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Metaplasticity: Dark exposure boosts local excitability and visual plasticity in adult human cortex

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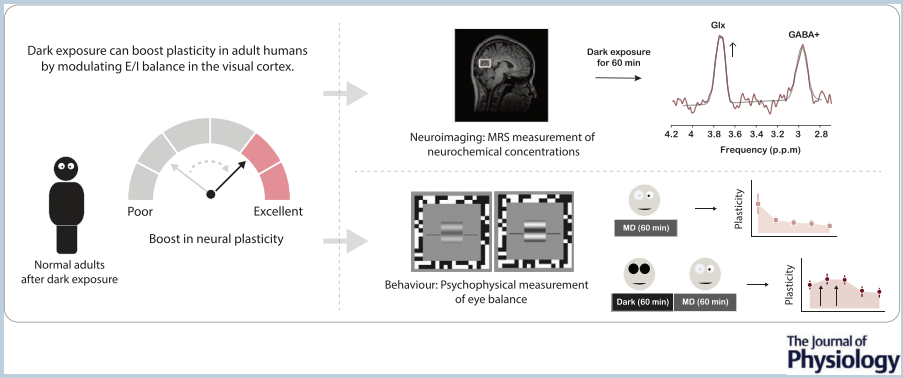

Abstract An interlude of dark exposure for about 1 week is known to shift excitatory/inhibitory (E/I) balance of the mammalian visual cortex, promoting plasticity and accelerating visual recovery in animals that have experienced cortical lesions during development. However, the translational impact of our understanding of dark exposure from animal studies to humans remains elusive. Here, we used magnetic resonance spectroscopy as a probe for E/I balance in the primary visual cortex (V1) to determine the effect of 60 min of dark exposure, and measured binocular combination as a behavioural assay to assess visual plasticity in 14 normally sighted human adults. To induce neuroplastic changes in the observers, we introduced 60 min of monocular deprivation, which is known to temporarily shift sensory eye balance in favour of the previously deprived eye. We report that prior dark exposure for 60 min strengthens local excitability in V1 and boosts visual

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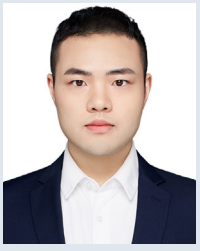

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plasticity in normal adults. However, we show that it does not promote plasticity in amblyopic adults. Nevertheless, our findings are surprising, given the fact that the interlude is very brief. Interestingly, we find that the increased concentration of the excitatory neurotransmitter is not strongly correlated with the enhanced functional plasticity. Instead, the absolute degree of change in its concentration is related to the boost, suggesting that the dichotomy of cortical excitation and inhibition might not explain the physiological basis of plasticity in humans. We present the first evidence that an environmental manipulation that shifts cortical E/I balance can also act as a metaplastic facilitator for visual plasticity in humans.

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Abstract figure legend In human adults, neural plasticity is known to be limited. However, a brief period of darkness boosted plasticity in the visual system by shifting the cortical balance between excitation and inhibition in the primary visual cortex. Specifically, the concentration of the excitatory neurotransmitters glutamate and glutamine (Glx) significantly increased but not that of the inhibitory neurotransmitter GABA. The concentrations were measured using magnetic resonance spectroscopy. Behavioural measurements were conducted using psychophysics. Monocular deprivation (MD) was introduced to shift a significant change in eye balance, an assay for visual plasticity. The boost in visual plasticity significantly increased when MD was followed by a brief period of dark exposure.

Key points

- A brief interlude (60 min) of dark exposure increased the local concentration of glutamine/glutamate but not that of GABA in the visual cortex of adult humans.
- After dark exposure, the degree of the shift in sensory eye dominance in favour of the previously deprived eye from short-term monocular deprivation was larger than that from only monocular deprivation.
- The neurochemical and behavioural measures were associated: the magnitude of the shift in the concentration of glutamine/glutamate was correlated with the boost in perceptual plasticity after dark exposure.
- Surprisingly, the increase in the concentration of glutamine/glutamate was not correlated with the perceptual boost after dark exposure, suggesting that the physiological mechanism of how E/I balance regulates plasticity is not deterministic. In other words, an increased excitation did not unilaterally promote plasticity.

Introduction

Neural plasticity, a defining feature of the nervous system that permits the brain to modify its response and development based on sensory experience, prevails during the critical period and has been believed to irreversibly decline into adulthood. However, molecular and physiological evidence from the animal literature suggests that visual plasticity in the mammalian adult cortex can be reinstated after the animals experience an interlude of dark exposure (Bavelier et al., 2010; Montey & Quinlan, 2011). Specifically, dark exposure increases cortical plasticity by perturbing the expression of various proteins that are better known as molecular brakes on plasticity, such as chondroitin sulfate proteoglycans (Pizzorusso et al., 2002), perineuronal nets (Hensch & Fagiolini, 2005) and neurofilament (Duffy & Mitchell, 2013), acting as a metaplastic facilitator.

The effect of dark exposure has been studied in cats and rodents. For instance, in rats dark rearing from birth could delay and prolong the critical period (Cynader & Mitchell, 1980; Fagiolini et al., 1994) and reactivate it in the adult (He et al., 2006) even if animals had experienced a cortical stroke (Stodieck et al., 2014), enabling their cortical recovery. Furthermore, amblyopic rats and kittens display a faster and a more complete recovery in visual functions after a 10-day immersion in the dark (Duffy & Mitchell, 2013; He et al., 2007), but this does not occur

in adult cats (Holman et al., 2018). The studies show that there is a temporal window of 8 months after birth during the critical period in which dark exposure can effectively increase neural plasticity in kittens (Mitchell et al., 2019). A recent study shows that binocular intravitreal injection of tetrodotoxin (TTX), which is a blocker of the sodium channel, promotes the visual recovery of kittens and mice more potently (Fong et al., 2016) than 7 days of darkness (Erchova et al., 2017), suggesting that the silencing of the retina using TTX as a pharmacological manipulation can be more effective than introducing dark exposure as an environmental manipulation. In sum, silencing the visual input at the level of either the eye or a population of neurons seems to promote plasticity in the visual cortex, more so in younger animals.

Inspired by the animal literature, human studies have employed short-term monocular deprivation (MD) to induce plasticity in adults in the last decade (Lunghi et al., 2011). This protocol mirrors that of animal studies, which assess the susceptibility of the visual cortex to long-term MD as an index of cortical plasticity. The susceptibility in humans to short-term MD (15 min to 5 h) is measured as the degree of shift in ocular dominance that is observed in binocular combination after MD (Min et al., 2018), which favours the deprived eye and is thought to involve homeostatic plasticity (Mower et al., 1981 p.19) that is regulated by intracortical excitatory/inhibitory (E/I) balance (Ip et al., 2021). This effect has been observed with multiple visual tasks in adults (Bai et al., 2017; Baldwin & Hess, 2018; Min et al., 2021), suggesting that neural plasticity still exists after the critical period. Moreover, GABA (γ-aminobutyric acid) is decreased in regions within the primary visual cortex (V1) associated with the previously deprived eye (Lunghi et al., 2015), suggesting a clear relationship between the perceptual gain of the deprived eye and reduced GABAergic inhibition in its pertinent areas within adult V1.

