

Altered visual cortex excitatory/inhibitory ratio following transient congenital visual deprivation in humans

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Abstract

Non-human animal models have indicated that the ratio of excitation to inhibition (E/I) in neural circuits is experience dependent, and changes across development. Here, we assessed 3T Magnetic Resonance Spectroscopy (MRS) and electroencephalography (EEG) markers of cortical E/I ratio in ten individuals who had been treated for dense bilateral congenital cataracts, after an average of 12 years of blindness, to test for dependence on early visual experience. First, participants underwent MRS scanning at rest with their eyes opened and eyes closed, to obtain visual cortex Gamma-Aminobutyric Acid (GABA+) concentration, Glutamate/Glutamine (Glx) concentration, and the concentration ratio of Glx/GABA+, as measures of inhibition, excitation, and E/I ratio respectively. Subsequently, EEG was recorded to assess aperiodic activity (1-20 Hz) as a neurophysiological measure of the cortical E/I ratio, during rest with eyes open and eyes closed, and during flickering stimulation. Across conditions, sight recovery individuals demonstrated a significantly lower visual cortex Glx/GABA+ ratio, and a higher intercept and steeper aperiodic slope at occipital electrodes, compared to age-matched sighted controls. In the sight recovery group, a lower Glx/GABA+ ratio was associated with better visual acuity, and Glx concentration correlated positively with the aperiodic intercept in the conditions with visual input. We interpret these findings as resulting from an increased E/I ratio of the visual cortex as a consequence of congenital blindness, which required commensurately increased inhibition after restored visual input provided additional excitation.

eLife assessment

This potentially **useful** study involves neuro-imaging and electrophysiology in a small cohort of congenital cataract patients after sight recovery and age-matched control participants with normal sight. It aims to characterize the effects of early visual deprivation on excitatory and inhibitory balance in the visual cortex. While the findings are taken to suggest the existence of persistent alterations in Glx/GABA ratio and aperiodic EEG signals, the evidence supporting these claims is **incomplete**. Specifically, small sample sizes, lack of a specific control cohort, and other methodological limitations will likely restrict the usefulness of the work, with relevance limited to scientists working in this particular subfield.

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Introduction

Sensitive periods are epochs during the lifespan within which effects of experience on the brain are particularly strong (Knudsen, 2004 [↗](#)). Non-human animal work has established that structural remodelling (Bourgeois, 1997 [↗](#)) and the development of local inhibitory neural circuits strongly links to the timing of sensitive periods (Gianfranceschi et al., 2003 [↗](#); Hensch et al., 1998 [↗](#); Hensch & Bilimoria, 2012 [↗](#); Hensch & Fagiolini, 2004 [↗](#); Takesian & Hensch, 2013 [↗](#)). Early visual experience has been shown to fine-tune local inhibitory circuits (Benevento et al., 1992 [↗](#); Chattopadhyaya et al., 2004 [↗](#); Gandhi et al., 2008 [↗](#); Toyozumi et al., 2013 [↗](#)), which dynamically control feedforward excitation (Tao & Poo, 2005 [↗](#); Wu et al., 2022 [↗](#)). The end of the sensitive period has been proposed to coincide with the maturation of inhibitory neural circuits (Hensch, 2005 [↗](#); Wong-Riley, 2021 [↗](#); H. Zhang et al., 2018 [↗](#)). Within this framework, neural circuit stability following the sensitive period is maintained via a balance between excitatory and inhibitory transmission across multiple spatiotemporal scales (Froemke, 2015 [↗](#); Haider et al., 2006 [↗](#); Maffei et al., 2004 [↗](#); Takesian & Hensch, 2013 [↗](#); Wu et al., 2022 [↗](#)). Such an excitatory/inhibitory (E/I) ratio has been studied at different organizational levels, including the synaptic and neuronal levels, as well as for neural circuits (Van Vreeswijk & Sompolinsky, 1996 [↗](#); Wu et al., 2022 [↗](#)).

The early experience-dependence of local inhibitory circuit tuning is supported by a large body of work in non-human animals. In particular, studies of the mouse visual cortex has demonstrated a disrupted tuning of local inhibitory circuits as a consequence of lacking visual experience at birth (Hensch & Fagiolini, 2004 [↗](#); Levelt & Hübener, 2012 [↗](#)). In addition, dark-reared mice have been shown to have spontaneous neural firing in adulthood (Benevento et al., 1992 [↗](#)) and a reduced magnitude of inhibition, particularly in layers II/III of the visual cortex (Morales et al., 2002 [↗](#)), suggesting an overall higher level of excitation.

