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Neurochemical Changes Within Human Early Blind Occipital Cortex

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Abstract

Early blindness results in occipital cortex neurons responding to a wide range of auditory and tactile stimuli. These changes in tuning properties are accompanied by an extensive reorganization of occipital cortex that includes alterations in anatomical structure, neurochemical and metabolic pathways. Although it has been established in animal models that neurochemical pathways are heavily affected by early visual deprivation, the effects of blindness on these pathways in humans is still not well characterized. Here, using ^{1}H magnetic resonance spectroscopy in nine early blind and normally sighted subjects, we find that early blindness is associated with higher levels of creatine, choline and *myo*-Inositol and indications of lower levels of GABA within occipital cortex. These results suggest that the cross-modal responses associated with early blindness may, at least in part, be driven by changes within occipital biochemical pathways.

Introduction

Since the 1960's early blindness from peripheral causes has been a useful model system for understanding developmental plasticity (Wiesel and Hubel, 1965a, b). In animal models, early blindness is associated with dramatic changes in the functional responses of occipital neurons (Movshon and Van Sluyters, 1981, Fregnac and Imbert, 1984, Benevento et al., 1992) that includes enhanced responses to auditory and tactile stimuli – known as *`cross-modal plasticity'* -within visual cortex (Rauschecker et al., 1992, Kahn and Krubitzer, 2002). Similar enhancements of cross-modal responses (for reviews see Noppeney, 2007, Collignon et al., 2008) and higher levels of functional connectivity with auditory (Klinge et al., 2010), tactile (Fujii et al., 2009) and lateral prefrontal (Bedny et al., 2010) cortex have also been found within human occipital cortex within individuals who become blind early in life.

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However it is remains unclear how the `cross-modal' changes in functional responsiveness that occur in early blind individuals are mediated. One hypothesis is that these responses are due to stronger and/or novel white matter connections to other areas, perhaps due to a lack of pruning (Bourgeois et al., 1989). However, while white matter hypertrophy has been found within prefrontal areas (Lepore et al., 2010) within early blind individuals, white matter connections between V1 and other pre-cortical and cortical areas (including prefrontal cortex) have so far been shown to be unaffected or attenuated by blindness (Shimony et al., 2006, Bridge et al., 2009, Shu et al., 2009, Lepore et al., 2010).

Another, non-exclusive, possibility is that the changes in functional response that have been found in early blind individuals are driven by changes within inhibitory, excitatory and neuromodulatory biochemical pathways within occipital cortex itself. As detailed in the *Discussion*, inhibitory GABAergic, excitatory glutamatergic, and neuromodulatory cholinergic biochemical pathways affect short-term tuning and responses in heterogeneous ways, as well as influencing both developmental and adult plasticity (for review see Hensch, 2005, Bavelier et al., 2010). Within animal models it has been established that these pathways are heavily affected by early visual deprivation. However, little is known about changes in biochemical pathways as a result of early blindness in humans. Here, we used ^{1}H MRS to examine the effects of early blindness on occipital metabolite concentrations. MRS concentrations of choline (a peak containing multiple choline containing compounds), creatine (a peak containing both creatine and phosphocreatine), *myo*-Inositol, N-acetyl aspartate (NAA), GABA and glutamate were compared across nine early blind and nine individually gender and age-matched sighted control subjects.

Experimental Procedures

Participants

Data shown here are from nine early blind (EB; mean age, 52.8, range, 40–69) and nine individually gender and age-matched sighted control (SC; mean age 52.3, range, 44–61) subjects. We scanned 10 blind and 10 sighted subjects. One blind individual (and their matched sighted control) was excluded from main analyses due to significant head motion in all scans (data was used from the sighted control for test-retest validation measures). EB was defined as an age of blindness onset of less than 1 year and no memory of vision. All EB individuals were blind due to peripheral rather than cortical damage (see Table 1), and were excluded if they had light perception that included form, color or object perception. All procedures were in agreement with the Institutional Review board regulations of the University of Washington.

Magnetic Resonance Spectroscopy

Data were acquired using the Philips Achieva 3T scanner (version 2.5.3) and an 8-channel SENSE Philips head coil. Spectra were acquired from each of two voxels placed along the left and right medial occipital wall, centered on the calcarine sulcus, and one control voxel placed in the frontal lobe, anterior to the central sulcus, Fig. 1A. Because cross-modal plasticity within early blind individuals is found across wide regions of occipital cortex e.g. (Sadato et al., 1998, Burton et al., 2002, Burton, 2003) large voxel sizes were used to

maximize signal to noise. The occipital voxel was positioned to cover much of V1 and V2 (primary visual areas) and may have included parts of additional visual areas (V3–V4) in some subjects (Van Essen et al., 2005). In visually normal subjects these areas are primarily visual and demonstrate novel responses to a variety of auditory and tactile tasks in early blind individuals (Lewis and Fine, 2011). The precentral gyrus voxel was primarily centered on motor areas, but likely included motor planning, supplementary motor and somatosensory areas in some subjects (Van Essen et al., 2005).

A short echo PRESS sequence (TR = 2000 ms; TE = 32 ms, FID points = 2048; spectral width = 2000Hz; voxel size = 3×2×3 cm; number of averages = 64) allowed for the absolute quantification of the hydrogen spectrum including the resonances associated with N-acetyl Asparatate, choline, creatine and *myo*-Inositol, Fig. 1B.