However, our understanding of the role of E/I balance on plasticity is incomplete (Bavelier et al., 2010; Hensch & Fagiolini, 2005; Mitchell & Maurer, 2022). Some propose that the effect of shift in E/I balance on the physiology of plasticity is deterministic: a shift in favour of excitation enhances plasticity, whereas a shift in favour of inhibition regulates it (Ip et al., 2021; Lunghi et al., 2015). Another school of thought believes that the simple dichotomy of excitation and inhibition from the deterministic view is misleading because it can originate from simple experimental designs that have limited application to the natural physiology of the cortex (Fritschy, 2008). Findings that inhibition is important for both triggering and regulating plasticity (Blatow et al., 2005; Floyer-Lea et al., 2006; Frangou et al., 2019) argue against the deterministic view, suggesting that the direction of the shift is not important. Instead, the view advocates that if the change in E/I balance is significantly different from the normal range of transient fluctuation of the balance across time, it can be physiologically purposeful (Dehghani et al., 2016; Steel et al., 2020). Thus far, these two conflicting views have not been resolved.

Using magnetic resonance spectroscopy (MRS), we show that a brief interlude of darkness (analogous to dark rearing in animals) increases Glx (the aggregate of glutamate and glutamine) concentration of the visual cortex in humans. Furthermore, we report with supporting behavioural evidence that it boosts visual plasticity in the form of an increased magnitude and longevity of the perceptual gain from the deprived eye after MD. Finally, our evidence indicates that the influence of E/I balance on plasticity is not deterministic but rather a complex and dynamic process, where the degree of deviation can be more physiologically relevant to metaplasticity in the visual system. Our findings present the first direct evidence that an environmental manipulation that shifts cortical E/I balance can also act as a metaplastic facilitator for visual plasticity in adult humans, providing insights into the neurophysiological role of E/I balance in promoting visual plasticity and highlighting clinical applications of metaplasticity.

Methods

Ethics approval

All experimental procedures in the study followed the most recent guidelines of the *Declaration of Helsinki* and were approved by the Ethics Committee of Wenzhou Medical University (ethics approval number: 2022KY220) and the University of Science and Technology of China (ethics approval number: 2022KY204). However, the procedures were not registered in a database. All observers were naïve to the purpose of the experiment and provided written informed consent.

Participants

Sixteen observers (11 females, mean ± SD age = 24.4 ± 3.1 years) with normal or corrected-to-normal vision, including two authors, participated in the MRS experiment. Fourteen of them participated in the behavioural experiment across all four conditions. Seven adults with amblyopia (4 females, mean ± SD age = 24.7 ± 3.5 years) participated in two of the four conditions of the behavioural experiment. Using Miles's test, we established the eye dominance of each subject (Miles, 1930). With their hands, the participants were asked to form a peephole with their index finger and thumb, place it at arm's length, and focus on a visual target that was in the centre of the peephole. By alternately closing each eye, they were then asked to report when the target's

position was displaced the most. There would be a larger lateral displacement of the target during the closure of the dominant eye.

Visual deprivation

In this study, there were two forms of visual deprivation. First, monocular deprivation involved the occlusion of the dominant eye in controls using a translucent patch that transmitted 80% of incidentlight; the amblyopic eye was deprived in amblyopic participants. Second, binocular deprivation in the form of dark exposure was achieved by the use of opaque black patches that were worn in front of both eyes while the subjects were placed in a room that had no light, and asked to keep their eyes open and stay awake. The black eye patches (0% light transmittance) worn in front of both eyes ensured that the subjects did not experience any residual or intermittent stray light in the darkened room.

Magnetic resonance spectroscopy

Data acquisition. Magnetic resonance imaging (MRI) was conducted on a 3.0-tesla MRI scanner (Discovery MR750, GE Healthcare, Milwaukee, WI, USA). The equipment had an eight-channel high-resolution radio-frequency head coil. Prior to the scanning procedure, all observers were asked to lie down with eyes open and stay awake during scanning. Structural 3D T1-weighted MRI of brain was acquired using the T1-3D BRAVO sequence with the following parameters: repetition time (TR)/echo time (TE): 8.16 ms/3.18 ms, flip angle = 12°, 252 axial slices with no gap, matrix: 256 × 256, field of view (FOV): 256 × 256 mm, slice thickness: 1 mm.

Glx and $GABA^+$ were quantitatively measured using ^1H MRS technology. The spectra were acquired with a MEGA-PRESS sequence (Mescher et al., 1998): TE = 68 ms, TR = 2000 ms, NEX = 8256 transients of 4096 data points obtained at 5 kHz. The duration of the scan was 9 min 20 s. A 17 ms Gaussian editing pulse was applied at 1.9 (ON) and 7.46 (OFF) ppm. The voxel size was $20 \times 30 \times 20$ mm^3 at the primary visual cortex (V1) near the calcarine sulcus. The water suppression band of the editing pulses was applied at 4.68 ppm using chemical shift selective saturation (CHESS) imaging (Haase et al., 1985) and outer volume suppression (OVS) (Felmlee and Ehman, 1987). The very selective suppression (VSS) method in OVS technology was used for the volume of interest extra-spatial signal suppression (Edden et al., 2014).

The ON spectral line was the spectral line that was edited by the spectrum, and the OFF spectral line was the opposite spectral line. During the scanning process of the MEGA-PRESS sequence, an empty sweep period of eight repetition times (TRs) was required at the beginning of the sequence to allow the signal to reach a steady state during the acquisition. In addition, the reference spectrum containing the water signal was acquired before the ON and OFF spectral line acquisitions. During acquisition of the phase correction reference signal, we turned off the CHESS module in the MEGA-PRESS sequence was turned off for 16 TRs.

Data processing. We quantitatively measured the concentrations of $GABA^+$ and Glx by editing the MRS spectra using Gannet 3.1.5 (GABA Analysis Toolkit) (Edden et al., 2014) and inhouse MATLAB scripts (The MathWorks, Natick, MA, USA). All MRS data were analysed within the .7 file format. The water reference and the edited spectrum signals were isolated from the MRS data, and the water reference signal was used to correct the edited spectral signal. The end of the edited spectrum signal was filled with zeros for Fourier transform. Next, a phase correction was performed on the spectrum signal in the frequency domain. The edited spectral signals were aligned to frequencies; the chemical shifts of the Cr signals were aligned to the standard value of 3.02 ppm.

The remaining water signals of the average ON and OFF spectra were isolated and removed from the edited spectral signal, and their baselines were corrected. The ON and OFF spectra were subtracted to produce the DIFF (difference) spectrum, from which $GABA^+$ (3 ppm) and Glx (3.8 ppm) signal intensities were modelled using a Gaussian model. The area under the peak of the GABA signal was calculated to obtain a quantitative measure of GABA. The areas under the peak of the creatine (Cr) signal were used to assess the fitting error of the edit spectrum signal. All the neurochemical signal intensities were considered as the area of the fitted peak(s) and expressed in institutional units (i.u.). Creatine was used as the reference metabolite to derive the internal concentrations of Glx and $GABA^+$.

We calculated the proportions of grey matter and white matter in the voxel of interest for each subject by performing segmentation analysis because the concentrations of the neurotransmitters can vary based on the grey matter proportion rather than the experimental intervention, which in this case is a brief interlude of dark exposure. To control for this, we performed statistical analysis by treating the proportion of grey matter as a covariate in subsequent statistical analyses (Kolasinski et al., 2019).

