



Pupillometry indexes ocular dominance plasticity

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ABSTRACT

Short-term monocular deprivation in normally sighted adult humans produces a transient shift of ocular dominance, boosting the deprived eye. This effect has been documented with both perceptual tests and through physiological recordings, but no previous study simultaneously measured physiological responses and the perceptual effects of deprivation. Here we propose an integrated experimental paradigm that combines binocular rivalry with pupillometry, to introduce an objective physiological index of ocular dominance plasticity, acquired concurrently with perceptual testing. Ten participants reported the perceptual dynamics of binocular rivalry, while we measured pupil diameter. Stimuli were a white and a black disk, each presented monocularly. Rivalry dynamics and pupil-size traces were compared before and after 2 h of monocular deprivation, achieved by applying a translucent patch over the dominant eye. Consistent with prior research, we observed that monocular deprivation boosts the deprived-eye signal and consequently increases ocular dominance. In line with previous studies, we also observed subtle but systematic modulations of pupil size that tracked alternations between exclusive dominance phases of the black or white disk. Following monocular deprivation, the amplitude of these pupil-size modulations increased, which is consistent with the post-deprivation boost of the deprived eye and the increase of ocular dominance. This provides evidence that deprivation impacts the effective strength of monocular visual stimuli, coherently affecting perceptual reports and the automatic and unconscious regulation of pupil diameter. Our results show that a combined paradigm of binocular rivalry and pupillometry gives new insights into the physiological mechanisms underlying deprivation effects.

1. Introduction

During binocular rivalry, incompatible images are simultaneously presented to the two eyes and perception alternates between them (Bartels & Logothetis, 2010, Blake & Logothetis, 2002, Levelt, 1967). At any given moment, the reported perceptual dominance mirrors the fluctuating contribution of each eye to conscious visual perception, providing a reliable index of ocular dominance (Acquafredda, et al., 2023). Ocular dominance is generally considered to develop during a “critical period” in early life (Hensch & Quinlan, 2018, Hubel & Wiesel, 1965); however, multiple studies over the last decade have shown that a form of ocular dominance plasticity may still be elicited in adult volunteers (for reviews see: Castaldi, Lunghi & Morrone, 2020, Hess & Hyun Min, 2023), where occluding vision in one eye for few hours leads to a transient boost of the occluded (deprived) eye, and a consequent shift of ocular dominance (Lunghi, Burr & Morrone, 2011, Lunghi, Burr & Morrone, 2013, Zhou, Clavagnier & Hess, 2013).

The transient post-deprivation boost has often been measured with psychophysical techniques, mainly linked with binocular competition (Lunghi, Burr & Morrone, 2011, Lunghi, Burr & Morrone, 2013, Virathone, et al., 2021, Wang, McGraw & Ledgeway, 2020) or binocular summation (Zhou, Clavagnier & Hess, 2013), with partially compatible results (Bai, et al., 2017, Chen, Bobier & Thompson, 2023). Monocular deprivation effects have also been documented by measuring physiological responses to stimuli in either eye, before/after deprivation, through EEG (Lunghi, et al., 2015, Lyu, et al., 2020, Schwenk, VanRullen & Bremmer, 2020, Wang, et al., 2023, Zhou, et al., 2015), MEG (Chadnova, et al., 2017) or fMRI (Binda, et al., 2018, Kurzawski, et al., 2022). Although the physiological and behavioral indices of ocular dominance plasticity may be correlated across participants (Binda et al., 2018), the conditions under which they are acquired are markedly different. Stimuli for EEG and fMRI measurements are typically presented to a passive observer, with no perceptual report required; they often cover large portions of the visual field and in some cases (Binda

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et al., 2018, Kurzawski et al., 2022, Lunghi et al., 2015) they are presented monocularly. All this contrasts with the conditions of the behavioral experiments, where small foveal stimuli in the two eyes interact (competitively or cooperatively) while participants keep their attention focused on the stimuli to report their visual perception. These methodological differences imply that the effects revealed by EEG and fMRI could in principle be distinct from those subtending the changes in binocular competition/summation and limit the possibility of combining the physiological and perceptual results. Here we aim to overcome this limitation, introducing a new objective physiological index of ocular dominance plasticity that can be collected during its perceptual assessment – through pupillometry.

The diameter of the eye-pupils is not only set by light levels and sympathetic activity; when light is constant and task demands controlled for, there are still small pupil-size changes that can be systematically associated with visual features (Binda & Murray, 2015, Mathôt, 2018). For example, during binocular rivalry, pupil dilations accompany the act of reporting perceptual shifts and/or the decision-making process that precedes it (Brascamp, et al., 2021, Einhauser, et al., 2008). In addition, when the rivalry stimuli have different brightness, pupil size varies in-synch with perceptual alternations and a relative constriction accompanies dominance of the brighter percept (Acquafredda, Binda & Lunghi, 2022, Fahle, Stemmler & Spang, 2011, Lowe & Ogle, 1966, Naber, Frassle & Einhauser, 2011), even though the stimuli (hence luminance) remain exactly the same at all times. Crucially, the amplitude of these rivalry-related pupil changes depends on the contrast of the rivaling stimuli. For example, doubling the contrast of one of the monocular stimuli produces a 100 % increase in the pupil-size change (Acquafredda, Binda & Lunghi, 2022).