A long echo J-Coupled MegaPress sequence (TR = 1500 ms; TE = 87 ms, FID points = 2048; spectral width = 2000Hz; voxel size = 3×2×3 cm; number of averages = 200) was used to isolate GABA and glutamate (Mescher et al., 1998, Mullins et al., 2012). The MegaPress acquisition consists of two parts: *edit-on* where GABA is refocused using an editing pulse of 15ms duration at 1.9 ppm, and *edit-off* where the editing pulse is moved on the other side of water. Glutamine dephasing during the edit-off part of the acquisition allows for the isolation of glutamate, Fig. 1C (Schubert et al., 2004). Edit-on and edit-off measurements were conducted in blocks of 200 averages, one after the other.

SC participants were instructed to keep their eyes closed during the scanning sessions. Although this was not specifically monitored, it is unlikely that eye opening during scanning would measurably alter concentration values (Mangia et al., 2006, Mangia et al., 2007).

Analyses

For most metabolites (with the exception of GABA) basis sets, spectra fits and absolute quantification were conducted offline using LCModel software, which analyzes an in vivo spectrum as a linear combination of in vitro spectra estimated from individual metabolite solutions (Provencher, 1993, 2000). Using LCmodel, the residual water signal was subtracted, free-induction decays (FIDs) field inhomogeneities and eddy currents were accounted for and free-induction delays were zero- and first-order phase corrected. Absolute concentrations for each metabolite were obtained by scaling the in vivo spectrum to the unsuppressed water peak and are reported in units that approximate millimolar (mM).

FSL tissue segmentation software (FEAT; http://www.fmrib.ox.ac.uk/fsl/feat5/detail.html) was used to classify the contents of each MRS voxel (left and right occipital and frontal lobe control) into three tissue types: grey matter (GM), white matter (WM) and cerebral spinal fluid (CSF) using whole-brain intensity estimates. All metabolite values were normalized to = 100% brain tissue using C=Co × (1/(1-FCSF)), where C=concentration, Co=metabolite concentration, and FCSF=estimated fraction CSF in order to correct for CSF partial volume effects. Voxel reconstructions were made for each individual metabolite to correct for individual chemical shifts.

In the case of GABA, where differences in the acquired spectra provided an edited spectrum, basis sets were generated using a calibrated phantom with known concentrations. Custom software was used to realign (in frequency space, referenced to the choline peak) the long echo pre-subtraction spectra and to correct GABA concentration estimates to take account of residual signals from macromolecules with overlapping spectra. Remaining analyses were carried out using LCModel.

Measures of fit quality

A variety of measures were used to determine spectra quality, including phantom measurements, model fit values and test-retest reliability.

Validation of GABA measurements using an echo time of 87 ms

The 87 ms TE that we used to measure GABA concentrations is longer than the echo time of 68ms that is more commonly used in the literature (Rothman et al., 1993). Recent in vivo and phantom studies (Edden et al., 2012a, Puts et al., 2012) suggest that modulation of the GABA signal drops off fairly slowly as a function of echo time, so an echo time of 87ms does not result in a dramatic drop of signal. It has also been shown that short editing pulses are relatively unselective at 3T, thereby causing non-trivial suppression of the GABA signal. Using a longer TE allowed us to use a longer (more selective) editing pulse, at a relatively small cost in signal to noise within the GABA signal itself (Edden et al., 2012b).

While use of a long TE and longer echo times for measuring GABA has been previously described (Edden et al., 2012b), the use of longer TE/longer editing pulses is still relatively novel and our particular choice of TE and editing pulses (constrained by our scanner) had not yet been validated. We therefore measured our ability to estimate GABA concentrations using an echo time of 87ms in the presence of other metabolites and macromolecules using three brain tissue mimicking head phantoms containing: (1) metabolites NAA, creatine, choline, lactate, glutamate and 5mM GABA in an aqueous solution, (2) these metabolites within a mixture of Difco Bacto agar (Difco Laboratories, Detroit MI) the concentration of which influences the water *T*2 value, and animal-hide gelatin (Sigma Aldrich: Gelatin from Bovine Skin, 225-bloom) which contributes a protein background spectrum that creates an environment more similar to brain tissue than agar alone, and (3) a phantom identical to 2 but only containing 1mM GABA. These methods were based on (Rice et al., 1998) with the difference that instead of using formaldehyde to provide thermal stability we used a water bath. (There was a possibility that formaldehyde, by cross-linking the proteins, would increase the molecular weight of the macromolecules, which might result in less interference with the GABA signal.) These phantoms demonstrated that the GABA signal at 3.0 ppm was modulated by the editing pulses, and was detectable both with and without macromolecules. However these phantoms did reveal some contribution of neighboring macromolecules with spectra that overlapped the GABA signal.

We estimated and compensated for neighboring macromolecules by comparing spectra from two acquisition sequences. The first was the sequence we used to measure GABA, as described above. The second had a TE of 87ms but used a pulse editing sequence of 1.9ppm and 1.5ppm. Subtraction of these spectra produces a difference spectrum that isolates GABA

by minimizing the modulation of macromolecules at 1.7 ppm. Comparison of these two difference spectra provided an estimate of the factor by which the macromolecule signal inflated the GABA signal (.0302) in our original measurements. Correction for each individual subject was then carried out by multiplying their individual concentration of MM17 (the LCmodel defined proportion of the fit that includes macromolecules with peak resonances at 1.7ppm) by this factor and subtracting the resulting estimate of the macromolecule inflation of the GABA signal for that individual subject from his/her uncorrected GABA signal.

