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Purpose. Recent studies on adults have shown that short-term monocular deprivation boosts the deprived eye signal in binocular rivalry, reflecting homeostatic plasticity. Here we investigate whether homeostatic plasticity is present also during occlusion therapy for moderate amblyopia.

Methods. Binocular rivalry and visual acuity (using Snellen charts for children) were measured in 10 children (mean age 6.2 ± 1 years) with moderate anisometropic amblyopia before the beginning of treatment and at four intervals during occlusion therapy (2 hours, 1, 2, and 5 months). Visual stimuli were orthogonal gratings presented dichoptically through ferromagnetic goggles and children reported verbally visual rivalrous perception. Bangerter filters were applied on the spectacle lens over the best eye for occlusion therapy.

Results. Two hours of occlusion therapy increased the nonamblyopic eye predominance over the amblyopic eye compared with pretreatment measurements, consistent with the results in adults. The boost of the nonamblyopic eye was still present after 1 month of treatment, steadily decreasing afterward to reach pretreatment levels after 2 months of continuous occlusion. Across subjects, the increase in nonamblyopic eye predominance observed after 2 hours of occlusion correlated (rho = −0.65, $P = 0.04$) with the visual acuity improvement of the amblyopic eye measured after 2 months of treatment.

Conclusions. Homeostatic plasticity operates during occlusion therapy for moderate amblyopia and the increase in nonamblyopic eye dominance observed at the beginning of treatment correlates with the amblyopic eye recovery rate. These results suggest that binocular rivalry might be used to monitor visual cortical plasticity during occlusion therapy, although further investigations on larger clinical populations are needed to validate the predictive power of the technique.

Keywords: homeostatic plasticity, amblyopia, occlusion therapy, binocular rivalry, psychophysics

Binocular rivalry is a form of perceptual bistability that engages strong competition between the monocular inputs.[1–3] When dissimilar images are simultaneously displayed on each retina, the resulting percept is not a fusion of the two, but a continuous alternation between the monocular images in a "winner-takes-all" dynamic: while one of the two images is perceived (dominant), the other is suppressed from awareness until a switch occurs and the previous suppressed image becomes visible again. The dominance duration of one eye relative to the other depends on the strength of the stimulus (e.g., increasing stimulus contrast in one eye will produce longer dominance periods of that eye compared with the other one[2]), and is an index of the strength of the monocular signals.[4] We have recently introduced binocular rivalry as a probe to measure neuroplasticity of the visual cortex in adult humans by showing that after 150 minutes of monocular deprivation, the deprived eye surprisingly dominates visual perception during binocular rivalry. The boost in dominance of the deprived eye does not increase the time of piecemeal rivalry, where a patchwork of the two rivalrous images occurs, but lengthens the phase duration of the deprived eye to the disadvantage of the nondeprived eye. This boosting effect is consistent with homeostatic plasticity,[5,6] where the lack of visual input may set high the gain of the neuronal responses. The effect originates in the primary visual cortex (V1), as we have shown that short-term monocular deprivation alters the earliest component of the Visual Evoked Potential, C1 in an eye-specific manner.[7] Overall, these results reinforce evidence coming from perceptual learning studies in normally sighted and amblyopic subjects (reviewed in Refs. 8, 9) pointing to a residual plastic potential of the primary visual cortex in adult humans.

Homeostatic plasticity is a compensatory reaction of the nervous system that involves a dynamic rescaling of synaptic activity to maintain constant average neural activity.[10–12] Evidence from in vitro measurements of cortical networks activity shows that this form of plasticity is mediated by an

TABLE. Visual Acuity Before and During Occlusion Therapy

				Visual Acuity, logMAR							
		Refractive Error, Diopters		Before Treatment		1 Month of Treatment		2 Months of Treatment		5 Months of Treatment	
Subject	Age, y	Right Eye	Left Eye	Right Eye	Left Eye	Right Eye	Left Eye	Right Eye	Left Eye	Right Eye	Left Eye
S1	5	+1.25 +0.50 (90)	+5 (80)	0.046	0.222	—	—	0.046	0.046	0	0
S2	6	+1.25 +4.00 (110)	+2.00 +0.75 (90)	0.398	0	—	—	0.155	0	0.097	0
S3	5	+2.25	+3.25 (75)	0	0.301	0	0.155	0	0.046	0	0
S4	6	+2.50	+4.25 +0.50 (90)	0	0.398	0	0.097	0	0.046	0	0
S5	7	+0.25 +0.50 (100)	−2.00+3.00 (110)	0	0.222	0	0.155	0	0.097	—	—
S6	6	+1 +3.75 (95)	+1 +1.25 (90)	0.097	0	0	0	0	0	—	—
S7	7	−1 −3 (15)	−5.25 −2.50 (45)	0.398	0	0.097	0	0.155	0	0.097	0
S8	7	+4	+5 +1.50 (80)	0	0.097	0	0	0	0	0	0
S9	5	+0.75	−4.25 −0.75 (75)	0	0.155	0	0.097	0	0	0	0
S10	8	+4.50 +1 (90)	+1.50	0.398	0	0.398	0	0.301	0	0.301	0

Refractive errors (expressed in diopters) and visual acuity (converted from decimals to logMAR according to this conversion logMAR of 0 corresponds to a visual acuity of 20/20, or 1.0 decimals, or 1 arc min) is reported for each eye. Visual acuity is reported at different times: before the beginning of treatment, 1 month, 2 months, and 5 months after occlusion therapy onset. Dashes represent missing binocular rivalry measurements due to subject's unavailability. The numbers in the parentheses are the ax of astigmatism.

adjustment of excitatory and inhibitory activity within recurrent cortical networks.[10–12] The unexpected perceptual boost of the deprived eye activity found in adults after short-term monocular deprivation[5–7] could represent the first short-term homeostatic response of the visual system to visual deprivation, probably acting through an upregulation of contrast-gain control mechanisms of the deprived eye,[5] mediated by changes in the intracortical excitation/inhibition balance.[13]

Here we use binocular rivalry to investigate neuroplasticity during occlusion therapy in unilateral amblyopic children. Amblyopia is a neurodevelopmental disorder of vision provoked by abnormal visual experience early in childhood that leads to a loss of visual acuity mainly in one eye and a consequent impairment of binocularity.[14–18] The most used treatment for anisometropic amblyopia during childhood is occlusion therapy, which consists of patching the nonamblyopic eye for extended periods of time.[17–20] Because sensitivity to high spatial frequency is a crucial factor for the development of amblyopia,[21] a less aggressive form of occlusion can also be used for the treatment of mild forms of amblyopia that consists of applying Bangerter filters on the spectacle lens of the nonamblyopic eye rather than using an eye patch. Bangerter filters (Ryser Optik, Gallen, Switzerland) are translucent diffuser filters made of scattering micro-elements designated to reduce visual acuity by different levels, depending on the strength of the filter. They act by attenuating the high and mid-range of spatial frequencies without introducing spurious spatial frequencies or alteration of the phase spectra.[22] Occlusion therapy with Bangerter filters, which we used in this study, has been previously shown to be effective in the treatment of moderate amblyopia[23–26] and also as effective as patching, while causing less treatment burden.[27]

The homeostatic boost of the occluded eye that we have previously reported (increased strength of the occluded eye reflected in increased predominance over the nonoccluded eye after short-term monocular deprivation)[5,6] may appear to be in contrast with the outcome of occlusion therapy, in which the nonoccluded eye is strengthened. However, it is possible that homeostatic plasticity coexists with structural neuroplasticity involved in the recovery of visual function of the amblyopic eye[28–30] without interfering with it, as suggested by animal work showing that both homeostatic and Hebbian mechanisms regulate neural activity during ocular dominance plasticity.[31] Here we investigate the relationship between these two forms of neuroplasticity by measuring binocular rivalry at different times during occlusion therapy in amblyopic children, and we use the homeostatic response to monocular occlusion to predict the recovery rate of the amblyopic eye.