This observation generates a clear prediction on how monocular deprivation should affect the dynamics of pupil size during binocular rivalry. If monocular deprivation boosts the strength of the deprived eye (transiently after patch removal), then it should increase the size of pupil changes observed during binocular rivalry (over the same post-deprivation period). If confirmed, this phenomenon could present pupillometry as a novel, objective index of ocular dominance plasticity, acquired during the perceptual assessment of the effect with simple and non-obstructive apparatus. Pupillometry would thereby introduce a new tool to probe visual representations and monitor potential changes of their effective strength with experience, e.g. deprivation.

2. Methods

2.1. Ethics statement

The experiment was conducted in accordance with the principles of the 1964 Declaration of Helsinki and its protocol was approved by the local ethics committee (Comitato Etico Pediatrico Regionale—Azienda Ospedaliero-Universitaria Meyer—Firenze [FI]). All participants gave written informed consent prior to beginning the study.

2.2. Subjects

We recruited 10 participants (6 females, mean age 26.9 ± 0.77 years) with normal or corrected-to-normal vision and normal color vision (based on the most recent oculist assessment that participants reported), no known history of amblyopia, eye surgery or other active eye diseases. Sample size was set considering that monocular deprivation effects are generally large ($d \geq 0.9$) and reliably detected with $N \leq 10$ (given standard two-tailed α of 0.05 and power of 0.8). Like in previous studies (Acquafredda et al., 2023), candidate participants were screened with a preliminary binocular rivalry test (with the same task used in the main experiment and averaging across the four possible stimulus configurations) and recruited only if the log-ratio of dominant to non-dominant eye mean phase durations was ≤ 0.25 , implying well-balanced eyes.

2.3. Apparatus, stimuli and procedures

Experiments took place in a quiet experimental booth with no illumination other than the display screen. Visual stimuli were generated in Matlab (MATLAB r2010a, The MathWorks Inc., Natick, MA) using PsychoPhysics Toolbox routines (Brainard, 1997) housed in a Mac Pro 4.1. They were displayed on a 52.5 cm-wide LCD screen, driven at a resolution of 1920 x 1080 pixels. The display was seen through a four-mirror stereoscope which enabled dichoptic viewing of two display areas of 12 x 8 deg each; a chin rest was used to stabilize head position at 57 cm from the display. In each display area, a central red fixation point (0.15 deg in diameter) surrounded by a square frame (3.5 x 3.5 deg) was shown against a uniform grey background (luminance: 12.3 cd/m²). The mirrors were carefully adjusted at the beginning of each session to ensure accurate alignment of the dichoptically presented squares. Participants were asked to keep their gaze on the fixation point and to refrain from blinking while the stimuli were on. Dichoptic presentations consisted of two disks, 3 deg in diameter, one white (28.9 cd/m², Michelson contrast: 0.31) and one black (1.7 cd/m², Michelson contrast: 0.8). To discourage binocular fusion, the disks were overlaid with thin orthogonal grey lines (45° clockwise or counter-clockwise, 1 pixel wide, corresponding to 0.033 deg, and 0.5 deg apart, with the same luminance as the background). Perception alternated between exclusive dominance of the white and the black disk, seldom interspersed with mixed percepts (either a piecemeal combination of the two stimuli or their fusion). Participant continuously reported their perception throughout the duration of each trial (defined below) by keeping one of three keys pressed (right or left arrows to report dominance of the stimulus with clockwise or counter-clockwise tilted lines; down-arrow key to report mixed percepts).

Participants undertook four monocular deprivations, performed on separate days. On each day, binocular rivalry was measured twice, before and after monocular deprivation. They were performed in 12-minute blocks, comprising four 3-minute trials, each with a different combination of disk color and line orientation presented to the two eyes (combinations were varied pseudo-randomly across trials, Fig. 1A). The first trial was always discarded, due to reduced signal-to-noise ratio of the pupil measurements, especially evident in the post-deprivation blocks. Thus, the dynamics of binocular rivalry and pupil size were analyzed from 3 min to 12 min after the end of deprivation and compared with measurements taken over the same amount of time, immediately before the beginning of deprivation. As a result, analyses included a total of 24 trials per participant (3 trials x 2 blocks before/after deprivation x 4 deprivations).

We analyzed participants' keypresses by extracting dominance phases, i.e. the duration of periods during which the image in either eye dominated perception. Dominance phase durations shorter than 0.3 s (finger errors) were discarded; we verified that no phase exceeded the duration of 30 s (failed rivalry). We also checked that phase durations followed the expected gamma distribution (Levelt, 1967):

$$f(x|\alpha, \beta) = \frac{1}{\beta^\alpha \Gamma(\alpha)} x^{\alpha-1} e^{-\frac{x}{\beta}} \quad \text{for } x, \alpha, \beta > 0 \quad (1)$$

where Γ is the gamma function and x is the duration of dominance phases. The function was fit to the distributions in Fig. 1D, which were obtained by normalizing each participant's phase durations to their average, then pooling across participants while keeping white and black dominance phases separate.