Human neuroimaging studies have similarly demonstrated that visual experience during the first weeks and months of life is crucial for the development of visual circuits (Baroncelli et al., 2011 [↗](#); Lewis & Maurer, 2005 [↗](#); Maurer & Hensch, 2012 [↗](#); Röder et al., 2021 [↗](#); Röder & Kekunnaya, 2021 [↗](#); Singh et al., 2018 [↗](#)). As studies manipulating visual experience are impossible in human research, much of our understanding of the experience-dependence of visual circuit development comes from patients who underwent a transient period of congenital blindness due to dense bilateral congenital cataracts. If human infants born with dense bilateral cataracts are treated later than a few weeks from birth, they suffer from a permanent reduction of not only visual acuity (Birch et al., 1998 [↗](#); Khanna et al., 2013 [↗](#)) and stereovision (Birch et al., 1993 [↗](#); Tytla et al.,

1993) but additionally from impairments in higher-level visual functions such as face perception (Le Grand et al., 2001; Putzar et al., 2010; Röder et al., 2013), coherent motion detection (Bottari et al., 2018; Hadad et al., 2012; Maurer & Lewis, 2017), visual temporal processing (Badde et al., 2020) and visual feature binding (McKyton et al., 2015; Putzar et al., 2007). These visual deficits in congenital cataract-reversal individuals have been attributed to altered neural development due to the absence of vision, as individuals who suffered from developmental cataracts do not typically display a comparable severity of impairments (Lewis & Maurer, 2009; Sourav et al., 2020). While extant literature reported correlations between structural changes and behavioral outcomes in congenital cataract-reversal individuals (Feng et al., 2021; Guerreiro et al., 2015; Hölig et al., 2023; Pedersini et al., 2023), functional brain imaging (Heitmman et al., 2023; Racz et al., 2022) and electrophysiological research (Bottari et al., 2016; Ossandón et al., 2023; Pant et al., 2023; Pitchaimuthu et al., 2021) have started to unravel the neural mechanisms which rely on visual experience during sensitive periods for development.

Resting-state activity measured via fMRI suggested an increased E/I ratio in the visual cortex of congenital cataract-reversal individuals (Racz et al., 2022): The amplitude of low frequency (<1 Hz) (blood oxygen level-dependent) fluctuations (ALFF) in visual cortex was increased in congenital cataract-reversal individuals compared to normally-sighted controls when they were scanned with their eyes open. Since similar changes were observed in permanently congenitally blind humans, the authors speculated that congenital visual deprivation resulted in increased E/I ratio of neural circuits due to impaired neural tuning, which was not reinstated after sight restoration (Racz et al., 2022). Other studies measured resting-state electroencephalogram (EEG) activity and analyzed periodic (alpha oscillations) (Bottari et al., 2016; Ossandón et al., 2023; Pant et al., 2023) as well as aperiodic activity (Ossandón et al., 2023). Both measures pointed towards a higher E/I ratio of visual cortex in congenital cataract-reversal individuals (Ossandón et al., 2023). In recent research, authors have interpreted the slope of the aperiodic component of EEG power spectral density as an indication of the relative level of excitation; the flatter the slope, the higher the assumed E/I ratio (Gao et al., 2017; Lombardi et al., 2017; Medel et al., 2020; Muthukumaraswamy & Liley, 2018; Nanda et al., 2023). In fact, an increasing number of prospective studies in children have reported a flattening of this slope that was interpreted as higher levels of excitation with increasing age (Favaro et al., 2023; Hill et al., 2022). Ossandón et al. (2023), however, observed that in addition to the flatter slope of the aperiodic power spectrum in the high frequency range (20-40 Hz), the slope of the low frequency range (1-19 Hz) was steeper in both, congenital cataract-reversal individuals, as well as in permanently congenitally blind humans. The low frequency range has often been associated with inhibition (Jensen & Mazaheri, 2010; Lozano-Soldevilla, 2018; Lozano-Soldevilla et al., 2014). However, it remains unclear how to reconcile EEG resting-state findings for lower and higher frequency ranges.

Two studies with permanently congenitally blind humans employed Magnetic Resonance Spectroscopy (MRS) to investigate the concentration of both, the inhibitory neurotransmitter Gamma-Aminobutyric Acid (GABA), and the excitatory neurotransmitters Glutamate/Glutamine (Glx) as proxy measures of visual cortex inhibition and excitation, respectively (Coullon et al., 2015; Weaver et al., 2013). Glutamate/Glutamine concentration was significantly increased in the visual cortex of anophthalmic compared to normally-sighted individuals, suggesting increased excitability (Coullon et al., 2015). Preliminary evidence in congenitally permanently blind individuals suggested a decreased GABA concentration in the visual cortex compared to normally-sighted individuals (Weaver et al., 2013). Thus, these MRS studies corroborated the hypothesis that a lack of visual input at birth enhances relative excitation in visual cortex. However, the degree to which neurotransmitter levels recover following sight restoration after a phase of congenital blindness, and how they related to electrophysiological activity, remained unclear.

