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RESEARCH ARTICLE

A new counterintuitive training for adult amblyopia

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Abstract

Objectives: The aim of this study was to investigate whether short-term inverse occlusion, combined with moderate physical exercise, could promote the recovery of visual acuity and stereopsis in a group of adult anisometropic amblyopes. **Methods**: Ten adult anisometropic patients underwent six brief (2 h) training sessions over a period of 4 weeks. Each training session consisted in the occlusion of the amblyopic eye combined with physical exercise (intermittent cycling on a stationary bike). Visual acuity (measured with ETDRS charts), stereoacuity (measured with the TNO test), and sensory eye dominance (measured with binocular rivalry) were tested before and after each training session, as well as in follow-up visits performed 1 month, 3 months, and 1 year after the end of the training. **Results**: After six brief (2 h) training sessions, visual acuity improved in all 10 patients (0.15 ± 0.02 LogMar), and six of them also recovered stereopsis. The improvement was preserved for up to 1 year after training. A pilot experiment suggested that physical activity might play an important role for the recovery of visual acuity and stereopsis. **Conclusions**: Our results suggest a noninvasive training strategy for adult human amblyopia based on an inverse-occlusion procedure combined with physical exercise.

Introduction

Amblyopia is a neurodevelopmental disorder of vision[1,2] with a prevalence ranging from 1% to 5% depending on the population tested.[3] The most frequent causes of amblyopia are strabismus, anisometropia, and deprivation (e.g., due to cataracts) early in life,[1,2] within the so-called *critical period*.[4,5] If the retinal image is degraded during development, the visual cortex disregards the eye providing degraded input and responds primarily to the eye providing the better signal. Amblyopia can be ameliorated in young children by the long-term occlusion of the better (non-amblyopic) eye.[3] However, in humans, treatment after the closure of the critical period has a dramatically lower therapy efficacy, requiring extensive occlusion of the non-amblyopic eye to produce only a modest improve of visual acuity.[6] New therapeutic strategies recently proposed for adult amblyopia involve perceptual learning,[7–10] antisuppression dichoptic training[11–15] and brain stimulation.[16,17] The results of these studies are very important, indicating that the adult amblyopic visual system retains some degree of plasticity both at the monocular (amblyopic eye visual acuity) and binocular (stereopsis) level. However, these paradigms involve either very prolonged visual training to be performed in the laboratory, or invasive manipulations necessitating medical supervision, rendering them hardly applicable for clinical purposes.

Recently, new hope has emerged from evidence that the early visual cortex retains some degree of plasticity in adult humans.[18–26] Many studies have demonstrated a plastic

response to visual deprivation: a few hours[18,19] or a few days[20,21] of binocular deprivation modulate excitability of the primary visual cortex. However, monocular occlusion of one eye for a few hours paradoxically boosts the deprived eye signal, shifting ocular dominance in favor of the deprived eye.[22–25] This counterintuitive effect of monocular deprivation reflects a compensatory reaction of the visual cortex to deprivation aimed at maintaining the average cortical activity constant, indicating that some form of homeostatic plasticity[27] is also present in adult humans. Importantly, this plasticity is retained also in amblyopic patients, both for the fellow[28] and the amblyopic eye,[29] suggesting that inverse occlusion might be used to potentiate the amblyopic eye signal.

Recent evidence has shown that physical activity enhances homeostatic plasticity in adult humans,[30] and the modulatory effect of physical activity on visual plasticity is corroborated by several recent studies on animal models.[31–34] Given that physical exercise appears as a potentially suitable and noninvasive therapeutic strategy for adult amblyopia, we performed an apparently counterintuitive experiment which combined short-term deprivation of the amblyopic eye with physical exercise on selected adult anisometropic patients with no microstrabismus. Patching the amblyopic eye is commonly referred to as "inverse occlusion"[35] and has been historically used as an alternative treatment for cases of amblyopia with eccentric fixation, aimed at preventing the reinforcement of the eccentric fixation point in the amblyopic eye.[36–39] Abandoned for several decades, this approach has been recently reintroduced in a study involving occlusion of the amblyopic eye[40] over more than 2 months. The results are encouraging suggesting some recover of visual acuity after a prolonged occlusion. Here, we found that after six short training sessions (2 h each) of physical activity and inverse occlusion, visual acuity and stereopsis improved in adult patients, and that the improvement persisted for up to 1 year after the end of the treatment.

Methods

Subjects

Adult anisometropic patients (aged between 20 and 40 years old) were enrolled in the study after an ophthalmic screening examination in which detailed ocular and systemic anamnesis has been investigated (see Procedures section). Patients with a history of chronic ocular diseases other than amblyopia have been excluded from the study as long as patients presenting any chronic systemic disease. Similarly, patients with neurological or psychiatric disorder or under medication of any sort were excluded.

Out of 40 patients screened, 10 met the inclusion criteria and were enrolled in the study (4 males, mean age 32.9 ± 1.5, clinical data reported in Table 1).

Ethical statement

The experimental procedures are in line with the declaration of Helsinki and were approved by the regional ethics committee (Comitato Etico Pediatrico Regionale—Azienda Ospedaliero-Universitaria Meyer—Firenze [FI]), under the protocol "Plasticità del sistema visivo" (3/2011).