Experimental procedure. As previously mentioned, magnetic resonance spectra were obtained with a MEGA-PRESS sequence (Mescher et al., 1998) from the primary visual cortex (V1) near the calcarine sulcus

with a voxel size of 20 × 30 × 20 mm^3 (Fig. 1*A*). Each observer completed three MRS sessions. The first MRS scan was a practice session and was not included in the analysis. The data from the scan were only examined to evaluate whether changes in MRS data across sessions were due to differences in the quality of data. Next, the second MRS scan was conducted after the subjects had spent 60 min in a brightly lit lab and stayed awake. Finally, the third MRS scan was completed after the subjects had undergone 60 min binocular deprivation in the form of dark exposure. The protocol of dark exposure in the MRS procedure was identical to that in the psychophysical experiment. Figure 1*B* illustrates an observer's spectra before and after 60 min dark exposure.

Behavioural measurement

Apparatus. The experiments were coded with MATLAB and Psychtoolbox, and were conducted on a MacBook Pro laptop. Head-mount goggles (Goovis pro, NED Optics, Shenzhen, China) with a resolution of 1920 × 1080, a pixel per degree of 41.6, and a refresh rate of 60 Hz in each eye were used to dichoptically show the visual stimulus. The mean luminance of OLED goggles was 75 cd/m^2. The experiment was conducted in Wenzhou Medical University.

Psychophysical method. In this study, we used a binocular phase combination task to quantify the change in eye balance and induced visual plasticity (Fig. 1*C*). This procedure is described in detail in previous studies (Ding & Sperling, 2006; Min et al., 2019; Zhou et al., 2013). In brief, a sinusoidal grating was shown to each eye with offset phases (−22.5° for one eye, +22.5° for the other eye), a spatial frequency of 0.3 cycles/degree and a size of 6.6 × 6.6 degrees. When the two eyes contributed equally to binocular vision, the darkest strip of the fused grating would be at the middle of the grating; in other words, the perceived phase would be 0° (the sum of +22.5° and −22.5°). However, if one eye that was shown with a +22.5° grating contributed more to binocular vision, then the fused grating would have a positive, rather than negative, perceived phase. The observers were asked to move the flanking reference line (a one-pixel movable line that the user can control; see Fig. 1*D*) next to where they perceived the darkest strip of the fused grating using the keyboard.

The sinusoidal gratings from both eyes were shown at five interocular contrast ratios (dominant eye/non-dominant eye: 1/2, $1/\sqrt{2}$, 1/1, $\sqrt{2}/1$, 2/1) for baseline measurement and three interocular contrast ratios ($1/\sqrt{2}$, 1/1, $\sqrt{2}/1$) for measurements that were made after the deprivation (i.e. post-patch measurement), and the base contrast was set at 60%. Using the data of perceived phase, a psychometric function was fitted to estimate the contrast ratio that resulted in equal contribution between the eyes (Min et al., 2019). In the case of a normal observer, whose dominant eye was patched, a positive ratio (dB) would indicate a stronger dominant (i.e. patched, PE) eye, and the negative ratio a stronger non-dominant (non-patched, NPE) eye. The ratio was computed in decibel units using this formula, 20 × log(contrast of NPE/contrast of PE).

Two configurations of the visual stimuli were used to remove response bias. The first configuration showed a grating of +22.5° to the dominant eye and a grating of −22.5° to the non-dominant eye. In the second configuration, the dominant eye was shown with a grating of −22.5° and the non-dominant eye with a grating of +22.5°.

There were eight and five repetitions (i.e. trials) for each interocular contrast ratio during the baseline and post-patch tests, respectively; therefore, there were 80 trials during the baseline measurement (5 interocular contrast ratios × 8 repetitions × 2 configurations) and 30 trials during the post-patching measurement (3 interocular contrast ratios × 5 repetitions × 2 configurations).

Experimental procedure. The subjects completed three tests of baseline measurement (see Fig. 1*D*). The baseline data were within a range of ±1 dB, suggesting that the data had a minimal margin of error. Next, one of the four conditions of visual deprivation (monocular or binocular) was introduced to the subjects. After the deprivation, ocular dominance was measured using the same psychophysical task at 0, 3, 6, 12 and 24 min after the patch had been removed from the subjects. The order of the condition was randomized for each observer with a random number generator in R. Each condition was tested on a separate day to prevent any perceptual changes after MD from being carried over to a subsequent session. Based on a previous study that shows that the effect does not last for more than a day (Min et al., 2019), we decided to separate two sessions by at least 24 h.

Adults with amblyopia. Seven adults with amblyopia were recruited in two of the four conditions from the behavioural experiment (MD and Dark → MD). The clinical information about the amblyopic observers is included in Table 1. The amblyopic eye was deprived using a translucent patch. The rationale for testing them was to see whether the potentiation from dark exposure that was observed in normal observers (see Results below) would also be elicited in adults with amblyopia. The contrast ratios of the sinusoidal gratings were set differently because the amblyopes already had an abnormal eye balance, requiring us to use contrast ratios that strongly favoured the amblyopic eye. For example, the contrast of