Here, we filled this gap: we assessed Glutamate/Glutamine (Glx) and Gamma Aminobutyric Acid (GABA+) concentrations using the MEGA-PRESS sequence (Mescher et al., 1998 [DOI](#)) in individuals whose sight had been restored, on average, after 12 years of congenital blindness. The ratio of Glx/GABA+ concentration was used as a proxy for the ratio of excitatory to inhibitory neurotransmission (Grent-'t-Jong et al., 2022 [DOI](#); Liu et al., 2015 [DOI](#); Steel et al., 2020 [DOI](#); Takei et al., 2016 [DOI](#); L. Zhang et al., 2020 [DOI](#)). Ten congenital cataract-reversal individuals were compared to age-matched, normally sighted controls at rest, both with eyes open and with eyes closed. To link MRS and EEG markers of cortical excitation/inhibition, we subsequently assessed the aperiodic slope of the EEG spectrum in the same subjects across three conditions: at rest with eyes closed, at rest with eyes open, and while viewing visual stimuli which changed in luminance at frequencies ranging from 1 to 30 Hz (Pant et al., 2023 [DOI](#)). Additionally we calculated the aperiodic intercept, which has previously been linked to broadband neuronal firing (Manning et al., 2009 [DOI](#); Musall et al., 2014 [DOI](#); Winawer et al., 2013 [DOI](#)). We predicted an altered visual cortex Glx/GABA+ concentration ratio in the edited MRS signal in congenital cataract-reversal individuals, and a higher intercept as well as altered slope of the EEG aperiodic component. We further hypothesized that neurotransmitter changes would relate to changes in the slope and intercept of the EEG aperiodic activity in the same subjects.

Methods

Participants

We tested two groups of participants. The first group consisted of 10 individuals with a history of dense bilateral congenital cataracts (CC, 1 female, Mean Age = 25.8 years, Range = 11 – 43.5). Participants in this group were all recruited at the LV Prasad Eye Institute (Hyderabad, India) and the presence of dense bilateral cataracts at birth was confirmed by ophthalmologists and optometrists based on a combination of the following criteria: clinical diagnosis of bilateral congenital cataract, drawing of the pre-surgery cataract, occlusion of the fundus, nystagmus, a family history of bilateral congenital cataracts, and a visual acuity of fixating and following light (FFL+) or less prior to surgery, barring cases of absorbed lenses. Absorbed lenses occur specifically in individuals with dense congenital cataracts (Ehrlich, 1948 [DOI](#)) and were diagnosed based on the morphology of the lens, anterior capsule wrinkling, and plaque or thickness of stroma.

Duration of deprivation was calculated as the age of the participant when cataract removal surgery was performed on the first eye. Two participants were operated within the first year of life (at 3 months and 9 months of age), all other participants underwent cataract removal surgery after the age of 6 years (Mean Age at Surgery = 11.8 years, SD = 9.7, Range = 0.2 – 31.4). All participants were tested at least 1 year after surgery (Mean Time since Surgery = 14 years, SD = 9.1, Range = 1.8 – 30.9) (**Table 1** [DOI](#)).

The second group comprised of 10 normally sighted individuals (SC, 8 males, Mean Age = 26.3 years, Range = 12 – 41.8). Participants across the two groups were individually age matched (± 2 years, $t_{(9)} = -0.12$, $p = 0.91$). One additional individual was tested in each group, however, they were excluded from data analysis as their data files were corrupted due to inappropriate file transfer from the scanner. All participants (as well as legal guardians for minors) gave written and informed consent. This study was conducted after approval from the Local Ethical Commission of the LV Prasad Eye Institute (Hyderabad, India) as well as of the Faculty of Psychology and Human Movement, University of Hamburg (Germany).

Data Collection and Analysis

The present study consisted of three data acquisition parts: Magnetic Resonance Spectroscopy (MRS), Electroencephalography (EEG), and visual acuity assessment.

Table 1

Clinical and demographic information of the participants with a history of dense bilateral congenital cataracts (CC).

NA indicates that patient's data for the field were not available. FFL: Fixating and Following Light; CF: Counting Fingers; PL: Perceiving Light. Duration of visual deprivation was calculated by subtracting the date of birth from the date of surgery on the first eye. Time since surgery was calculated by subtracting the date of surgery on the first eye from the date of testing. Visual acuity on the date of testing was measured with the Freiburg Vision Test (FrACT) (Bach 1996).