Apparatus and stimuli

Binocular rivalry

The experiment took place in a dark and quiet room. Visual stimuli were generated by the ViSaGe stimulus generator (CRS, Cambridge Research Systems), housed in a PC (Dell) controlled by Matlab programs. Visual stimuli were two Gaussian-vignetted sinusoidal gratings (Gabor Patches), oriented either 45° clockwise or counterclockwise (size: $2\sigma = 2°$, spatial frequency: 2 cpd), presented on a uniform background (luminance: 37.4 cd/m^2, C.I.E.: 0.442–0.537) in central vision with a central black fixation point and a common squared frame to facilitate dichoptic fusion. Visual stimuli were displayed on a 20-inch Clinton Monoray (Richardson Electronics Ltd., LaFox, IL) monochrome monitor, driven at a resolution of 1024 × 600 pixels, with a refresh rate of 120 Hz. Observers viewed the display at a distance of 57 cm through CRS Ferro-Magnetic shutter goggles that occluded alternately one of the two eyes each frame. Responses were recorded through the computer keyboard.

Procedures

Screening examination

Uncorrected visual acuity (ETDRS charts), best corrected visual acuity (BCVA, ETDRS charts, scored letter by letter average of three different charts), intraocular pressure (IOP), color vision (evaluated with the Ishihara tables), and stereopsis (evaluated with TNO and Lang tests 1 and 2) have been evaluated and registered. All patients have also been subjected to pharmacological cycloplegia (one eye drop of 1% tropicamide administered three times with an interval of 5 min between the administrations) in order to register cycloplegic ametropia with an autorefractometer (Topcon RM8900; Topcon, Tokyo, Japan) and skiascopy (Heine Beta 200; Heine, Herrsching, Germany) after 20 min from last eye drop administration. During the screening visit, all patients

Table 1. Clinical data.

| Patient ID | Age/ sex | Refractive error | | Visual acuity (LogMar) | | Stereoacuity (ArcSec) | Fixation AE | Treatment history |
		Right eye	Left eye	Right eye	Left eye			
S1	28/F	+0.25 +0.25 × 75	+2.25 +2.25 × 55	−0.06	0.62	Stereoblind	Central unsteady	Patched at age 5
S2	29/M	+0.00	−1.00 × 60	−0.2	0.16	Stereoblind	Central	No treatment
S3	27/F	−6.50 −3.00 × 10	−0.25 −0.75 × 165	0.1	−0.14	600	Central	Patched at age 11
S4	36/F	+0.25 −7.00 × 10	−1.00	0.2	−0.2	Stereoblind	Central	No treatment
S5	35/M	+0.00	+3.00 × 90	−0.2	0.54	240	Central unsteady	No treatment
S7	34/F	−0.25 × 180	+2.00 −1.50 × 180	−0.1	0.78	Stereoblind	Central	Patched at ages 7–10
S8	38/F	−3.25 −1.50 × 10	−1.00 −2.75 × 180	0.0	0.46	600	Central	Patched at age 5
S9	40/M	+0.00	+2.00	−0.2	0.14	240	Central	Patched at age 5
S10	34/M	+0.50	−0.50 −0.50 × 90	0.38	−0.2	240	Central	Patched at ages 7–13
S11	28/F	+5.00 +1.00 × 80	+0.00	0.52	−0.1	600	Central unsteady	No treatment

underwent noncontact ocular biometry (IOLMaster 500; Carl Zeiss, Oberkochen, Germany), and axial length (AL), anterior chamber depth, and white to white signal have been registered. A complete examination of ocular motility has also been performed in order to exclude muscular causes of amblyopia: cover and uncover far and near tests, versions and objective convergence evaluation, Irvine test and 8 diopters Paliaga test for microstrabimus. Fixational quality was also assessed using microperimetry (Nidek MP-1 Professional, Créteil, France). The screening examination was completed with endothelial cell count (Tomey EM 3000; Tomey, Nürnberg, Germany), corneal topography (Sirius System, CSO, Firenze, Italy) to study keratometric parameters, slit lamp biomicroscopy (SL 9900; CSO) and complete phundus examination with both 90D and 20D lenses have also been performed.

After the aforementioned examinations, subjects presenting any ophthalmic pathology other than anisometropic amblyopia (difference between the two eyes visual acuity >0.2 LogMar) have been excluded from the study. Subjects presenting abnormal or unclear ocular findings (e.g., elevated IOP, the presence of epiretinal membranes, low endothelial cell count, etc.) have been excluded from the study, as long as subjects presenting abnormal ocular motility that may have caused amblyopia. No new optical correction was provided after the screening examination.

BCVA (ETDRS charts) and stereoacuity (TNO test) were also measured in the follow-up tests performed 1 month, 3 months, and 1 year after the end of the training procedure.

Training procedure

Pilot experiment

Each training session lasted 2 h and consisted in the occlusion of the amblyopic eye (same procedure as main experiment) without simultaneous physical exercise. During the 2 h of monocular occlusion, patients sat at a distance of 90 cm in front of a 20″ monitor (LG) and watched a movie. This training was repeated three times in three consecutive days. Before and after each training session, visual acuity (ETDRS charts), stereoacuity (TNO test), and sensory eye dominance (binocular rivalry) were assessed for each patient.

