Report

Short-Term Monocular Deprivation Alters GABA in the Adult Human Visual Cortex

Graphical Abstract

Highlights

- In adult humans, 2.5 hr of monocular deprivation strongly boosts vision in deprived eye

- Primary visual cortex resting GABA is decreased after 2.5 hr of monocular deprivation

- The change in resting GABA strongly correlates with deprived eye perceptual boost

- A decrease in resting GABA triggers homeostatic plasticity in adult primary visual cortex

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In Brief

Lunghi et al. show that short-term monocular deprivation drives homeostatic plasticity in adult humans, favoring input from the deprived eye. Using 7T MR spectroscopy, they show that resting GABA concentration decreases after deprivation and that the decrease in GABA strongly correlates with the individual plastic change, implying a causal effect.
Short-Term Monocular Deprivation Alters GABA in the Adult Human Visual Cortex

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SUMMARY

Neuroplasticity is a fundamental property of the nervous system that is maximal early in life, within the critical period [1–3]. Resting GABAergic inhibition is necessary to trigger ocular dominance plasticity and to modulate the onset and offset of the critical period [4, 5]. GABAergic inhibition also plays a crucial role in neuroplasticity of adult animals: the balance between excitation and inhibition in the primary visual cortex (V1), measured at rest, modulates the susceptibility of ocular dominance to deprivation [6–10]. In adult humans, short-term monocular deprivation strongly modifies ocular balance, unexpectedly boosting the deprived eye, reflecting homeostatic plasticity [11, 12]. There is no direct evidence, however, to support resting GABAergic inhibition in homeostatic plasticity induced by visual deprivation. Here, we tested the hypothesis that GABAergic inhibition, measured at rest, is reduced by deprivation, as demonstrated by animal studies. GABA concentration in V1 of adult humans was measured using ultra-high-field 7T magnetic resonance spectroscopy before and after short-term monocular deprivation. After monocular deprivation, resting GABA concentration decreased in V1 but was unaltered in a control parietal area. Importantly, across participants, the decrease in GABA strongly correlated with the deprived eye perceptual boost measured by binocular rivalry. Furthermore, after deprivation, GABA concentration measured during monocular stimulation correlated with the deprived eye dominance. We suggest that reduction in resting GABAergic inhibition triggers homeostatic plasticity in adult human V1 after a brief period of abnormal visual experience. These results are potentially useful for developing new therapeutic strategies that could exploit the intrinsic residual plasticity of the adult human visual cortex.

RESULTS

Binocular Rivalry Dynamics Change after Monocular Deprivation

We tested binocular rivalry between oriented gratings in 19 healthy volunteers (monocular deprivation group, mean age 24.3 ± 5.4 years) before and after 150 min of monocular deprivation. Before deprivation (Figure 1B), all observers showed similar durations in which they perceived the stimulus presented to one or the other eye (called mean phase duration), as shown by the scatter of the individual subject’s data around the unity line in Figure 1B. The average dominant to non-dominant eye duration ratio was 1.23 ± 0.03 (Figure 1D), indicating a slight preference for one eye. Consistent with previous reports [11, 12], 150 min of monocular deprivation of the dominant eye resulted in increased perceptual dominance of this eye during binocular rivalry (Figure 1C). The average dominant to non-dominant eye mean phase duration ratio was 1.82 ± 0.16 (Figure 1D). The increase in eye-dominance ratio is highly significant (paired t test, t(18) = 3.48, p = 0.003). The red symbols in Figures 1B and 1C show the data of control subjects (control group, n = 7, mean age 26.2 ± 6 years) that followed the same procedure but did not undergo monocular deprivation. The procedure of performing the binocular rivalry task twice, therefore, did not induce any change in performance.

Resting GABA Concentration Decreases in V1 after Monocular Deprivation

Magnetic resonance (MR) spectra were acquired at 7T from an occipital voxel (2 × 2 × 2 cm3), centered bilaterally on the calcarine sulcus (visual cortex, V1), and a control voxel of the same size, centered on the bilateral posterior cingulate cortex (PCC). An example spectrum is shown in Figure 2A, and the average spectrum from all participants and conditions can be seen in Figure S1. A diagram of the experimental paradigm is shown in Figure 1A: each observer participated in two magnetic resonance spectroscopy (MRS) sessions separated by a 150-min interval during which the main group of observers wore a translucent eye patch over the dominant eye (monocular deprivation group). GABA levels (quantified using LCModel [13]) were
measured during four different viewing conditions: eyes closed, non-deprived eye stimulated, deprived eye stimulated, and eyes open (see Supplemental Experimental Procedures for further information about MRS acquisition and analysis).