Our GABA concentration estimates, made using a TE of 87ms, are somewhat smaller than those of previous studies carried out at 3T using a 68ms TE. For example Yoon et al. (Yoon et al., 2010) reports mean GABA/Creatine ratios of 0.105 and Near et al. (Near et al., 2011) reports mean ratios of 0.093 for occipital cortex in visually normal control subjects, while our GABA/creatine ratio, using an appropriate correction factor (0.85) to take account for our longer TE (Edden et al., 2012a), was 0.087.

Measures of fit

In the case of GABA, custom software was used for realignment of the long echo pre-subtraction spectra and model fitting of the spectroscopic signal. Consequently Cramer-Rao bounds were not available for these spectra and quality was assessed using S/N (the height of the GABA signal compared to the estimated spectral noise amplitude, calculated as the standard deviation of a noise region of the spectrum) and FWHM (full width at half maximum of the GABA spectroscopic signal, which gives a measure of the spectroscopic signal quality based on the magnetic field homogeneity). The mean S/N was 10.7 and the mean FWHM was 0.14.

For the remainder of the metabolites that we measured, an initial quality control was carried out by viewing spectra using in house software. Spectra with very high lipid concentrations (implying inaccurate voxel placement) or extremely broad line widths (implying head motion) were discarded. After analysis, we used three parameters of fit quality from LCModel software: percent standard deviation (Cramer-Rao bound from the LCmodel least squares analysis), full-width at half maximum and the signal to noise ratio (S/N). We used all three outputs as measures of spectra quality (Kanowski et al., 2004). Spectra were considered to be of adequate quality if they had an unsuppressed NAA+ Cramer-Rao bound of less than 20, FWHM of less than 0.05ppm, and S/N estimates greater than 15. Almost all spectra passed both these quality control checks, and those spectra that did not were re-collected in a later scanning session. For the short-echo data that passed quality control measures the Cramer-Rao bound of the NAA+ peak was never above 3% and had a mean of 2.18%.

Test-retest reliability

In a small number of scans (2 blind and 2 sighted subjects: one of these subjects was the 10th sighted subject, excluded from main analyses due to significant head motion in his associated blind subject) signal to noise was low or there were excessive subject head movements during the collection of the control voxel. We brought these subjects back for a

second scanning session in which we recollected additional data for the left occipital voxel so as to quantify re-test reliability for metabolite concentration and tissue volume estimates (Fayed et al., 2009, Gasparovic et al., 2011). Repeated estimates were calculated using $\%\mathrm{diff}=200\times {}^{\mathrm{abs}(m1-m2)}/_{(m1+m2)}$, where *m1* and *m2* are measurements taken on the same subjects in separate sessions on different days Test-retest variation was likely to be due to any/all of the following: differences in voxel alignment (voxels were realigned across scanning session by eye, so voxel location differed slightly across sessions), neural and scanner noise, fluctuations in metabolite concentrations and susceptibility of the fitting procedure to these various noise sources. Metabolites measured using the short echo PRESS sequence showed reasonable re-test reliability: N-acetyl aspartate= 4.9%, creatine = 8.1%, choline = 6.6%, and *myo*-Inositol = 12.9%. Measurements based on the long echo J-Coupled MegaPRESS sequence were less reliable: GABA = 46.2%, glutamate = 42.4%. One reason for this is that GABA and glutamate have low concentrations with associated low signal-to-noise. Moreover, GABA requires subtraction from MEGAPRESS frequency selective on/off pulses.

No differences in fit quality between early blind and sighted subjects

Of the 18 subjects used in the main data set only 3 (2 blind, 1 sighted) out of 108 acquisitions (18 subjects × 3 locations × 2 scan protocols) failed to meet the fit criteria described above, generally due to head motion. These data were recollected for that particular sequence and voxel in a later scanning session.

We explicitly tested for differences in fit quality between early blind and sighted subjects. No differences in S/N or FWHM were found for GABA within any voxel or when data were combined across all voxels treating each voxel as an independent measure (two-sample t-test). Examining the Cramer-Rao bound for short echo metabolites, there was no main effect of group [$F(1,240) = 1.79$, $p=0.18827$]. There was a significant interaction between group and chemical [$F(4,240) = 7.25$, $p<0.001$]: post-hoc tests based on Tukey's honestly significant difference criterion found that *myo-Inositol* fits were significantly worse for sighted subjects, likely driven by sighted subjects having significantly lower concentration values for that metabolite. No other group effects were significant.

Results

Because there were no significant differences between left and right hemisphere occipital voxels for either subject group, occipital data were combined across hemispheres (treating each hemisphere as an independent measurement).