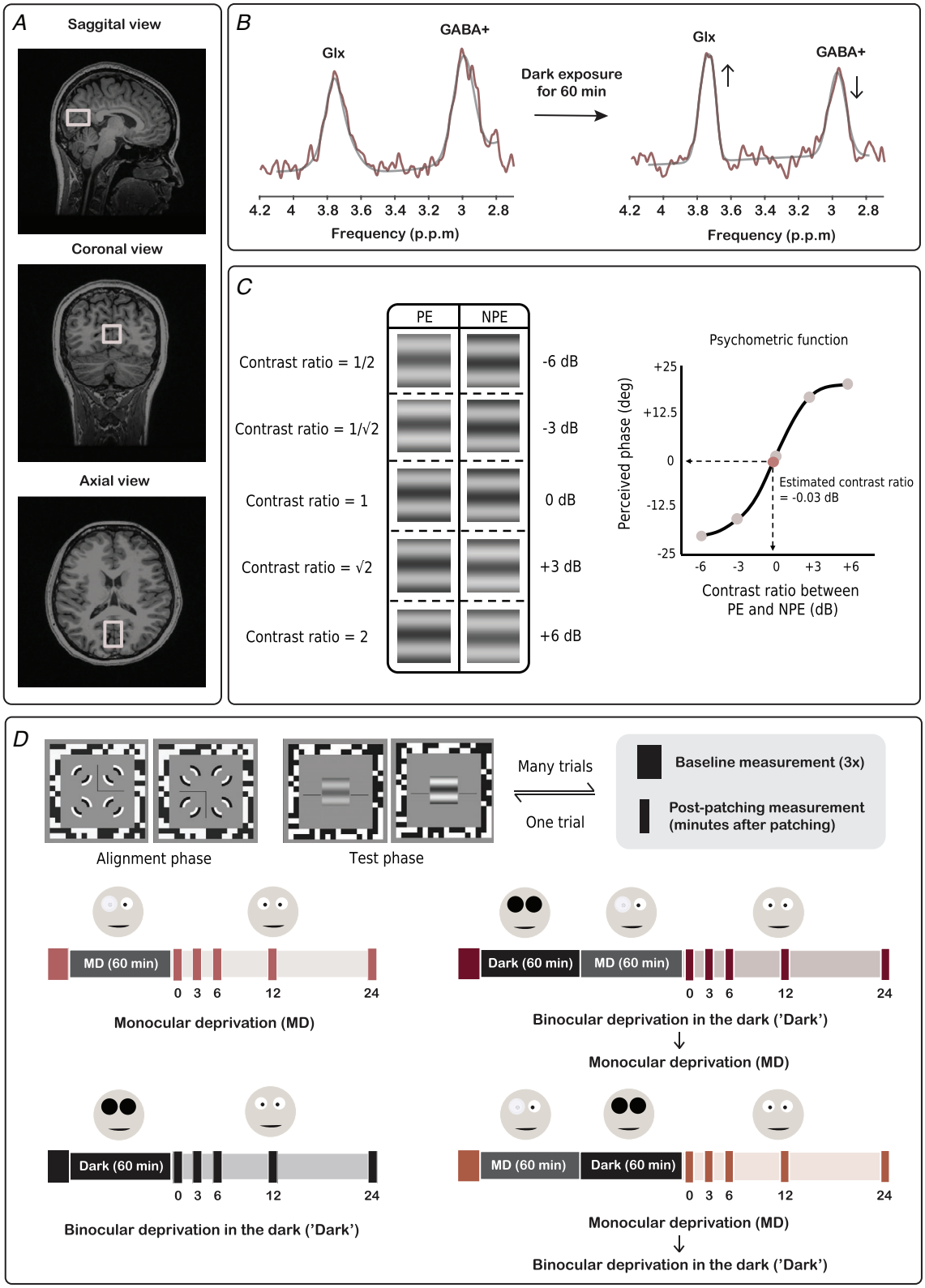

Figure 1. MRS and behavioural experiments
A, sagittal, coronal, and axial views of the representative MRS voxel in the primary visual cortex (V1). *B*, representative observer's edited spectra from the visual cortex. Data from two MRS sessions (before and after 60 min dark exposure) were included in data analysis. The arrows indicate hypothetical results after dark exposure. *C*, binocular combination protocol. Sinusoidal gratings were dichoptically presented at five contrast

ratios for baseline measurement (pre-MD) and at three ratios for post measurements (post-MD). Using the data, we fitted a psychometric function to estimate the eye balance. *D*, behavioural experiment design. Each trial consisted of an alignment phase and a test phase. During the alignment phase, a dichoptically displayed cross appeared; the subject was asked to fuse and align them into a whole cross. During the test phase, the subject was asked to move the reference line that flanks the grating to pinpoint where they perceive the darkest area of the fused grating. There were three sessions of baseline tests, and then either 60 min MD (top-left), 60 min dark exposure followed by 60 min MD ('Dark → MD'; top-right), 60 min dark exposure ('Dark'; bottom-left) alone, or 60 min MD followed by 60 min dark exposure (bottom-right) and finally post-MD tests at 0, 3, 6, 12 and 24 min after patch removal. MD, monocular deprivation of the dominant (in controls) or poor (in amblyopes) eye.

Table 1. Clinical information for amblyopic patients

Subject	Age/sex	Refraction (OD/OS)	logMAR VA (OD/OS)	RDS (Arc seconds)	History of orthoptic treatment
A1	22/M	+3.00/−0.50 × 130° +0.25/−0.25 × 35°	0.40 0.16	400	Detected at the age of 8, had been patched and received red light therapy for less than 1 month
A2	27/F	−2.50/−0.50 × 42° +2.25/−0.25 × 175°	−0.10 0.24	200	Detected at the age of 9, received red light therapy for 2 years
A3	24/F	−0.75/−0.25 × 180° +3.00/−0.75 × 60°	0.00 0.20	200	Detected at the age of 13, wore glasses for 1 year, no history of patching
A4	28/F	Plano +3.00/−2.25 × 10°	−0.04 0.32	200	Detected at the age of 18, no history of treatment and had not worn glasses consistently
A5	19/M	−8.25/−1.75 × 5° 0/−2.50 × 175°	0.08 0.58	200	Detected at the age of 8, had been patched for 1 year, had worn glasses since the diagnosis
A6	29/M	+4.00 +2.25	0.42 0.14	400	Detected at the age of 13, no history of treatment, had worn glasses occasionally when viewing near objects
A7	24/F	−3.75/−1.50 × 180° −3.50/−1.00 × 165°	−0.06 0.36	800	Detected at the age of 16, no history of patching, had worn glasses since the diagnosis

A1–A6 are anisometropic amblyopes with no squint. logMAR, logarithm of the minimum angle of resolution; OD, right eye; OS, left eye; plano, emmetropia; RDS, Randot stereoacuity; VA, visual acuity.

the amblyopic eye was fixed at 100%. The contrast ratios (fellow eye/amblyopic eye) were $1/(9 \times \sqrt{3})$, 1/9, $1/(3 \times \sqrt{3})$, 1/3 and 1 for the baseline measurement, and they were $1/(9 \times \sqrt{3})$, $1/(3 \times \sqrt{3})$, and 1 for the post-patching measurement. However, some amblyopes had a normal eye balance. So, for them, the stimuli were shown at the contrast ratios that were shown to the normally sighted subjects.

Statistical analysis. Fourteen subjects with normal vision completed all psychophysical and MRS experiments. Creatine was used as a reference metabolite to compute the relative concentrations of Glx and $GABA^{+}$. Student's two-sample paired *t* test of the ratios between Glx and GABA (20 × log(Glx/GABA)) was conducted between before and after dark exposure while the proportion of the grey matter was set as a covariate. Inclusion of the covariate was necessary because there was chance that the concentrations of the two neurotransmitters could be correlated with the volume of the grey matter within the voxel of interest. Therefore, the volume of the grey matter had to be controlled for. The ratios between Glx and GABA were scaled in logarithmic units (dB) to assume symmetry. An effect size (Cohen's *d*) was computed by dividing the mean difference of the concentration between the two time points with their pooled standard deviation.

As for the behavioural data, we integrated each item of data of shift in perceptual eye dominance as an areal measure using the trapezoidal integration method. This enabled us to capture the longevity and magnitude of changes followed by short-term MD. After performing one-way repeated measures analysis of variance (ANOVA), we performed a *post hoc* pairwise *t* test with Bonferroni *P*-value correction to determine whether there was a difference within each pair from the four behavioural conditions. To examine whether there was a relationship between neurochemical and

behavioural measures, we performed a permutation test by forming a linear model, where the dependent variable was the metaplasticity index (see the subsection below) and the predictor variable was the Glx deviation index. The correlation test was performed after controlling for the volume of the grey matter as the grey matter index. After the permutation test, a 95% confidence interval of the correlation coefficient was obtained to determine statistical significance. Other correlation analyses were also coupled with a permutation test. All data analyses and visualizations were conducted using MATLAB, R and Python.

Metaplasticity index, Glx index and Glx deviation index. The metaplasticity index (i.e. metaplasticity induced by prior dark exposure) was computed from areal measures $(\text{Area}_{\text{Dark}\rightarrow\text{MD}} - \text{Area}_{\text{MD}})/(\text{Area}_{\text{Dark}\rightarrow\text{MD}} + \text{Area}_{\text{MD}})$ of all observers. The Glx index was computed from the Glx concentration relative to creatine using the equation $(\text{Glx}_{\text{after}} - \text{Glx}_{\text{baseline}})/(\text{Glx}_{\text{after}} + \text{Glx}_{\text{baseline}})$, where $\text{Glx}_{\text{after}}$ represents the referenced concentration after dark exposure. The Glx deviation index (see Fig. 4 in Results) is the *absolute* value of Glx index, and captures the magnitude of change in the concentration. Similar indices have been used in a previous study (Lunghi et al., 2015). The grey matter index was computed from the grey matter proportion before and after dark exposure using the equation $(\text{grey}_{\text{after}} - \text{grey}_{\text{baseline}})/(\text{grey}_{\text{after}} + \text{grey}_{\text{baseline}})$, and it was used as a covariate.