Figure 2C shows the concentration of GABA:H₂O, and Figure 2D shows the more standard normalized concentration of GABA:tNAA acquired before and after deprivation while observers kept their eyes closed (this is considered to be a measure of resting GABA level). A significant decrease in concentration was found both for GABA:H₂O (paired-samples t test: t(18) = 2.57, p = 0.019) and for GABA:tNAA (paired-samples t test: t(18) = 2.9, p = 0.009) concentration (see Figure S3 for additional bootstrap statistics on two independent samples of subjects). The decrease in resting GABA concentration following monocular deprivation is also evident from inspection of the LCModel fits for the GABA spectra, examples of which are shown in Figures 2A and 2B. Resting GABA concentrations for all subjects are reported in Table S1. Although the primary hypothesis is a reduction of resting GABA, a non-significant decrease in GABA:H₂O and GABA:tNAA concentration was observed between pre- and post-deprivation measurements in the other viewing conditions (see Figure S4). That the effect of GABA reduction is more easily measurable during rest is to be expected since GABA is believed to play a role in many aspects of early visual processing [14]. The strength of these inhibitory interactions elicited by the stimuli may mask any effects of deprivation on GABA. No difference in spectral linewidth is observed across monocular deprivation, indicating no major blood-oxygenation-level-dependent (BOLD) effect on GABA quantification [15].

The significant decrease of both resting GABA:H₂O and resting GABA:tNAA is about 8% (one-sample t test H₀ X s₁, Bonferroni corrected α = 0.0125; GABA:H₂O: t(18) = 2.89, p = 0.01; GABA:tNAA: t(18) = 2.98, p = 0.008) (Figure 3, black bars). Furthermore, the decrease is specific for the V1 voxel, and it is not present for a control voxel positioned in PCC (Figure 3, etched bars; one-sample t test H₀ X s₁; GABA:H₂O: t(12) = 1.29, p = 0.22; GABA:tNAA: t(12) = 1.21, p = 0.25). The solid gray bars of Figure 3 show the GABA:H₂O and the GABA:tNAA ratios for the V1 voxel during the control experiment when there is no monocular deprivation. While there is a trend for increased GABA:H₂O in the later MRS session, the ratios do not differ significantly from one (one-sample t test H₀ X s₁; GABA:H₂O: t(6) = 1.65, p = 0.15; GABA:tNAA: t(6) = 1.7, p = 0.14). Furthermore, in each case, the GABA ratio in the main experiment is significantly lower than that measured from the PCC (independent-samples t test, GABA:H₂O: Bonferroni corrected α = 0.0167, t(30) = 2.78, p = 0.01; GABA:tNAA: Bonferroni corrected α = 0.0167, t(30) = 2.76, p = 0.01) and from V1 in the control experiment (GABA:H₂O: Bonferroni corrected α = 0.0167, t(24) = 3.31, p = 0.003; GABA:tNAA: Bonferroni corrected α = 0.0167, t(24) = 3.43, p = 0.002).

Taken together, these results indicate that monocular deprivation induces a change in resting GABA that is specific to V1 and...
does not depend on performing the behavioral task and the scanning procedure twice.

**Decrease in Resting GABA Concentration Strongly Correlates with Changes in Binocular Rivalry**

Having shown both a behavioral change using binocular rivalry dominance and a reduction in resting GABA concentration in visual cortex following monocular deprivation, we measured the relationship between these changes. For each subject, the ratio of deprived and non-deprived eye balance in phase duration observed before and after monocular deprivation (deprivation index; Equation 1 in Supplemental Experimental Procedures) was correlated with the ratio of resting GABA:H$_2$O (Figure 3C) and GABA:tNAA (Figure 3F) measured after and before deprivation. Changes in both GABA:H$_2$O and GABA:tNAA concentration correlated significantly with the change in perceptual predominance of the deprived eye during binocular rivalry (GABA:H$_2$O, Figure 3C; Spearman’s rank correlation coefficient $\rho = 0.78$, two-tailed exact permutation test $p < 0.001$, confidence intervals [CIs], Fisher’s Z transformed, CI = 0.38–0.93; GABA:tNAA, Figure 3F; $\rho = 0.62$, $p = 0.006$, CI = 0.23–0.84). These strong correlations indicate that the greater the behavioral plasticity effect, the greater the decrease of resting GABA, suggesting a link between the two measures as previously demonstrated in animals [6–10].

