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Perceptual learning improves contrast sensitivity, visual acuity, and foveal crowding in amblyopia

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Abstract

Background

Amblyopic observers present abnormal spatial interactions between a low-contrast sinusoidal target and high-contrast collinear flankers. It has been demonstrated that perceptual learning (PL) can modulate these low-level lateral interactions, resulting in improved visual acuity and contrast sensitivity.

Objective

We measured the extent and duration of generalization effects to various spatial tasks (i.e., visual acuity, Vernier acuity, and foveal crowding) through PL on the target's contrast detection.

Methods

Amblyopic observers were trained on a contrast-detection task for a central target (i.e., a Gabor patch) flanked above and below by two high-contrast Gabor patches. The pre- and post-learning tasks included lateral interactions at different target-to-flankers separations (i.e., 2, 3, 4, 8λ) and included a range of spatial frequencies and stimulus durations as well as visual acuity, Vernier acuity, contrast-sensitivity function, and foveal crowding.

Results

The results showed that perceptual training reduced the target's contrast-detection thresholds more for the longest target-to-flanker separation (i.e., 8λ). We also found generalization of PL to different stimuli and tasks: contrast sensitivity for both trained and untrained spatial frequencies, visual acuity for Sloan letters, and foveal crowding, and partially for Vernier acuity. Follow-ups after 5-7

months showed not only complete maintenance of PL effects on visual acuity and contrast sensitivity function but also further improvement in these tasks.

Conclusion

These results suggest that PL improves facilitatory lateral interactions in amblyopic observers, which usually extend over larger separations than in typical foveal vision. The improvement in these basic visual spatial operations leads to a more efficient capability of performing spatial tasks involving high levels of visual processing, possibly due to the refinement of bottom-up and top-down networks of visual areas.

Keywords

Amblyopia; Perceptual Learning; Visual Acuity; Crowding; Contrast Sensitivity

1. Introduction

Amblyopia is a developmental visual disorder characterized by reduced visual acuity (VA), contrast sensitivity function (CSF), Vernier acuity, and as well as presence of foveal crowding (see Polat, 2009 for a review). These abnormalities cannot be improved by refractive correction of the amblyopic eye and occur in the absence of ocular diseases that could contribute to reduced visual acuity. Anomalous spatial interactions (Bonneh, Sagi, & Polat, 2007; Levi, Hariharan, & Klein, 2002; Polat, Bonneh, Ma-Naim, Belkin, & Sagi, 2005; Polat, Ma-Naim, Belkin, & Sagi, 2004; Polat, Sagi, & Norcia, 1997) have also been observed in the amblyopic eye; these are mainly suppressive and extend over larger distances than in normal foveal vision, similar to the peripheral vision of non-amblyopic observers (Levi et al., 2002; Maniglia et al., 2011).

The evidence that perceptual learning (PL), defined as improved performance after repeated practice in a sensory task, is associated with neural plasticity in human adults (see Sagi, 2011, for a review) has led several studies to inquire about the potential of PL as a non-invasive treatment for amblyopia.

Specificity –improvement regarding the trained stimulus that is considered to be encoded by low-level brain mechanisms – vs. generalization are debated aspects of perceptual learning that have become crucial for translational research. Although specificity is desirable because it reflects plasticity in the adult visual system, it is troublesome from the point of view of rehabilitative outcomes because effective rehabilitation should be generalized to visual tasks performed in daily life. This is why several studies have investigated the conditions under which a trained task transfers to some related untrained tasks (Chen, Chen, Fu, Chien, & Lu, 2008; Chung, Li, & Levi, 2008; Chung, Li, & Levi, 2006; Huang, Zhou, & Lu, 2008; Levi & Polat, 1996; Levi, 2005; Levi, Polat, & Hu, 1997; Li & Levi, 2004; Li, Provost, & Levi, 2007; Li, Young, Hoenig, & Levi, 2005; Li, Howard, Parrish, & Gottfried, 2008; Polat, 2009; Polat et al., 2004; Zhou et al., 2006). Amblyopic patients have been trained through several PL tasks, such as Vernier acuity (Levi & Polat, 1996; Levi et al., 1997), position discrimination in noise (Li, Klein, & Levi, 2008; Li et al., 2007, 2005; Li & Levi, 2004), identification of either contrast-defined or luminance-defined letters (Chung et al., 2008; Chung et al., 2006; Levi, 2005), and contrast detection of sinusoidal stimuli presented either isolated (Huang et al., 2008; Zhou et al., 2006) or together with high-contrast collinear flankers (Campana, Camilleri, Pavan, Veronese, & Giudice, 2014; Chen et al., 2008; Polat, 2009; Polat et al., 2004). Although PL-dependent improvement in visual acuity has been shown after almost all of these tasks, a benefit for other visual tasks has only been found with some PL procedures but not others. For example, PL on Vernier acuity – a task based on discrimination

between stimuli – does not transfer to detection (Levi & Polat, 1996; Levi et al., 1997), and PL on identification of contrast-defined letters does not transfer to luminance-defined letters (Chung et al., 2008). However, PL on contrast detection of sinewave gratings or Gabor patches generalizes to both visual acuity and untrained spatial frequencies (Huang et al., 2008; Polat et al., 2004; Zhou et al., 2006), although transfer to Vernier acuity has not been tested yet.

To be truly effective in improving visual functions, PL should reduce foveal crowding in the amblyopic eye. Indeed, amblyopic participants often exhibit strong crowding effects, i.e., impaired discriminability of target objects when presented in clutter, either assessed directly (Bonneh et al., 2007; Levi et al., 2002; Polat et al., 2005; Polat et al., 1997) or demonstrated by poorer visual acuity when letters are presented in a line than when in isolation (Flom, Weymouth, & Kahneman, 1963; Giaschi, Regab, Kraft, & Kothe, 1993; Li & Levi, 2004; Li et al., 2007; Simmers, Gray, McGraw, & Winn, 1999). Wong et al. (2005) and Polat et al. (2004) suggested that predominantly inhibitory cortical interactions may account for foveal crowding in the amblyopic eye. Li and Levi (2004) and Li and colleagues (2007) interpreted greater improvement in discriminating the misalignment between pairs of line segments than in single Snellen letter acuity as PL-dependent reduced crowding. However, a direct measure of the effect of PL in reducing crowding – as demonstrated by the reduced minimum distance between the target and flankers needed to cancel out the detrimental effect of flankers on target recognition (i.e., critical spacing) – has never been provided.

Another critical concern is *how* PL improves abnormal visual functions in the amblyopic eye. One line of research attempts to distinguish the effect of PL on spatial uncertainty (internal positional noise) from its effect on sampling efficiency (the ability to extract stimulus information) – the two main hypotheses for reduced spatial vision in amblyopia (Li et al., 2008, 2005; Li & Levi, 2004). Polat and colleagues (2004, 2009) focused on anomalous spatial interactions using a lateral masking paradigm to train amblyopic adults and children to detect Gabor patches presented with collinear high-contrast Gabor flankers. They found that training on increasingly high spatial frequencies significantly improved the contrast sensitivity function (CSF) and visual acuity. These improvements are compatible with a modulation of lateral interactions. Indeed, the initial spatial suppression that amblyopes manifested at intermediate target-to-flanker separations (at which normal-sighted observers present facilitation) was strongly reduced or cancelled out by PL (Polat et al., 2004).