	COMORBIDITIES			GENDER	VISUAL ACUITY PRE SURGERY		DURATION OF VISUAL DEPRIVATION (YEARS)	TIME SINCE SURGERY (YEARS)	VISUAL ACUITY ON DATE TESTED (LOGMAR)	FAMILY HISTORY
	ABSORBED LENSES	STRABISMUS	NYSTAGMUS		OD	OS				
1	No	Yes	Yes	Male	FFL -	FFL +	0.2	16.8	0.17	No
2	Yes	Yes	Yes	Male	1.18	1	20.8	22.7	0.9	Yes
3	Yes	Yes	Yes	Male	1.48	1.77	15.6	3.1	0.9	No
4	Yes	NA	Yes	Male	CF at 1.5 m	CF at 3 m	7.0	8.2	0.62	No
5	No	Yes	Yes	Male	NA	NA	14.0	18.4	0.88	Yes
6	No	Yes	Yes	Male	NA	NA	6.0	17.9	0.78	Yes
7	No	Yes	Yes	Male	PL+	PL+	0.7	12.4	0.54	No
8	Yes	No	Yes	Male	1.2	1.3	16.4	1.8	0.66	Yes
9	No	NA	Yes	Male	NA	NA	6.0	30.9	1.34	Yes
10	Yes	Yes	Yes	Female	1.48	1.48	31.4	7.4	1.04	Yes

Magnetic Resonance Spectroscopy

Participants underwent MRI and MRS scanning at LUCID Diagnostics in Hyderabad (India) with a 3T GE SIGNA Pioneer MRI machine employing a 24-channel head coil. An attendant was present in the scanning room for the duration of the scan to ensure that participants were comfortable and followed the instructions.

A T1 weighted whole brain image was collected for each participant (Repetition Time (TR) = 14.97 ms, Echo Time (TE) = 6.74 ms, Matrix size = 512×512 , In-plane resolution = 0.43×0.43 mm, Slice thickness = 1.6 mm, Axial slices = 188, Interslice interval = -0.8 mm, Inversion time = 500 ms, Flip angle = 15°). This structural scan enabled registration of every MRS scan to the participants' anatomical landmarks (**Figure 1**). For this scan, participants were instructed to keep their eyes closed and stay as still as possible.

The MRS scans consisted of single-voxel spectroscopy data that were collected using the MEGA-PRESS sequence, which allows for in-vivo quantification of the low-concentration metabolites GABA and glutamate+glutamine (Glu+Gln) (Mescher et al., 1998; Mullins et al., 2014). Due to the spectral overlap of GABA (3.0 ppm) and Glu/Gln (3.75 ppm) with the higher concentration peaks of N-Acetyl Aspartate (NAA) and Creatine (Cr), accurate quantification of GABA and Glu/Gln is challenging. MEGA-PRESS uses spectral editing to obtain these measurements. Spectroscopy data consist of an edit-ON and an edit-OFF spectrum for each voxel, wherein the “ON” and “OFF” refer to whether the frequency of the editing pulse applied is on- or off-resonance with the signal coupled to the GABA complex (applied at approximately 1.9 ppm). Therefore, subtracting repeated acquisitions of the edit-ON and edit-OFF spectra allows for measurement of the magnitude of signals differing in their response to the editing pulse (e.g. GABA), while cancelling out signals that do not (e.g. Cr) (Mescher et al., 1998). Each MEGA-PRESS scan lasted for 8 minutes and was acquired with the following specifications: TR = 2000 ms, TE = 68 ms, Voxel size = $40 \text{ mm} \times 30 \text{ mm} \times 25 \text{ mm}$, 192 averages (each consists of two TRs). Additionally, 8 unsuppressed water averages were acquired, allowing all metabolites to be referenced to the tissue water concentration. Concentrations of GABA and Glu/Gln quantified from these acquisitions are respectively referred to as GABA+, due to the presence of macromolecular contaminants in the signal (Mullins et al., 2014), and Glx, due to the combined quantification of the Glu, Gln and Glutathione peaks.

Two MRS scans were collected from the visual cortex, centered on the calcarine sulcus of every participant (**Figure 1**). The scans were recorded with the participants' eyes open (EO condition) and eyes closed (EC condition), respectively. During the scans, participants were instructed to lie as still as possible.