**GABA Concentration during Monocular Stimulation Correlates with Eye Dominance after Deprivation**

The individual effect of plasticity can be indirectly measured by the change in ocular dominance of binocular rivalry after deprivation. The previous results indicate that GABAergic inhibition is decreased at rest, suggesting the potential for increased neuronal responses during visual stimulation, predicted to be stronger in observers showing greater plasticity. In agreement with this prediction, we found that, after monocular deprivation, the concentration of both GABA:H$_2$O and GABA:tNAA measured during monocular stimulation (Figure 4) correlated with eye dominance (ratio between mean phase duration measured after monocular deprivation). When the non-deprived eye was stimulated, correlation of both GABA:H$_2$O (rho = –0.51, $p = 0.038$, CI = 0.039–0.795) and GABA:tNAA (rho = –0.56, $p = 0.022$, CI = 0.026–0.819) with rivalry was strong. Similar results were obtained when the deprived eye was stimulated (correlation of GABA:H$_2$O with rivalry: rho = –0.5, $p = 0.043$, CI = 0.026–0.79; correlation of GABA:tNAA with rivalry: rho = –0.53, $p = 0.035$, CI = 0.053–0.8).

**DISCUSSION**

By combining MRS with psychophysical measures of eye dominance, we have demonstrated the importance of GABAergic mechanisms for homeostatic plasticity in adult humans. Specifically, we report two important findings: first, resting GABA concentration decreases in visual cortex of adult humans after 150 min of monocular deprivation; second, and more importantly, there was a high correlation between a reduction in GABA concentration in the visual cortex and the perceptual boost of the deprived eye induced by monocular deprivation. This indicates a possible functional role of the neurochemical change in mediating the perceptual boost of the deprived eye.
Our result of a homeostatic boost of the deprived eye induced by a few hours of monocular deprivation is surprising, particularly given that the modulation occurs at such short timescales. In mice, only after several days of monocular deprivation during the critical period is there an increase in the spontaneous neuronal responses of a subset of cells devoted to the deprived eye [16]. This is a compensatory neural reaction that dynamically readjusts neuronal excitability in order to keep the average neural activity constant, known as homeostatic plasticity [17]. Interestingly, homeostatic plasticity, which involves changes in the balance between excitation and inhibition at the synaptic level [18], has never been observed in the intact adult visual cortex [19] or after short-term monocular deprivation. We therefore provide the first direct evidence in favor of a specific, important role of resting GABAergic inhibition in driving homeostatic plasticity in adult human visual cortex.

Figure 3. Decrease in Resting GABA Concentration Following Monocular Deprivation and Correlation with Change in Binocular Rivalry Eye Dominance
(A) Ratio of resting GABA:H₂O measured after and before monocular deprivation in visual cortex (black bar) and PCC (etched bar) and the ratio of resting GABA:H₂O measured in the second and first scan in the visual cortex for the control group of observers (gray bar). Error bars represent SEM.
(B) Same as (A), but for GABA:tNAA.
(C) Correlation of GABA:H₂O ratio measured after and before monocular deprivation in visual cortex with the change in ratio of dominance of the patched eye (deprivation index) (see Equation 1 in Supplemental Experimental Procedures).
(D) Same as (C), but for GABA:tNAA.
(E) Location of V1 voxel from which MRS data were acquired.
(F) Location of PCC voxel from which MRS data were acquired.

Intracortical balance between excitation and inhibition plays a critical role in mediating experience-dependent plasticity during development [10]. In particular, the maturation and activity of the GABAergic inhibitory interneurons parvalbumin (PV)-expressing basket cells regulates ocular dominance plasticity [20, 21]. In juvenile mice, 1 day of monocular deprivation induces a transient reduction of responsiveness in these PV cells [22]. Furthermore, studies manipulating the balance between intracortical excitation and inhibition, either by increasing excitation [7, 8] or decreasing inhibition [6], have suggested that similar mechanisms could act in the adult brain (reviewed in [10]). There is, however, no direct evidence for a reduction of inhibitory responses during visual plasticity in adult animals or direct evidence of ocular dominance plasticity after short-term visual deprivation, as observed here. Indirect evidence in support of a role for GABAergic inhibition in human visual cortex plasticity comes from administration of benzodiazepine, which potentiates GABAergic inhibition and has been shown to block plasticity induced by light deprivation, as measured by decreased transcranial magnetic stimulation phosphene thresholds [23].