Moreover, although a long-term follow-up is required to determine the actual efficacy of PL as a treatment, few studies have assessed whether the PL-related improvement and its transfer are maintained at follow-up to show that PL is an effective tool for rehabilitation of amblyopia. These

studies found sustained improvement lasting from 3 to 12 months after the tratment (Li et al., 2013; Liu, Zhang, Jia, Wang, & Yu, 2011; Polat et al., 2004; To et al., 2011). Interestingly, in Polat et al. (2004), the 12-month follow-up testing showed not only complete retention but also additional improvement in CSF at spatial frequencies of 12 and 18 cycles per degree (cycles/°).

In the present study, ten participants with either strabismic amblyopia (binocular misregistration) or anisometropic amblyopia (due to high refractive error), who were aged between 6 and 53 years, participated in a perceptual-learning protocol based on a contrast-detection task with lateral masking. Many of them underwent occlusion treatment that was ineffective, either because it was discontinuous or due to little compliance (Table 1). We used a contrast-detection paradigm because previous studies showed greater improvement, good maintenance, and a high degree of generalization (Levi, 2012). The training sessions consisted of measuring contrast thresholds for detecting, in the amblyopic eye, small and foveally presented Gabor patches, displayed along with collinear flankers with the same spatial frequency but higher contrast than the central target. For all of the trained observers, we assessed whether the PL-dependent improvement varied with spatial frequency and target-to-flanker separations, expressed in multiples of the carrier's wavelength (λ) of the Gabor patch. Transfer of PL was assessed in visual acuity of isolated letter recognition, visual hyperacuity, CSF, and foveal crowding. Differently from previous studies, we did not test CSF with a standard contrast sensitivity chart or use the same contrast-detection task employed for the training (Huang et al., 2008; Zhou et al., 2006). Instead, we measured CSF with an orientation-discrimination task to assess whether PL based on contrast detection enhanced the CSF, as measured with a different task. Moreover, previous studies have not directly assessed whether PL on contrast-detection tasks with lateral masking stimuli is suitable for reducing critical spacing in an untrained foveal crowding task. Maniglia and coworkers (2011) showed that after PL in a contrast-detection task with a lateral masking paradigm, peripheral crowding was strongly reduced in both normal-sighted observers and patients with macular degeneration (Maniglia et al., 2016). Since anomalous foveal interactions are thought to be similar to those in the peripheral vision of non-amblyopic observers, we asked whether PL in contrast detection of lateral masking may reduce foveal crowding in the amblyopic eye.

Additionally, visual acuity (for 7 participants) and CSF (for 6 participants) were tested 5 to 7 months after the training. We aimed not only to replicate the result that PL transfer effects were retained but also to determine whether, in the absence of further training, there was an additional improvement in visual acuity and CSF at high spatial frequencies, as previously reported by Polat et

al. (2004). In fact, adding evidence on this topic would provide some insight on the involvement of long-term neural plasticity in PL (Levi, 2012).

2. Methods

2.1. Participants

Ten amblyopic and ten age-matched non-amblyopic observers participated in the study. The amblyopic participants provided ophthalmological information before testing, including full refractive correction, a detailed ophthalmic history, diagnosis of amblyopia, and subjective refraction with best visual acuity (VA). To join the study, the patients must have had stable visual acuity and subjective refraction in both their amblyopic and non-amblyopic eyes for at least six months. The difference in acuity between the two eyes was > 0.2 logMAR for the monocular amblyopes, except for one subject, whose difference was 0.12 logMAR. Seven participants underwent occlusion therapy, which either failed or was ineffectively concluded because of poor compliance. Table 1 reports the clinical details for each participant. Two participants did not fully meet the inclusion criteria (absence of ocular diseases that could contribute to reduced visual acuity and learning difficulties): according to their ophthalmological reports, P1 had recovered from a corneal ulcer three years before the testing, and P9 was diagnosed with ametropic amblyopia (high hypermetropia with astigmatism) associated with albinism and nystagmus. One participant, P4, had a borderline score on a sustained attention assessment, although the participant's reduced attentional capacity did not compromise task performance.

Single letter monocular visual acuity (Table 1) was assessed before the PL sessions began, and corrective lens were not changed during the training. All of the participants were compliant during the training, although two of the children (P1 and P6) sometimes needed to be spurred by their parents. The research adhered to the tenets of the Declaration of Helsinki and was conducted under the experimental protocol for human subjects. Furthermore, it was approved by the Ethics Committee of the School of Psychology of the University of Padova (protocol no. 1931). Written informed consent was obtained from each participant or their parents after detailed explanation of the study.

[Table 1 about here]

2.2. Stimuli

2.2.1. PL stimuli

PL stimuli were generated using Matlab Psychtoolbox (Brainard, 1997; Pelli, 1997) and displayed on a LCD monitor with a refresh rate of 60 Hz and 8 bits of luminance resolution. The choice to use this basic hardware was made to accommodate participants through user-friendly home-based training, and it is justified by the high contrast thresholds of the participants in the amblyopic eye. In fact, the contrast thresholds in each case reached the limits of the hardware in use (i.e., 0.01 Michelson contrast). Stimuli were generated using a gamma-corrected lookup table (LUT) to ensure display linearity for the LCD monitors used at each participant's home or at the clinical centre. The gamma correction for each colour channel was applied through a LCD monitor-specific calibration with the Spyder 4 Elite colorimeter (DataColor, Lawrenceville, NJ, USA); the mean luminance was 50 cd/m^2. The stimuli software was set up for the screen resolution to ensure the correct representation of spatial frequencies at the viewing distance of 57 cm.

The stimuli were Gabor patches consisting of a cosinusoidal carrier enveloped by a stationary Gaussian. Each Gabor patch was characterized by its wavelength (λ), its phase (ϕ), and the standard deviation (σ) of the luminance Gaussian envelope in the (x, y) space of the image. Formally, each Gabor patch can be expressed as follows:

$$G(x,y) = \cos\left(\frac{2\pi}{\lambda}x + \varphi\right) e^{\left(-\frac{x^2+y^2}{\sigma^2}\right)}, \qquad \text{Eq. 1}$$

with $\sigma = \lambda$ and $\phi = 0$ (even symmetric). The Gabors' sensitivity function (SF) used for the PL session was within the range of 3 to 12 cycles/°. The configuration used consisted of a centrally presented Gabor target flanked above and below by two collinear high-contrast Gabor patches (0.6 Michelson contrast) oriented as the target (Campana et al., 2014; Chen et al., 2008; Polat, 2009; Polat et al., 2004). Four configurations of collinear Gabors were trained, each with different global orientations (horizontal, vertical, diagonal 135° and diagonal 45°). Flankers were located at various distances from the target: 2λ, 3λ, 4λ, and 8λ, corresponding to 0.67, 1, 1.33 and 2.67 deg for 3 cycles/° and 0.17, 0.25, 0.33 and 0.67 deg for 12 cycles/° (Figure 1).

[Figure 1 about here]

2.2.2 Transfer stimuli

2.2.2.1. Visual acuity stimuli

We used the FrACT (Freiburg Visual Acuity and Contrast Test) software (Bach, 1996) to measure the visual acuity of single letters, as derived from the measurement of the threshold font size of 10 Sloan letters: C, D, H, K, N, O, R, S, V, and Z. In response, the appropriate letter is typed by the experimenter. Any non-Sloan letter was ignored. Observers viewed the letters monocularly for a maximum of 30 s at a viewing distance of 200 cm. Acoustic feedback was present for both correct and incorrect answers.