Main experiment

Each training session lasted 2 h and consisted in the combination of amblyopic eye occlusion and physical exercise. Occlusion of the amblyopic eye was performed using eyepatching. The eye-patch was custom-made of a translucent plastic material that allowed light to reach the retina (attenuation 15%) but completely prevented pattern vision, as assessed by the Fourier transform of a natural world image seen through the eye-patch. During the 2 h of monocular occlusion, patients sat on a stationary bike equipped with a chair, and a computer monitoring physical activity parameters (cycling speed, distance). Heart rate was monitored through a wireless chest band connected to the bike computer. Patients were instructed to cycle intermittently maintaining a heart rate between 110 and 120 bpm for 10 min, interleaved with 10 min rest;

the experimenter controlled that physical exercise was performed according to these parameters. A 20″-monitor (LG) was placed in front of the bike at a distance of 90 cm; patients watched a movie projected onto the monitor during the training. Before and after each training session, visual acuity (ETDRS charts), stereoacuity (TNO test), and sensory eye dominance (binocular rivalry) were assessed for each patient. This training was repeated six times over a 4-week period: on three consecutive days during the first week of training and 1 day per week during the following 3 weeks (Fig. 1).

Binocular rivalry

Each binocular rivalry experimental block lasted 180 sec. After an acoustic signal (beep), the binocular rivalry stimuli appeared. Subjects reported their perception (clockwise, counterclockwise, or mixed) by continuously pressing with the right hand one of the three keys (left, right, and down arrows) of the computer keyboard. At each experimental block, the orientation associated to each eye was randomly varied so that neither subject nor experimenter knew which stimulus was associated with which eye until the end of the session, when it was verified visually. Because of anisometropia, when rivalrous stimuli having equal contrast were presented, amblyopic patients do not report perceptual alternation, as vision is completely dominated by the non-amblyopic eye. In order to adjust the monocular stimuli contrast to compensate for the patients' anisometropia, before the first training session, a few 60-sec preliminary experimental blocks were performed. At each block, contrast of monocular stimuli was varied, increasing contrast of stimuli presented to the amblyopic eye (maximum contrast: 100%) and decreasing contrast of the stimulus presented to the non-amblyopic eye (minimum contrast: 20%), aimed at inducing perceptual alternations and equal predominance (if possible) of the two eyes. By performing this contrast adjustment procedure, binocular rivalry could be measured in six of the 10 patients tested. In these six subjects, two binocular rivalry experimental blocks were acquired before and after each training session. The monocular contrast assessed in the preliminary test was kept constant throughout the experiment and various testing sessions.

Analyses

Binocular rivalry

The perceptual reports recorded through the computer keyboard were analyzed using Matlab. Mean phase durations (the average perceptual duration of each rivalrous stimulus) were computed for the two eyes. To quantify sensory eye dominance, we obtained an index of ocular
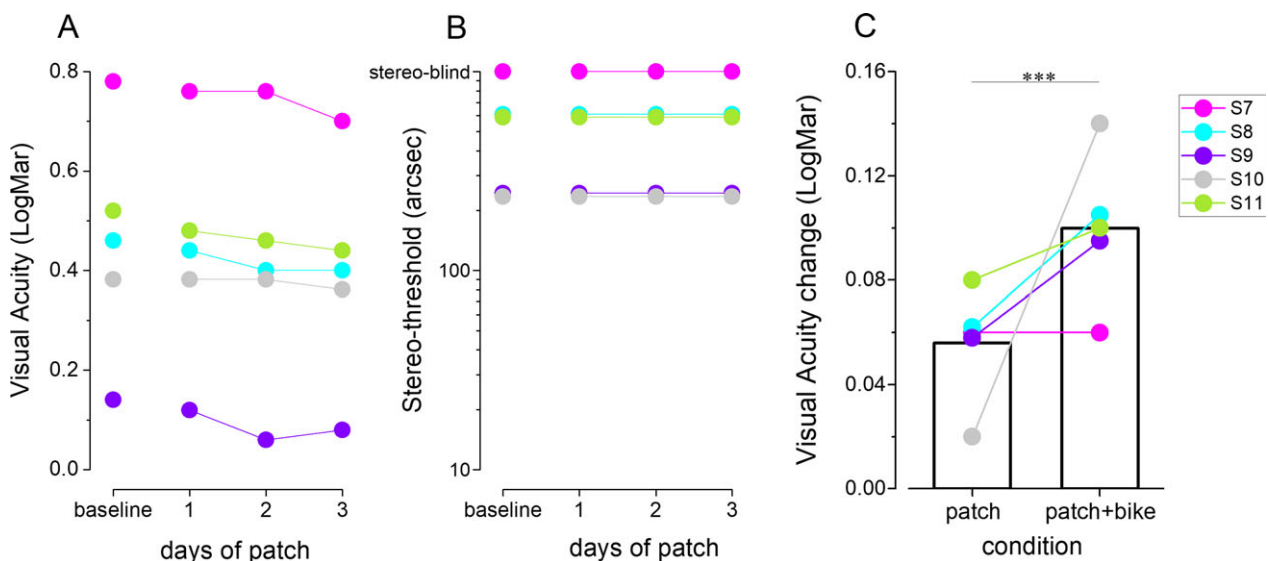
Figure 1. Pilot experiment results. Five out of the 10 patients recruited for the study performed a pilot experiment. In three consecutive days, the non-amblyopic eye was patched without the simultaneous physical exercise training. (A) LogMar visual acuity plotted as a function of time from the beginning of the pilot experiment (different symbols colors represent individual patient's performance). Only a small improvement in visual acuity was observed. (B) LogMar stereo-threshold plotted as a function of time from the beginning of the pilot experiment (C) Comparison between the visual acuity improvement observed in the pilot experiment and after the first 3 days of training in the main experiment combining monocular occlusion and physical exercise (different symbols for single subjects, bars: average visual acuity). The visual acuity improvement is significantly larger (bootstrap sign test, ***$P < 0.001$) for the main experiment.