METHODS

Subjects

Given the developmental time course of binocular rivalry,[32,33] we tested children between 5 and 7 years of age. After a complete ophthalmologic examination, we selected children affected by unilateral anisometropic amblyopia without additional ocular or neurologic pathologies and without any previous treatment. Ten children (three female), mean age 6.2 ± 1.0 years (±1 SD), were included in the study. The Table reports refractive errors and monocular visual acuity for each subject. Cycloplegic refraction was measured 30 minutes after administration of cyclopentolate and tropicamide (cyclopentolate was administered twice, the two administrations were separated by a 5-minute interval, and tropicamide was administered once during the first ophthalmologic visit). Full spectacle correction was then prescribed. After 2 months of optical correction, all patients presenting at least an interocular acuity difference of 0.2 decimals and a stereo acuity of at least 600 seconds of arc on Lang stereotest were considered eligible for the study. For the amblyopia treatment, a Bangerter filter

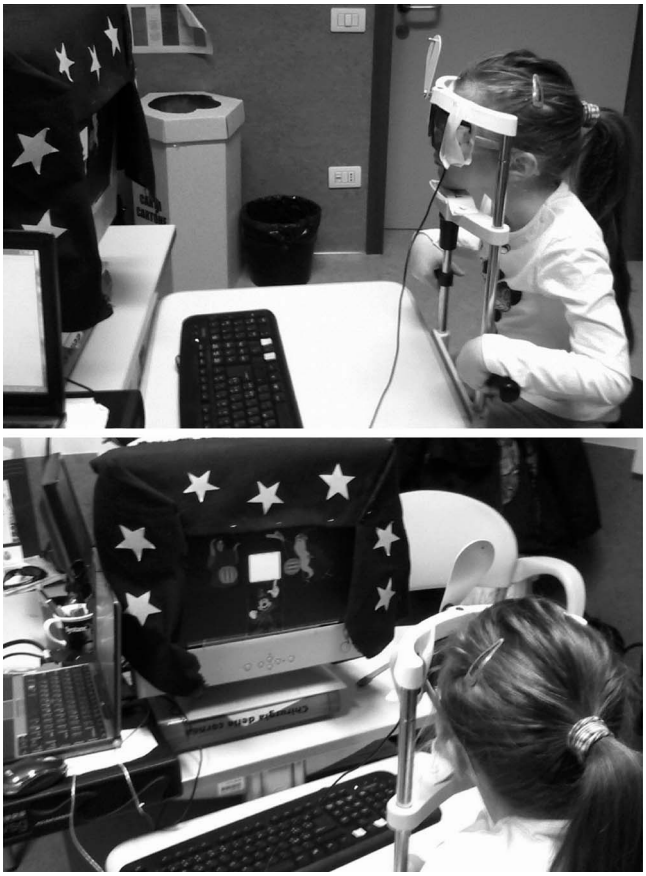

FIGURE 1. Child-friendly experimental setup. Children were told to be the referees of a magic contest between the characters stuck on the monitor.

(strength 0.4) was placed on the spectacle lens over the nonamblyopic eye and worn the whole waking time, as reported and monitored by the parents. Visual acuity of the amblyopic eye improved during the treatment, indicating that the occlusion therapy was effective for all patients (see results section for statistics).

Ethics Statement

The experiment was performed according to the principles of the Declaration of Helsinki and was approved by the ethics committee of the hospital (Azienda Ospedaliero-Universitaria Meyer, Florence). Children were accompanied by their parents, who were also present during the test. Written informed consent was obtained from the participants' parents.

Apparatus and Stimuli

The experiment was set up in a dark and quiet room. Participants were first tested for best corrected visual acuity (Snellen acuity) of their two eyes using vector Snellen charts for children. Visual acuity was measured in decimals and then converted to logMAR acuity, where 0 logMAR corresponds to 1 arc minute or 20/20. The Table reports children's visual acuity measured at different time intervals from the beginning of the occlusion therapy (missing values in the chart indicate missing data due to patient unavailability on testing dates).

Visual stimuli were generated by a portable VSG 2/5 (CRS; Cambridge Research Systems Limited, Rochester, Kent, UK) housed on a laptop (DELL, Round Rock, TX, USA) and controlled by Matlab programs (The Mathworks, Inc., Natick, MA, USA). They were displayed on a gamma-corrected cathode ray tube monitor (LG, Seoul, South Korea) driven at a resolution of 600×400 pixels, with a refresh rate of 120 Hz. The patient's head was stabilized with a chinrest placed at a distance of 57 cm from the monitor. Participants viewed the visual stimuli through CRS FE-Shuttering Goggles that were fastened to the chinrest. The goggles were synchronized with the monitor refresh rate through the VSG 2/5 and occluded alternatively the two lenses at each frame. Visual stimuli presentations were synchronized at the same frequency of the shuttering goggle, so each eye was presented with one of the two stimuli allowing dichoptic viewing with no leakage between the eyes.

Visual stimuli were achromatic orthogonal (horizontal and vertical) Gabor patches (size 2°, spatial frequency 2 cpd, contrast 75%) presented on a uniform average gray background (luminance 37 cd/m^2) in central vision, with a black fixation point and a black common squared frame (size 2.5°). Observers reported verbally their visual perception and responses were recorded through the computer keyboard manipulated by the experimenter.

Occlusion therapy was achieved with a Bangerter 0.4 filter glued on the spectacle lens of the nonamblyopic eye. A full characterization of Bangerter filters optics, including the 0.4 strength filter used in this study, has been reported elsewhere[22]: Bangerter filters decrease contrast monotonically with increasing spatial frequency, approximating a Gaussian filter, without producing phase distortions. A 50% attenuation of the 0.4 filter is reached at spatial frequency of 2 cycles per degree.[22] Because the optical features of the Bangerter foils are not homogeneous and often differ from the labeled density designation,[22] before being applied on the patient's appropriate spectacle lens, each filter was first measured by the experimenter for the correct level of blur. As reported by Perez et al.,[22] visual acuity measured through a 0.4 Bangerter filter should be equivalent to 0.4 decimals: the experimenter viewed vector Snellen charts through the filter and tested that the acuity attenuation produced by the filter was correct before applying it to the patient's spectacles. The patients were asked to wear the spectacles all the time with the filter applied on the best eye's lens until the control visit when visual acuity was checked again so as to measure the result of treatment. The full-time use of the spectacles and filter was confirmed by the report of the patients' parents.