Figure 2 shows group median tissue concentrations for sighted control (light gray bars) and early blind subjects (dark gray bars). Because we had repeated measures (Hurlbert, 1984), a nonparametric three way ANOVA (Wobbrock et al., 2011) rather than a Chi-Square test was used to examine whether there were significant differences in tissue type across subject groups. We found a significant main effect of tissue type [$F(2,150) = 249.13$, $p<0.001$], but no significant main effect of group [$F(1,150) = 0.01$, $p=0.9243$] or voxel location [$F(1, 150) = 02$, $p = 0.8791$]. Nor did we see significant interactions between group × location

[F(1,150) = 0.01, p=0.9084], group × tissue type [F(2,150) = 1.91, p=0.1518], location × tissue type [F(2,150) = 0.03, p<0.9745] or group × location × tissue type [F(2,150) = 0.54, p=0.584]. Thus, there was no statistical indication of differences in the proportion of gray, white and CSF tissue types within either the MRS occipital or the precentral voxels between sighted and blind subject groups.

Metabolite concentrations are shown in Figure 3 and Table 2. Across all quantified metabolites, a three way ANOVA found a significant main effect of group [F(1,293) = 25.01, p<0.001] and metabolite [F(5, 293) = 2146.91, p < 0.001] with significant interactions between metabolite × voxel location [F(5,293) = 2.57, p<0.05] and metabolite × subject group, [F(5,293) = 7.54, p<0.001]. A non-parametric ANOVA (Wobbrock et al., 2011) produced similar results, though with an additional significant main effect for location.

Traditional paired Student t-test comparisons were then used to examine group differences for each metabolite for occipital and precentral cortex Using uncorrected paired t-tests, early blind subjects showed significantly higher concentrations of choline (p<.01), creatine (p<0.01), *myo*-Inositol (p<0.001) and significantly lower concentrations of GABA (p<0.05) within occipital cortex. Statistical results *after* Bonferroni-Holm correction for multiple comparisons are shown in Table 2: The result for GABA failed to pass multiple correction comparison. Very similar results were found using Wilcoxon signed rank parametric statistics. No significant group differences were found within the precentral gyrus control voxel for any metabolite.

Discussion

Because early blind are somewhat difficult to recruit, this study relies on a relatively small number of subjects, and consequently lacks power. However it should be noted that a significant result based on a small sample is no more susceptible to false positives than a study based on a larger sample. Indeed, finding significant effects with a small sample requires a treatment effect that is larger than would be required to reach the same level of significance with a large sample. Our finding of higher levels of creatine, choline and *myo*-Inositol, and possibly lower levels of GABA in a relatively under-powered study suggest that the effects of blindness on biochemical pathways are relatively large (Friston, 2012).

No difference in white/gray/CSF ratios within our voxel

We saw no significant difference in the proportion of gray, white and CSF tissue between sighted and blind subject groups within either occipital or precentral cortex. This finding means that group differences in metabolites described below are unlikely to be due to macroscopic differences in tissue composition across groups.

Previous studies have shown a smaller volume and greater thickness of gray matter in V1 in early blind subjects (Jiang et al., 2009). However, these anatomical differences are relatively subtle and are localized to V1, which likely explains why we did not see significant differences in the relative ratios of white/gray/CSF within our large fixed-size voxel (which likely included V2 as well as V1, as well as portions of V3–V4).

Higher concentrations of choline

Our finding of higher levels of choline differs from the results found in an animal MRS model of low vision (due to glaucoma) that found a lower choline/creatine ratio (Chan et al., 2009) since we found higher levels for both metabolites that resulted in no significant change in ratio across blind and sighted subjects. This discrepancy may reflect improved measurement sensitivity in our study, differences between early human and adult animal models of visual loss, and/or degeneration specific to a glaucoma model.

In the case of choline, there are multiple contributors to the choline peak of which phospholipids are the main component (Kirkwood et al., 1999, Boulanger et al., 2000). In disease states higher levels of choline is generally interpreted as being a marker for membrane breakdown and/or an increase in phospholipid membrane precursors (Gujar et al., 2005). However, such membrane turnover in adulthood is generally due to pathology (Sabatier et al., 1999, Cordoba et al., 2002). We are not aware of any papers examining membrane turnover or synthesis in animal models of blindness.

Elevated levels of creatine

We see significantly higher levels of creatine in early blind individuals. The ^{1}H MRS creatine peak contains both creatine and phosphocreatine, metabolites intimately involved with energy usage within both neurons and glia: by acting as an ATP buffer, the reversible phosphorylation of creatine allows for the relatively continuous access to ATP needed for moment-to-moment energy requirements (Gujar et al., 2005). Previous PET studies have demonstrated that while early blind subjects exhibit roughly similar oxygen-to-glucose metabolic ratios as sighted subjects (De Volder et al., 1997), primary and extrastriate visual cortical areas exhibit greater levels of glucose metabolism and regional cerebral blood flow than sighted subjects during (silent) rest as well as when performing an auditory or tactile task (Wanet-Defalque et al., 1988, Veraart et al., 1990, Uhl et al., 1993, De Volder et al., 1997). These previous PET results might have been due to either ongoing differences in neural activity or long-term changes in metabolism. In contrast, our results are unlikely to be due to higher resting firing states or responses to acoustic scanner noise: intensive visual stimulation in the normally sighted barely alters the concentrations (Mangia et al., 2006) and has no effect on the creatine/NAA ratio (Mangia et al., 2007). Thus, as described below, our results are likely to reflect chronic metabolic adaptations.