dominance, ranging from −1 (complete dominance of the non-amblyopic eye) to 1 (complete dominance of the amblyopic eye), by computing the contrast between the mean phase duration of the two eyes:

$$\text{Ocular dominance index} = \frac{\text{Amblyopic eye} - \text{Nonamblyopic eye}}{\text{Amblyopic eye} + \text{Nonamblyopic eye}}. \quad (1)$$

Statistics

Statistical analyses were performed using SPSS20 (IBM Corp., Armonk, NY, USA) and Matlab softwares (Math-Works, Natick, MA, USA). BCVA, stereoacuity, and sensory eye dominance before, during, and after training were compared using repeated measures ANOVA (Greenhouse–Geisser correction was applied when the assumption of sphericity was violated) and paired-sample t-tests (α error fixed at 0.05). Correlations were computed using the Spearman's correlation coefficient (rho); a statistical significance was assessed using a permutation test. The effect of training on visual acuity in the main and control experiments, as well as the effect of monocular occlusion on binocular rivalry were compared using a within-subjects bootstrap sign test (10^6 repetitions).

Results

Ten adult anisometropic amblyopes (four males and six females), selected for good fixation and with no other neurological deficit, were recruited for the study. The mean age at the time of the enrollment was 32.9 ± 1.5 years. To assure a high homogeneity of the sample, only purely anisometropic amblyopes were included, discarding all patients showing microstrabismus. Mean visual acuity of enrolled subjects was -0.14 ± 0.07 LogMar for the dominant eye (range −0.2 to 0.0 LogMar) and 0.37 ± 0.2 LogMar for the amblyopic eye (range 0.14–0.78 LogMar). The mean spherical equivalent of amblyopic eyes measured in cycloplegia was 2.8 ± 2.3 (range −8 to 5.5) while mean AL was 22.7 ± 1.6 mm (range 20.5–24.9 mm). Microperimetry was performed in all subjects: three of them presented a central unsteady fixation and the others central fixation. Fundus appearance was examined in all subjects with both a 90D lens (slit lamp examination) and a 28D lens (indirect fundoscopy). No main fundus alterations were found with the exception of small areas of latex dystrophy that were found in one subject (refractive error −6.50 −3.00 × 10). Corneal topography and slit lamp biomicroscopy did not show any significant alteration and the mean IOP was 14 ± 1.9 mmHg. Stereopsis was present in six patients (range 240–600 ArcSec) while it was completely absent in four subjects (stereoblind) due to the deep amblyopic status.

Five patients underwent a pilot experiment consisting in three training sessions of short-term inverse occlusion in consecutive days, without physical exercise. At each session, the amblyopic eye was occluded for 2 h with a translucent eye-patch while patients watched a movie sitting on a chair. Visual acuity (ETDRS charts) slightly improved after 3 days of training (average visual acuity improvement: 0.06 ± 0.01 LogMar, repeated measures ANOVA: $F(3, 12) = 11.18$, $P = 0.005$, $\eta^2=0.74$, Fig. 1A), but no changes were observed in stereopsis (TNO test, Fig. 1B). Given that the effect of short-term inverse occlusion was promising but modest, patients repeated exactly the same patching procedure (three consecutive 2 h sessions at least 1 month after the pilot experiment) with the addition of physical exercise consisting of an intermittent cycling on a stationary bike. When physical exercise was combined with short-term inverse occlusion, the visual acuity improvement observed after 3 days of training was about double (average visual acuity improvement 0.1 ± 0.01 LogMar, Fig. 1C, bootstrap sign test, 10^6 repetitions, $P < 0.001$,) than that measured after occlusion alone. In one patient (Fig. 2B), we also measured an improvement in stereopsis.

In order to consolidate the effect of the training, patients underwent one session per week during the following 3 weeks, for a total of six 2 h training sessions over a 4-week period (Fig. 2). As the addition of physical exercise to the training was more effective, the other five patients recruited for the study performed only the complete training (main experiment) combining inverse occlusion and physical exercise.