Task and Procedures

To make the setup and the task appealing for children, we transformed the monitor into a "magic box" by covering it with a black cloth with yellow stars and some cartoon characters. A picture of the setup is shown in Figure 1. We told the children that they were required to act as referees of a magic contest, telling them that the magic tricks could be seen only through the goggles. Children were very keen to perform the task and look through the goggles. They were trained to report verbally whether the "stripes" (Gabor patches) on the monitor were "standing up" (vertical) or "lying down" (horizontal). The experimenter held the appropriate key of the computer keyboard according to the visual perception reported by the observer. Children were motivated to perform accurately the task because at the end of each 3-minute experimental block they were asked to judge which of two characters had won the magic contest (one was associated with the horizontal, the other with the vertical orientation). The orientation presented to each eye was swapped at every session as well as the orientation associated with a particular character to reduce the possibility of response bias in favor of

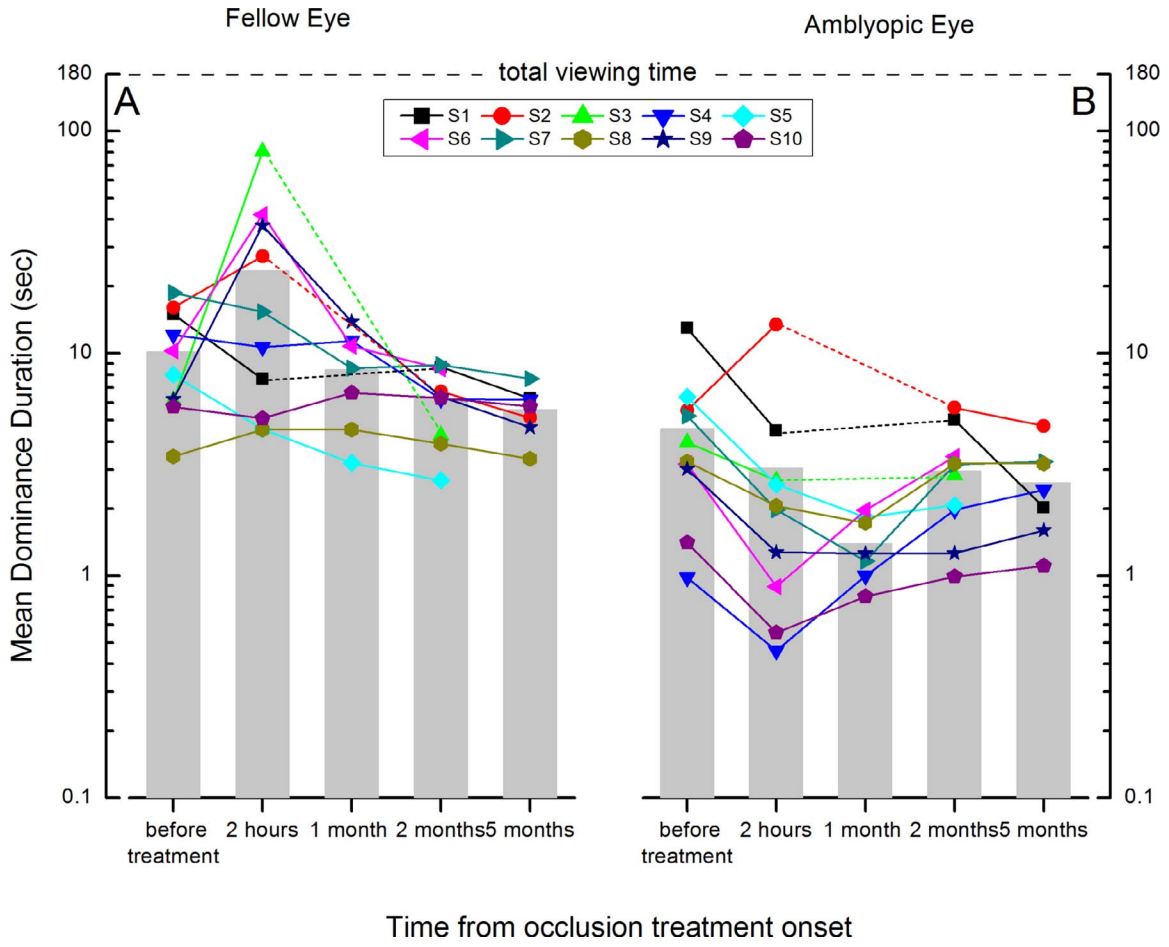

Figure 2. Nonamblyopic and amblyopic eye mean phase durations measured during 5 months of occlusion therapy. (**A**) Mean phase duration of the nonamblyopic eye measured during binocular rivalry before and during 5 months of occlusion therapy. Single patient data are represented by different *symbols* and *colors*, the *dashed line* connects the measurements acquired 2 hours and 2 months after occlusion therapy onset for three patients whose measurements at 1 month after therapy onset are missing. *Gray bars* represent the average mean phase duration. (**B**) Same as (**A**), but for the amblyopic eye.

one or the other orientation or character. We did not train the children to report the time of piecemeal rivalry, which is a difficult task to perform at this age. However, we asked after the experimental session if they observed a patchwork or a superposition stimulus, and none of the observers reported periods of patchy rivalry.

During the first training session, binocular rivalry was simulated by presenting the same orientation to both eyes and changing it at random intervals mimicking the dynamics of binocular rivalry. During the training session, the experimenter could check the accuracy of the child and ended the training when the observer reported the changes in visual orientation correctly. Children who were not able to perform the task were excluded and did not take part in the binocular rivalry experiment. These children could not maintain attention on the task and were reporting the simulated alternations unreliably (a total number of three children were excluded for this reason).

After the training session, a short (90 seconds) binocular rivalry session was recorded to determine the quality of binocular rivalry for the different children. Observers who did not show binocular rivalry (either not alternating at all or fusing the two visual images reporting to perceive a plaid) were excluded. In total, two children were excluded because they did not report perceptual alternations during the binocular rivalry training session and were only perceiving the stimulus presented to the nonamblyopic eye. These two children had a history of strabismus that had been surgically treated 6 months before the test. Binocular rivalry was measured before the placement of the Bangerter foil on the lens of the nonamblyopic eye (baseline measurements) and at different time intervals following the onset of the occlusion therapy: 120 minutes, 1 month, 2 months, and 5 months. Experimental blocks of 2 × 180 seconds were recorded for each session and child. Visual acuity was also measured at each of these testing times.