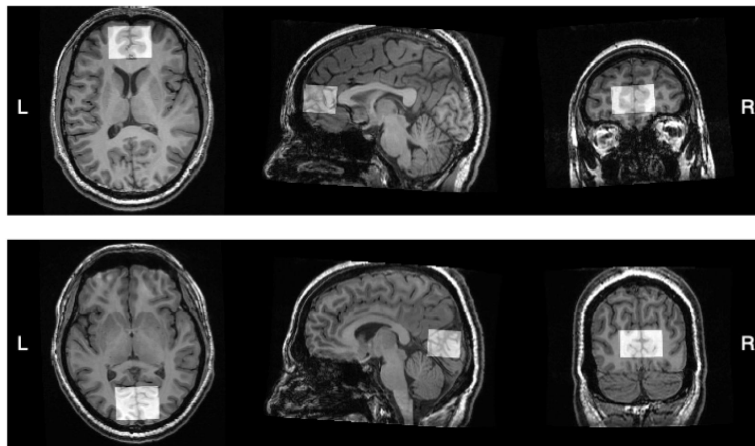
To ensure that we were identifying neurochemical changes specific to visual regions, we selected the frontal cortex as a control region (**Figure 1**) and collected two scans (EO and EC) from the frontal cortex too. The order of the MRS scans was counterbalanced across individuals for both locations and conditions. Two SC subjects did not complete the frontal cortex scan for the EO condition and were excluded from the statistical comparisons of frontal cortex neurotransmitter concentrations.

MRS Data Analysis

All data analyses were performed in MATLAB (R2018b, The MathWorks Inc.). For MRS data analyses we used Gannet 3.0, a MATLAB based toolbox specialized for the quantification of GABA+ and Glx from edited spectrum data (Edden et al., 2014).

GABA+ and edited Glx concentration values were obtained and corrected using the *GannetFit*, *GannetCoRegister*, *GannetSegment* and *GannetQuantify* functions (Edden et al., 2014). Briefly, reported concentration values were corrected for the differences in GABA concentration and

a. Voxel Placement



b. Electrode Placement

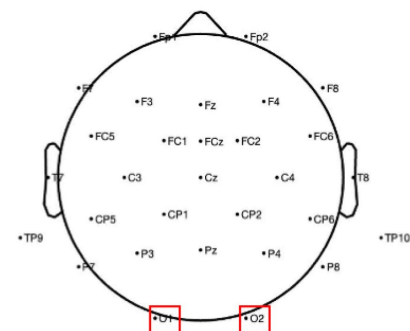


Figure 1

Voxel placement for Magnetic Resonance Spectroscopy and electrode placement for Electroencephalography.

a. Position of the frontal cortex (top) and visual cortex (bottom) voxels in a single subject. Figures output from GannetCoRegister. b. Electrode montage according to the 10/20 electrode system with marked occipital electrodes (red) preselected for analysis.

relaxation times between different kinds of tissue in the voxel (grey matter, white matter and cerebrospinal fluid) (Harris et al., 2015 [DOI](#)). Gannet uses SPM12 to determine the proportion of grey matter, white matter and cerebrospinal fluid in each individual participant's voxel (Penny et al., 2007 [DOI](#)). Note that the tissue fraction values did not differ between groups or conditions (all p 's > 0.19, see Supplementary Material S2). GABA+, Glx and Glx/GABA+ values were compared across groups as proxy measures of inhibition, excitation and E/I ratio respectively. The use of Glx/GABA+ as a proxy measure of E/I neurotransmission is supported by a study that observed a regional balance between Glx and GABA+ at 3T (Steel et al., 2020 [DOI](#)). Further, the Glx/GABA+ ratio has been employed in prior studies of visual (Takei et al., 2016 [DOI](#); L. Zhang et al., 2020 [DOI](#)), cingulate (Bezalel et al., 2019 [DOI](#)), frontal (Liu et al., 2015 [DOI](#)) and auditory cortex (Grent-'t-jong et al., 2022 [DOI](#)).

To control for potential unspecified visual cortex changes due to eye pathology, as opposed to genuine changes in neurotransmitter ratio, we compared N-Acetyl Aspartate (NAA) concentrations in the visual cortex of CC vs SC individuals. NAA forms one of the most prominent peaks in the MR spectrum (2.0 ppm chemical shift). NAA has been quantified with high reproducibility in the visual cortex (Brooks et al., 1999 [DOI](#)) and medial-temporal cortex (Träber et al., 2006 [DOI](#)) of neuro-typical individuals as well as in various pathologies across visual, frontal and temporal cortex (Paskalis et al., 2014 [DOI](#)), for example, schizophrenia (Mullins et al., 2003 [DOI](#)). We did not expect to find differences in NAA concentration between CC and SC individuals as it has not been demonstrated to vary in anophthalmia (Coullon et al., 2015 [DOI](#)) or permanent early blindness (Weaver et al., 2013 [DOI](#)) in humans. TARQUIN 4.3.11 was employed to analyze the OFF-spectrum data (Wilson et al., 2011 [DOI](#)) to assess NAA concentration. FID-A toolbox was used to correct the data for phase errors across acquisitions arising from temporal changes in the magnetic field strength or participant motion (Simpson et al., 2017 [DOI](#)).