2.4. Monocular deprivation

Monocular deprivation was achieved by applying a translucent patch over the dominant eye, defined as the eye with longer mean dominance-phase duration. As in previous studies (Binda et al., 2018, Binda & Lunghi, 2017, Lunghi, Burr & Morrone, 2011, Lunghi, Burr & Morrone,

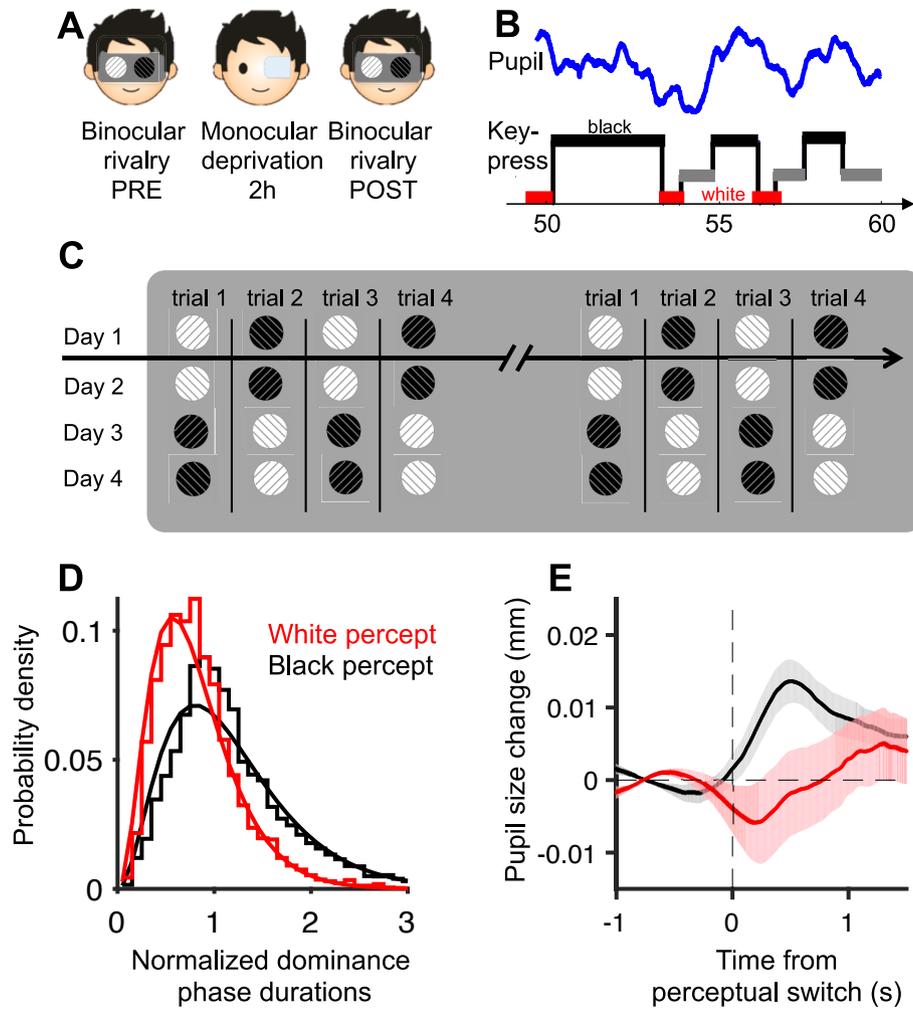


Fig. 1. Experimental procedure and indices of perceptual alternations before monocular deprivation. **A:** Structure of the experiment, where binocular rivalry dynamics were measured before and after 2 h of monocular deprivation, achieved by applying a translucent patch on the dominant eye. **B:** Example traces from a segment of the experiment, where participants used keypresses to report the dominant percept (square wave) and we recorded pupil size modulations (blue wave). **C:** Experimental procedure. Each participant was tested on four separate days, pre- and post-deprivation, and each block comprised four trials with pseudo-random orientation-color-eye assignments (maintained before and after monocular deprivation). **D:** Probability density function of the phase durations for each percept (black and white), measured before deprivation and pooled across participants after normalizing to each participant's mean phase duration. Both functions conform to the typical gamma distribution (Equation (1) in Methods; $R^2 = 0.97$ and 0.99 for black and white percepts), with shape α and scale β parameters: $\alpha = 3.20$; $\beta = 0.37$ for the black percept and $\alpha = 3.39$ and $\beta = 0.24$ for the white percept. **E:** Pupil-size segments aligned to the time of a perceptual switch toward a black or white percept (black and red traces). Shadings are mean \pm s.e.m across participants. The difference between the two curves is used as a measure of the pupil modulations, and it is plotted as a blue curve in Fig. 2C.