Analyses

The average time during which each stimulus was perceived (mean phase duration) was computed for each observer and each experimental session. For the baseline measurement, the second 180-second experimental block was used in the analyses as the first block served as training, whereas for measurements following occlusion treatment onset the first experimental block was used in the analyses because, as previously reported,[5,6] the effect of monocular occlusion on the dynamics of binocular rivalry is maximum during the first 3 minutes after occlusion removal.

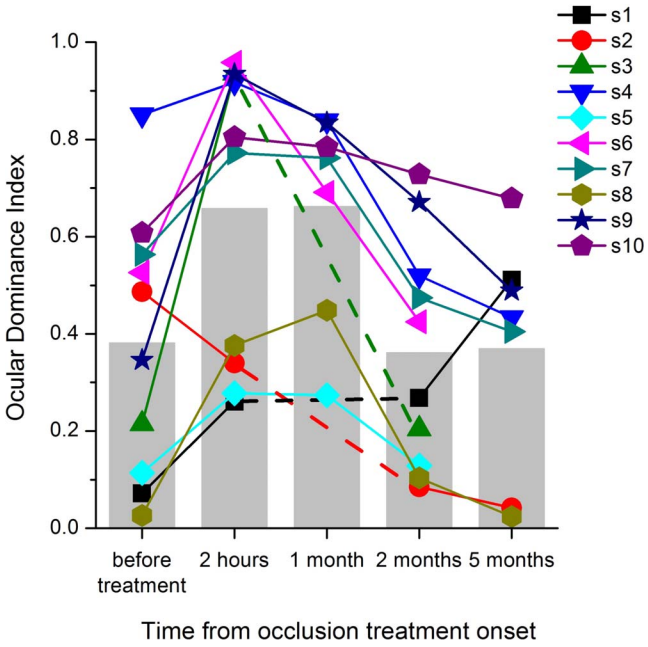

FIGURE 3. Nonamblyopic eye predominance in binocular rivalry over the amblyopic eye during occlusion therapy. The index of ocular dominance obtained by Equation 1 is plotted before occlusion therapy and during 5 months of therapy. According to this index, the value 0 represents balance between the two eye durations, and 1 represents total dominance of the nonamblyopic eye. Single patient data are represented by different *symbols* and *colors*, the *dashed line* connects the measurements acquired 2 hours and 2 months after occlusion therapy onset for three patients whose measurements at 1 month after therapy onset are missing. *Gray bars* represent the average mean phase duration.

Because of unavailability, not all 10 children were tested at each time interval. Binocular rivalry was measured in all 10 children at three time intervals: before treatment, 2 hours after treatment onset, and after 2 months of treatment. One month after treatment, patients S1 and S2 did not show up for the measurement. Five months after treatment onset, patients S5, S6, and S3 did not show up for the measurement. Five of 10 children provided data for the entire testing period.

Exclusions

One month after treatment onset, one subject (S3) never switched during the 180-second binocular rivalry test, reporting only the stimulus presented to the nonamblyopic eye. For this reason, S3 data acquired 1 month after treatment onset were excluded from the analyses, although consistent with the effect reported here.

Statistics

A repeated measures ANOVA was performed on the first four time intervals tested (before treatment, 2 hours, 1 month, and 2 months after treatment onset) on seven subjects (S4-S5-S6-S7-S8-S9-S10). Statistical tests were performed on the log transform of the mean phase durations. In addition, pairwise comparisons between measurements (dominance index) obtained at different time intervals were performed on all subjects available (see the Results section in the text for details). Correlations between binocular rivalry and visual acuity measurements were performed on data of all 10 subjects' data.

RESULTS

Occlusion Therapy

Monocular visual acuity was measured before occlusion therapy and at different time intervals after treatment onset (1, 2, and 5 months), as shown in the Table. Visual acuity of the amblyopic eye improved for all of them during the first 2 months of treatment (mean ± 1 SEM, visual acuity before treatment = 0.269 ± 0.042 logMAR, 2 months after treatment = 0.085 ± 0.032 logMAR, paired samples, 2-tailed *t*-test, $\alpha = 0.05$, $t(9) = 6.73$, $P < 0.0001$). Six of 10 children fully recovered visual acuity within 5 months from the beginning of treatment.

Binocular Rivalry

A child-friendly experimental setup was used to test binocular rivalry (Fig. 1). Binocular rivalry dynamics were measured before occlusion therapy onset and at four different time points during therapy: 2 hours, 1 month, 2 months, and 5 months. Mean phase durations, defined as the average duration in which observers perceived the stimulus presented to either eye during binocular rivalry, are reported in Figure 2. As expected from the difference in visual acuity between the eyes, dominance durations of the nonamblyopic eye were overall considerably higher compared with the amblyopic eye, mean phase duration in binocular rivalry being a proxy for eye dominance (Fig. 2). Interestingly, during occlusion therapy, a different trend was observed for the nonamblyopic (Fig. 2A) and amblyopic (Fig. 2B) eye: phase durations of the nonamblyopic eye increased after 2 hours of occlusion, whereas durations of the amblyopic eye decreased, both reverting to pretherapy levels after 2 months of therapy retaining the same balance at 5 months (comparison 2 versus 5 months: paired-sample *t*-test, $n = 7$ [S1, S2, S4, S7, S8, S9, and S10], $\alpha = 0.05$, $t[6] = 0.9$, $P = 0.4$). Because complete data are available for only five patients, we performed ANOVA tests on the first four intervals tested (from pretreatment to 2 months after treatment). The Shapiro-Wilk test indicated that the data distribution followed a normal distribution (all *P*s < 0.17). A 2(EYES) × 4(TIME) repeated measures ANOVA (performed on log mean phase durations of the seven patients for which all measurements were acquired: S4, S5, S6, S7, S8, S9, and S10) confirmed a significant effect of the factor EYES, $F_{1,6} = 28.072$, $\eta^2 = 0.82$, $P = 0.002$, and a significant EYES*TIME interaction, $F_{3,18} = 8.812$, $\eta^2 = 0.595$, $P = 0.001$ (Mauchly's Test of Sphericity indicated that the assumption of sphericity had not been violated, Mauchly's $w = 0.49$, $\chi^2[5] = 7.26$, $P = 0.21$), however no significant effect of the factor TIME, $F_{3,18} = 2.538$, $\eta^2 = 0.297$, $P = 0.09$ (Mauchly's $w = 0.49$, $\chi^2[5] = 3.33$, $P = 0.65$). A polynomial test on the EYES*TIME interactions revealed a quadratic component of the effect, $F_{1,6} = 22.436$, $\eta^2 = 0.79$, $P = 0.003$, reflecting that the effect of occlusion on binocular rivalry mean phase duration had a curvilinear trend.