In adults, neural plasticity has been consistently induced in structures such as the hippocampus [24] and the primary somatosensory cortex [25], and this type of plasticity appears to persist throughout life. Furthermore, changes in GABA concentration in adult human primary motor cortex have been shown following motor learning [26, 27], pointing to a pivotal role of intracortical inhibition in mediating motor cortical plasticity. The fact that we found a modulation of GABAergic balance in a cortical region that primarily comprises V1 is particularly...
important, as it indicates that the types of plasticity seen in other adult neural systems (e.g., long-term potentiation or long-term depression [28]) may also be present in the visual cortex. In recent years, several functional MRS studies at ultra-high field have demonstrated small, but significant, variations in the concentration of some brain metabolites in the activated human visual cortex during prolonged visual stimulation [29–31]. These studies, however, have not found a significant change in GABA concentration during visual stimulation [31, 32]. Here, we show that GABA measured in response to visual stimulation is a sensitive measure to probe plasticity. Ocular dominance after deprivation is a measure of plasticity, and it is interesting that it correlates with GABA concentration during stimulation of either the deprived or non-deprived eye. The most straightforward interpretation of this finding is that the reduction of resting GABA leads to a local increase in cortical excitability (resting GABA concentration has been previously shown to correlate with BOLD responsiveness [33, 34]). This finding is supported by the demonstration that GABA concentration measured during visual stimulation correlates negatively with the switching rate of three different forms of bistable perception (binocular rivalry, motion-induced blindness, and structure from motion [35]), simulating the effect of pharmacological stimulation of GABA receptors [35]. It is plausible that the reduction of resting GABA could also induce a reduction of interocular suppression during stimulation (multiplicative inhibition). This is consistent with animal studies showing that application of the GABA antagonist bicuculline abolishes interocular suppression [36, 37] and with the suggestion that the dynamics of interocular suppression determine binocular rivalry at a cortical level [38].

We previously found that, following 150 min of monocular deprivation, the perceptual advantage of the deprived eye observed during binocular rivalry was accompanied by a boost in apparent contrast [11], suggesting an involvement of contrast gain control mechanisms in mediating short-term homeostatic plasticity. The decreased GABA concentration that we found in V1 is consistent with our hypothesis of deprivation upregulating homeostatic contrast gain of the deprived eye. Evidence from animal studies suggests that contrast gain is GABA mediated in V1 [39–41]. Interestingly, contrast gain control mechanisms have been shown to modulate neuronal activity in humans (measured both by visual evoked potentials [40] and BOLD [42]) in a multiplicative way and to be involved in regulating both the dynamics of binocular rivalry [43] and eye dominance [44] during binocular combination (binocular combination also being altered after monocular deprivation in adult humans [45]). Furthermore, as monocular patching of the fellow eye is currently used as treatment for amblyopia in children, our results suggest that GABAergic inhibition could be involved in the plastic recovery of acuity in the amblyopic eye observed after occlusion therapy. Taken together, our results show a critical role for GABAergic inhibition in triggering visual plasticity, thus suggesting potential for medium-term intervention for disorders of binocular vision even beyond the critical period in humans.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, four figures, and one table and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2015.04.021.

AUTHOR CONTRIBUTIONS

C.L., M.C.M., and H.B. designed the research. U.E.E. and C.L. performed MRS and analyzed the data. M.C.M. and H.B. supervised the project. All authors wrote the paper.

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Supplemental Figures and Legends

Figure S1. Related to Figure 2.

An example spectrum and the individual metabolite peaks as fitted by the LCModel both for a full spectrum (A) and a spectrum chopped at 1.8 ppm (B). C: Mean (blue) ± standard deviation (shade) of summed spectra (TR = 8 s, TE = 30 ms, number of transients = 32) from the V1 voxel of all subjects and conditions (19 MD subjects x2 scans + 7 control subjects x2 scans). The spectra were normalized based on the unsuppressed water signal acquisition from the same voxel. The spectral range excluded from LCModel analysis (1.8-0.5) is highlighted as a gray box. tNAA, total N-acetylaspartate; tCho, total choline; tCr, total creatine; myo-Ins, myo-inositol, Asp, aspartate; Asc,
ascorbate; GSH, glutathione; Glu, glutamate; Gln, Glutamine; Lac, lactate. D: the correlation between GABA concentrations obtained from full and chopped spectra is highly significant, with GABA concentrations obtained from chopped spectra being proportionally higher. Data shown are for scans from the 26 subjects included in the main experiment (monocular deprivation and control group). 33/52 values are reported because of the poorer quality of GABA quantification using full spectra (GABA quantifications with CRLB >30% were discarded).

