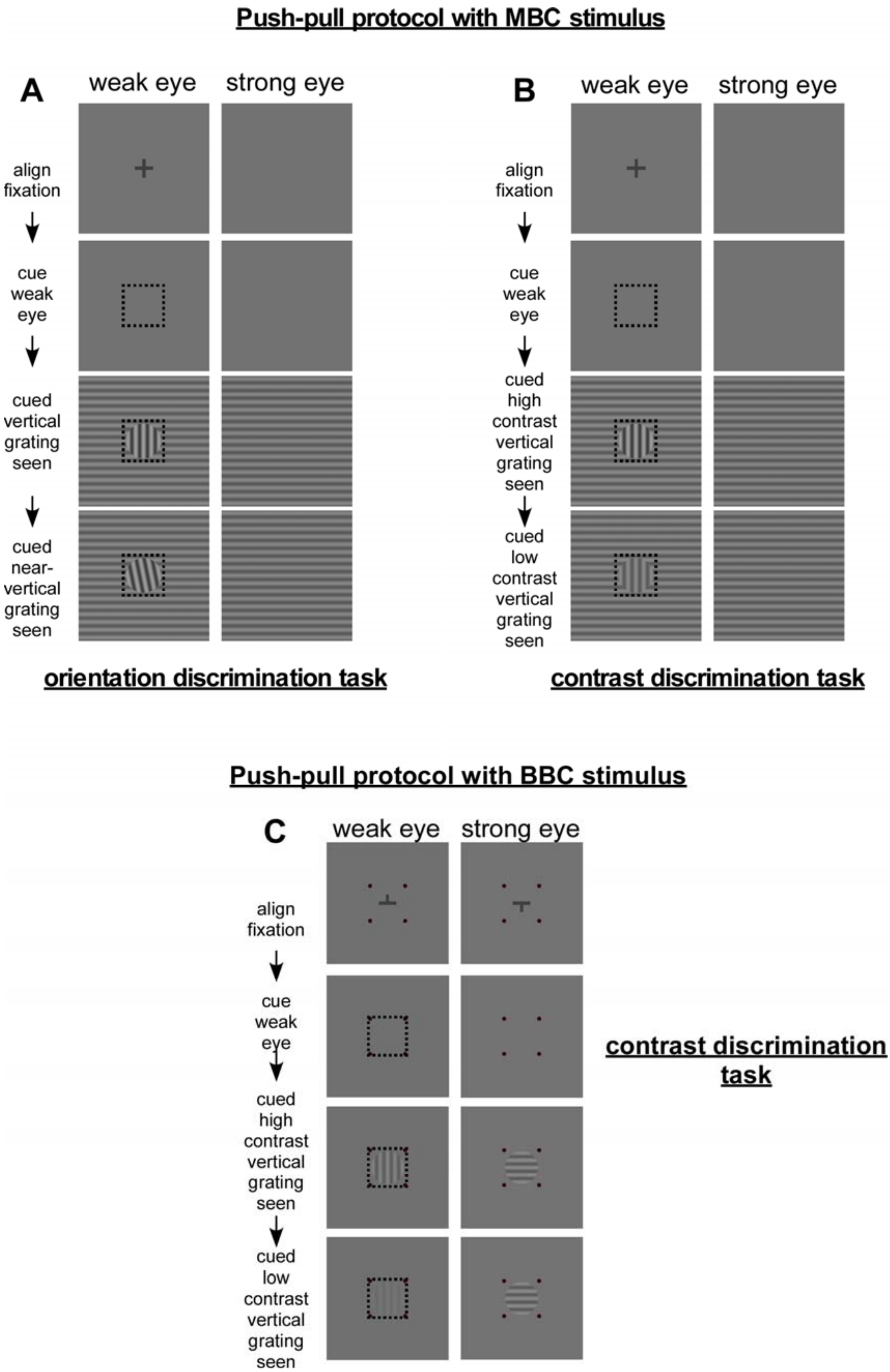

A. Binocular rivalry stimulus for SED
LE
RE
B. Monocular contrast threshold stimulus
LE
RE
C. Contour stereogram stimulus
LE
RE
D. Random-dot stereogram stimulus
LE
RE

**Figure S2.** Presentation sequences of the push-pull stimuli used during the training phase. The stimulation leads to complete perceptual suppression of the strong eye (pull) and excitation of the weak amblyopic eye (push), so that the observer is able to perceive the two sequential gratings viewed by the weak eye. This allows the observer to perform an orientation discrimination task as in (A), or contrast discrimination task as in (B) and (C). The binocular rivalry stimuli in (A) and (B) have an MBC design, while that in (C) has a BBC design.


Push-pull protocol with MBC stimulus
A
weak eye
strong eye
align fixation
cue weak eye
cued vertical grating seen
cued near-vertical grating seen
orientation discrimination task
B
weak eye
strong eye
align fixation
cue weak eye
cued high contrast vertical grating seen
cued low contrast vertical grating seen
contrast discrimination task
Push-pull protocol with BBC stimulus
C
weak eye
strong eye
align fixation
cue weak eye
cued high contrast vertical grating seen
cued low contrast vertical grating seen
contrast discrimination task