During the 4 weeks of training, visual acuity significantly improved in all patients (Fig. 2A), with an improvement already evident after the first 2 h of patching ($t(9) = 2.37$, $P = 0.04$), and then increasing after each session. The average improvement after the sixth (and last) training session was 0.15 ± 0.02 LogMar ($t(9) = 7.7$, $P < 0.001$, Fig. 2A, repeated measures ANOVA, $F(6, 54) = 24.9$, $P < 0.001$, $\eta^2 = 0.73$). This improvement was maintained in the follow-up measurements (Fig. 3) obtained 1 month ($t(9) = 4.04$, $P = 0.003$), 3 months ($t(9) = 5.22$, $P < 0.001$), and 1 year after the end of training ($t(5)=3.81$, $P = 0.012$). The initial visual acuity of the amblyopic eye and the acuity improvement observed at the end of the training were not correlated (Spearman's rho = −0.16, $P = 0.67$, Fig. 4A), indicating that the effect of the training was independent of anisometropia severity. The visual acuity improvement observed in the five patients who performed both the pilot and the main experiment was comparable (independent-samples t-test $t(8) = 1.18$, $P = 0.26$) to that observed in the five patients who only performed the main experiment. Interestingly, the improvement in visual acuity after the pilot study and
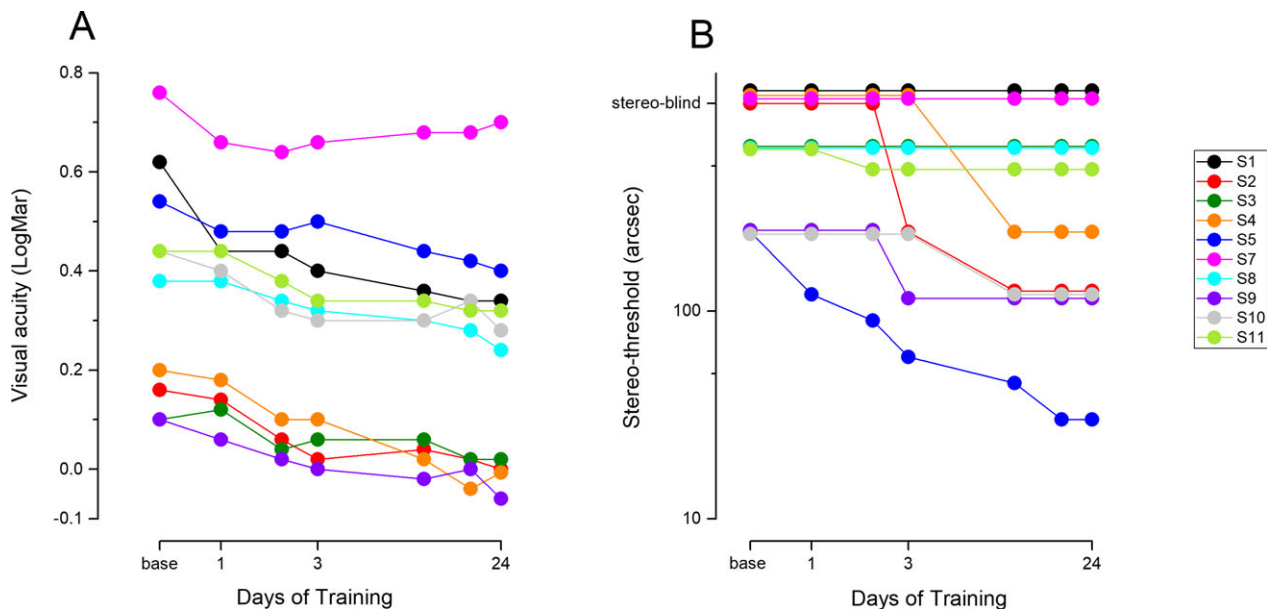
Figure 2. Training induces a recovery of visual acuity and stereo-threshold. (A) LogMar visual acuity plotted as a function of time from the beginning of training. To achieve a robust quantification of visual acuity, each point represents the average of three different ETDRS charts. Different symbol colors represent the individual subjects' performances after each training session. (B) Stereo-thresholds plotted as a function of time from the beginning of training. Stereo-thresholds were obtained using the TNO test. For subjects showing course stereopsis with the TNO test (S3, S8, S11), stereo-thresholds were obtained using the LANG stereo-test.

after the full training did not correlate significantly (Pearson's $r = -0.4$, $P = 0.5$), suggesting that there was no carryover of the effect between the two experiments.

The training was also effective enough to induce a significant improvement of stereo-thresholds (TNO test) in six out of the 10 patients, independently of their occlusion therapy history (Fig. 2B). This was a significant improvement for the sample (repeated measures ANOVA, $F(6, 54) = 6.2$, $P = 0.02$, $\eta^2 = 0.7$). Two of these patients were completely stereoblind before training (Fig. 2B). The patients who improved stereopsis during training also maintained the improvement in the follow-up measurements up to 1 year (Fig. 3B). Fixation quality of the amblyopic eye did not change after training (Fig. 4B, fixations within $2°$ pretraining $= 84.6 \pm 4.8\%$, after training $= 85.8 \pm 4.9\%$, $t(8) = 0.26$, $P = 0.8$), indicating that the improvement in visual function was not attributable to a change in ocular motility. Interestingly, the sensory dominance of the amblyopic eye, measured with a binocular rivalry task, also significantly increased after short-term occlusion (Fig. 5A, bootstrap sign test, 10^6 repetitions, $P < 0.001$), and ocular dominance measured before deprivation became progressively more balanced during the training (Fig. 5C). Consistently the proportion of mixed percepts during binocular rivalry (periods in which neither monocular image dominates, but a mixture of the two is perceived) progressively decreased during the

4 weeks of training (measurements acquired before deprivation, Fig. 5D), while increased following short-term monocular deprivation (Fig. 5B, bootstrap sign test, 10^6 repetitions, $P < 0.05$). These results suggest a change in the binocular neuronal circuitry mediating the ocular dominance.

The post-training changes in visual acuity, stereoacuity, and ocular dominance did not correlate with each other (all $Ps > 0.77$). Other ocular parameters such as IOP, AL, cycloplegic refractive error, and corneal topography remained stable during the study suggesting that the registered visual function improvement was not referable to variations of ocular conditions. In debriefing questionnaires, all patients reported a qualitative improvement of vision in the amblyopic eye ("I have a sharper/more contrasted/brighter vision from the amblyopic eye"). Two patients also reported a significant reduction in the occurrence of headaches/migraines usually experienced after prolonged exposure to screens, three patients reported an improvement in depth perception and one reported a reduction in crowding effects ("before the training I used to confuse adjacent letters with each other").