2.2.2.2. Visual acuity and foveal crowding stimuli

Thresholds for central visual acuity (eccentric VA) and for the critical space of crowding – i.e., the inter-letter distance at which target (i.e., the central letter) identification was unaffected by the presence of flankers – were measured before and after the training period. Stimuli were generated using Matlab Psychtoolbox (Brainard, 1997; Pelli, 1997) and presented at 57 cm. The stimuli were the same 10 white Sloan letters used for the visual acuity test, randomly presented for 133 ms on a black background. The size of the letters for acuity and the letter-to-letter distance for crowding varied according to an adaptive procedure – the maximum likelihood procedure (Grassi & Soranzo, 2009; Green, 1990, 1993) – that tracked 79% of correct responses. Participants had to verbally report the target letter, and the experimenter registered the answer. Each session was terminated after 60 trials.

For foveal crowding, a central target letter was flanked by two different random letters. The size of both the target and flanking letters was set 30% higher than the VA threshold to have correct identification at the largest target-to-flankers separation. The initial font size for acuity was 40 (streak width of arcmin), while the initial distance between the letters for crowding was set at 3 degrees. In both tasks, an acoustic cue was presented before the stimulus presentation to reduce temporal uncertainty. No feedback was provided.

2.2.3. CSF stimuli

CSF was measured using the FrACT software package. The stimuli were Gabor patches of 2 deg (full width at half maximum) with four different orientations (horizontal, vertical, and diagonal at 45° and 135°). The observers performed a four-alternative forced choice (4AFC) orientation discrimination task monocularly. Each stimulus disappeared immediately after the observer's response. The stimuli were displayed for a maximum of 30 s. The contrast of the stimulus was varied according to a BEST PEST procedure and by using FrACT's built-in bit-stealing technique

to extend the luminance resolution at 10 bits (Tyler, 1997). The viewing distance was 200 cm, and acoustic feedback was provided for incorrect trials. The spatial frequencies tested were 1, 3, 5, 7, 11 and 15 cycles/°.

2.2.4. Vernier acuity

Vernier acuity was measured using FrACT. The stimuli were two line segments (15 x 1.4 arcmin) with a vertical separation between them of 0.5 arcmin. The observers' task was to discriminate with their amblyopic eye (AE) whether the upper line was displaced to the left or right with respect to the lower line. The stimuli were displayed for a maximum of 30 s. The viewing distance was 200 cm, and acoustic feedback was provided for incorrect trials.

2.3 Procedure

2.3.1. Pre- and post-training evaluation

The pre-training and post-training measurements of visual acuity, CSF, Vernier acuity, and foveal crowding were obtained in the same ways with the same apparatuses and under the same viewing conditions (best optical correction) at the clinical centre. The evaluation of VA was done for all amblyopic eyes (AEs) and non-amblyopic eyes (NAEs). Vernier acuity was measured for the AE of six observers, foveal crowding was measured for the AE of seven participants, and CSF was measured for the AE of all of the observers. The order of testing was counterbalanced. For most of the participants, VA and CSF were also measured once or twice during the training. Moreover, visual acuity (for 7 participants) and CSF (for 6 participants) were tested 5–7 months after the training was terminated.

2.3.2 PL procedure

After completing pre-training testing, the LCD monitor calibration with the Spyder 4 Elite colorimeter was applied to the computer used for the training, and the training conditions were set based on the pre-training evaluation. Except for P9, the PL paradigm involved supervised patching of the fellow eye. Note that the daily occlusion duration (30–45 min) was insufficient to explain the effects of PL as being due to patching the fellow eye, even in patients with mild amblyopia (Pediatric Eye Disease Investigator Group, 2003). Multicentre-based training was administrated under supervision of the leading university centre. All of the training sessions were performed either at the clinical centre (for five participants: P2, P3, P7, P8, and P9) or at the clinical centre for

the first week and at the participants' home afterwards (for five participants: P4, P6, P1, P5, and P10).

The contrast threshold of the target was varied according to a 1-up/3-down staircase (Levitt, 1971). The participants performed a temporal-2AFC. The target was presented in one of the two temporal intervals, whereas the flankers were always presented in both temporal intervals. A fixation mark (0.18 deg) indicated the location of the target on the blank screen before and after each interval.

The observers had to report the temporal interval at which the target was presented. Acoustic feedback was provided for incorrect trials. Each block was terminated after 120 trials or 16 reversals. The contrast thresholds were estimated by averaging the contrast values corresponding to the last 8 reversals. During the training, the target-to-flankers separation was randomly varied within a daily session, whereas the global orientation of the stimulus configuration was varied across four daily sessions in the following sequence: horizontal, diagonal 45°, vertical, and diagonal 135°. The stimulus duration varied from 133 to 500 ms as reported in Table 2 according to each subject's performance. The participants completed between 21 and 93 sessions in 16–43 weeks. The Gabors' spatial frequency used for the PL session was chosen within the range of 3 to 12 cycles/°. The initial spatial frequency that was trained was set as the highest spatial frequency for which the contrast threshold could be measured in the pretest CSF with FRACT. The next spatial frequency was changed to a higher value, either according to the improvement obtained in the contrast-sensitivity function at the intermediate evaluation or according to PL performance (Polat, 2009), when observers consistently obtained a 2-fold threshold reduction (or more) in the conditions of the highest (8λ) and lowest (2λ) target-to flanker separation.

[Table 2 about here]

3. Results

3.1 PL effect on trained task

Figure 2 shows the results of PL expressed as contrast threshold (Figure 2A, B) and PL enhancement (i.e., threshold modulation; Figure 2C, D) as a function of the target-to-flankers separations (TtFS), for low (3 cycles/°, N = 7) and high spatial frequencies (≥ 6 cycles/°, N = 7). We performed a two-way repeated-measures ANOVA separately for low and high spatial frequencies, including as factors PL (pre- vs. post-training sessions) and TtFS (2λ, 3λ, 4λ and 8λ). The results showed that PL reduces contrast thresholds at both low ($F_{1,6} = 8.5$, $p = 0.027$, *partial-*η^2

= 0.59) and high spatial frequencies ($F_{1,7}$ = 6.2, p = 0.041, *partial-*η^2 = 0.47). The effect of TtFS is never significant and the interaction PL x TtFS is significant only for the low spatial frequency ($F_{3,18}$ =3.5, p = 0.037, *partial-*η^2 = 0.37). Bonferroni corrected post hoc reported lower contrast threshold at 8λ than at 3λ (p < 0.04) and 2λ (p < 0.047).

The reduction of thresholds by PL is confirmed by the negative PL modulation illustrated in Figures 2C and D. A one-way repeated-measures ANOVA on PL modulation showed that the effect of TtFS was only significant for the low spatial frequency ($F_{3,18}$ = 7.7, p = 0.02, *partial-*η^2 = 0.56; high spatial frequency: $F_{3,21}$ = 0.98, p = 0.42, *partial-*η^2 = 0.13). Bonferroni corrected post hoc comparisons between TtFS revealed that the PL modulation (negative values) was generally stronger at 8λ than at the others TtFS: 8λ vs 4λ (p = 0.033), 8λ vs 3λ (p = 0.069) and 8λ vs 2λ, (p = 0.1).