Figure S2. Related to Figure 2.

Matrix showing the correlation coefficients between all metabolite concentrations in V1, averaged across subjects and conditions (19 MD subjects x 2 scans + 7 control subjects x 2 scans). The values were determined from the LCModel fitting of semi-LASER spectra acquired at 7 T. Non-zero off-diagonal elements of the correlation coefficients matrix indicate the level of covariance between the fitted results for the two metabolites. Two metabolites with a high mean inverse correlation coefficient (correlation coefficient < −0.5) were reported as a sum. For example, since the mean correlation between Cr and PCr, and GPC and PCho were -0.75 and -0.85 respectively, these pairs were reported as the sum, total creatine (tCr) and total choline (tCho) whereas the mean correlation between GABA and the other metabolites was always greater than -0.3.
Figure S3. Related to Figure 2. Comparisons across two independent samples of subjects.

The 19 subjects were divided into two groups, subjects included in each group were selected according to the scanning time: group one included 10 subjects who were scanned within a 2-week period (Subjects S4-S15 in Table S3), whereas group two included the remaining subjects who were scanned at different periods (the first two and the last seven subjects scanned with 1 year gap). Scatter plot of the concentrations of resting GABA:H2O (A) and resting GABA:tNAA (B) measured before and after monocular deprivation for the individual subjects belonging to the two groups. The arrows represent average concentrations for group 1 (black symbols) and group 2 (gray symbols). In order to test for the effect of monocular deprivation on the two independent groups we performed a bootstrap with replacement (100,000 repetitions). The resulting distributions are plotted for the two groups (black line for group 1, gray line for group 2). We then tested the difference between pre- and post-deprivation on the resampled means using a sign-test (two tailed, α=0.05) and found that for both groups the decrease in GABA observed after deprivation was significant (GABA:H2O: group 1: p=0.03, group 2: p=0.006, GABA:tNAA: group 1: p=0.024, group 2: p=0.0008).
Figure S4. Average GABA concentrations measured before and after deprivation during different viewing conditions. Related to Figure 2.

Average GABA:H₂O (left panels) and GABA:tNAA (right panels) measured before (gray bars) and after (black bars) deprivation are reported for three different viewing conditions: non-deprived eye stimulated (A-B), deprived eye stimulated (C-D), both eyes stimulated (E-F). Error bars represent s.e.m. Within each condition pre- and post-deprivation concentrations were compared using paired-samples t-tests.
**Supplemental Tables**

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<td>S8</td>
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S9 The second scan was interrupted because of subject head movements.

| S10      | 50       | 8.917      | 2.61         | 17        | 14.64       | 1         | 45       | 8.917      | 2.33        | 20        | 14.79       | 1         |
| S11      | 41       | 8.917      | 2.80         | 16        | 13.65       | 1         | 39       | 8.917      | 2.92        | 18        | 14.66       | 2         |
| S12      | Excluded because of strong eye dominance |

S13 41 8.917 3.39 20 15.39 2 40 8.917 1.94 26 14.37 2
S14 54 8.917 2.12 22 13.53 1 48 8.917 2.16 17 12.63 1
S15 48 7.431 3.05 14 13.56 1 39 7.431 2.56 20 14.33 2
S16 33 8.917 0.54 96 12.57 2 38 8.917 1.839 27 13.53 2
S17 50 5.944 2.34 20 14.58 1 45 5.944 2.01 21 14.80 1
S18 47 8.917 2.94 15 15.39 1 45 8.917 3.12 15 15.89 1
S19 46 8.917 2.06 24 15.59 2 45 8.917 1.95 25 15.08 2
S20 53 8.917 2.68 15 13.16 1 58 10.403 2.64 14 13.61 1
S21 33 7.431 2.53 24 14.61 2 36 7.431 2.16 24 14.56 2

S22 The subject felt uncomfortable during the second scan.

S23 53 7.431 2.25 16 13.26 1 56 7.431 2.17 16 12.78 1
S24 54 8.917 2.44 15 13.44 1 51 8.917 2.18 18 13.63 1

Average ±1s.e.m 51.26 ±1.91 8.06 ±0.24 2.42 ±0.12 18.12 ±0.85 13.91 ±0.22 1.18 ±0.09 49.7 ±1.9 8.35 ±0.3 2.23 ±0.11 19.4 ±0.9 13.85 ±0.24 1.31 ±0.1

t-test p=0.14 p=0.1 *p=0.02 *p=0.04 p=0.99 p=0.08

Control group

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Average ±1s.e.m 50.86 ±1.93 8.70 ±0.39 2.28 ±0.184 18.29 ±1.7 13.83 ±0.27 1 49.7 ±2.7 8.70 ±0.21 2.67 ±0.26 15.9 ±1 13.79 ±0.41 1

T-test P=0.67 P=0.99 P=0.17 P=0.19 P=0.82

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Table S1 related to figure 2. LCMModel fit of the individual spectra.