Higher concentrations of myo-Inositol

Our findings of higher concentrations of *myo*-Inositol are consistent with those of Bernabeu et al. who found higher levels of *myo*-Inositol in late blind subjects with no or limited light perception (Bernabeu et al., 2009). *Myo*-Inositol (the most common biological stereoisomer of inositol) is synthesized mainly within astrocytes, and heightened concentrations are generally interpreted as indicating increased glial number or size (Pellerin, 2005, Soares and Law, 2009).

No significant differences in N-acetyl aspartate concentrations

We find no significant differences between blind and sighted subjects for N-acetyl aspartate. It has been suggested that blind individuals have thicker gray matter within V1 (Jiang, 2009 #5589, though see Rakic et al., 1991, Dehay et al., 1996). However it is not clear that NAA would be sensitive to such a difference; lower N-acetyl aspartate concentrations is generally considered as an indicator of neuronal loss or dysfunction (Rossner et al., 1994, Gujar et al., 2005), however its decrease is mostly observed at the moment when the disease/cell loss is in progression. Results across all metabolites did not differ qualitatively, or in statistical significance when referenced to NAA as compared to the unsuppressed water signal, as might be the case if differences between blind and sighted individuals were driven by differences in cell numbers.

Marginally significant lower concentrations of GABA

Two previous studies (containing 5 & 6 blind subjects respectively) failed to find reductions in $GABA_A$ receptors in humans using PET (Sanabria-Bohorquez et al., 2001, Mishina et al., 2003). In contrast, we found indications of lower concentrations of GABA in our early blind subjects. The poor reliability of our GABA measurements, and the fact that our findings did not pass post-hoc Bonferroni-Holm correction means that this result remains provisional. However, it should be noted that the effect of high variance in a sample is to decrease power (increase the likelihood of false-negatives) rather than to increase the likelihood of false-positives because outlier values tend increase to sample error variance more than the sample mean (Zimmerman, 1994).

One possible explanation for this discrepancy is that these previous PET studies compared responses across smaller regions of interest, and some subjects reported being deprived of form vision during early childhood rather than infancy. A second difference is that we measured a marker for the presence of the GABA molecule, whereas previous PET studies measured the synaptic density of benzodiazepine receptors.

Our finding suggesting potentially lower levels of GABA is also consistent with a large animal literature (including primate) which shows a down-regulation of the GABAergic network consequent on bilateral and unilateral deprivation that include effects on $GABA_A$, $GABA_B$ and receptor distribution (Hendry and Jones, 1986, Fosse et al., 1989, Benevento et al., 1995, Tigges et al., 1997, Morales et al., 2002, Jones, 1994 #5126, for a review see Desgent and Ptito, 2012).

No significant differences in glutamate concentrations

The most likely reason for our failure to find significant changes in glutamate was high variability in our measurements, as estimated by test-retest reliability. Animal models suggest that the organization of glutamatergic pathways is strongly affected by visual deprivation (Bear and Singer, 1986, McCoy et al., 2009), with effects of deprivation differing widely across receptor type. In some receptors (e.g. the NR2A subunit of the NMDA receptor) visual deprivation results in a decrease in expression, while other receptors (e.g. NR2B and AMPA) show increased expression (Gu, 2002). However, it is worth noting that a small study in dark-reared kittens show similar glutamate concentrations as normally

reared animals (as measured using uptake of D-aspartate, Fosse et al., 1989), suggesting that while individual receptor types are heavily affected by deprivation the effect of blindness on overall glutamate levels may be less dramatic.

Potential interpretations

These results suggest fundamental changes to occipital biochemical pathways as a result of blindness. Our finding of differences across markers associated with a variety of pathways make a number of non-exclusive interpretations possible, and suggest a number of interesting avenues for future research.

Increased glial cell numbers

Higher glial cell numbers in early blind individuals would be consistent with our observed higher concentration levels of *myo*-Inositol, choline and creatine, and can therefore provide a parsimonious explanation for several of our findings. While a variety of studies of dark-rearing show a *decrease* in the astrocyte population in dark-reared animals (Gabbott et al., 1986, Argandona et al., 2003) these effects are thought to be predominantly due to the impoverishment of a dark-rearing environment. Indeed, adult rats dark-reared from birth through adulthood in a highly enriched environment that included exercise show higher levels of astrocytes than control animals in a standard environment (Argandona et al., 2009). This suggests the intriguing possibility that meeting the challenge of becoming highly independent and proficient at the use of auditory and tactile cross-modal technologies (as was the case for all our subjects) might not merely prevent the decrease in astrocyte levels that is normally seen in dark-reared animals, but might even have an effect analogous to the effects of environmental enrichment, resulting in an increase in the astrocyte population local to occipital cortex.

Altered neural-hemodynamic coupling

The neurochemical differences between blind and sighted subjects described here (higher levels of GABA, lower levels of choline, higher levels of creatine) suggest that it may be unwise to presume that neurovascular coupling is unaltered by early blindness. Blood flow is mediated by GABAergic and cholinergic pathways not only through direct interaction between neurons and vessels but also indirectly via the perivascular astrocytes (Cauli et al., 2004, Hamel, 2006, Donahue et al., 2010, Kleinfeld et al., 2011, Muthukumaraswamy et al., 2012). Our finding of higher levels of creatine, in conjunction with previous PET results showing differences in metabolic activity in early blind subjects, further suggest group differences within metabolic processes themselves. In Alzheimer's disease the importance of considering potential metabolic differences between groups when interpreting BOLD signals has already been demonstrated: disease-related metabolic down-regulation has effects on neurovascular coupling that in turn influences task-related BOLD activity (Herminghaus et al., 2003, Lecrux and Hamel, 2011, Nicolakakis and Hamel, 2011).