To represent the interaction between eyes and time, we obtained an index of ocular dominance, ranging from −1 to 1, by computing the contrast between the mean phase duration of the two eyes:

$$\text{Ocular Dominace Index} = \frac{NonAmblyopicEye - AmblyopicEye}{NonAmblyopicEye + AmblyopicEye}. \quad (1)$$

An index value of 0 represents balance between the eyes, negative values represent dominance of the amblyopic eye, positive values dominance of the nonamblyopic eye. Figure 3 shows how the index varies with occlusion time. Before therapy onset, the ocular dominance index was significantly

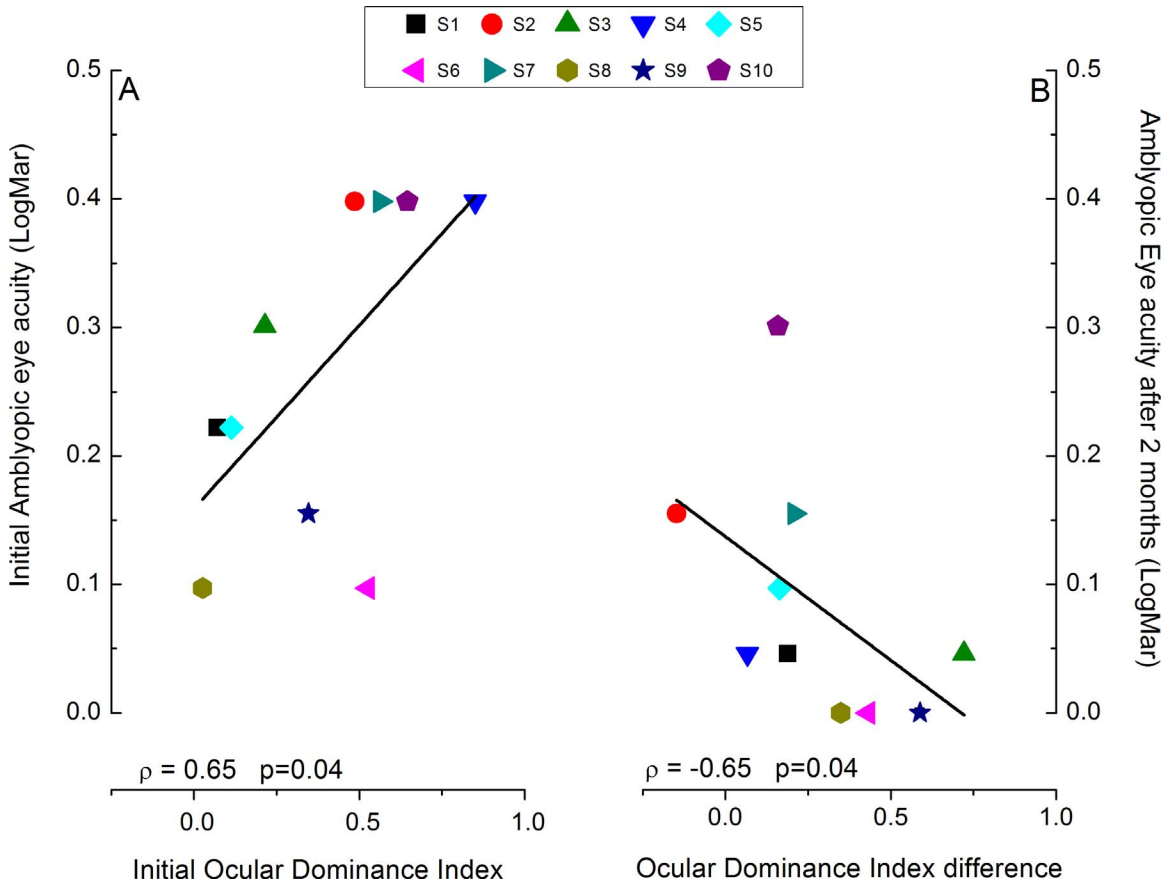

FIGURE 4. Binocular rivalry dynamics correlation with amblyopic eye acuity. (**A**) Correlation between visual acuity of the amblyopic eye and the ocular dominance index given by Equation 1 before treatment. Different *symbols/colors* represent different patients. (**B**) Correlation between visual acuity of the amblyopic eye measured after 2 months of therapy and the increment in nonamblyopic eye predominance over the amblyopic eye observed after 2 hours of occlusion (ocular dominance index measured after 2 hours of occlusion − ocular dominance index measured before occlusion).

higher than 0 (mean ± 1 SEM = 0.38 ± 0.09, one sample, 2-tailed *t*-test, $N = 10$ [all subjects], H_0: $\mu = 0$, $\alpha = 0.05$, $t[9] = 4.43$, lower 95% confidence interval [CI] = 0.19, Upper 95% CI = 0.58, $P = 0.002$), indicating that nonamblyopic eye mean dominance durations were longer than those of the amblyopic eye. After 2 hours of occlusion, the nonamblyopic eye predominance significantly increased (paired samples, 2-tailed *t*-test, Holms-Bonferroni corrected $\alpha = 0.0125$, $N = 10$ [all subjects], $t[9] = 3.38$, $P = 0.008$), reflecting a homeostatic boost of the occluded eye similar to that observed in adults.[5,6,13] The effect stabilizes to a similar value obtained after 1 month of occlusion therapy (1 month versus 2 hours occlusion: paired samples, 2-tailed *t*-test, $N = 7$ [S4, S5, S6, S7, S8, S9, and S10], Holms-Bonferroni corrected $\alpha = 0.025$, $t[6] = 1.43$, $P = 0.2$; 1 month versus baseline: $N = 7$ Holms-Bonferroni corrected $\alpha = 0.0167$, $t[6] = 3.4$, $P = 0.014$). After 2 months of occlusion therapy, the predominance of the nonamblyopic eye returns to pretherapy level (2 months versus baseline: paired samples, 2-tailed *t*-test, $N = 10$, Holms-Bonferroni corrected $\alpha = 0.05$, $t[9] = 0.34$, $P = 0.74$; 2 months versus 2 hours: Holms-Bonferroni corrected $\alpha = 0.01$, $t[9] = 4.34$, $P = 0.002$; 2 months versus 1 month: $N = 7$, Holms-Bonferroni corrected $\alpha = 0.0083$, $t[6] = 5.64$, $P = 0.001$).

Both eye predominance and acuity recovery are indices of neuronal plasticity. An interesting question is how the nonamblyopic eye predominance is related to the amblyopic eye acuity before and during treatment. Before treatment onset, the ocular dominance index correlated across subjects with the amblyopic eye acuity (Fig. 4A, $N = 10$ [all subjects], Spearman's rank correlation coefficient rho = 0.65, 2-tailed exact permutation test $P = 0.04$, CIs, Fisher's Z transformed, ranging from 0.35–0.91), indicating that the dynamics of binocular rivalry reflect interocular differences in visual acuity, although the spatial frequency of the stimuli is at least 10 times lower than grating acuity. Interestingly, the increase in nonamblyopic eye predominance measured 2 hours after treatment onset correlated across subjects with visual acuity of the amblyopic eye measured after 2 months of treatment (Fig. 4B, $N = 10$ [all subjects], Spearman's rank correlation coefficient rho = –0.65, 2-tailed exact permutation test $P = 0.04$, CIs, Fisher's Z transformed, ranging from –0.907 to –0.32), suggesting that the homeostatic boost of the nonamblyopic eye observed after 2 hours of occlusion can predict recovery rate of the amblyopic eye.