2013, Steinwurz, et al., 2020) we used a translucent plastic material that eliminated pattern vision while producing minimal light attenuation (about 15 %). Participants kept the patch on for 2 h, during which time they were free to read, work at the computer, or walk around the laboratory (but not to eat or sleep). We quantified the effect of monocular deprivation using the standard indices used in previous studies. These include: quantifying the deprived-eye dominance, defined as the proportion of total testing time spent seeing through the deprived eye minus the proportion of time seeing through the other eye; comparing mean phase durations for the (to be) deprived and non-deprived eye, before and after deprivation; and computing a Deprivation Index DI:

$$DI = \frac{DepPost}{NDepPost} / \frac{DepPre}{NDepPre} \quad (2)$$

DI compares the dominance-phase durations for the two eyes, pre- and post-deprivation, by taking the ratio of these values; we log-transformed it to better approximate a normal distribution. A deprivation index > 1

(or $\log DI > 0$) defines a shift of ocular dominance in favor of the deprived eye, which is the expected effect of monocular deprivation.

2.5. Pupillometry and eye-tracking data acquisition and analysis

Throughout the rivalry trials, we monitored pupil diameter and two-dimensional eye position with an infrared camera (EyeLink 1000 system, SR Research, Canada) mounted below the monitor screen and behind the stereoscope. EyeLink data were streamed to the main computer through the EyeLink toolbox for Matlab (Cornelissen, Peters & Palmer, 2002) and thereby synchronized with participant's keypresses. Pupil diameter measurements were transformed from pixels to millimeters using an artificial 4 mm pupil positioned at the approximate location of the subject's eye. Pupil and gaze tracking data consisted of 180 x 1000 time points (180 s at 1000 Hz). These included signal losses, eyeblinks and other artifacts, which we cleaned out employing the following steps (all implemented with in-house Matlab software):

- Identification and removal of major artifacts: removal of time-points with unrealistically small pupil size (<0.2 mm, corresponding to blinks or other signal losses).
- Identification and removal of finer artifacts: identification of samples where pupil size varied at unrealistically high speeds (larger than 10 mm per second) and removal of any resulting isolated data point.
- Removal of any linear trend by fitting a linear function to pupil data from each 180 s-long trial.

After this cleaning procedure was applied, we verified fixation stability by measuring the dispersion of (valid) eye position samples around the mean of each trial as the bivariate confidence ellipse area (BCEA), defined as:

$$BCEA = 2 * k * \sigma_H * \sigma_V * (1 - \rho)^{0.5} \quad (3)$$

where σ_H and σ_V are the standard deviation of eye positions in the horizontal and vertical meridian respectively, ρ is the product-moment correlation of these two position components and $k = 1.14$, implying that the ellipse included 68 % ($1 - e(-k)$) of the distribution.

Valid pupil size samples and continuous recordings of perceptual reports were down-sampled to 100 Hz, by taking the median of the retained time-points in non-overlapping 10 ms long time windows. If no retained sample was present in a window, that window was set to “NaN” (MATLAB code for “not a number”). Down-sampled pupil traces were finally parsed into epochs locked to each perceptual switch (when the subject changed perceptual report) and labeled according to the color (black or white) of the stimulus that dominated perception after the switch. To minimize the impact of pupil size changes unrelated to the perceptual switches, we also baseline-corrected pupil traces in each epoch, by subtracting the pupil value in the $[-1, -0.5]$ s interval preceding the switch. The resulting time courses were finally averaged across epochs for each participant and then averaged across participants to generate the traces in Fig. 1E. We quantified the size of pupil modulations linked to perceptual alternations as the difference between pupil traces corresponding to epochs of black vs. white stimulus dominance (Fig. 3C). In Fig. 3B, we compared pupil size across dominance phases (white vs. black) and conditions (pre vs. post deprivation) by taking the average pupil size in the $[-0.5, 1]$ s interval around the switch. All these steps were the same as in our previous study of pupil modulations during binocular rivalry (Acquafredda, Binda & Lunghi, 2022).

In addition to these measures of pupil-size modulation, we also included a measure of the absolute pupil diameter, which we obtained from the median of all valid pupil-size values over an entire trial (irrespective of which percept was being reported). This primarily reflects the slow and sustained pupil constriction related to retinal illumination (Loewenfeld, 1993).

2.6. Statistical approach

Shapiro-Wilk normality tests were performed before statistical comparisons for each of our measures (Shapiro & Wilk, 1965). For non-normal distributions, we performed nonparametric Wilcoxon signed-rank tests (Wilcoxon, 1946). Normally distributed variables were evaluated with Student t-tests or ANOVAs.

Significance was evaluated using p-values, following Bonferroni-Holm correction (Holm, 1979) in case of multiple comparisons ($\alpha = 0.05$). We also report log-transformed JZS Bayes Factors, computed with the default scale factor of 0.707 (Wagenmakers, et al., 2012). The Bayes Factor is the ratio of the likelihood of the two models $H1/H0$, where $H1$ is the experimental hypothesis (effect present) and $H0$ is the null hypothesis (effect absent). By convention, a base 10 logarithm of the Bayes Factor (\lgBF) > 0.5 is considered substantial evidence in favor of $H1$, and $\lgBF < -0.5$ substantial evidence in favor of $H0$. Bayesian ANOVAs were run in JASP, and the corresponding Bayes Factors

represent the change from prior to posterior inclusion odds (BFinclusion) computed across matched models.