Mean Signal-to-Noise Ratio values for GABA+ and Glx in all groups and conditions were above 19 in the visual cortex and above 8 in the frontal cortex (Supplementary Material S3). A recent study has suggested that an SNR value above 3.8 allows for reliable quantification of GABA+ (Zöllner et al., 2021 [DOI](#)), in conjunction with considering a given study's sample size (Mikkelsen et al., 2018 [DOI](#)). Cramer-Rao Lower Bound (CRLB) values, that is, the theoretical lower limit of estimated error, were 30% or lower for NAA quantification in both groups and conditions (Cavassila et al., 2001 [DOI](#)). Note that CRLB values above 50% are considered unreliable (Wilson et al., 2019 [DOI](#)). We confirmed the within-subject stability of metabolite quantification by testing a subset of the sighted controls ($n=6$) 2-4 weeks apart (Supplementary Material S5).

All reported values are water-normalized.

Prior to in-vivo scanning, we confirmed the GABA+ and GABA+/Glx quantification quality with phantom testing (Henry et al., 2011 [DOI](#); Jenkins et al., 2019 [DOI](#)). Imaging sequences were robust in identifying differences of 0.02 mM in GABA concentration. This 0.02 mM difference was documented by Weaver et al (2013) [DOI](#) between the occipital cortices of early blind and sighted individuals. The known vs. measured concentrations of both GABA ($r = 0.81$, $p = 0.004$) and GABA/Glx ($r = 0.71$, $p = 0.019$) showed significant agreement. The detailed procedure and results are described in the Supplementary Material (Supplementary Material S4). The spectra from all individual subjects are shown in Supplementary Material S10.

MRS Statistical Analysis

All statistical analyses were performed using MATLAB R2018b.

We compared the visual cortex concentrations of 3 neurochemicals (GABA+ and Glx from the DIFF spectrum, NAA from the edit-OFF spectrum) between the two groups. For each metabolite we submitted the concentration values from the visual cortices of CC and SC individuals to a group (2 Levels: CC, SC)-by-condition (2 Levels: EO, EC) ANOVA model. To compare the Glx/GABA+ ratio

between groups, we additionally submitted this ratio value to a group-by-condition ANOVA. Identical analyses were performed for the corresponding frontal cortex neurotransmitter values. Wherever necessary, post-hoc comparisons were performed using t-tests.

Electrophysiological recordings

EEG data were collected to investigate aperiodic activity in the same participants and on the same day. Data were acquired in three conditions: at rest with eyes open (EO, 3 minutes), at rest with eyes closed (EC, 3 minutes) and during visual stimulation with stimuli that changed in luminance (LU) (Pant et al., 2023 [↗](#)). We used the slope of the aperiodic (1/f) component of the EEG spectrum as an estimate of E/I ratio (Gao et al., 2017 [↗](#); Medel et al., 2020 [↗](#); Muthukumaraswamy & Liley, 2018 [↗](#)). Further, we compared the intercept of the aperiodic component in the human EEG between groups, as an estimate of broadband neuronal firing activity (Haller et al., 2018 [↗](#); Manning et al., 2009 [↗](#); Miller, 2010 [↗](#)).

The EEG was recorded using Ag/AgCl electrodes attached according the 10/20 system (Homan et al., 1987 [↗](#)) to an elastic cap (EASYCAP GmbH, Herrsching, Germany) (Figure 1 [↗](#)). We recorded 32 channel EEG using the BrainAmp amplifier, with a bandwidth of 0.01–200 Hz, sampling rate of 5 kHz and a time constant of 0.016 Hz $/(10\text{ s})$ (<http://www.brainproducts.com/> [↗](#)). All scalp recordings were performed against a left ear lobe reference.

Participants were asked to sit as still as possible while EEG was being recorded. First, resting-state EEG data were collected. During the EO condition, participants were asked to fixate on a blank screen. During the EC condition, participants were instructed to keep their eyes closed. The order of conditions was randomized across participants. The EEG data sets reported here were part of data published earlier (Ossandón et al., 2023 [↗](#); Pant et al., 2023 [↗](#)).