[Figure 2 about here]

3.2 Transfer to visual functions

Table 3 summarizes individual transfer data for SLOAN acuity, CSF, Vernier acuity, and foveal crowding before PL, after PL, and at the follow-up. Test-retest data for an age-matched control group were also included in Table 3. It should be noticed that for one amblyopic participant (P1), PL did not affect either acuity or CSF. Although, according to the ophthalmological report, she had recovered from corneal ulcer three years before testing, the small corneal leucoma may have justified the inefficacy of PL in improving VA and CSF. Moreover, for one participant (P9) who had bilateral amblyopia from nystagmus, we reported data obtained in binocular vision that were similar to those obtained in monocular testing.

Overall, visual acuity was substantially better after than before PL, for both trained amblyopic eyes (AEs) and for the five tested untrained non-amblyopic eyes (NAEs). Additionally, for the AEs, visual acuity was higher at the follow-up than after PL. Contrast sensitivity for trained SF range (3-7 cycles/°) was higher in the trained AEs after PL than before PL and higher at the follow-up than after PL. Contrast sensitivity for untrained low SF was not increased either after PL or at the follow-up with respect to the baseline. Conversely, contrast sensitivity for untrained high spatial frequencies (11 and 15 cycles/°) was higher after than before PL. VA and CW in the trained AEs were reduced by PL.

[Table 3 about here]

3.2.1 Transfer to VA

Figure 3 shows individual and mean SLOAN visual acuity, obtained before and after PL in the amblyopic eye (Figure 3A) and fellow-eye (Figure 3B). Follow-up data were also collected for the amblyopic eye after three months for P6 and after at least six months for P1, P3, P4, P5, P7, P9, and P10.

One-sample *t*-tests showed a significant higher visual acuity in the amblyopic eye after PL than before PL (difference: 0.18 logMAR; $t(9) = 3.8$, $p = 0.004$) and at the follow-up with respect to the performance after PL (difference: 0.098 logMAR; $t(7) = 2.7$, $p = 0.03$). The improvement was also significant when comparing before and after PL in the fellow eye (difference: 0.13 logMAR; $t(7) = 2.4$, $p = 0.049$). Moreover, neither before ($t(8) = 0.042$, $p = 0.97$) nor after PL ($t(8) = 0.57$, $p = 0.58$), VA differed between juvenile and adult amblyopia.

[Figure 3 about here]

3.2.2 Transfer to CSF

Figure 4A shows the mean normalized CSF measured before and after PL for all participants and at the follow-up for 6 participants. For each participant, CSFs were normalized for the maximum value (i.e., by scaling the contrast sensitivity value with respect to the maximum), which was set to the value of 1. Contrast sensitivity enhancement after PL expressed as $\log(CS_{post}) - \log(CS_{pre})$, and at follow-up $\log(CS_{f\text{-}up}) - \log(CS_{post})$ is also shown in Figure 4B. Results indicate that CSF, measured with an orientation discrimination task (see the Methods section), was enhanced by PL on contrast detection task at medium and high spatial frequencies, but not at low spatial frequencies. Moreover, CSF function at follow-up showed further improvement at medium and high spatial frequencies.

Contrast sensitivity enhancement was evaluated for each spatial frequency, with one-sample two-tailed *t*-tests. Results revealed a significant enhancement of contrast sensitivity at intermediate spatial frequencies, both after PL (1 cycles/°: $t(8) = -0.34$, $p = 0.17$; 3 cycles/°: $t(9) = 1.3$, $p = 0.22$; 5 cycles/°: $t(9) = 2.1$, $p = 0.069$; 7 cycles/°: $t(8) = 3.2$, $p = 0.012$; 11 cycles/°: $t(6) = 2.63$, $p = 0.039$; 15 cycles/°: $t(4) = 1.5$, $p = 0.21$) and at the follow-up with respect to the post-test (1 cycles/°: $t(4) = 0.51$, $p = 0.64$; 3 cycles/°: $t(5) = -0.55$, $p = 0.61$; 5 cycles/°: $t(5) = 2.33$, $p = 0.067$; 7 cycles/°: $t(5) = 3.5$, $p = 0.017$; 11 cycles/°: $t(3) = 2.1$, $p = 0.12$). The average contrast sensitivity enhancement did not differ amongst juvenile and adult amblyopic patients ($t(8) = 1.15$, $p = 0.28$).

[Figure 4 about here]

3.2.3 Transfer to Vernier acuity

The transfer of PL to Vernier acuity is shown in Figure 5, which compares individual and average Vernier acuity obtained before and after PL. Four out of six participants showed increased Vernier acuity following PL. A paired sample *t*-test conducted on the six observers did not reach significance ($t(5) = 2.44$, $p = 0.058$).

[Figure 5 about here]

3.2.3 Transfer to foveal crowding

The transfer of PL to foveal crowding is shown in Figure 6, which compares center-center critical spacing (expressed in stroke-units) before and after-PL for each of the seven tested participants and at follow-up for three participants. All tested participants, except P9, showed reduction of crowding following PL on contrast detection with lateral masking configurations, and the difference in critical spacing before and after PL was significant ($t(7) = 2.64$, $p = 0.039$).

[Figure 6 about here]

4. Discussion

Our results indicate a general PL-dependent reduction of contrast threshold that was stronger at the largest target-to-flanker separation for the low spatial frequency. Besides, PL transferred to untrained tasks, such as orientation discrimination of both trained and untrained spatial frequencies, visual acuity with Sloan letters, Vernier acuity, and foveal crowding. More importantly, follow-up after 5 to 7 months showed not only an almost complete retention of PL effects, but also further improvement in visual acuity and contrast sensitivity with respect to observers' performances immediately after the training.

4.1. The effect of PL on lateral interactions

To our knowledge, only one study addressed the question of whether PL with a lateral masking paradigm modulates lateral interactions in the amblyopic eye (Polat et al., 2004). These

authors showed, before training, inhibition with TtFS < 2λ and no modulatory effect by the flankers at TtFS > than 6λ. Moreover, they showed that PL modulates foveal inhibitory spatial interactions in the amblyopic eye. In particular, for a Gabor target of 6 cycles/°, they found reduced inhibition exerted by flankers placed at a distance of 3λ (Bonneh et al., 2007; Levi et al., 2002; Polat et al., 2005; Polat et al., 2004, 1997). Considering that we did not measure contrast thresholds for central Gabors either isolated or flanked by orthogonal Gabors, we cannot make a direct comparison with previous results. However, our results show that, at low spatial frequencies, PL leads to lower contrast detection thresholds for 8λ than 2λ or 3λ; therefore, a larger threshold modulation was observed for the largest TtFS. These results may be taken as indirect evidence of PL modulation of long-range lateral interaction in the amblyopic eye. Since the initial spatial frequency trained was 3 cycles/° for most participants, it may be that PL affected lateral interactions more at the beginning of training. A more parsimonious explanation is one which considers the effect of TtFS before PL. Our results differ from those of Polat et al. (2005) and Levi et al. (2002), who found facilitation at 3 cycles/°, and instead are consistent with those of Polat and Norcia (1997), who found less facilitation at 3 cycles/° in particular for collinear configurations. In fact, the results of Polat and Norcia (1997) and our results showed that contrast thresholds do not depend on TtFS before PL. Under the assumption that the effect of TtFS depends on the interplay between two mechanisms (Polat, Mizobe, Pettet, Kasamatsu, & Norcia, 1998)—one responsible for inhibition, relying on suppressive effects within one receptive field, and the other responsible for facilitation, relying on long-range lateral interactions—the higher PL modulation at the largest TtFS suggests that, at low spatial frequency, PL results in more efficient long-range facilitation of lateral interactions.