3. Results

In 10 normally sighted adult observers, we measured perceptual alternations and pupil-size modulations during binocular rivalry, before and after short-term monocular deprivation. Measurements collected before deprivation recapitulated our recent findings (Acquafredda, Binda & Lunghi, 2022). Pupil size modulated in synchrony with perceptual alternations (Fig. 1E), producing a relative dilation when participants reported seeing black compared to when they reported seeing white. The black stimulus dominated rivalry for a larger proportion of time than the white one (Fig. 1D), coherent with its higher Michelson contrast and despite identical Weber contrast.

Comparing rivalry dynamics before and after the 2 h of monocular deprivation, we replicated the established phenomenon of transiently increased dominance of the deprived eye. Fig. 2 quantifies the effect using standard indices, collapsing trials in which the black or white stimulus was presented to the dominant eye (please see Fig. 3 for separate analyses of the two stimulus configurations). Fig. 2A shows deprived-eye dominance, defined as the proportion of total testing time spent seeing through the deprived eye, minus the proportion of time seeing through the other eye. This value is positive before deprivation, reflecting our choice to patch the dominant eye; it significantly increases after deprivation (Wilcoxon $z = 2.80$, $p < 0.01$, $\lgBF = 2.14$), revealing the expected systematic ocular dominance shift following eye-patch removal (in the short period of about 12 min during which we performed our tests). The difference post-pre deprivation averaged 0.12 ± 0.03 (mean \pm s.e.m.), implying an ocular dominance shift slightly over 10 %.

Coherent conclusions are obtained by examining the mean duration of dominance phases, separately for the deprived and non-deprived eye (Fig. 2B). Deprived-eye mean phase durations increased markedly after deprivation (Wilcoxon $z = 2.70$, $p = 0.012$, $\lgBF = 1.54$); however,

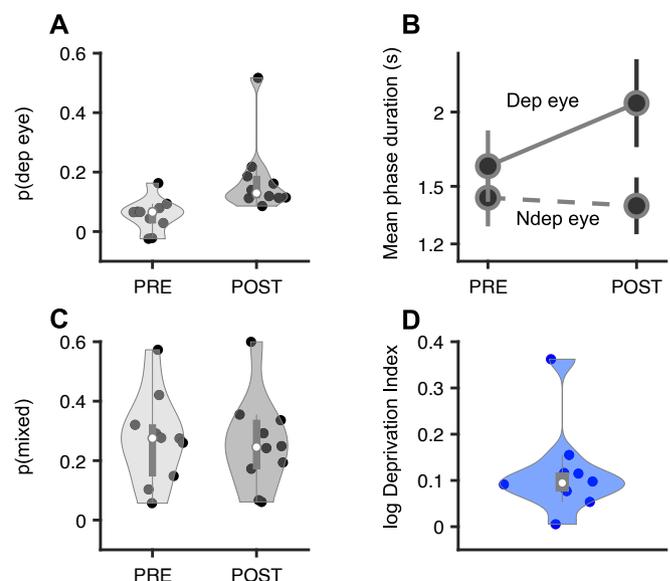


Fig. 2. Monocular deprivation effects on binocular rivalry dynamics. A: Deprived eye dominance before and after monocular deprivation. B: Mean phase durations for each eye (deprived and non-deprived) before and after monocular deprivation. Error bars are s.e.m. C: Mixed proportions before and after monocular deprivation. D: Deprivation Index values, computed from mean phase durations with Equation (2) in Methods. In panels A, C and D, data are presented as violin plots, with dots showing individual participants' values and the central element is a box-and-whisker plot of the same data.

phase durations for the non-deprived eye decreased by a smaller amount (Wilcoxon $z = 2.80$, $p = 0.43$, $\lg\text{BF} = -0.37$). We also combined these values in a Deprivation Index that compares the duration of dominance phases of the deprived/non-deprived eye, after/before deprivation (Fig. 2D, Equation (2) in Methods). After log-transform, this value averaged 0.11 ± 0.03 (mean \pm s.e.m.), significantly larger than 0 (one sample t -test: $t_{(9)} = 3.82$, $p = 0.004$, $\lg\text{BF} = 1.10$).

Finally, Fig. 2C examines the third type of perceptual phases, mixed percepts. Their proportion was relatively high, probably due to the nature of the stimuli (homogenous black and white disks would normally fuse into a gray percept; we overlaid thin orthogonal lines to oppose this tendency). Importantly, the proportion of mixed percepts was indistinguishable across sessions ($t_{(9)} = 1.01$, $p = 0.33$, $\lg\text{BF} = -0.33$) suggesting that monocular deprivation did not affect the probability of binocular fusion, and selectively affected the proportion of exclusive dominance of the monocular stimuli.

In summary, these observations confirm that our stimuli and set-up were adequate for measuring the typical ocular dominance shift produced by a 2-hour monocular deprivation.