SNR, signal to noise ratio of non-weighted spectra calculated by LCMModel; FWHM, spectral linewidth expressed as full-width at half maximum calculated by LCMModel; CRLB, Cramér-Rao lower bound. Subjects S16 and subject C2 were excluded because of poor quality of the GABA signal (CRLB >30). T-tests between
the baseline and the deprivation parameters. Only GABA concentration and the Cramer-Rao bands are significantly different in the two conditions. The latter result is expected since the reduction in GABA makes detection of the metabolite more challenging.

**Supplemental Experimental Procedure**

**Subjects**

Twenty-three observers (ten females, mean age 23.8±5 years), including two of the authors, participated in the main study involving monocular deprivation. With the exception of the two authors all subjects were naïve to the purpose of the study, and all had normal or corrected-to-normal vision. Eight observers (four males, mean age 26±6 years) also participated in a control condition which did not involve monocular deprivation. The data from two participants in the monocular deprivation group were discarded from the analyses because of discomfort in the scanner leading to excessive head movements during the scan (identified through significant reduction in water suppression efficiency). Two participants were excluded (one from the monocular deprivation and one from the control group) as the Cramér-Rao lower bound (CRLB) LCMModel analysis exceeded the criterion value of 30%. One observer from the monocular deprivation group was discarded because of strong eye dominance (7-fold difference in binocular rivalry dominance duration between the eyes). The naïve subjects were reimbursed for their time at a rate of £75 per day.

**Ethical Statement**

The experiment was approved by the Central University Research Ethics Committee (CUREC) of the University of Oxford and the ethical committee of the Scientific Institute Stella Maris. It was conducted in agreement with the principles of the Declaration of Helsinki.
Binocular Rivalry

Apparatus and Stimuli

The psychophysical experiment took place in a dark and quiet room. Visual stimuli were created in MATLAB running on a laptop (Asus) using PsychToolbox [S1], and displayed on a 15-inch monitor (BenQ). Observers viewed the visual stimuli presented on the monitor at a distance of 57 cm through anaglyph red-blue goggles (right lens blue, left lens red). Responses were recorded with the computer keyboard. Visual stimuli were two oblique orthogonal red and blue gratings (orientation: ±45°, size: 3°, SF 2 cpd, contrast 50%), surrounded by a white smoothed circle, presented on a black uniform background in central vision. Peak luminance of the red grating was reduced to match the peak luminance of the blue one using photometric measures.

Task and Procedure

Each observer participated in four experimental sessions of 150 seconds each. After an acoustic signal (beep) the visual stimuli appeared on the screen. Observers were instructed to report their visual percept by continuous alternate key-press (arrow keys). They were trained to hold the appropriate key when the red or the blue grating was fully dominant and a third key when they perceived a mixture of the two visual stimuli. Two experimental sessions (baseline measurements) recorded before the first MRI scan and two sessions (deprivation measurements) recorded before the second MRI scan. Only the second session of the baseline measurements and the first session of the deprivation measurements were considered for analyses as the first session of the baseline measurements served as training on the psychophysical task and the first session of the deprivation measurements captured the maximum effect of monocular deprivation. The orientation associated to the colors and eyes was changed at each experimental session and counterbalanced between subjects. The two scans were separated by a 150 minute interval during which observers were free to perform their normal activities; one group of observers wore a translucent eye-patch over the
dominant eye during this period (monocular deprivation condition). The eye-patch was made of a translucent plastic material that allowed light to reach the retina (attenuation 15%), but no pattern information, as assessed by the Fourier transform of a natural world image seen through the eye-patch. Ocular dominance was assessed for each observer using the Porta’s test and for all but one observer (figure 1B) the designated dominant eye also slightly prevailed during binocular rivalry in baseline measurements.

*Analyses*

The perceptual reports recorded through the computer keyboard were analyzed using Matlab, and the resulting phase durations were compared with a paired-sample t-test. Correlations between metabolite concentrations and behavioral measurements (binocular rivalry phase durations) were computed with Spearman’s Rank correlation index, the p-value for which was computed using exact permutation distributions in Matlab.