4.2. Transfer of PL to other visual tasks

Our results confirm previous findings that PL on contrast detection tasks transfers to visual acuity and CSF. The new finding is the transfer of PL to foveal crowding, a phenomenon often reported in amblyopic patients (Bonneh et al., 2007; Levi et al., 2002; Polat et al., 2004, 2005, 1997). On the other hand, there was little transfer to Vernier acuity. The improvement in visual acuity was also found in the untrained fellow-eyes, consistently with previous results (Levi & Polat, 1996; Levi et al., 1997; Li et al., 2008; Zhou et al., 2006).

Though the clinical relevance of transfer of PL to untrained tasks, previous studies did not always find that PL transfers across tasks. For example, Levi et al. (1996, 1997) found that PL on detecting the lateral displacement of two high contrast lines (Vernier acuity task) did not transfer to

contrast detection of one of these lines. Other studies showed that PL to recognize contrast defined letters did not transfer to either luminance-defined letters (Chung et al., 2006) or visual acuity (Chung et al., 2008). However, with exception of Vernier acuity in which the transfer was weak, we found that PL generalizes to different tasks and stimuli. In fact, we found a transfer to visual acuity and CSF tested with a different task than the one used during PL (i.e., 4AFC orientation discrimination vs. 2AFC contrast detection). Additionally, we found that PL transferred to foveal crowding and substantially reduced the critical spacing for crowding. However, although overall significant, the transfer effect is not ubiquitous: only participants having high crowding before PL (P1, P4, P8, and P10) have high transfer effect on this task. Moreover, the level of initial crowding did not depend on either initial acuity or on the type of amblyopia; indeed, different from previous studies (Bonneh, Sagi, & Polat, 2004; Polat, 2005), strabismic amblyopic were not more likely to show crowding. It should be noted that—different from Hussain et al. (2012)—in our paradigm the reduction of crowding was not a direct consequence of training crowded letters. Instead, it occurred across tasks, since it was a generalization effect of practicing on contrast detection with lateral masking configurations. These across-task transfer effects cannot be explained by task-learning and, indeed, test-retest data for aged matched control group (Table 3) did not show no-task learning.

These transfer effects point to a common mechanism (or mechanisms) that, when strengthen by PL and rendered more efficient, exert their beneficial influence on untrained tasks and stimuli. The result whereby the improvement in visual acuity following PL was not specific for the eye suggests that this mechanism acts beyond the modulatory effect of anomalous lateral interactions in the cortical representation of the amblyopic eye. Based on the assumption that vision is attained by intra-cortical (within visual areas) and inter-cortical (between visual areas) connections (Lamme, Supè, & Spekreijse, 1998; Robol, Grassi, & Casco, 2013), it can be hypothesized that improved low level contextual influences, mediated by inter-cortical connections, allow integration of visual responses into a coherent representation via the recurrent intra-cortical processing (both feedforward and feedback). Recurrent intra-cortical processing may translate the low-level improvement of lateral interactions into a better capability of segmenting and binding together incoming local sensory signals into meaningful perceptual structures. Recurrent processing may allow the PL-dependent refinement of lateral interactions at early cortical level to result, at higher stage of visual processing, in more efficient binding operations. In this case, letters identification would be improved both when they are presented as isolated or flanked by other letters. Indeed, it has been shown that the same binding process may underlie feature integration in letter identification and crowding: whereas binding within-target elements subserves contour integration,

binding together target and distractor elements disrupts contour integration (Chakravarthi & Pelli, 2011). In addition, inter-cortical recurrent processing allowing feedback from higher to lower processing levels may mediate a more efficient top-down and attention-driven modulation of low-level high-resolution tasks, such as Vernier acuity and orientation discrimination of high spatial frequency gratings. The follow-up data discussed below also point to a PL effect not limited to the improvement of low level visual processing but extended to top-down driven improvement in combining these signals into salient percepts.

4.3. Follow-up

We found that visual acuity and contrast sensitivity for the trained spatial frequencies were further increased at follow-up several months after the training. This result indicates that not all the learning in the amblyopic eye is concurrent with practice. Previous studies have shown a latent phase lasting several hours during which the ability to perform a visual task further improves in the absence of concomitant training (Gais, Plihal, Wagner, & Born, 2000; Karni & Sagi, 1993; Stickgold, Whidbee, Schirmer, Patel, & Hobson, 2000; Walker, Stickgold, Jolesz, & Yoo, 2005). However, our transfer tests were performed several months after the training and it is unlikely that overnight consolidation of learning may be the explanation of the further improvement in visual acuity and CSF found at the follow-up with respect to the post-training evaluation. Four patients (P1, P5, P7, and P8) had new glasses after post-test, at least three months before follow-up, but the follow-up data were collected for two of them (P5 had full evaluation and P7 had only VA measured) and these data may have been affected at least partially by the new correction. We speculate that the observed improvement at the follow-up may instead reflect a more efficient recurrent inter- and intra-cortical processing. Follow-up improvement may reflect long-term consolidation of the positive influence of the low-level mechanisms being trained on higher level spatial processing. Moreover, it could, in turn, account for top-down modulation of the central low-level visual processing. From our data it could be suggested that recurrent processing in vison may be adaptive in nature as suggested by the result that the improvement at follow-up occurs for the tasks more used in daily life (i.e., visual acuity and contrast sensitivity).

Conclusions

Amblyopia is a disorder of spatial vision which can be reduced by refining the lateral interconnections between neurons in the primary visual cortex through perceptual learning. Perceptual learning can improve low-level spatial vision modulating facilitatory and inhibitory

lateral interactions between spatial filters in the primary visual cortex (Gilbert & Wiesel, 1989). Additionally, we showed that training protocols based on lateral interactions are not stimulus and task specific, but they generalize to a variety of more complex spatial tasks (e.g., contrast sensitivity function, foveal, and peripheral crowding). This suggest that improved lateral interactions by PL may trigger complex cortico-cortical feedforward and feedback processing (Lamme et al., 1998; Robol et al., 2013), thus improving perceptual grouping and segmentation of forms at higher cortical levels, so to achieve salient perception of visual forms.

Acknowledgements

This research was supported by the University of Padova (Italy, authors MB, GC, LB, and CC) and the University of Lincoln (UK, author AP).