Discussion

Together, our results show that six brief sessions of short-term deprivation of the amblyopic eye combined with
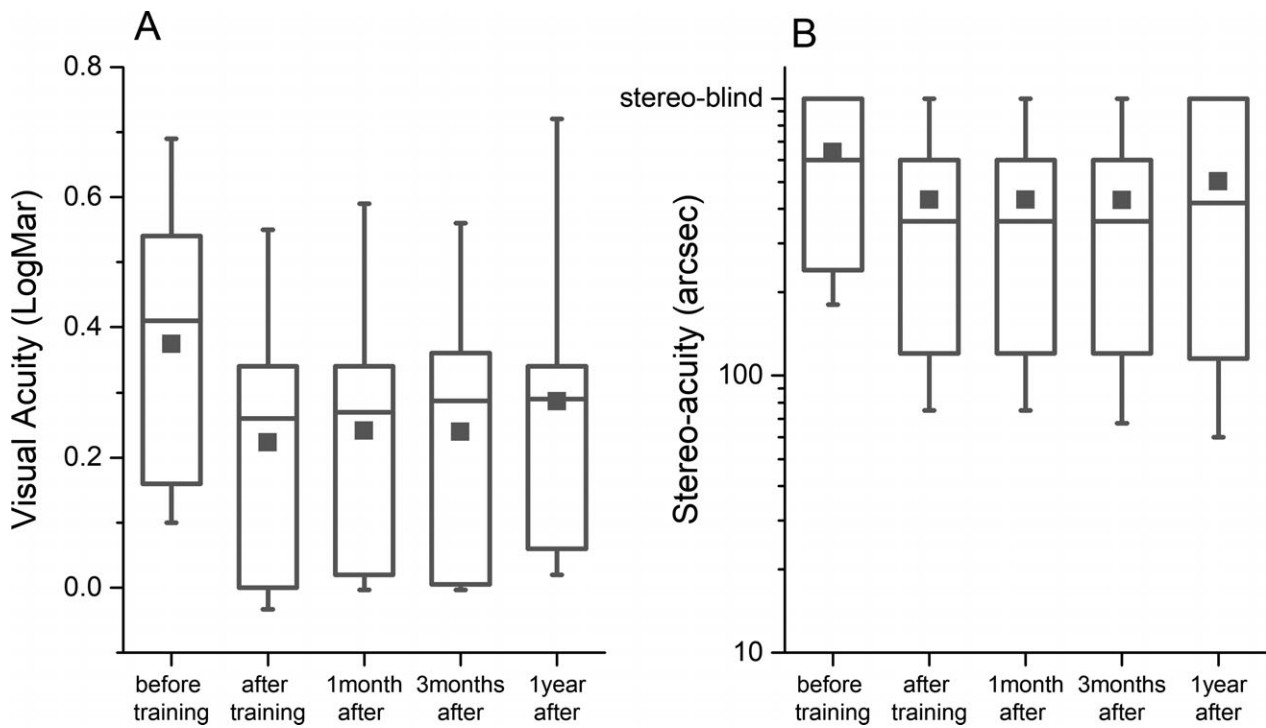
Figure 3. Visual acuity and stereo-threshold in follow-up measurements. Box plots showing visual acuity (A) and stereo-thresholds (B) measured before and after 4 weeks of training and in follow-up measurements obtained 1 months, 3 months, and 1 year after the end of training. Box plot explanation: upper horizontal line of box, 75th percentile; lower horizontal line of box, 25th percentile; whiskers, 10th and 90th percentile. Squares represent means.
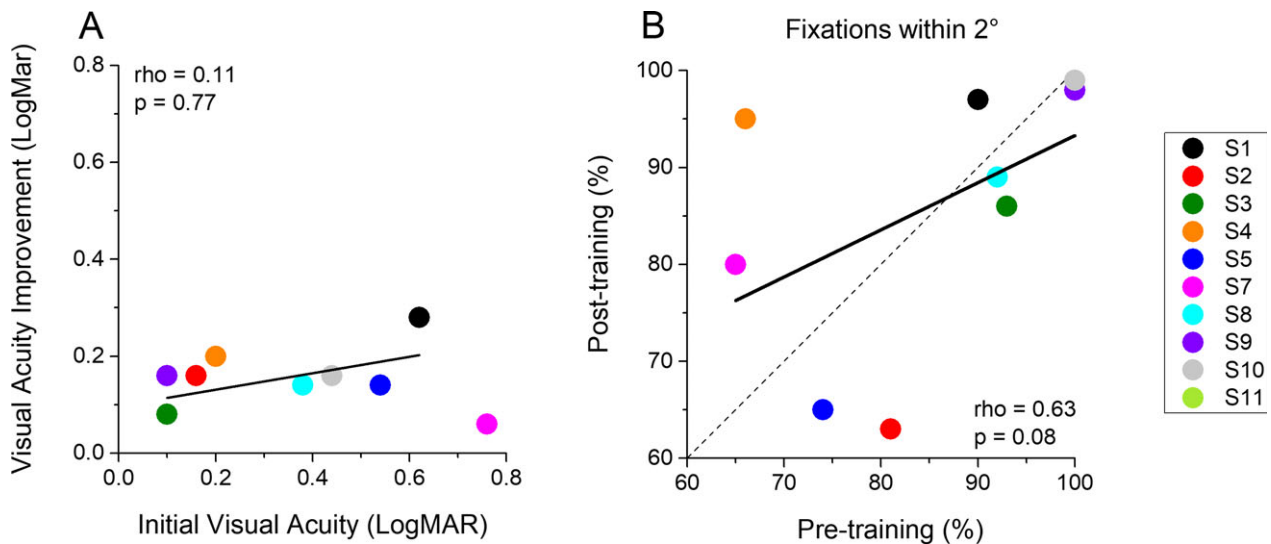
Figure 4. Correlations between anisometropia, visual acuity improvement, and fixation quality. (A) Scatter plot of the LogMar visual acuity measured for each subject (different symbol colors) before training (x-axis) and the difference between visual acuity measured after and before the 4-week training (y-axis). No correlation is observed between initial anisometropia and visual acuity. (B) Scatter plot reporting the percentage of fixations falling within a 2° radius from the fixation cross presented inside a microperimeter obtained before and after training. No consistent change in fixation quality is observed across subjects.