Rationale for the experimental design. In the MRS procedure, 60 min dark exposure was introduced and subsequent changes in the concentrations of Glx and GABA^+ were obtained in the primary visual cortex of all normal observers. Our motivation for the design was to see how the sensory deprivation could shift the E/I status of the visual cortex. Conversely, the procedure for the behavioural experiment was designed so that the increased visual plasticity from dark exposure could be computed from the difference in data between the two conditions (Dark → MD *vs.* MD). Note that in the MRS procedure, MD was not included because it can directly reduce GABAergic inhibition (Lunghi et al., 2015). If it were included in the MRS procedure, it would introduce an interaction between the effects of MD and dark exposure on E/I balance rather than the lone effect of dark exposure.

Results

Resting concentration of Glx shifts after dark exposure

Figures 2*A* and *B* show the normalized concentrations of Glx and GABA^+ relative to creatine before and after 60 min dark exposure in 16 normal subjects. Glx is a primary marker for excitatory neurotransmitters and includes both glutamine and glutamate since MRS cannot distinguish the two molecules. GABA^+ is a key marker for the inhibitory neurotransmitter GABA, which plays a pivotal role in cortical inhibition (Hertz & Rothman, 2016).

A paired two-sample *t* test with the covariate (grey matter proportion in the voxel of interest) showed that there was a significant increase in Glx:creatine between before and after exposure to darkness (Fig. 2*A*; $P = 0.005$, Cohen's $d = 1.01$) but not a significant decrease of the GABA concentration ($P = 0.51$, Cohen's $d = 0.66$). Ten of the 16 subjects experienced an increment in Glx after the dark exposure, whereas six of them experienced a decrement in Glx. Conversely, 10 subjects (with some different individuals) experienced a decrease in GABA after the dark exposure, whereas six of them underwent an increase in GABA. Lastly,

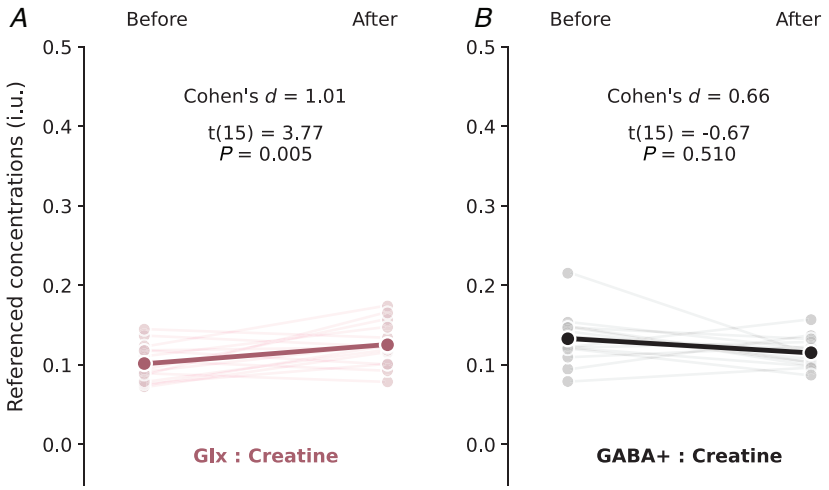

Figure 2. Effects of dark exposure on E/I balance in V1 in 16 normal observers
A, a slope chart showing that Glx:creatine significantly increased after dark exposure. *B*, a slope chart showing that GABA:creatine mildy decreased after dark exposure.

we computed the concentration ratio between Glx and GABA in decibels unit, 20 × log(Glx/GABA). The ratio significantly increased ($P < 0.001$) after dark exposure. However, it is important to point out that the ratio was heavily driven by the changes in Glx rather than GABA as there was no significant change in GABA. In sum, these results show that a brief dark exposure (60 min) can introduce a shift in the concentration of Glx within V1. Also, we found no significant difference in $GABA^{+}$ and Glx levels between the first (practice) and second MRS scanning sessions, indicating that the change in the neurochemical concentrations were not due to the measurement error.

Perceptual changes of MD are boosted with dark exposure

The psychophysical data from 14 normal observers are illustrated in Fig. 3*B–D*. In line with previous reports (Lunghi et al., 2011; Zhou et al., 2013), we found that the deprived eye experienced an increase in its contribution to binocular combination as a result of short-term MD. This is shown in Fig. 3*B*, which shows that changes in eye balance were positive. The deprived eye did not experience a gain after binocular deprivation ('Dark') for 60 min (see Fig. 2*D*) as the change in eye balance was around 0 (one sample *t* test, $P > 0.05$). This suggests that change due to a period of dark exposure was only effective after MD and not effective in its own right. Surprisingly, the magnitude of shift in eye balance in Dark → MD measured at 24 min is comparable to the counterpart in MD at between 0 and 3 min, indicating that the change was more long-lasting and potent after a brief dark exposure.

In addition, we computed the area under a curve (AUC) of changes in eye balance at various time points after patch removal relative to baseline (Fig. 3*A*). This enabled us to capture both the magnitude and the longevity of the induced visual plasticity, and compare it across the four deprivation conditions. Figure 3*A* indicates that the area is largest in Dark → MD, followed by MD and the Dark. A one-way repeated measures ANOVA verified this observation by showing a significant difference in the AUCs across the three conditions ($F(2,51) = 16.06$, $P < 0.001$, $\eta^2 = 0.39$). A *post hoc* comparison using Tukey's Honestly Significant Difference (HSD) test indicated that there was a significant difference in the areal measure between MD and Dark → MD ($P = 0.016$), MD and Dark ($P = 0.024$), and Dark → MD and Dark ($P < 0.001$). A one sample *t* test showed that the area was significantly different from 0 in MD, Dark → MD conditions ($P < 0.001$) and Dark → MD ($P < 0.05$), but not so in Dark ($P > 0.05$). Moreover, the *post hoc* comparison revealed that there was a notable difference between MD and MD → Dark, and MD and Dark. From the data, we can make three observations. First, changes in eye balance that are typically observed after MD were driven by interocular competition. Only monocular deprivation induced a shift in perceptual balance. Second, the fact that the dominant eye was deprived for 120 min in Dark → MD does not explain the boosted change in visual plasticity because the control condition MD → Dark did not show a similar increase; this indicates that a prior dark