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Table 1

Partici-pants	Gen-der	Age	Refractive error	Visual acuity logMAR	Stra-bism	Suppres-sion	Stereopsis	Eye alignment	Cause of Amblyopia	Occlusion therapy
P1	F	10,8	RE +10.00 +2.00x170 LE plano	**RE** .78, LE -.02	No	No	Absent	Ortho	Aniso-metropia	No
P2	F	9,9	RE +6.75-1.50x180 LE +6.75-1.75x160	RE .06, **LE** .18	Yes	No	70" with Randot	Exotropia	Strabismus	failed
P3	F	7,8	RE +2.00, LE +2.00	RE .05, **LE** .42	Yes	LE	Not tested	Exotropia	Strabismus	interrupted
P4	M	25,3	RE +0.50-4.50x140 LE +2.00-4.75x30	RE .18, **LE** .42	Yes	LE	Coarse	Exotropia	Strabismus	irregualar
P5	M	11,7	RE +4.25+1.50x135 LE +4.25+2.50x94	RE .02, **LE** .73	Yes	LE	Absent	Esotropia	Strabismus	irregular
P6	M	7,3	RE +3.00+1.25x85, LE +7.75+0.75x88	RE -.15, **LE** .23	Yes	LE	Absent	Exotropia	Strabismus & aniso-metropia	irregualar delated
P7	M	7,5	RE -8.25-2.50x150 LE plano	**RE** .88, LE -.12	No	No	240"	Ortho	Aniso-metropia	irregualar delated
P8	F	47,2	RE plano LE -0.25+1.00x80,	**RE** .37, LE .009	No	No	Unknown	Ortho	Unclassified	No
P9	M	21,8	RE +4.50+2.50X110 LE +5.00+2.50X70	**RE** .63, **LE** .40	No	No	Unknown	Nystag-mus	Bilateral ametropia	No
P10	M	53,0	RE +0.50-1.00x140 LE +0.25-0.75x25	RE -.07, **LE** .6	Yes	No	No	Esotropia	Strabismus	irregular interrupted
Controls (M+F) n.10		7-51 (±16.9)			No	No	Yes	Regular	No	No

Table 1. Clinical details of each amblyopic observer. Visual acuity is expressed in logMAR (MAR, minimum angle of resolution in arc min); LE, left eye (in bold and underlined if is the amblyopic eye); RE, right eye (in bold and underlined if is the amblyopic eye).

Table 2

Partici-pant	SF (cycles/°)	Stimulus duration (ms)	N. sess. per SF	Tot. n. sess.	PL duration per SF (month)	Tot. PL duration (months)
P1	3, 3, 6	500, 250, 250	18, 9, 27	54	1.4, 0.5, 2.1	4
P2	3, 11, 9, 11	133, 133, 133, 133	8, 20, 25, 19	72	1.2, 3.4, 2.3, 3.8	10.7
P3	3, 8	133, 133	28, 8	36	3.0, 0.9	3.9
P4	3, 6	250, 250	33, 31	64	4.2, 1.4	5.6
P5	3, 3, 6, 6, 8	133, 250, 133, 250, 250	22, 11, 19, 19, 18	89	1.3, 0.6, 1.2, 1.3, 1.1	5.5
P6	3, 5, 6, 6	133, 133, 133, 133	27, 30, 22, 14	93	1.2, 1.5, 1.3, 4.1	8.1
P7	3, 4	133, 133	24, 13	37	2.7, 2.2	4.9
P8	6	133	21	21	4,2	4.2
P9	3, 3, 4, 6	250, 133, 133, 133	7, 4, 12, 20	43	0.5, 0.3, 1.2, 2.1	4.1
P10	10, 11, 12, 15	200, 200, 200, 200	9, 12, 16, 46	83	0.5, 1, 2.3, 3.1	6.9

Table 2. The spatial frequencies (SF) and stimulus duration used during training. Details of training duration and number of training session are also reported.

Table 3

Partici-pants	Date	VA (logMAR)		CONTRAST SENSITIVITY (cycles/°)						Vernier	CW
		F Eye	A Eye	1	3	5	7	11	15	arcsec	Gap c-c
P1	03/06/2013	-0.02	0.78	18.76	7.531	2.44	0.00	0.00			11.5
	07/10/2013		0.86	27.17	17.75	5.69	3.57	7.82			6.9
f-up	18/01/2016	-0.27	0.82	31.45	18.69	5.00	2.57				7.7
P2	25/07/2013	0.06	0.18	39.68	91.74	30.86	20.37	13.26	3.13	249.2	6.00
	03/12/2014	-0.30	-0.23	44.64	109.8	222.2	103	106.3	91.74	62.4	5.0
P3	18/02/2014	-0.05	0.42	153.8	104.1	28.98	14.4	7.86	2.56		
	13/06/2014	-0.08	0.29	86.95	222.2	30.39	25.9	13.07	5.065		
f-up	07/12/2015		0.03	153.0	336	33	36	19	7		
P4	19/06/2014	0.18	0.52	135.1	192.3	39.84	88.50	16.05	3.21		19.6
	19/02/2015	-0.02	0.13		135.1	85.47	86.95	19.8	4.99		6.9
f-up	29/09/2015	0.14	0.13	86.95	156.2	204.0	147.0	31.74			7.7
P5	26/06/2014	0.02	0.73	46.30	21.28	8.46	4.81			131.6	7.1
	22/04/2015		0.55	120.5	51.81	17.42	19.53	7.42		69.1	5.0
f-up	21/10/2015	-0.15	0.31	33.67	74.63	52.36	37.04	7.60		50.4	
P6	01/09/2014	-0.15	0.23	93.46	39.22	37.59	14.60	5.44	1.89		7.8
	16/06/2015		0.18	54.95	80.00	24.21	30.40	9.80		93.7	
f-up	29/09/2015	-0.06	0.05	131.5	131.5	158.7	79.37	31.45		23.3	
P7	30/04/2014	-0.12	0.88	434.7	86.96	16.05	6.61			281	5.0
	15/05/2015		0.72	163.9	39.06	14.60	6.52			277	
f-up	15-12-2015		0.62								
P8	25/09/2014	0.01	0.37	29.15	43.80	18.72	18.51	4.08	2.44	250.7	13.3
	08/04/2015	-0.01	0.17	50.50	40.48	20.28	25.00	6.27	3.47	250.7	9.8
P9	15/09/2015		0.63	107.5	38.57	5.34	0			60.8	5.0
	28/01/2016		0.40	72	69	5	1.68			48	5.0
f-up			0.42	141 2	24	8	5			33	7.7
P10	08/02/2016	-0.07	0.6	78.13	98.04	69.44	78.74	18.98	8.29	95	21.6
	05/05/2016	-0.14	0.5	67.67	90.91	133.3	92.59	20	7.7	64	9,9
	19/12/2016		0.46								
Control group (N=10)	Test	-0.15		88.46	150.4	114.3	68.11	42.93	30.51	4.89	0.20
	Retest	-0.15		83.75	128.2	114.3	74.25	48.35	29.03	5.62	0.24

Table 3. Transfer data for the amblyopic eye measured before PL, after, and at follow-up (f-up): SLOAN acuity, CSF for spatial frequencies of 1, 3, 5, 7, 11, and 15 cycles/°, Vernier acuity, and foveal crowding expressed as center-to-center critical spacing in stroke units (Gap c-c). SLOAN data were also measured for the fellow eye. Data from the control group refers to the Test-Retest.

Figure Captions

Figure 1. Representation of the stimuli used in the training phase and in pre- and post-training to test for lateral interactions. Only the vertical global configuration is shown, from left to right 2λ, 3λ, 4λ, and 8λ are shown. See text for more details about the stimuli.

Figure 2. Top panels (A and B) show contrast thresholds as a function of the target-to-flankers separation obtained before and after PL to low (A) and high spatial frequencies (B). Bottom panels show threshold modulation as a function of the target-to-flankers separation, resulting from PL to low (C) and high spatial frequencies (D). Zero indicates no modulation, whereas negative values indicate reduced suppression. Error bars ±SEM.