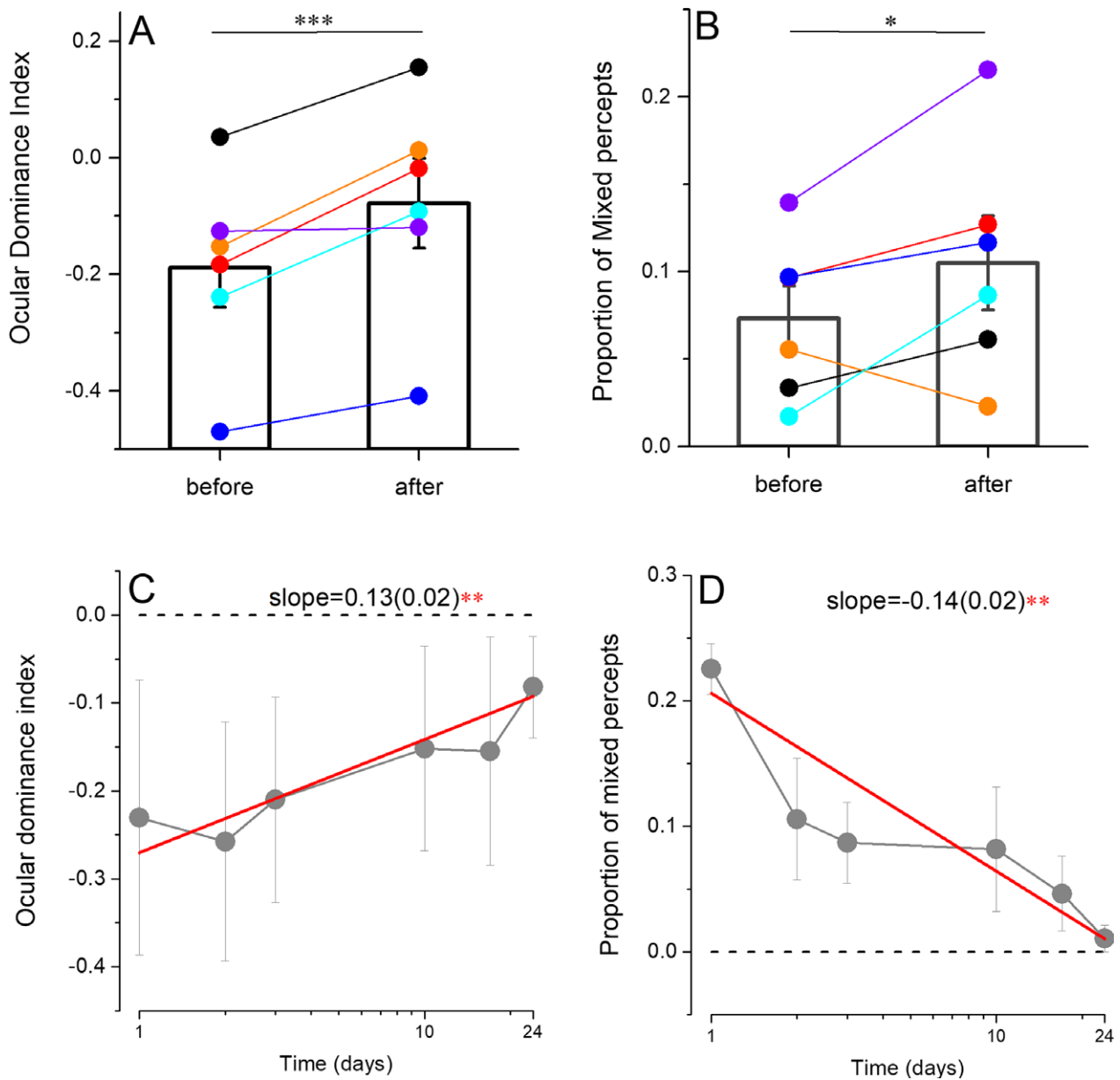
Figure 5. Sensory eye dominance changes after training. (A) Sensory eye dominance assessed before and after each 2 h training session (amblyopic eye occlusion + physical exercise), averaged for each subject across training sessions, different symbols colors for different subjects. (B) Proportion of mixed percepts measured before and after short-term monocular deprivation combined with physical exercise, different symbols colors for different subjects (paired-samples bootstrap sign test, ***$P < 0.001$, *$P < 0.05$). (C) Ocular dominance index measured at each training session before deprivation. The red line represents the linear fit of the data, the slope is significantly ($F(1, 4) = 39.9$, $P = 0.004$) higher than 0, indicating a positive trend. (D) Same as (C) but for the proportion of mixed percepts, the slope is significantly ($F(1, 4) = 65.5$, $P = 0.0013$) lower than 0, indicating a negative trend. Error bars represent $1 \pm$ SEM.