Finally, one important characteristic of binocular rivalry is the peculiar asymmetric distribution of phase durations (the duration of each epoch of one eye predominance) that is considered to be an hallmark of binocular rivalry and is usually well approximated by a two-parameter (r, λ) *gamma distribution* of the form:

$$g(x) = \frac{\lambda^r x^{r-1}}{\Gamma(r)} e^{-\lambda x} \qquad (2)$$

where Γ is the *gamma function*, r is the shape parameter, and

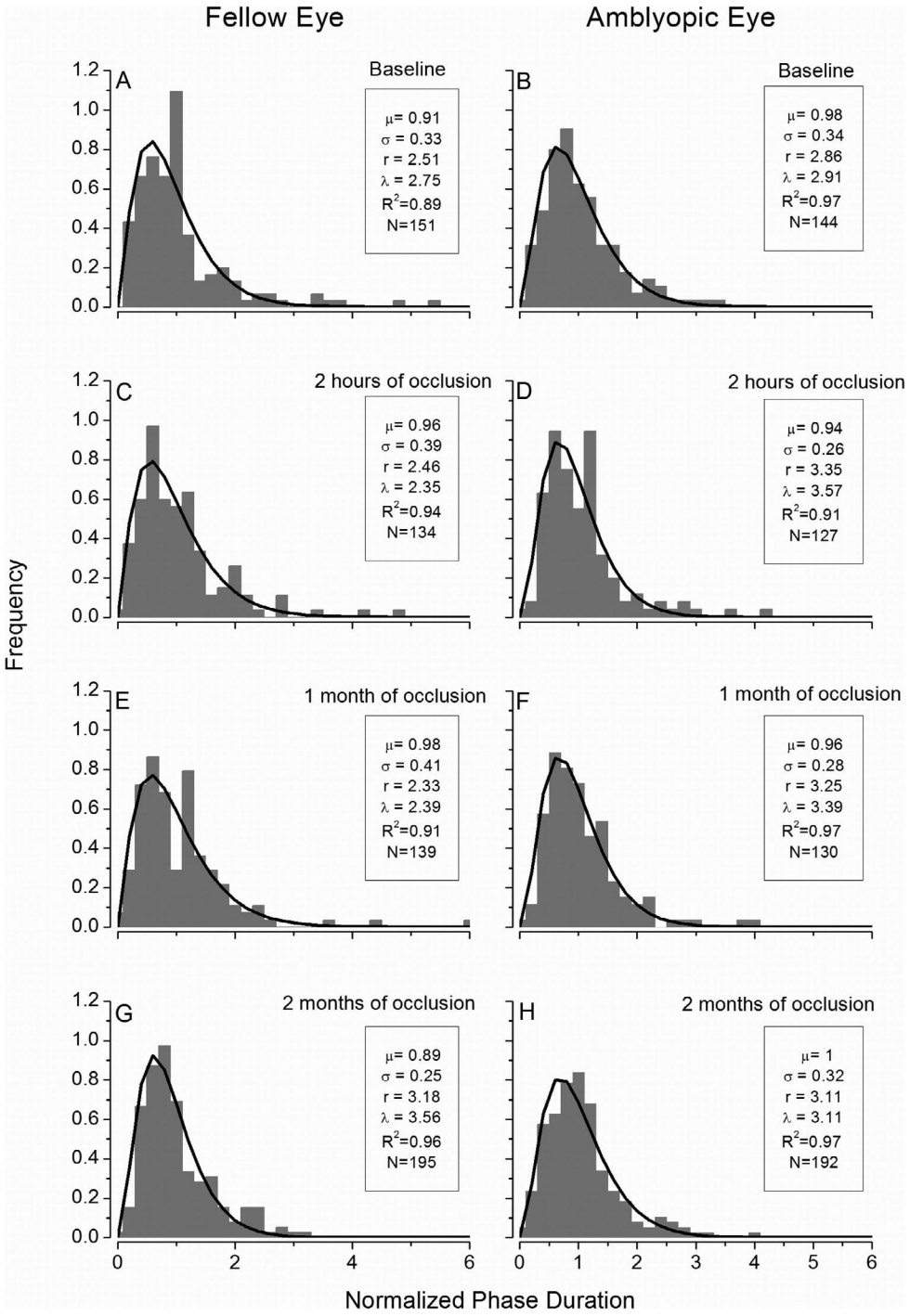

Figure 5. Phase duration distributions and relative gamma distribution fits for the nonamblyopic and amblyopic eye. Distribution of phase durations of the nonamblyopic (*left*) and amblyopic (*right*) eye normalized for each subject to the mean phase duration are reported for measurements acquired before the onset of occlusion therapy (**A**, **B**), after 2 hours (**C**, **D**), 1 month (**E**, **F**), and 2 months (**G**, **H**) of therapy. Phase duration distributions are well fit by two parameters (r, λ) *gamma distribution* of the form given by Equation 2.

λ is the scale parameter.[34] Figure 5 reports the distribution of the nonamblyopic and amblyopic eye phase durations normalized for each subject to the mean phase duration and the relative gamma distribution fits for the 3-minute blocks acquired before (Figs. 5A, 5B) and during 2 months of occlusion therapy (Figs. 5C–H). All phase-duration distributions are well fitted by the model (R^2 ranging from 0.89 and 0.97), confirming typical binocular rivalry dynamics in amblyopic observers and during treatment.

Discussion

By testing binocular rivalry during 5 months of occlusion therapy in unilateral amblyopic children, we have found that despite the recovery of visual acuity in the amblyopic eye, a homeostatic boost of the nonamblyopic eye (increased predominance of the nonamblyopic eye during binocular rivalry compared with pretreatment measurements) occurs during the first month of treatment. We also showed that the

homeostatic response measured soon after occlusion therapy onset predicts the recovery rate of the amblyopic eye: the increase in nonamblyopic eye predominance observed after 2 hours of occlusion correlated with visual acuity of the amblyopic eye measured after 2 months of treatment.

Traditionally, amblyopia has been used as a model to study visual cortical plasticity and its underlying mechanisms.[28–30] Early in life, within the temporal window of maximal neuroplasticity, called the *critical period*,[35–37] experience-dependent changes in visual cortical organization are massive: even a short period of monocular deprivation produces amblyopia. The major structural changes are observed in the primary visual cortex, where most neurons respond only to the open eye.[28–30,35,38] Importantly, if "reverse deprivation" occurs within the critical period (i.e., the nonamblyopic eye is deprived, similarly to occlusion therapy), ocular dominance shifts toward the previously amblyopic eye that reacquires the capability of driving cortical neurons.[28,38] Interestingly, one of the key mechanisms involved in neuroplasticity both for the induction of amblyopia and for the subsequent recovery of visual function is the balance between excitation and inhibition in the visual cortex.[39–44] A particularly important role in regulating experience-dependent plasticity during the critical period is played by GABAergic inhibition (reviewed in Ref. 45).