Subsequently, EEG data were recorded during 100 trials of a target detection task with stimuli that changed in luminance (LU). Stimuli were presented with a Dell laptop, on a Dell 22 inch LCD monitor with a refresh rate of 60 Hz. They were created with MATLAB r2018b (The MathWorks, Inc., Natick, MA) and the Psychtoolbox 3 toolbox (Brainard, 1997 [↗](#); Kleiner et al., 2007 [↗](#)). On each trial, participants observed a circle at the center of a black screen, subtending a visual angle of 17 degrees. The circle appeared for 6.25 s and changed in luminance with equal power at all frequencies (0–30 Hz). At the end of every trial, participants had to indicate whether a target square, subtending a visual angle of 6 degrees, appeared on that trial. The experiment was performed in a darkened room (for further details, see (Pant et al., 2023 [↗](#))).

EEG Data Analysis

Data analysis was performed using the EEGLab toolbox on MATLAB 2018b (Delorme & Makeig, 2004 [↗](#)). All EEG datasets were filtered using a Hamming windowed sinc FIR filter, with a high-pass cutoff at 1 Hz and a low-pass cutoff at 45 Hz. Eye movement artifacts were detected in the EEG datasets via independent component analysis using the *runica.m* function's *Infomax* algorithm in EEGLab. Components corresponding to horizontal or vertical eye movements were identified via visual inspection and removed (Plöchl et al., 2012 [↗](#)).

The two 3 minutes long resting-state recordings (EC, EO) were divided into epochs of 1 s. Epochs with signals exceeding $\pm 120\text{ }\mu\text{V}$ were rejected for all electrodes. We then calculated the power spectral density of the EO and EC resting-state data using the *pwelch* function (window length = 1000, overlap = 0).

Datasets collected while participants viewed visual stimuli that changed in luminance (LU) were down-sampled to 60 Hz (antialiasing filtering performed by EEGLab's *pop_resample* function) to match the stimulation rate. The datasets were divided into 6.25 s long epochs corresponding to each trial. Subsequently, baseline removal was conducted by subtracting the mean activity across

the length of an epoch from every data point. After baseline removal, epochs with signals exceeding a threshold of $\pm 120 \mu\text{V}$ were rejected in order to exclude potential artifacts. Finally, we calculated the power spectral density of the LU data using the *pwelch* function (window length = 60 samples, overlap = 0).

We calculated the aperiodic (1/f) component of the power spectrum for the EO, EC and LU conditions (Donoghue, Haller, et al., 2020; Schaworonkow & Voytek, 2021 [↗](#)). First, we fit the 1/f distribution function to the frequency spectrum of each participant, separately for each electrode. The 1/f distribution was fit to the normalized spectrum converted to log-log scale (range = 1-20 Hz) (Donoghue, Dominguez, et al., 2020; Gyurkovics et al., 2021 [↗](#); Schaworonkow & Voytek, 2021 [↗](#)). We excluded the alpha range (8-14 Hz) for this fit to avoid biasing the results due to documented differences in alpha activity between CC and SC individuals (Bottari et al., 2016 [↗](#); Ossandón et al., 2023 [↗](#); Pant et al., 2023 [↗](#)). This 1/f fit resulted in a value of the aperiodic slope, an aperiodic intercept value corresponding to the broadband power of 1-20 Hz, and a fit error value for the spectrum of every participant, individually for all electrodes. The visual cortex aperiodic slope and intercept values were obtained by averaging across pre-selected occipital electrodes O1 and O2, giving one value of broadband slope and intercept per participant for the EO, EC and LU conditions (**Figure 1** [↗](#)).

EEG Statistical Analysis

We compared the average visual cortex aperiodic slope and intercept in separate group (2 Levels: CC, SC) by condition (3 levels: EC, EO, LU) ANOVA models.

Visual acuity

Visual acuity was measured for every participant on the date of testing, using the Freiburg Visual Acuity Test (FrACT) (Bach 1996, Bach 2007, <https://michaelbach.de/fract/> [↗](#)). Visual acuity is reported as the logarithm of the mean angle of resolution (logMAR, **Table 1** [↗](#)), wherein higher values indicate worse vision (Elliott, 2016 [↗](#)). As in previous studies, we ran a number of exploratory correlation analyses between GABA+, Glx and Glx/GABA+ concentrations, and visual acuity at the date of testing, duration of visual deprivation, and time since surgery respectively in the CC group (Birch et al., 2009 [↗](#); Guerreiro et al., 2015 [↗](#); Kalia et al., 2014 [↗](#); Rajendran et al., 2020 [↗](#)). We additionally tested the correlation between the aforementioned metrics and chronological age across the CC and SC groups.