The reason for these metabolic changes is not clear, but one possibility is that the higher rates of spontaneous neural firing that have been associated with visual deprivation (Movshon and Van Sluyters, 1981), might lead to long-term compensatory changes in metabolic processing. Consistent with the notion of task demands affecting metabolic

demand, cognitive training is associated with (resting state) creatine increases within the hippocampus (Valenzuela et al., 2003).

Upregulation of cholinergic phospholipid pathways

While acetylcholine is a very small component of the choline peak, MRS ^{1}H choline measurements are a surprisingly reliable surrogate marker of acetylcholine: in animal models the correlation across individuals between measured choline and acetylcholine levels is above 0.8 across multiple brain regions (Satlin et al., 1997, also see Frederick et al., 2002, Wang et al., 2008). These high correlations may be because phospholipids (such as phosphocholine and/or glycerophosphocholine, major components of the ^{1}H choline peak) play a key role in mediating activity within cholinergic pathways (Cantley, 2002, Suh and Hille, 2002, Beloueche-Babari et al., 2010) by providing choline for acetylcholine synthesis (Wurtman et al., 1985, MacKay et al., 1996, Satlin et al., 1997, Boulanger et al., 2000, Valenzuela et al., 2003). In the case of *myo*-Inositol, cholinergic activity might stimulate phosphoinositide hydrolysis and thereby raise the level of intracellular inositol (Wess, 1996). Thus, higher levels of choline and myo-Inositol might both possibly reflect long-term up-regulation of cholinergic phospholipid pathways consequent on early blindness, as has been found in one animal model (Fosse et al., 1989).

Tuning and plasticity

Cholinergic phospholipid and GABAergic pathways are associated with changes in sensitivity and selectivity across a variety of time-scales. Short-term modulation of cholinergic pathways affect tuning and responses in heterogeneous way (Sillito and Kemp, 1983, Sato et al., 1987, Murphy and Sillito, 1991, Zinke et al., 2006) which are not fully understood, though it is believed that one of the roles of this pathway is mediating the relative effectiveness of bottom-up thalamic vs. top down and attentional input (Disney et al., 2006, Disney et al., 2007, Disney and Aoki, 2008, Herrero et al., 2008, Rokem and Silver, 2010). Both short-term modulation (Katzner et al., 2011) and longer term individual differences in GABA levels have similarly been shown to be associated with changes in response gain and tuning across a variety of time scales (Edden et al., 2009, Muthukumaraswamy et al., 2009, Yoon et al., 2010, Muthukumaraswamy et al., 2012).

As far as plasticity is concerned, it is thought that a homeostatic balance (Desai et al., 2002, Turrigiano and Nelson, 2004, McCoy et al., 2009, Tropea et al., 2009) between excitatory, inhibitory and modulatory (Morales et al., 2002) pathways mediates developmental and adult synaptic plasticity across multiple time scales via a combination of feed-forward `Hebbian' (Hensch, 2005, Hensch and Fagiolini, 2005) and feedback `synaptic scaling' (Desai et al., 2002) mechanisms. Modulatory cholinergic pathways appear to play a strong role, such that up-regulation of these pathways can shift the balance towards excitation (for reviews see Gu, 2003, Morishita and Hensch, 2008, Bavelier et al., 2010). For example, in adult humans pharmacological enhancement of GABA receptor function, reduction of muscarinic acetylcholine receptor function, and reduction of NMDA receptor function all have similar effects in blocking temporary increases in responsiveness as a result of short term visual deprivation (Boroojerdi et al., 2001). Similarly, in animal models, prolongation of the critical period in development and a `reawakening' of plasticity in adulthood are not

only mediated by glutamatergic (e.g. Fagiolini et al., 2003, McCoy et al., 2009) and GABAergic pathways (e.g. Hensch et al., 1998, Iwai et al., 2003), but also by modulatory cholinergic pathways (Greuel et al., 1988, Morishita et al., 2010), see Gu (2003) and Bavelier et al. (2010) for reviews.

If it is indeed the case that early blindness affects GABAergic and/or cholinergic phospholipid pathways then this would suggest that that the cross-modal responses associated with early blindness may, at least in part, be mediated by a complex collection of changes within occipital biochemical pathways themselves. If so, this this offers the intriguing possibility that `re-awakening plasticity' in late blind individuals might allow them to make better use of cross-modal technologies (Bavelier et al., 2010).