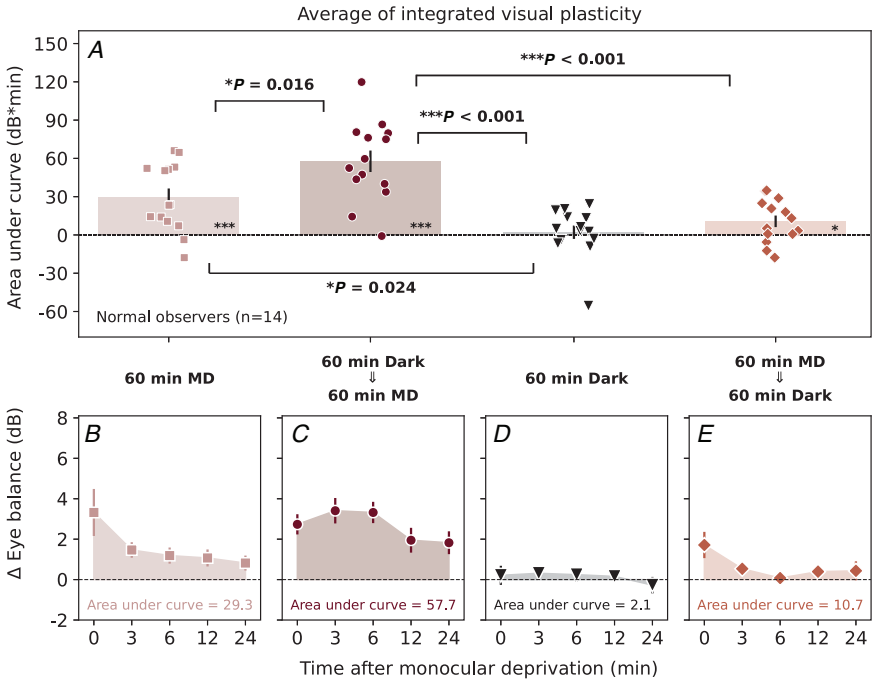

Figure 3. Behavioural results from visual deprivation on binocular balance in 14 normal observers
A, averaged AUCs across all observers. Asterisks denote a statistical significance based on *post hoc* Tukey HSD test or one-sample *t* test (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). 60 min MD is shown as light brown, dark → MD as mahogany, 60 min dark by black, and MD → dark as orange. *B–D*, changes in binocular balance after short-term monocular deprivation (averaged across all observers). Same colours are used as those in panel *A*. The error bars indicate standard errors.

exposure, rather than a period of 120 min deprivation of the dominant eye, can boost the perceptual change after MD. Third, the change in sensory eye dominance did not get stored (maintained) in the dark, as shown by the data in Dark → MD. If it was stored during in darkness, its rate of decay would be slower and the areal value of visual plasticity larger.

The relationship between MRS and behavioural data

We hypothesized that a shift in the magnitude of neurotransmitter concentrations after dark exposure could be associated with metaplasticity. First, according to a permutation test, we found a significant correlation between changes in Glx and $GABA^+$ after the brief dark exposure ($r = 0.49$, $P = 0.041$). This could be because the concentrations of GABA and glutamate are correlated and balanced (Steel et al., 2020). Instead, a large deviation of a neurotransmitter's release from its natural fluctuation can be physiologically significant and meaningful (Dehghani et al., 2016). For instance, we can deduce that those with a negative Glx index experienced a decrease in $GABA^+$ as well. Therefore, if the Glx index is increased after dark exposure as we show in our data, we cannot directly infer if the local excitation in the cortex has increased. To capture the absolute degree of change of the neurotransmitter's concentration, we computed the Glx deviation index (see Methods for its derivation). Then, we evaluated the correlation between the Glx deviation index and the metaplasticity index (see Methods for more details). The larger the Glx deviation index, the larger the absolute change in the concentrations of the neurotransmitter (Glx) after the dark exposure. The larger the metaplasticity index, the larger the difference of visual plasticity (in AUC units from Fig. 3) between Dark → MD and MD conditions. $GABA^+$ was not included in the analysis because short-term MD is known to directly reduce $GABA^+$ in the visual cortex (Lunghi et al., 2015), and MD was not included in the procedure of the MRS experiment (see Methods for the rationale of the design).

We calculated the correlation between the Glx deviation index and the metaplasticity index while controlling for the grey matter volume in the voxel of interest as a covariate, and found a significant correlation across all subjects from a Pearson correlation test ($r = 0.60$, $P = 0.005$; see Fig. 4*A*). The correlation was still robust even without the apparent outlier (Pearson's $r = 0.66$, $P = 0.048$, Fig. 4*B*). Our results support that there is a relationship between shifts in the neurotransmitter concentrations and visual metaplasticity.

However, we did not find a significant correlation between changes in Glx (raw, not absolute) and the metaplasticity index across all subjects ($r = 0.58$, $P = 0.22$, Fig. 5*A*). Even without the outlier, we did not find a significant correlation between Glx index and metaplasticity ($r = 0.28$, $P = 0.34$, Fig. 5*B*). The results argue against the deterministic view, which states that the simple dichotomy of excitation and inhibition is adequate to explain the basis of plasticity.

Behavioural results from adults with amblyopia

We also performed a pilot experiment by recruiting seven adults with amblyopia and observed that there was no potentiation from dark exposure in adult amblyopes. As Fig. 6*A* indicates, a two-sample paired *t* test between

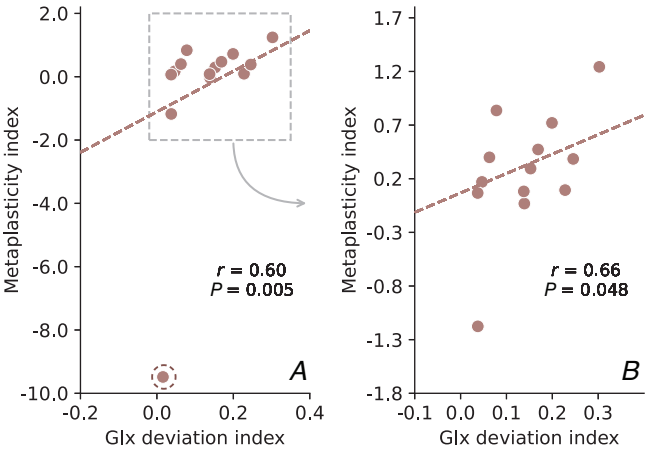

Figure 4. Relationship between the Glx deviation index and the metaplasticity index after dark exposure
The Glx deviation index denotes the absolute degree of concentration change of Glx after dark exposure. *A*, a correlation plot across all subjects ($n = 14$). *B*, a correlation plot without the apparent outlier ($n = 13$).

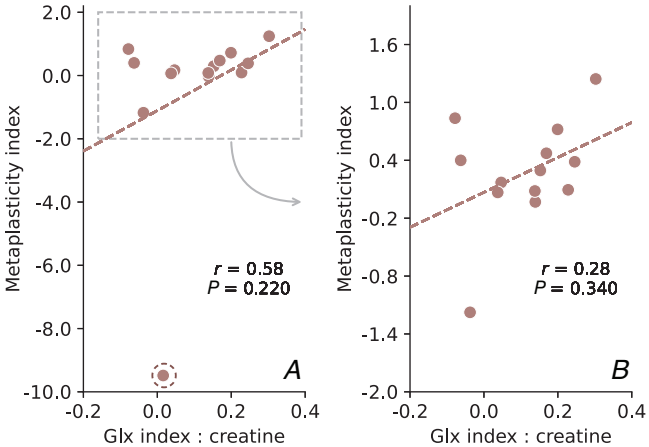

Figure 5. Relationship between the changes in Glx concentration and metaplasticity from dark exposure
A, a correlation plot from 14 normal observers who participated in both MRS and behavioral experiments. The Glx index was computed from changes in concentration of Glx after dark exposure. *B*, a correlation plot after excluding the apparent outlier ($n = 13$).

the areas from the two experimental conditions revealed that there was no significant difference between them ($t(6) = -0.20$, $P = 0.85$). The areas of both behavioural conditions were significantly different from 0 based on one-sample t test ($P < 0.05$). According to Fig. 6*B* and *C*, the peak of change in eye balance (at 3 and 6 min after patching) was higher in Dark → MD than in MD as it was the case in the data of normal adults (see Fig. 3).