To further quantify the effect of monocular deprivation while accounting for the marked dominance of the black stimulus, we repeated the analyses after separating trials where the black stimulus was presented to the (to be) deprived or non-deprived eye. Fig. 3A compares the deprived eye dominance before and after deprivation; these values were entered a two-way ANOVA for repeated measures with factors “deprived eye stimulus” (black or white) and “time” (before or after monocular deprivation). Values were positive when the deprived eye was presented with the black stimulus (black dots), and negative when it was presented with the white stimulus (magenta dots), leading to a significant main effect of the “deprived eye stimulus” factor ($F_{(1,9)} = 48.85$, $p < 0.001$) and indicating that the dominance of the black stimulus was sufficient to overcome ocular dominance in our (fairly balanced) participants. After the 2-hour monocular deprivation (y-axis), the dominance of the deprived eye increased overall, as indicated by all data-points (black and magenta alike) laying above the bisection of the axes and by a significant main effect of factor “time” ($F_{(1,9)} = 17.64$, $p = 0.002$). This reiterates the observation that monocular deprivation increased the dominance of the deprived eye. There was no significant interaction ($F_{(1,9)} = 0.01$, $p = 0.90$), indicating that the effect of monocular deprivation was similar irrespectively of whether the deprived eye was presented with the white or the black stimulus.

We next asked whether this dominance shift also affected the pupil-size changes that accompanied perceptual alternations during binocular rivalry. Results from a control experiment in Acquafredda, Binda and Lunghi (2022) showed that increasing the effective strength of the stimulus in one eye (by manipulating its contrast) enhances pupil-size modulations during rivalry. It follows that, if monocular deprivation boosts the dominance of the deprived eye by increasing its effective contrast, this should result in enhanced pupil-size differences. Inspection of pupil-difference traces in Fig. 3C suggests that this is indeed the case, as the size of pupil modulations during rivalry (difference of the black and red traces in Fig. 1E, shown as the blue curve in Fig. 3C) increased after monocular deprivation (cyan curve in Fig. 3C). Fig. 3B presents a quantification of the effect, obtained by extracting the average pupil-difference in the $[-0.5 \text{ } 1]$ s interval relative to perceptual switches – the same interval that we previously found to be sensitive to contrast manipulations (Acquafredda, Binda & Lunghi, 2022). These values were obtained separately for the two stimulus configurations (black or white stimulus presented in the deprived eye) and reported in Fig. 2A to show post-deprivation against pre-deprivation values. These values were entered the same 2x2 ANOVA used for perceptual dominance; they are also reported in Appendix Table A.1. Note that values are numerically small (in the order of few microns); however, they represent averages over many repetitions (the many perceptual phases) and reveal systematic effects. Although such small pupil-size modulations are not likely to affect the quality of retinal images, they are informative of how

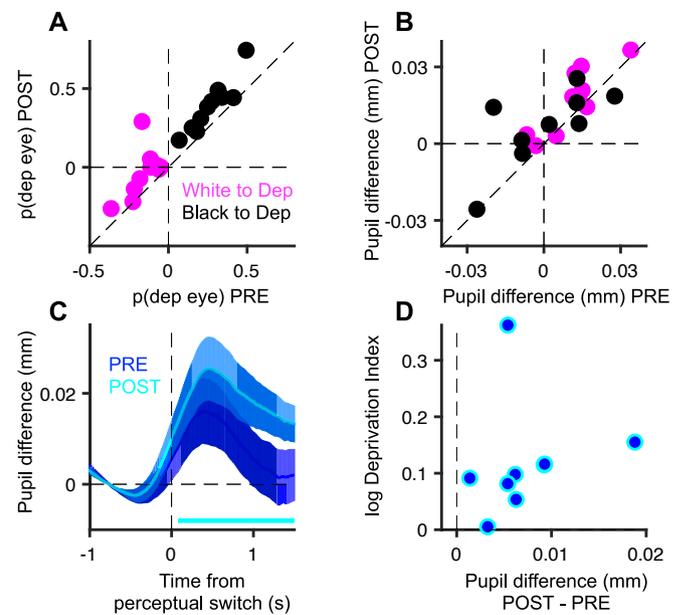


Fig. 3. Monocular deprivation effects on perceptual dominance and pupil modulations. **A:** Deprived-eye dominance measured before (x-axis) and after (y-axis) monocular deprivation. Dots show individual participants' values when the white (magenta dots) or the black stimulus (black dots) was presented to the deprived eye. **B:** Mean pupil-size difference in the interval $[-0.5 \text{ } 1]$ s around perceptual switch, measured before (x-axis) and after (y-axis) monocular deprivation. Same conventions as in panel A. **C:** Time course of the pupil-size difference during black and white percepts, computed before (blue) and after monocular deprivation (cyan). Shadings report mean \pm s.e.m. and the cyan horizontal line marks the time points where the post- and pre-deprivation curves differ (paired t-tests, $p < 0.05$ FDR corrected). **D:** Monocular deprivation effect on pupil-size differences in each participant (pooling across trials with the white or black stimulus presented to the deprived eye), plotted against the corresponding behavioral effects (log-transformed Deprivation Index, computed with Equation (2); y-axis).