A deprivation index summarizing in one number the change in the ratio between deprived and non-deprived mean phase duration following MD compared to baseline measurements was computed as follows:

\[
\text{deprivation index} = \left( \frac{\text{base}\text{MPD}_{\text{dep}_\text{eye}}}{\text{dep}\text{MPD}_{\text{dep}_\text{eye}}} \right) \times \left( \frac{\text{dep}\text{MPD}_{\text{non}_\text{dep}_\text{eye}}}{\text{base}\text{MPD}_{\text{non}_\text{dep}_\text{eye}}} \right)
\]

Eq.1

In Eq. 1, *MPD* represents Mean Phase Duration computed in seconds, *base* stands for baseline measurements, *dep* for measurements acquired after monocular deprivation. A deprivation index value equal to 0 represents no change in the ratio between dominant and non-dominant eye mean phase duration, while a value >1 represents a decrease in dominant-eye predominance and a value <1 an increase in dominant eye predominance during binocular rivalry.
Magnetic Resonance Spectroscopy

Experimental Procedure

Each subject participated in two 1h scans separated by an interval of 150 minutes. When entering the scanner observers wore a translucent eye-patch over the dominant eye; in the baseline scan observers were required to keep their eyes closed during the scan setup prior to data acquisition, while in the deprivation scan observers were asked to keep their eyes open while wearing the eye-patch on the deprived eye during this period in order to top up the monocular deprivation (total monocular deprivation time: 150 minutes + 15 minutes top up). MR experiments were performed using a 7T whole body MR system (Siemens, Erlangen) with a Nova Medical 32-channel receive array head-coil. Images acquired with a 1-mm isotropic resolution MPRAGE sequence (repetition time TR = 2.3 s, inversion time TI = 1.05 s, echo time TE = 2.8 ms, 192 partition-encode steps, 256 phase-encode steps, 256 data points in the read direction, nominal flip angle = 7°, total acquisition time = 3 min) were used for the selection of VOIs. First- and second-order shims were adjusted using FASTMAP (fast, automatic shimming technique by mapping along projections) with echo-planar imaging (EPI) readout [S2]. Spectra were measured with semi-localization by adiabatic selective refocusing (semi-LASER) pulse sequence (TE = 30 ms, TR = 8 s) with VAPOR water suppression and outer volume suppression [S3]. Briefly, the sequence consisted of a 4.2 ms asymmetric slice-selective 90° pulse (bandwidth 3.7kHz) [S4] followed by two pairs of slice selective adiabatic full passage (AFP) pulses (4.5 ms duration, HS4 modulation, R25, bandwidth 5.27 kHz) [S5]. After the initial 20-minute scan (adjustments, voxel location, water suppression) MRS data (number of transients NT = 32, number of dummy scans = 2, spectral bandwidth = 6 kHz, data point = 2048) were acquired in four different viewing conditions of about 5 minutes each. In the eyes closed condition observers were asked to close their eyes; in the non-deprived eye stimulated condition observers were asked to open their eyes while the eye-patch was over the dominant eye; in the deprived eye stimulated condition observers were asked to swap the
eyepatch to the non-dominant eye and then look at the screen with both eyes open and in the eyes
open condition observers were asked to remove the eyepatch and look at the screen with both eyes. Conditions were always presented in the above order. Before entering the scanner observers were trained to change the position of the eye-patch gently to minimize head-movements. After the eyes open condition observers were asked to close their eyes and a final 20 minute scan started in which the voxel in the Posterior Cingulate Cortex was located and MRS acquired from that region (NT = 32).

Visual Stimulation

The visual stimuli presented in the scanner were generated with the software Presentation, and consisted of four luminance-modulated checkerboards (each of size 9.6° x 7.2°, SF 0.4 cpd, Contrast 50%), one in each quadrant. The checkerboards were presented on a mid-gray background with a central white fixation spot. During each 5-minute MRS acquisition all the visual stimuli were present on the screen and every 8 seconds, with a pseudo-random order, one of the four checkerboards flickered at 10 Hz.