Discussion

Together, MRS and behavioural evidence from normal observers reveals that a brief dark exposure boosts Glx concentration and visual plasticity in adult V1. Importantly, we also provide the first direct evidence that an environmental substrate that boosts excitability in V1 also increases visual plasticity in humans. Our data also indicate that the role of E/I balance on functional plasticity is not deterministic, such that an increase in excitation unilaterally potentiates plasticity while its decrease diminishes it (Lunghi et al., 2015; Shibata et al., 2017). Instead, they support the view that the influence of E/I balance on functional plasticity is a complex, dynamic process where the roles of excitation and inhibition can be blurred and the deviation of the E/I balance from its natural range can be more physiologically important (Fritschy, 2008; Steel et al., 2020).

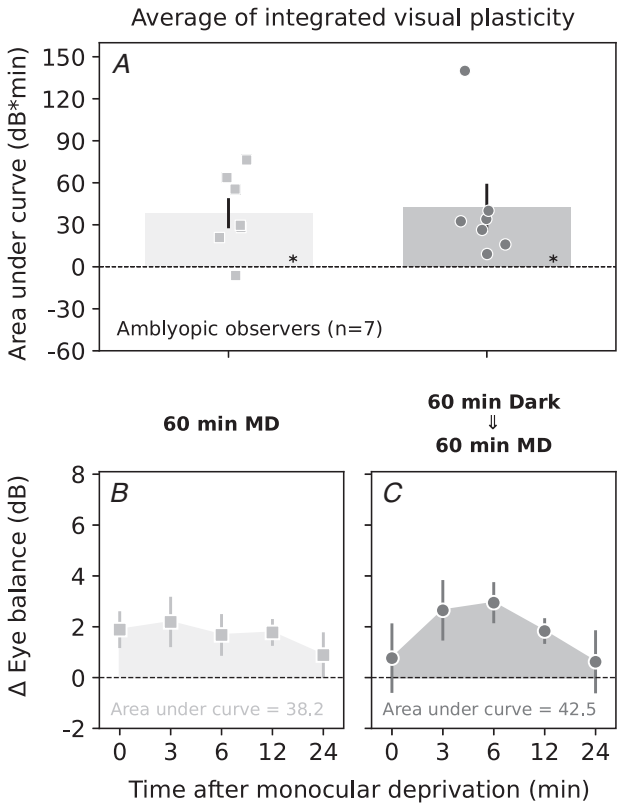

Figure 6. Behavioural results from visual deprivation on binocular balance in seven adults with amblyopia
A, averaged AUCs across all amblyopic observers. *B*, averaged changes in eye balance after 60 min MD condition over time. The asterisk (*) within each bar denotes a statistical significance ($P < 0.05$) of the areas based on one-sample t test. *C*, averaged changes in eye balance after 60 min Dark → 60 min MD over time. The error bars indicate standard errors.

The metaplastic property of darkness has been observed from cats and rodents in other forms, such as an extended critical period and boosted visual recovery after an artificial induction of amblyopia through long-term MD or cortical damage upon birth (Cynader, 1983; Fox et al., 1992; Mower et al., 1985). Here, we show for the first time that dark exposure also possesses the metaplastic quality in normally sighted adults by potentiating the neuroplastic changes after short-term MD. Our finding that visual plasticity can be increased after a 60 min interlude of darkness in adult humans is surprising, given that the interlude is brief and the participants are adults. In studies of juvenile mice and rats, an interval of darkness of up to 10 days has been explored. Our findings indicate that to induce a metaplastic facilitation for adult humans, if an interlude of darkness is introduced in a clinical setting, there might be no need for it to have a duration of days or months, which could disrupt the circadian rhythm and raise endocrinological consequences beyond the nervous system.

However, according to the pilot data of seven amblyopic adults, visual plasticity was not potentiated after dark exposure. There are several possibilities for why this was. First, the shift in eye balance after monocular deprivation has been shown to be smaller in amblyopes than that in normal observers (Min et al., 2022). To illustrate, 5 h patching induces a larger shift in eye balance in binocular combination than 2 h patching in normal but not in amblyopic observers. This finding indicates that there might be a ceiling effect for plasticity in amblyopia, where the effect of a metaplastic facilitator, such as an increased duration of patching or environmental manipulation, can be limited. Perhaps, a longer duration of dark exposure (rather than 1 h) or a more invasive form of visual deprivation, such as retinal silencing via TTX, is needed to observe such a boost in visual plasticity in amblyopic adults. Second, the psychophysical task that is used in the study might not have been sensitive enough to capture the boost in plasticity in amblyopia. In fact, previous studies show that the boost of plasticity from an increased patching duration was only present in one visual task but not another that measures binocular combination (Min et al., 2018, 2022). Lastly, it is well established that plasticity is markedly reduced in adults. Perhaps, the boost from potentiation can be better observed in children with amblyopia. The feline visual system also shows a boosted plasticity after 10 days of dark immersion in kittens but not in adult cats (Holman et al., 2018). In our study, due

to ethical concerns, we were not able to recruit children with amblyopia and submerse them in a darkened room because the deprivation of visual input for a significant duration could cause them to experience a feeling of anxiety and terror. In future, studies should examine whether the plateau of the boost in visual plasticity can be overcome in amblyopic adults, and then test whether the metaplastic facilitator of interest can be beneficial for amblyopic children with ethical protocols.

Interpreting the MRS data remains a challenge. Following the conventional interpretation of MRS data, we can infer from our data that the time spent in the dark raises the local excitation of the visual cortex after the release of glutamate has increased. Alternatively, we can deduce that the time spent in the dark can result in an accumulation but not in an active release of glutamate, thereby not shifting E/I balance in the extrasynaptic environment. Unfortunately, MRS captures the concentrations of Glx and GABA from both intrasynaptic and extrasynaptic environments (Belelli et al., 2009; Beppu et al., 2014; Okubo et al., 2010; Stagg, 2014). In addition, the concentrations of GABA and glutamate/glutamine are not independent because glutamine is a primary precursor of GABA (Rae, 2014; Stagg, 2014; Steel et al., 2020). In other words, GABA and glutamine (or Glx) are constantly cycled, so their concentrations are correlated and balanced in humans (Dehghani et al., 2016; Steel et al., 2020) across the sleep–wake cycle. Instead, a deviation of their concentrations can have a physiological influence (Steel et al., 2020). Therefore, whether an increase in the concentration of Glx truly refers to an increase in local excitation in the cortex remains unclear because it can also indicate that GABA has increased. For these reasons, some speculate that the E/I ratio describes behavioural data better than glutamate or GABA alone (Shibata et al., 2017) but, in our case, drawing a relationship between $GABA^+$ data and metaplasticity from the behavioural data was inappropriate because the proxy for functional plasticity that we have used (i.e. short-term MD) perturbs the $GABA^+$ concentration (Lunghi et al., 2015).