visual information is processed and perceived. Most pupil-difference values are positive, indicating that a relative dilation generally accompanied black percepts (as shown in Fig. 1E and as expected from previous studies). Values are generally larger when the white stimulus was presented to the (to be) deprived eye than in the opposite stimulus configuration (magenta vs. black dots, significant effect of factor “deprived eye stimulus”, $F_{(1,9)} = 10.57$, $p = 0.01$). This suggests that, while the black stimulus behaved like a stronger stimulus in perceptual alternations, the white stimulus behaved like a stronger driver for pupil modulations, as could be expected from previous studies (e.g., Binda & Murray, 2015). Thus, when presented to the dominant eye, the white stimulus elicited larger pupil-size modulations. This finding also implies that eye-dominance modulated the amplitude of pupil-size changes accompanying perceptual alternations. Coherently, the significant effect of factor “time” ($F_{(1,9)} = 6.46$, $p = 0.03$) indicates that, as eye-dominance became more marked after monocular deprivation, pupil-size changes became larger. The non-significant interaction ($F_{(1,9)} = 0.01$, $p = 0.92$) indicates that the effect of deprivation is insensitive to the stimulus configuration, as observed for perceptual reports. While the amplitude of pupil-size modulations changed depending on the stimulus configuration and before/after deprivation, we verified that absolute pupil diameter (median over the entire trials) was unaffected by either factor (“deprived eye stimulus”: $F_{(1,9)} = 0.07$, $p = 0.79$; “time”: $F_{(1,9)} = 0.31$, $p = 0.28$) or their interaction ($F_{(1,9)} = 0.09$, $p = 0.77$).

In summary, we found that monocular deprivation affects perceptual dominance and pupil-size changes during binocular rivalry in congruent ways. Fig. 3D supports this by plotting the perceptual effect of deprivation (log-transformed Deprivation Index) against the deprivation

effect on pupil-size change (post-pre difference of pupil-size modulations) across our 10 participants (values averaged across stimulus configurations). Except for one participant with an uncharacteristically large perceptual effect, the two values showed a trend for a positive association (note that our small sample size does not allow for a reliable assessment of correlations across-participant). We also examined the effects of deprivation separately for each of our 3-min long trial. Over the short time window that we monitored (between 3 and 12 min after eye-patch removal), we did not observe a decay of the behavioral effect (all $t < 1.5$, $p > 0.16$); similarly, there was no detectable change in the pupil-size modulation (all $t > 1.6$, $p > 0.13$).

A control analysis examined and excluded the possibility that changes in fixation stability mediated the effects of monocular deprivation on pupil size. We extracted an index of fixation stability via the BCEA (Equation (3) in Methods), i.e. the area of the bivariate confidence ellipse including 68 % of the gaze positions over trials for each time condition, and we found no significant difference between BCEA values before and after monocular deprivation (Wilcoxon $z = 1.68$, $p = 0.10$, $lgBF = 0.12$).

Finally, in analogy with our previous work on the effect of attention on pupil modulations during rivalry, we also tested for possible modulations of pupil size during mixed percepts. We found none (“deprived eye stimulus”: $F_{(1,9)} = 0.31$, $p = 0.59$; “time”: $F_{(1,9)} = 0.65$, $p = 0.44$; interaction: $F_{(1,9)} = 0.24$, $p = 0.62$).

4. Discussion

We investigated for the first time the effects of short-term monocular deprivation on the pupil-size changes accompanying perceptual alternations during binocular rivalry. We successfully replicated the effects of short-term monocular deprivation on binocular rivalry, which consists of an ocular dominance shift in favor of the deprived eye (Castaldi, Lunghi & Morrone, 2020, Hess & Hyun Min, 2023). We also confirmed that pupil size tracks perceptual alternations during binocular rivalry, despite constant stimulation. When participants reported seeing black, the pupils were relatively dilated compared to when they reported seeing white, suggesting a pupil modulation linked with perceived luminance (Acquafredda, Binda & Lunghi, 2022, Fahle, Stemmler & Spang, 2011, Lowe & Ogle, 1966, Naber, Frassle & Einhauser, 2011). This effect is consistent with a large body of work suggesting that the subcortical circuit generating the pupillary light response is modulated by perceptual signals, presumably generated within the visual cortex (Binda & Gamlin, 2017, Binda & Murray, 2015, Mathôt, 2018).

We found that, after monocular deprivation, the pupil-size modulations increased in magnitude, as the difference in pupil diameter between black and white percepts became larger. This increase was not accompanied by a change in the absolute pupil diameter, speaking against the possibility that monocular patching affected pupil-size modulations through changes in retinal sensitivity (e.g. via dark adaptation). Three other pieces of evidence make this unlikely: first, the translucent patch does not block luminance, but rather deteriorates pattern information (Lunghi, Burr & Morrone, 2011); second, the effects of monocular deprivation are generally inconsistent with retinal or thalamic adaptation (Kim, Kim & Blake, 2017, Lunghi, Burr & Morrone, 2013); third, these effects are first seen in the visual cortex and are undetectable at earlier processing stages, including LGN (Kurzawski et al., 2022). We propose that the post-deprivation enhancement of pupil-size modulations results from the enhanced representation of the deprived-eye in the visual cortex; if, as discussed above, this signal feeds into the subcortical circuit of the pupillary light response, its enhancement is bound to produce a modulation of pupil size.