We have recently demonstrated that, in adult humans, gamma-aminobutyric acid (GABA) concentration decreases in the primary visual cortex after 150 minutes of monocular deprivation[13] and that the decrease in GABA concentration correlates with the perceptual homeostatic boost of the deprived eye.[13] These results corroborate the developmental evidence, suggesting that similar neural mechanisms (i.e., changes in excitation/inhibition balance) underlie the structural plasticity involved in the recovery of visual function observed in amblyopic animals and homeostatic plasticity observed after short-term monocular deprivation in adults. Here we show for the first time that these two forms of neuroplasticity, despite the apparent contradiction (the perceptual boost of the nonamblyopic occluded eye, and the recovery of visual acuity of the amblyopic eye), are strongly linked in children during the critical period: one form of plasticity (homeostatic) can be used to predict the other. Importantly, the results presented here also suggest that changes in visual cortical excitation/inhibition balance are involved in neuroplasticity operating during occlusion therapy for amblyopia. Several models indicate that the strength of perceptual suppression during binocular rivalry depends on the excitation/inhibition balance in the primary visual cortex.[3,46,47] The amount of mixed periods during binocular rivalry is an index of weak suppression and has been shown to be a sensitive measure of visual cortical inhibition in autistic patients.[48] Because of the young age of the children participating in the study, and to keep the task as simple as possible, we did not measure periods of mixed perception during binocular rivalry. Further investigations may be needed to understand whether amblyopic patients show an abnormal amount of mixed perception during rivalry.

Several models (reviewed in Ref. 49) have suggested that together with increased neural noise due to the weakening of the amblyopic eye activity, one of the neural mechanisms involved in amblyopia is interocular suppression, the process by which signals from the nonamblyopic eye suppress the amblyopic eye,[50,51] particularly strong in strabismic amblyopia.[52] It has been shown that interocular suppression is mediated by GABAergic inhibition in the primary visual cortex,[53,54] as the application of the $GABA_A$ receptor antagonist bicuculline in the visual cortex of cats disrupts interocular suppression.[53,54] Interestingly, both interocular suppression[55] and GABAergic inhibition[56] are involved in driving the dynamics of binocular rivalry. These shared neural mechanisms may explain why binocular rivalry is a sensitive method to measure the effects of occlusion therapy in amblyopic observers. The method is so sensitive, that even after regaining visual acuity of the amblyopic eye, at 5 months of occlusion therapy, binocular rivalry is still dominated by the nonamblyopic eye. If interocular suppression and binocular rivalry use the same mechanisms and spatial frequency dependence,[52,57] even a small preference of the high spatial frequency visibility for the nonamblyopic eye might strengthen the suppression of the amblyopic eye and hence the imbalanced dominance observed here during binocular rivalry. It is well known that after treatment, the balance between the eyes is in a fragile state and that the children need to be followed in time to avoid relapses of acuity loss in the amblyopic eye even in patients with orthotropia and good stereoacuity that do not seem to have a protective effect on the risk of recurrences.

Taken together, the results presented here suggest that binocular rivalry could be used in the future as a rapid and noninvasive tool to monitor visual cortical plasticity during occlusion therapy in children with moderate unilateral amblyopia and to predict the recovery rate of the amblyopic eye at the beginning of treatment. However, because of the small sample size tested in our study, further experimental evidence is needed to validate the predictive power of the method.

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References

1. Blake R, Logothetis NK. Visual competition. *Nat Rev Neurosci*. 2002;3:13–21.
2. Levelt WJ. The alternation process in binocular rivalry. *Br J Psychol*. 1966;57:225–238.
3. Blake R, Camisa J. On the inhibitory nature of binocular rivalry suppression. *J Exp Psychol Hum Percept Perform*. 1979;5:315–323.
4. Blake R. A neural theory of binocular rivalry. *Psychol Rev*. 1989;96:145–167.
5. Lunghi C, Burr DC, Morrone C. Brief periods of monocular deprivation disrupt ocular balance in human adult visual cortex. *Curr Biol*. 2011;21:R538–R539.
6. Lunghi C, Burr DC, Morrone MC. Long-term effects of monocular deprivation revealed with binocular rivalry gratings modulated in luminance and in color. *J Vis*. 2013;13(6):1.
7. Lunghi C, Berchicci M, Morrone MC, Russo FD. Short-term monocular deprivation alters early components of Visual Evoked Potentials. *J Physiol*. 2015;593:4361–4372.
8. Levi DM, Li RW. Improving the performance of the amblyopic visual system. *Philos Trans R Soc Lond B Biol Sci*. 2009;364:399–407.