Figure 3. Individual and average visual acuity measured before and after PL and at the follow-up in the amblyopic eye (A) and fellow eye (B). Error bars ±SEM.

Figure 4. (A) Normalized contrast sensitivity function estimated before training (empty circles and dotted line), after training (filled black circles and continuous black line), and at follow-up (grey circles and continuous gray line). (B) PL-dependent enhancement in contrast sensitivity expressed as the log differences between contrast sensitivity before and after the training (empty squares and dotted line) and between contrast sensitivity estimated after training and at the follow-up (filled black squares and continuous line). Error bars ±SEM.

Figure 5. Individual and mean Vernier acuity measured in the amblyopic eye before and after PL. Error bars ±SEM.

Figure 6. Individual and mean critical spacing measured in the amblyopic eye before and after PL. The value reported compares center-center critical spacing expressed in stroke-units. Error bars ±SEM.

Figure 1

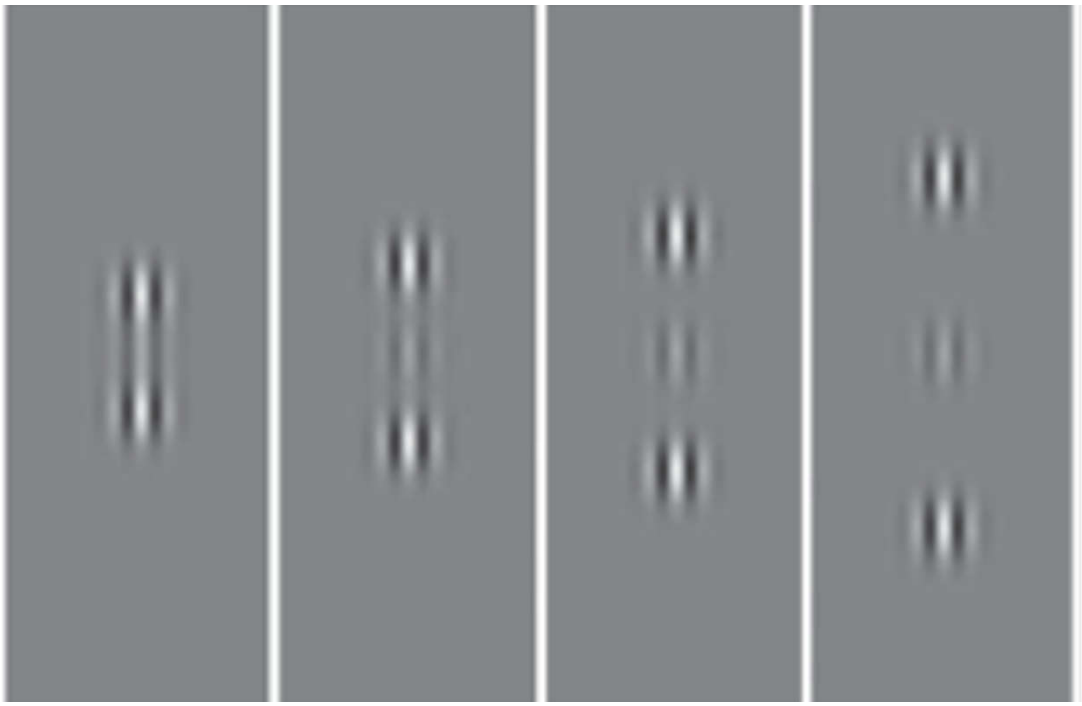


Figure 2

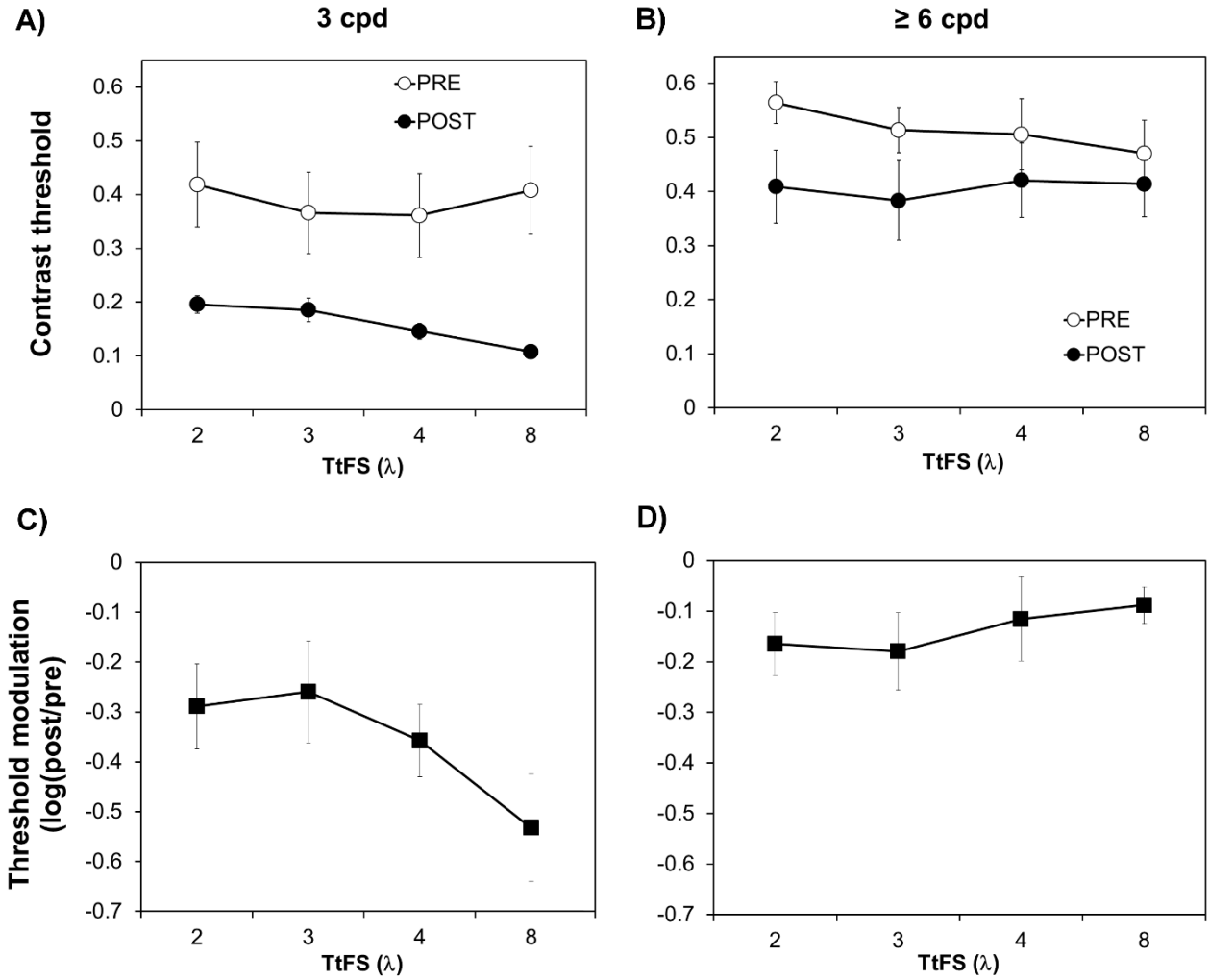

A)
3 cpd
B)
≥ 6 cpd
Contrast threshold
PRE
POST
TtFS (λ)
C)
D)
Threshold modulation (log(post/pre)