This logic is coherent with previous evidence that the magnitude of pupil-size modulations during rivalry reflects the strength of the rivaling stimuli. In particular, Acquafredda, Binda and Lunghi (2022) showed that increasing the contrast of the stimuli (in both eyes or selectively in one eye) leads to increased pupil-size modulations. Thus, we can

account for our observations by assuming that monocular deprivation produces an enhancement of the effective stimulus strength (mainly, in the deprived eye). On the other hand, our results are not consistent with either a selective decrease of the effective strength in the non-deprived eye (which would lead to reduced average contrast, and supposedly decreased pupil-size modulation) or a symmetric and opposite modulation of the effective strength in the two eyes (which would leave the average contrast unaffected, predicting unaltered pupil-size modulations). This is in line with the observed perceptual effect of monocular deprivation, which is clearly asymmetric and primarily consists of an elongation of deprived-eye dominance phases and a more modest shortening of non-deprived eye dominance phases (as often observed, e.g.: Binda et al., 2018, Lunghi, Burr & Morrone, 2011, Lunghi & Sale, 2015, Steinwurz et al., 2020, Wang, McGraw & Ledgeway, 2020).

In contrast, physiological studies typically reported a symmetric effect on visual evoked responses measured with EEG or fMRI, showing that the increase of responses to the deprived eye is similar in magnitude to the decrease of responses to the non-deprived eye (Binda et al., 2018, Kurzawski et al., 2022, Lunghi et al., 2015, Lyu et al., 2020, Schwenk, VanRullen & Bremmer, 2020) – except in two studies where the effect was primarily on the deprived eye (Chadnova et al., 2017, Zhou et al., 2015). This suggests that, under the conditions previously employed for physiological recordings, the neural mechanisms modulating the relative strength of the two eyes may be distinct from those engaged by binocular rivalry or binocular summation tests. The current study is a first attempt to directly compare the effects of monocular deprivation on physiological and perceptual responses to binocular rivalry stimuli – future studies may extend these observations with more direct measures of cortical responses, such as EEG and fMRI, acquired during perceptual testing.

Another standard parameter of binocular rivalry dynamics is the proportion of mixed percepts. In line with several previous studies, we find that monocular deprivation left this parameter unaffected (Lunghi et al., 2015, Lunghi, Burr & Morrone, 2011, Lunghi et al., 2019, Nguyen, Srinivasan & McKendrick, 2023, Steinwurz et al., 2020, Wang, McGraw & Ledgeway, 2021). One previous study did detect an increase of mixed percepts following deprivation (Sheynin, Proulx & Hess, 2019) and suggested that this was missed in other studies due to the low percentage of mixed percepts in their participant samples. Our results do not support this hypothesis, given that the proportion of mixed percepts in our dataset was higher than usual (probably due to the nature of the stimuli, black and white disks), yet unmodulated by deprivation. We acknowledge that the interpretation of this finding should be mitigated by the limited power achieved by our small sample size.

When we analyzed pupil-size modulations during mixed percepts, we found that these too were unaffected by monocular deprivation. These pupillometric results are exactly specular to those we obtained in our previous study (Acquafredda, Binda & Lunghi, 2022) where we used the same technique and stimuli (black/white disks) to measure the effects of endogenous attention during rivalry. These radically different manipulations – monocular deprivation and endogenous attention – produced remarkably similar effects on rivalry dynamics, both shifting dominance in favor of one eye (deprived or attended) by about 10 %. In contrast, the effects on pupil-size modulation were completely different; only monocular deprivation, not attention cueing, increased pupil-size modulations during exclusive dominance phases; only attention cueing, not monocular deprivation, affected pupil size during mixed percepts. This suggests that combining pupillometry with binocular rivalry provides a useful tool to discriminate phenomena that might act through different mechanisms yet produce similar perceptual effects.

4.1. Conclusions

There is increasing evidence for the usefulness of pupillometry in investigating a variety of perceptual and physiological phenomena, including neuroplasticity (Binda & Lunghi, 2017, Viglione, Mazzotti &

Pizzorusso, 2023). Here, for the first time, we were able to integrate the pupillometric technique into one of the standard tests for revealing short-term monocular deprivation effects – binocular rivalry. Our results give insights on the underlying physiological mechanisms, suggesting a change of effective stimulus strength that simultaneously affects perceptual reports and the automatic and unconscious control of pupil diameter.

Recent evidence indicates that homeostatic plasticity research holds significant translational value, as the transient ocular dominance shifts can (under specific conditions) be consolidated into long-term changes (Lunghi et al., 2019). Pupillometry could offer an objective quantification of the effect and thereby of its clinical usefulness, e.g. for amblyopia treatment in adults (reviewed in: Baroncelli & Lunghi, 2021, Castaldi, Lunghi & Morrone, 2020).

Our analysis relies on perceptual reports for parsing pupil traces; however, finding a systematic pupil-size modulation in phase with perceptual alternations represent a pre-condition for developing a pupillometry-only approach, where pupil-size changes are used to track the dynamics of binocular rivalry and thereby index ocular dominance and its plasticity. It remains an open question whether pupil-size traces afford sufficient signal-to-noise ratio for this application (Frassle, et al., 2014).

CRedit authorship contribution statement

Miriam Acquafredda: Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation. **Paola Binda:** Writing – review & editing, Supervision, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data are available at the following DOI: 10.5281/zenodo.12185903

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.visres.2024.108449>.

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