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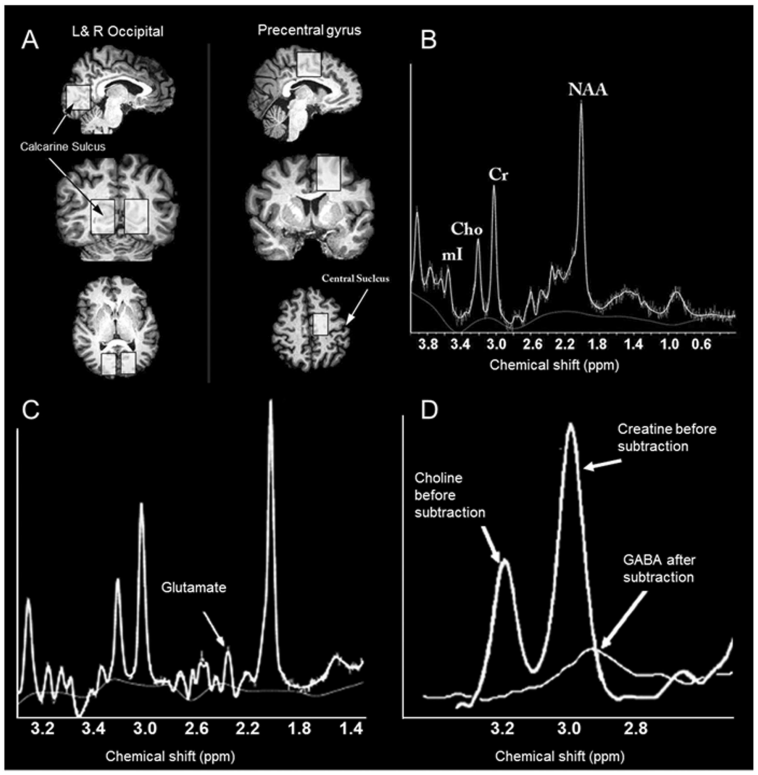

Figure 1.

(A) The locations of the three MRS voxels shown in a representative early blind individual. A high resolution T1 weighted anatomical scan (MPRAGE: TR = 7 ms; TE = 3.20 ms; flip angle = 8°; matrix size 240×240; 160 sagittal slices; slice thickness = 1 mm) was collected at the beginning of each session to guide voxel placement for the MRS sequences. 3×2×3 cm voxels were placed within visual cortex, along the left and right occipital wall centered around the calcarine sulcus and within a control frontal lobe region, anterior to the central sulcus and superior to the corpus callosum. Care was taken to ensure that voxels did not overlap with the pial surface as to avoid lipid contamination. **(B)** The short echo (TE =32 ms) spectrum, after model fitting and quantification procedures, showing the locations of several hydrogen MRS peaks: myo-Inositol (mI), choline (Cho), creatine (Cr) and N-acetyl aspartate (NAA). The smooth line below the spectrum is the LCmodel computed spectral baseline (subtracted from the resonance area under the curve when calculating chemical concentrations). **(C)** The long echo (TE=87ms) spectrum used to quantify GABA and glutamate. Longer echo times allow for the isolation of the glutamate peak due to dephasing of the glutamine signal at this echo time (Puts et al., 2012). The smooth line below the spectrum is the LCmodel computed spectral baseline. **(D)** The GABA peak was isolated with a frequency-selective saturation MEGAPRESS technique with subtraction across the two different frequency positions of the editing pulse. The thicker line shows the spectrum with the editing pulse positioned at 1.9 ppm with both creatine and choline visible. The thinner line shows the MEGAPRESS spectrum after subtraction of the two editing pulses (7.5 − 1.9 ppm), with GABA visible at 2.95 p.p.m.

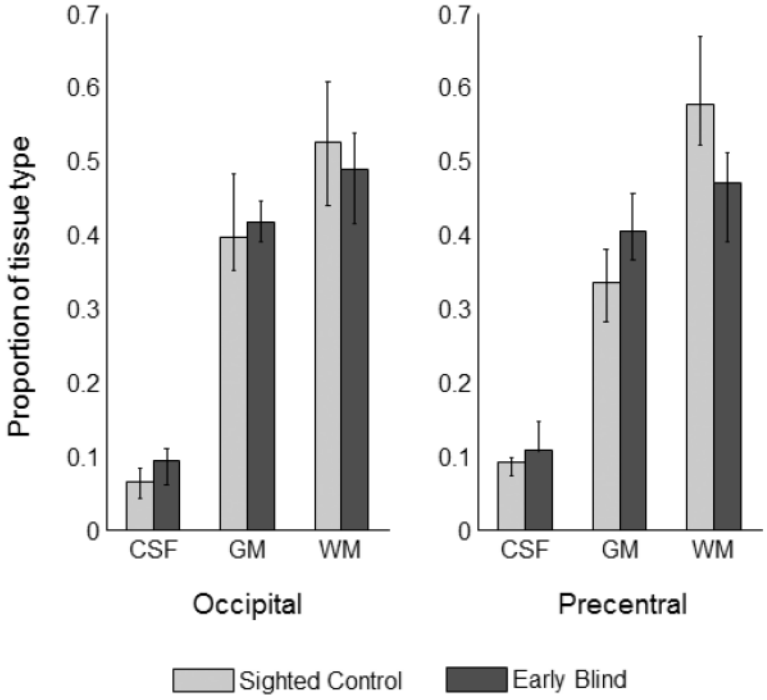

Figure 2.

Group median tissue concentrations for sighted control (light gray bars) and early blind subjects (dark gray bars). Error bars represent the 25–75% interquartile range.