physical activity promote a long-term recovery of both visual acuity and stereopsis in adult anisometropic patients. The first surprising aspect of our results is that patching the amblyopic eye is effective in improving visual acuity in anisometropic patients, a procedure that is opposite to the traditional occlusion therapy used for amblyopia.[3] Even though prolonged inverse occlusion (6–30 weeks) was found to be effective to some extent in this particular class of patients, its effect was weaker than the effect of traditional occlusion,[39] and this practice was abandoned. The inverse occlusion approach for the treatment of amblyopia has been recently reintroduced by a

study[40] showing comparable effects of traditional and inverse long-term (2 months) occlusion for the treatment of amblyopia. Our results are consistent with this literature, confirming that inverse occlusion has moderate beneficial effects on the amblyopic eye acuity. However, we show that when short-term inverse occlusion is combined with physical exercise, it might be more effective than traditional occlusion for the recovery of visual function in adult amblyopes (234 h of occlusion per 0.1 LogMar acuity improvement[6] for traditional occlusion vs. 12 h of occlusion per 0.15 LogMar acuity improvement in our study). Moreover, the effect of short-term inverse occlusion is not due to a change in ocular motility, as fixation quality of the amblyopic eye did not change after training. Rather, we propose that the effect is due to a genuine boost of the amblyopic eye signal, consistent with the homeostatic plasticity previously observed in adult emmetropes[22–25,41,42] and amblyopes.[28,29]

Our data suggest that moderate physical exercise might boosts the effect of short-term monocular deprivation, inducing a larger improvement of visual acuity and stereo-sensitivity compared to inverse occlusion alone, and might potentially play a role in promoting the efficacy of the training: 120 h of inverse occlusion[40] are needed to observe an improve in visual acuity similar to that observed here after 12 h of occlusion combined with exercise. Our findings are consistent with recent evidence that voluntary physical exercise enhances visual cortical activity[43] and promotes visual plasticity in adult rodents[31,33] and in normally sighted humans.[30,44,45] Voluntary physical activity endorses the recovery of visual acuity in adult amblyopic rodents[31,33] when performed during long-term reverse suture, a paradigm conceptually very different from ours involving short-term amblyopic eye deprivation and eliciting homeostatic plasticity. Nevertheless, these studies agree with our results in showing a beneficial effect of exercise on visual plasticity. A recent study on adult humans[46] failed to find an effect of physical exercise on perceptual learning; however, in this study, physical exercise was performed either before or after the visual task. The aforementioned paradigms on both animal models and human subjects showed that the simultaneity between physical exercise and visual stimulation is crucial to observe the effect.

Our results indicate for the first time that physical exercise might promote visual cortical plasticity in amblyopic human subjects, after the closure of the critical period. The modulation of visual cortical activity and plasticity by physical activity has been linked to a decrease in GABAergic inhibition in the primary visual cortex of animal models.[32,34] We have recently found that a decrease in GABAergic inhibition in the primary visual cortex is also one of the key mechanisms mediating the effect of short-term monocular deprivation in adult humans: GABA concentration decreases in V1 after 2.5 h of monocular deprivation and the change in GABA strongly correlates with the change in ocular dominance measured with binocular rivalry.[41] It is therefore plausible that the beneficial effect of exercise on visual plasticity hinted by the results presented here is mediated by a modulation of GABAergic inhibition, promoting ocular dominance plasticity.

More generally, physical exercise also increases neurotrophic factors (BDNF, IGF-1, and VEGF) and cardiovascular fitness, two factors that might be involved in mediating neuroplasticity.[47] Increased BDNF following exercise has been related to enhanced hippocampal plasticity and neurogenesis,[48,49] as well as with improved memory and executive functions in humans.[50] Importantly, BDNF is also one of the critical mechanisms underlying visual plasticity, regulating the critical period for ocular dominance plasticity.[51–53] On the other hand, whereas cardiovascular fitness has been related to higher cognitive performances, no consistent correlation between cardiovascular fitness and improved cognitive functions after physical exercise has been found[54] and there is no evidence at present of a role of cardiovascular fitness in mediating visual cortical plasticity.

In conclusion, we propose a new training paradigm that is totally noninvasive, does not require extensive supervision, and leaves the patients free to perform pleasant activities such as watching a movie or television. It is therefore a valid candidate for clinical applications that, however, needs to be validated on a larger sample of patients. Amblyopia is the principal cause of (predominantly monocular) visual loss in the pediatric population (prevalence up to 5%[3]). While we believe that our data can provide a valid training approach for adult amblyopic patients and adolescents resilient to the standard occlusion therapy, we are well aware that the methods may be not appropriate as a substitution of standard clinical therapy, especially in young children. Homeostatic plasticity of the patched eye has been reported in amblyopic children[28] suggesting that the same method in principle could be used, but given the high plasticity of young children visual cortex, it should be first carefully validated with a dose-dependent chart.

Acknowledgments

Authors Contributions

C.L., A.S., and M.C.M. designed the experiment; A.T.S., A.L., M.L., and D.L. performed patients' clinical examinations; C.L. collected and analyzed the data; all authors discussed the results; C.L., M.C.M., A.T.S., and A.S. wrote the paper.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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