Our understanding of how a shift of E/I balance in a particular direction can affect neural plasticity remains to be elucidated because animal studies show that both excitation and inhibition are important for both promoting and regulating plasticity. The sheer diversity of GABAergic interneurons in the cortex and their sophisticated functional organizations argue against the deterministic view (Blatow et al., 2005), which advocates that an increase in excitation unilaterally potentiates plasticity while its reduction diminishes it. Instead, E/I balance is a complex, dynamic status where the roles of excitation and inhibition in the cortex can be multifarious. To illustrate, GABAergic inhibition has dual purposes. First, it triggers plasticity. For instance, diazepam, which potentiates inhibitory signalling, can induce ocular dominance plasticity in juvenile mice (Hensch et al., 1998). In fact, preserving GABAergic circuitry is key to inducing juvenile-like ocular dominance plasticity in adult mice (Greifzu et al., 2014). Second, it regulates plasticity. To illustrate this, it has been observed that adult and old mice with a cortical lesion can recover if their intracortical inhibition is mitigated (Stodieck et al., 2014), demonstrating that a reduced inhibition is correlated with enhanced plasticity. Collectively, these studies do not strongly support the simple deterministic view about the role of E/I balance on plasticity.

From multiple angles, our data also show that the role of E/I balance on plasticity is not simple and that it cannot be simply reduced to a narrative of dichotomy where excitation boosts plasticity and inhibition regulates it (Dehghani et al., 2016; Fritschy, 2008; Lunghi et al., 2015; Shibata et al., 2017). For instance, we show that changes in Glx and $GABA^+$ after dark exposure were correlated. This means that Glx and $GABA^+$ could increase concurrently after dark exposure. In addition, our data indicate that the boost in plasticity after dark exposure was not strongly correlated with the concentration change of Glx, which is an aggregate signal for the excitatory neurotransmitters glutamate and glutamine (Steel et al., 2020). Instead, we show that the Glx deviation index, which captures the absolute magnitude of change in Glx's concentration, was strongly correlated with visual metaplasticity. Hence, our data support the notion that the degree of deviation from the natural range of E/I balance's fluctuation may be more physiologically relevant (Steel et al., 2020).

Surmising from previous studies and our data, we speculate that the direction of shift in E/I balance is not crucial for enhancing different types of functional plasticity in humans. To illustrate this, transient shifts of the GABA:Glx ratio or $GABA^+$ concentration have been reported during motor (Floyer-Lea et al., 2006; Kolasinski et al., 2019) and perceptual learning (Frangou et al., 2019; Shibata et al., 2017) in mixed directions. For example, motor learning has been shown to reduce the GABA level in the cortex (Floyer-Lea et al., 2006; Kolasinski et al., 2019), whereas enhanced visual perception is correlated with an increased GABAergic inhibition (Frangou et al., 2019). However, both motor learning and enhanced perception can be interpreted as functional plasticity (Bavelier et al., 2010). Importantly, fluoxetine, which is a drug that has been shown to increase cortical plasticity of the rat by reducing GABAergic inhibition, does not enhance perceptual learning performance in humans (Lagas et al., 2016). This translational study exemplifies that designing a treatment regimen by adopting the deterministic view of excitation and inhibition can be inadequate. There is a need to systematically coalesce the body of literature to review whether the direction of shift in E/I ratio is directly related to changes in behavioural

measures of functional plasticity. Rather than taking a snapshot of the transient shift at a specific moment, we could perhaps be better informed about the phenomenon by studying E/I balance across time in future using functional MRS (Mullins, 2018). Also, future work can explore whether a larger change in E/I balance, which could perhaps be induced by a longer duration of dark exposure or other interventions such as aerobic exercise (Sale et al., 2007) and drug treatment (Sale et al., 2007; Silver et al., 2008), could cause a larger shift in visual plasticity. Such investigations could confirm the possible causal role of E/I balance in plasticity and inform about the nature of the interaction between them in detail. Ideally, it would be preferable for one to implement a behavioural assay for plasticity that does not directly perturb excitatory or inhibitory transmission unlike short-term MD (Lunghi et al., 2015) and motor or perceptual learning (Floyer-Lea et al., 2006; Frangou et al., 2019; Kolasinski et al., 2019).

Thus far, the translational impact of intervention strategies that can reactivate cortical plasticity in adult animals (Morishita and Hensch, 2008; Bavelier et al., 2010) has been elusive in adults with cortical disorders, such as amblyopia (Mitchell & Maurer, 2022). One such strategy is aerobic exercise (Sale et al., 2007). However, the effect of exercise on visual plasticity in normal adults remains inconclusive (Baldwin et al., 2022; Finn et al., 2019; Lunghi & Sale, 2015; Virathone et al., 2021; Zhou et al., 2017). Although not demonstrated, exercise is believed to modulate E/I balance (Bavelier et al., 2010; Sale & Berardi, 2015) by reducing GABAergic inhibition. Another approach that works in animals is pharmacological manipulation. However, a cocktail of approved drugs such as cholinesterase inhibitors (Silver et al., 2008) that have been shown to facilitate cortical plasticity in mice has yet to demonstrate improved perceptual learning or visual recovery in humans with amblyopia (Levi, 2020). Perhaps, the drug dose that has been used in animal studies is much more physiologically potent for animals than what can be provided for humans, failing to elicit a putative effect. Moreover, directly interfering with the neurochemical milieu using drugs raises both ethical and safety issues. Therefore, combined with its disappointing outcome in humans, pharmacological intervention has a limited appeal. The purported mechanism by which these drugs operate to increase cortical plasticity in humans is believed to be through affecting the release of excitatory and inhibitory neurotransmitters (Sale et al., 2010; Silver et al., 2008). Our findings in normal adults highlight that na short period of dark exposure can not only tap into the same mechanism but also do so in a less invasive fashion as a metaplastic modulator. They may provide a novel and acceptable strategy to enhance plasticity in adult humans. Future work will explore whether an interlude of darkness before treatment can be therapeutically used to reinstate plasticity and bring larger perceptual improvements for both young and adult populations with cortical disorders.

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Additional information

Data availability statement

Data and an analysis code are uploaded online in a Github repository (https://github.com/smin95/JPhys2023).

Competing interests

There is no conflict of interest.

Author contributions

Funding acquisition: R.F.H., J.Z. Project administration: Z.H., X.W., R.F.H., J.Z. Resources: Z.H., X.W., R.F.H., J.Z. Supervision: X.W., R.F.H., J.Z. Conceptualization: S.H.M., Z.W., X.W., R.F.H., J.Z. Data curation: Z.W., M.C., R.H. Formal analysis: S.H.M., Z.W., M.C., R.H., X.W., R.F.H., J.Z. Investigation: S.H.M., Z.W., L.G., Z.H., X.W., R.F.H., J.Z. Methodology: S.H.M., Z.W., M.C., R.H., L.G., Z.H., X.W., R.F.H., J.Z. Software: S.H.M., R.H., L.G., X.W. Validation: S.H.M., Z.W., M.C., L.G., X.W., R.F.H., J.Z. Visualization: S.H.M., R.H. Writing – original draft: S.H.M., Z.W., M.C., R.F.H. Writing – review and editing: S.H.M., Z.W., M.C., R.H., L.G., Z.H., X.W., R.F.H., J.Z. All authors have read and approved the final version of this manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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Keywords

amblyopia, dark exposure, E/I balance, magnetic resonance spectroscopy, metaplasticity, visual plasticity

Supporting information

Additional supporting information can be found online in the Supporting Information section at the end of the HTML view of the article. Supporting information files available:

Statistical Summary Document
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