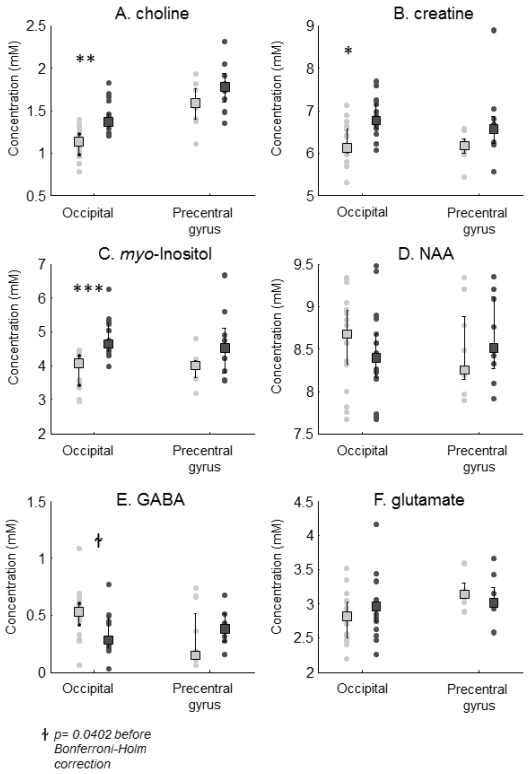

Figure 3.

Early visual deprivation has significant effects on choline, creatine and *myo*-Inositol and GABA concentrations in occipital but not precentral cortex. Absolute concentrations (in millimolar, mM) are shown across left and right occipital voxels and the precentral gyrus for A) choline, B) creatine, C) *myo*-Inositol, D) NAA. Group median metabolite concentrations are shown for sighted control (light gray bars) and early blind subjects (dark gray bars). Error bars represent the 25–75% interquartile range. * $p < 0.05$, ** $p<0.01$, *** $p < 0.001$ represent group differences using post-hoc Students t-tests corrected for 6 multiple comparisons using the Bonferroni-Holm method.

Table 1

Details of subjects (acuity is reported for the better eye; NLP – no light perception; LP – light perception; low LP implies no color, motion, or form perception; ROP – retinopathy of prematurity). EB – Early blind, SC – Sighted control._All individuals were free of co-morbid neurological and psychiatric concerns, heavy alcohol use and prescription/non-prescription drug use (self-report).

Subject	Gender	Age	Handedness	Age of Onset	Cause of Blindness	Prematurity	Visual acuity (best eye)	Braille wpm	Braille reading hand
EB01	F	56	L	1.5	Virus damaged optic nerve in both eyes	At term	Low LP	>300	Reads L
SC01	F	53	L						
EB02	M	40	R	0	Congenital cataracts	At term	NLP	120–150	Reads L guides R
SC02	M	44	L						
EB03	M	56	L	0	ROP	2 mo.	NLP	400–500	Reads L
SC03	M	55	L						
EB04	M	57	R	0	ROP	2 mo.	Low LP	150	Reads L index
SC04	M	58	R						
EB05	M	44	R	MinimalLP until 6–7	Congenital glaucoma	At term	Low LP	137	Reads R index
SC05	M	47	R						
EB06	F	49	R	MinimalLP until 25–30 when retina detached	ROP	< 1 mo.	NLP	110	Reads R index, guides L index
SC06	F	51	R						
EB07	M	56	R	0	ROP	2 mo.	NLP	225	Reads L & R
SC07	M	48	R						
EB08	M	57	R	MrnimalLP, gradually deteriorated, NLP @ 25–30	ROP/congenital glaucoma	2 mo.	NLP	120	Reads R index
SC08	M	61	L						
EB09	F	60	Amb	MinimalLP in left eye only until 5	Ruptured RE, detached retina @ 5 mo. Glaucoma secondary to surgery	At term	NLP	>300	Reads L & R
SC09	F	54	R						

Table 2

Early visual deprivation has significant effects on choline, creatine, *myo*-Inositol and possibly GABA concentrations in occipital but not precentral cortex. Median absolute concentrations (in millimolar, mM) and 25–75% interquartile range are shown for occipital and the precentral gyrus voxels. P-values represent Students t-tests corrected for 6 multiple comparisons using the Bonferroni-Holm method.

		Choline		***Creatine***		***myo-inositol***		***NAA***		***GABA***		***Glutamate***	
		median	**range**	**median**	**range**	**median**	**range**	**median**	**range**	**median**	**range**	**median**	**range**
Occipital	*SC*	1.137	0.987–1.229	6.113	5.994–6.551	4.080	3.420–4.292	8.670	8.320–8.959	0.532	0.417–0.609	2.822	2.509–3.03
	EB	1.372	1.29–1.458	6.769	6.568–7.153	4.649	4.43–5.253	8.388	8.159–8.674	0.286	0.217–0.483	2.959	2.738–2.996
	% difference	18.7%		10.2%		13.0%		−3.3%		−60.2%		4.8%	
	p	p>0.01		p<0.05		p<0.001				fails B-H correction			
Precentral gyrus	*SC*	1.588	1.393–1.756	6.172	5.994–6.309	3.997	3.646–4.177	8.248	8.129–8.881	0.150	0.112–0.508	3.140	3.075–3.29
	EB	1.770	1.591-1.935	6.539	6.221–6.868	4.524	3.782–5.089	8.511	8.267–9.108	0.377	0.276–0.512	3.014	2.941–3.227
	% difference	10.8%		5.8%		12.4%		3.1%		86.2%		−4.1%	
	p												