Analyses

Two non-suppressed water spectra were acquired: one for eddy current correction and reconstruction of the phased array spectra (the RF pulses of the VAPOR scheme were turned off, NT = 2, TR = 8 s, TE = 30 ms, number of dummy scans = 2, spectral bandwidth = 6 kHz, data point = 2048) and one for use as reference for metabolite quantification (VAPOR and OVS schemes turned off in order to eliminate magnetization transfer effects, NT = 2, TR = 8 s, TE = 30 ms, number of dummy scans = 2, spectral bandwidth = 6 kHz, data point = 2048). The reconstruction of the phased array spectra included weighting the spectra based on the sensitivity of each receive element at the VOI and correcting for the different constant phase shift terms of the complex spectra.
prior to the summation. Single scan spectra summed from 32 channels were corrected for frequency and phase variations induced by subject motion and then summed before LCModel analyses.

Metabolites were quantified using LCModel[S6]. The model spectra of aspartate (Asp), ascorbate/vitamin C (Asc), glycerophosphocholine (GPC), phosphocholine (PC), creatine (Cr), phosphocreatine (PCr), γ-amino-butyric acid (GABA), glucose (Glc), glutamine (Gln), Glutamate (Glu), glutathione (GSH), myo-inositol (myo-Ins), N-acetylaspartate (NAA), N-acetylaspartylglutamate (NAAG), phosphoethanolamine (PE), scyllo-inositol (scyllo-Ins) and taurine (Tau) were generated based on previously reported chemical shifts and coupling constants [S7, 8] by using GAMMA/PyGAMMA simulation library of VESPA for carrying out the density matrix formalism [SVersatile Simulation, Pulses and Analysis 9]. Simulations were performed with the same RF pulses and sequence timings as that on the 7T system in use.

A macromolecule spectrum acquired from the occipital cortex, using an inversion recovery sequence (TR = 3 s, TE = 30 ms, inversion time TI = 0.685 s), was included in the model spectra. Hankel singular value decomposition was used to remove the residual signal of the methylene of tCr at 3.93 ppm and the high-frequency noise was suppressed using a Gaussian filter (σ=0.05 seconds) before including the macromolecule spectrum into the LCModel basis set. LCModel analysis was performed on all spectra within the chemical shift range 1.8 to 4.2 ppm to avoid signal contamination (Figure S1), due to extraneous lipid artifact, observed in the spectral region 1.75–1.5 ppm as suggested by the LCModel Manual [S10]. Metabolite concentrations were obtained relative to an unsuppressed water spectrum acquired from the same VOI assuming a water content of 82% for occipital cortex and posterior cingulate, which primarily contain gray matter [S11]. The MPRAGE images were segmented using FAST (FMRI’s automated segmentation tool, part of the FSL toolbox) to determine CSF fraction (fCSF) in the voxels [S12]. Concentrations were then corrected for CSF fraction with the following formula: [Mcorr]=\([M] \times (1/\text{fCSF})\), where [Mcorr]=Corrected concentration and [M]=metabolite concentration from LCModel output.
It is important to ensure changes in GABA concentration are not an artifact of differences in spectral quality in the two sessions, so values for signal to noise ratio (SNR), full-width at half maximum (FWHM, the estimate of the linewidth) and CRLB of LCModel analysis were all compared for the two sessions (Supplemental Table S3). Neither SNR nor FWHM differed between the two conditions, but CRLB for baseline GABA was 18.12±0.85, and increased to 19.4±0.90 following deprivation. Although this value was significantly higher (paired t-test, t(18)=2.2 , p=0.041; no correction for multiple comparisons), the similar SNR and FWHM in the two conditions suggests higher CRLBs observed likely resulted from the reduction in the GABA signal in the voxel, which can lead to inappropriate spectral rejection [S13, 14]. A further analysis was performed to determine the extent to which the GABA concentration correlated with linewidth extracted from LCModel. Across all V1 data (deprivation and control experiments) there was a significant correlation between GABA and linewidth (r  = 0.34; p = 0.012). It is the case that a strong and significant negative correlation between GABA and linewidth could have explained the observed GABA decrease after deprivation; however, this was not observed in the current experiments.

Metabolites quantified with CRLB (estimated error of the metabolite quantification) > 30% were classified as not detected. The triangular table of correlation coefficients from the detailed LCModel output was used to evaluate the correlation between fitted concentrations of metabolites with high spectral overlap, which are expected to be inversely correlated [S15]. Thus, if the inverse correlation between two metabolites was consistently high (correlation coefficient < −0.5) in a given region, their sum was reported, e.g. total creatine (tCr), total choline (tCho), Glu+Gln and Glc+Tau (Figure S2).

The GABA concentrations are extracted as raw concentrations measured in umol/g, but throughout the paper the more standard measure of GABA normalised to tNAA is also provided (GABA:tNAA) for comparison to other studies.
Supplemental References