9. Fahle M. Perceptual learning: specificity versus generalization. *Curr Opin Neurobiol*. 2005;15:154–160.
10. Desai NS, Cudmore RH, Nelson SB, Turrigiano GG. Critical periods for experience-dependent synaptic scaling in visual cortex. *Nat Neurosci*. 2002;5:783–789.
11. Maffei A, Turrigiano GG. Multiple modes of network homeostasis in visual cortical layer 2/3. *J Neurosci*. 2008;28:4377–4384.
12. Turrigiano GG, Nelson SB. Homeostatic plasticity in the developing nervous system. *Nat Rev Neurosci*. 2004;5:97–107.
13. Lunghi C, Emir UE, Morrone MC, Bridge H. Short-term monocular deprivation alters GABA in the adult human visual cortex. *Curr Biol*. 2015;25:1496–1501.
14. Ciuffreda KJ, Levi DM, Selenow A. *Amblyopia: Basic and Clinical Aspects*. Stoneham, MA: Butterworth-Heinemann, 1991.
15. Levi DM, Carkeet A. Amblyopia: a consequence of abnormal visual development. In: Simons K, ed. *Early Visual Development, Normal and Abnormal*. New York, NY: Oxford University Press; 1993:391–408.
16. Levi DM, McKee SP, Movshon JA. Visual deficits in anisometropia. *Vision Res*. 2011;51:48–57.
17. Loudon SE, Simonsz HJ. The history of the treatment of amblyopia. *Strabismus*. 2005;13:93–106.
18. Webber AL, Wood J. Amblyopia: prevalence, natural history, functional effects and treatment. *Clin Exp Optom*. 2005;88:365–375.
19. Holmes JM, Clarke MP. Amblyopia. *Lancet*. 2006;367:1343–1351.
20. Stewart CE, Moseley MJ, Stephens DA, Fielder AR. Treatment dose-response in amblyopia therapy: the Monitored Occlusion Treatment of Amblyopia Study (MOTAS). *Invest Ophthalmol Vis Sci*. 2004;45:3048–3054.
21. Kiorpes L, Kiper DC, O'Keefe LP, Cavanaugh JR, Movshon JA. Neuronal correlates of amblyopia in the visual cortex of macaque monkeys with experimental strabismus and anisometropia. *J Neurosci*. 1998;18:6411–6424.
22. Perez GM, Archer SM, Artal P. Optical characterization of Bangerter foils. *Invest Ophthalmol Vis Sci*. 2010;51:609–613.
23. Agervi P, Kugelberg U, Kugelberg M, Simonsson G, Fornander M, Zetterstrom C. Treatment of anisometropic amblyopia with spectacles or in combination with translucent Bangerter filters. *Ophthalmology*. 2009;116:1475–1480.
24. Laria C, Pinero DP. Characterization of Bangerter filter effect in mild and moderate amblyopia associated with strabismus. *Binocul Vis Strabolog Q Simms Romano*. 2012;27:174–186.
25. Laria C, Pinero DP, Alio JL. Characterization of Bangerter filter effect in mild and moderate anisometropic amblyopia: predictive factors for the visual outcome. *Graefes Arch Clin Exp Ophthalmol*. 2011;249:759–766.
26. Iacobucci IL, Archer SM, Furr BA, Martonyi EJ, Del Monte MA. Bangerter foils in the treatment of moderate amblyopia. *Am Orthopt J*. 2001;51:84–91.
27. Rutstein RP, Quinn GE, Lazar EL, et al. A randomized trial comparing Bangerter filters and patching for the treatment of moderate amblyopia in children. *Ophthalmology*. 2010;117:998–1004.e6.
28. Blakemore C, Garey LJ, Vital-Durand F. The physiological effects of monocular deprivation and their reversal in the monkey's visual cortex. *J Physiol*. 1978;283:223–262.
29. Hubel DH, Wiesel TN. The period of susceptibility to the physiological effects of unilateral eye closure in kittens. *J Physiol*. 1970;206:419–436.
30. Wiesel TN, Hubel DH. Single-cell responses in striate cortex of kittens deprived of vision in one eye. *J Neurophysiol*. 1963;26:1003–1017.
31. Mrsic-Flogel TD, Hofer SB, Ohki K, Reid RC, Bonhoeffer T, Hubener M. Homeostatic regulation of eye-specific responses in visual cortex during ocular dominance plasticity. *Neuron*. 2007;54:961–972.
32. Hudak M, Gervan P, Friedrich B, Pastukhov A, Braun J, Kovacs I. Increased readiness for adaptation and faster alternation rates under binocular rivalry in children. *Front Hum Neurosci*. 2011;5:128.
33. Kovacs I, Eisenberg M. Human development of binocular rivalry. In: Alais D, Blake R, eds. *Binocular Rivalry*. Cambridge: MIT Press; 2005:101–116.
34. Levelt WJ. Note on the distribution of dominance times in binocular rivalry. *Br J Psychol*. 1967;58:143–145.
35. Wiesel TN. Postnatal development of the visual cortex and the influence of environment. *Nature*. 1982;299:583–591.
36. Berardi N, Pizzorusso T, Maffei L. Critical periods during sensory development. *Curr Opin Neurobiol*. 2000;10:138–145.
37. Pascual-Leone A, Amedi A, Fregni F, Merabet LB. The plastic human brain cortex. *Annu Rev Neurosci*. 2005;28:377–401.
38. Blakemore C, Van Sluyters RC. Reversal of the physiological effects of monocular deprivation in kittens: further evidence for a sensitive period. *J Physiol*. 1974;237:195–216.
39. Baroncelli L, Sale A, Viegi A, et al. Experience-dependent reactivation of ocular dominance plasticity in the adult visual cortex. *Exp Neurol*. 2010;226:100–109.
40. Fagiolini M, Fritschy JM, Low K, Mohler H, Rudolph U, Hensch TK. Specific GABAA circuits for visual cortical plasticity. *Science*. 2004;303:1681–1683.
41. Hensch TK, Fagiolini M, Mataga N, Stryker MP, Baekkeskov S, Kash SF. Local GABA circuit control of experience-dependent plasticity in developing visual cortex. *Science*. 1998;282:1504–1508.
42. Sale A, Maya Vetencourt JF, Medini P, et al. Environmental enrichment in adulthood promotes amblyopia recovery through a reduction of intracortical inhibition. *Nat Neurosci*. 2007;10:679–681.
43. Maya Vetencourt JF, Sale A, Viegi A, et al. The antidepressant fluoxetine restores plasticity in the adult visual cortex. *Science*. 2008;320:385–388.
44. Morishita H, Miwa JM, Heintz N, Hensch TK. Lynx1, a cholinergic brake, limits plasticity in adult visual cortex. *Science*. 2010;330:1238–1240.
45. Heimel JA, van Versendaal D, Levelt CN. The role of GABAergic inhibition in ocular dominance plasticity. *Neural Plast*. 2012;2011:391763.
46. Laing C, Chow CA. Spiking neuron model for binocular rivalry. *J Comput Neurosci*. 2002;12:39–53.
47. Seely J, Chow CC. Role of mutual inhibition in binocular rivalry. *J Neurophysiol*. 2011;106:2136–2150.
48. Robertson CE, Ratai EM, Kanwisher N. Reduced GABAergic action in the autistic brain. *Curr Biol*. 2016;26:80–85.
49. Hess RF, Thompson B, Baker DH. Binocular vision in amblyopia: structure, suppression and plasticity. *Ophthalmic Physiol Opt*. 2014;34:146–162.
50. Sengpiel F. Plasticity of the visual cortex and treatment of amblyopia. *Curr Biol*. 2014;24:R936–R940.
51. Sireteanu R, Fronius M. Naso-temporal asymmetries in human amblyopia consequence of long-term interocular suppression. *Vision Res*. 1981;21:1055–1063.
52. Fahle M. Non-fusable stimuli and the role of binocular inhibition in normal and pathologic vision, especially strabismus. *Doc Ophthalmol*. 1983;55:323–340.
53. Sengpiel F, Jirmann KU, Vorobyov V, Eysel UT. Strabismic suppression is mediated by inhibitory interactions in the primary visual cortex. *Cereb Cortex*. 2006;16:1750–1758.

Investigative Ophthalmology & Visual Science

54. Sengpiel F, Vorobyov V. Intracortical origins of interocular suppression in the visual cortex. *J Neurosci*. 2005;25:6394–6400.
55. Sengpiel F, Blakemore C, Harrad R. Interocular suppression in the primary visual cortex: a possible neural basis of binocular rivalry. *Vision Res*. 1995;35:179–195.
56. van Loon AM, Knapen T, Scholte HS, St John-Saaltink E, Donner TH, Lamme VA. GABA shapes the dynamics of bistable perception. *Curr Biol*. 2013;23:823–827.
57. Fahle M. Binocular rivalry: suppression depends on orientation and spatial frequency. *Vision Res*. 1982;22:787–800.

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