Figure 3

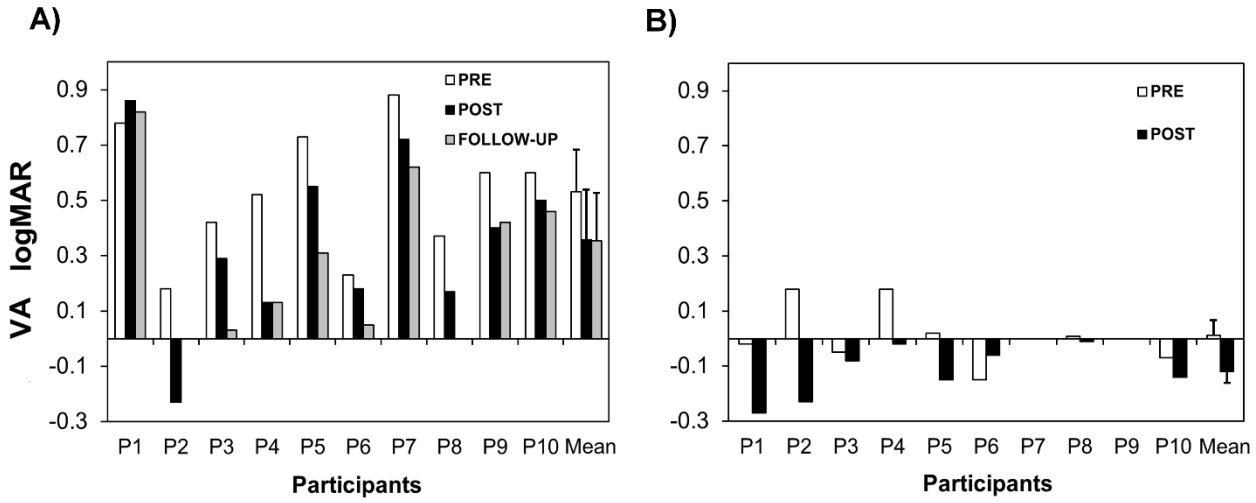

A)
B)
VA logMAR
0.9
0.7
0.5
0.3
0.1
-0.1
-0.3
PRE
POST
FOLLOW-UP
P1 P2 P3 P4 P5 P6 P7 P8 P9 P10 Mean
Participants

Figure 4

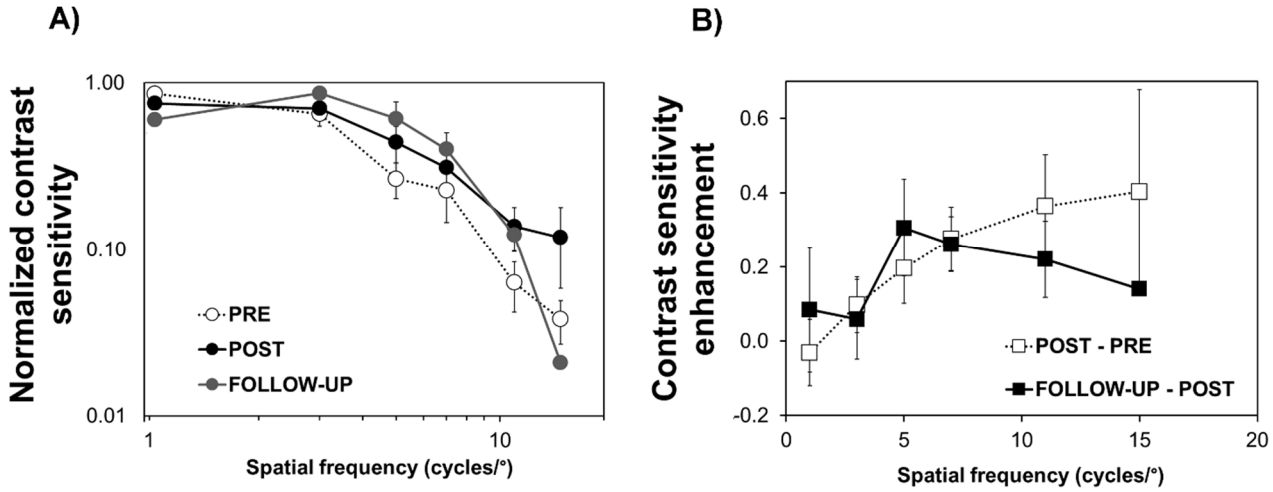

A)
Normalized contrast sensitivity
1.00
0.10
0.01
1
10
Spatial frequency (cycles/°)
PRE
POST
FOLLOW-UP
B)
Contrast sensitivity enhancement
0.6
0.4
0.2
0.0
-0.2
0
5
10
15
20
Spatial frequency (cycles/°)
POST - PRE
FOLLOW-UP - POST

Figure 5

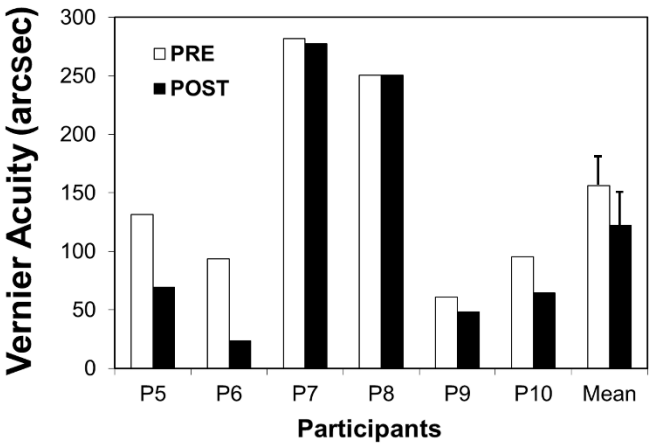
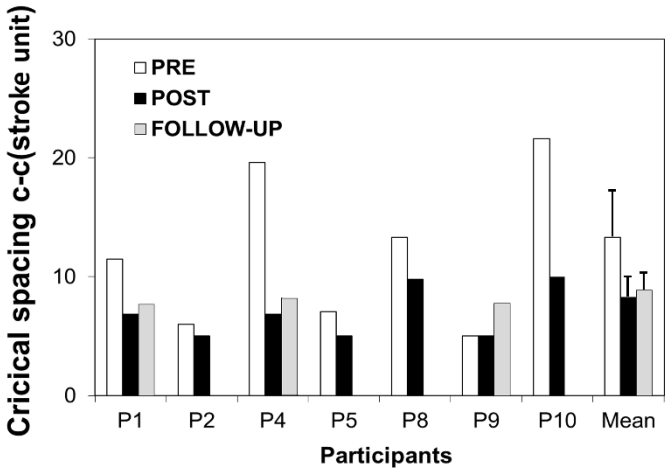

Vernier Acuity (arcsec)
300
250
200
150
100
50
0
PRE
POST
P5
P6
P7
P8
P9
P10
Mean
Participants

Figure 6


Cricical spacing c-c(stroke unit)
30
20
10
0
PRE
POST
FOLLOW-UP
P1
P2
P4
P5
P8
P9
P10
Mean
Participants