Correlation analyses between MRS and EEG measures

Exploratory correlation analyses between EEG and MRS measures were run separately for CC and SC individuals. We calculated correlations between the aperiodic intercept and GABA+, Glx and Glx/GABA+ concentrations. Further, correlations between aperiodic slope, and the concentrations of GABA+, Glx and Glx/GABA+ were assessed. MRS measures collected at rest with eyes open (EO) and eyes closed (EC) were correlated with the corresponding resting-state EEG conditions (EO, EC). The correlation between EEG data collected while participants viewed flickering stimuli (LU) calculated with GABA+, Glx and Glx/GABA+ concentration measured while participants' eyes were open at rest. We did not have prior hypotheses as to the best of our knowledge no extant literature has tested the correlation between aperiodic EEG activity and MRS measures of GABA+, Glx and Glx/GABA+. Therefore, we corrected for multiple comparisons using the Bonferroni correction (6 comparisons).

Results

Transient visual deprivation lowered Glx/GABA+ concentration in the visual cortex

The water-normalized Glx/GABA+ concentration ratio was significantly lower in the visual cortex of congenital cataract-reversal (CC) than age-matched, normally sighted control (SC) individuals (main effect of group: $F(1,39) = 5.80$, $p = 0.021$) (Figure 2). This effect did not vary with eye closure (main effect of condition: $F(1,39) = 2.29$, $p = 0.139$, group-by-condition interaction: $F(1,39) = 1.15$, $p = 0.290$). As a control for unspecified effects of surgery or visual deprivation on neurochemistry, the frontal cortex Glx/GABA+ concentration was compared between groups. There was no difference between CC and SC individuals in their frontal cortex Glx/GABA+ concentration (main effect of group: $F(1,37) = 0.05$, $p = 0.82$, main effect of condition: $F(1,37) = 2.98$, $p = 0.093$, group-by-condition interaction: $F(1,37) = 0.09$, $p = 0.76$) (Figure 2).

When separately comparing CC and SC individuals' GABA+ and Glx concentrations in the visual cortex, we did not find any group difference (GABA+ main effect of group: $F(1,39) = 2.5$, $p = 0.12$, main effect of condition: $F(1,39) = 0.6$, $p = 0.43$, group-by-condition interaction: $F(1,39) = 0.03$, $p = 0.86$; Glx main effect of group: $F(1,39) = 2.8$, $p = 0.103$, main effect of condition: $F(1,39) = 1.8$, $p = 0.19$, group-by-condition interaction: $F(1,39) = 1.27$, $p = 0.27$) (Figure 2). In the frontal cortex, GABA+ and Glx concentrations did not vary either with group or condition (all p values > 0.19) (Figure 2).

Glx/GABA+ concentration measured when CC individuals' eyes were closed correlated positively with visual acuity on the logMAR scale ($r = 0.65$, $p = 0.044$), indicating that CC individuals with higher Glx/GABA+ values had worse visual acuity (Figure 2C, Supplementary Material S5). The same correlation was not significant for the eyes opened condition ($r = -0.042$, $p = 0.908$) (Figure 2C). Duration of deprivation and time since surgery did not significantly predict Glx/GABA+, GABA+ or Glx concentrations in the CC group (all p values > 0.088 , Supplementary Material S6).

No difference in NAA concentration between CC and SC individuals' visual cortices

As a control measure to ensure that between-group differences were specific to hypothesized changes in Glx and GABA+ concentrations, we compared the NAA concentration between CC and SC individuals. The NAA concentration did not significantly differ between groups, neither in their visual (main effect of group: $F(1,39) = 0.03$, $p = 0.87$, main effect of condition: $F(1,39) = 0.31$, $p = 0.58$, group-by-condition interaction: $F(1,39) = 0.09$, $p = 0.76$) nor their frontal cortices (main effect of group: $F(1,37) = 1.1$, $p = 0.297$, main effect of condition: $F(1,37) = 0.14$, $p = 0.71$, group-by-condition interaction: $F(1,37) = 0.03$, $p = 0.86$) (Supplementary Material S1, Figure S1).

Transient visual deprivation resulted in a steeper aperiodic slope and higher aperiodic intercept at occipital sites

The aperiodic slope (1-20 Hz), measured via EEG as an electrophysiological estimate of the E/I ratio (Gao et al., 2017; Muthukumaraswamy & Liley, 2018), was compared between CC and SC individuals. The aperiodic slope was significantly steeper i.e. more negative, at occipital electrodes in CC than in SC individuals ($F(1,59) = 13.1$, $p < 0.001$) (Figure 3). Eye closure and visual stimulation did not affect the steepness of the aperiodic slope ($F(2,59) = 0.78$, $p = 0.465$, group-by-condition interaction: $F(2,59) = 0.12$, $p = 0.885$).

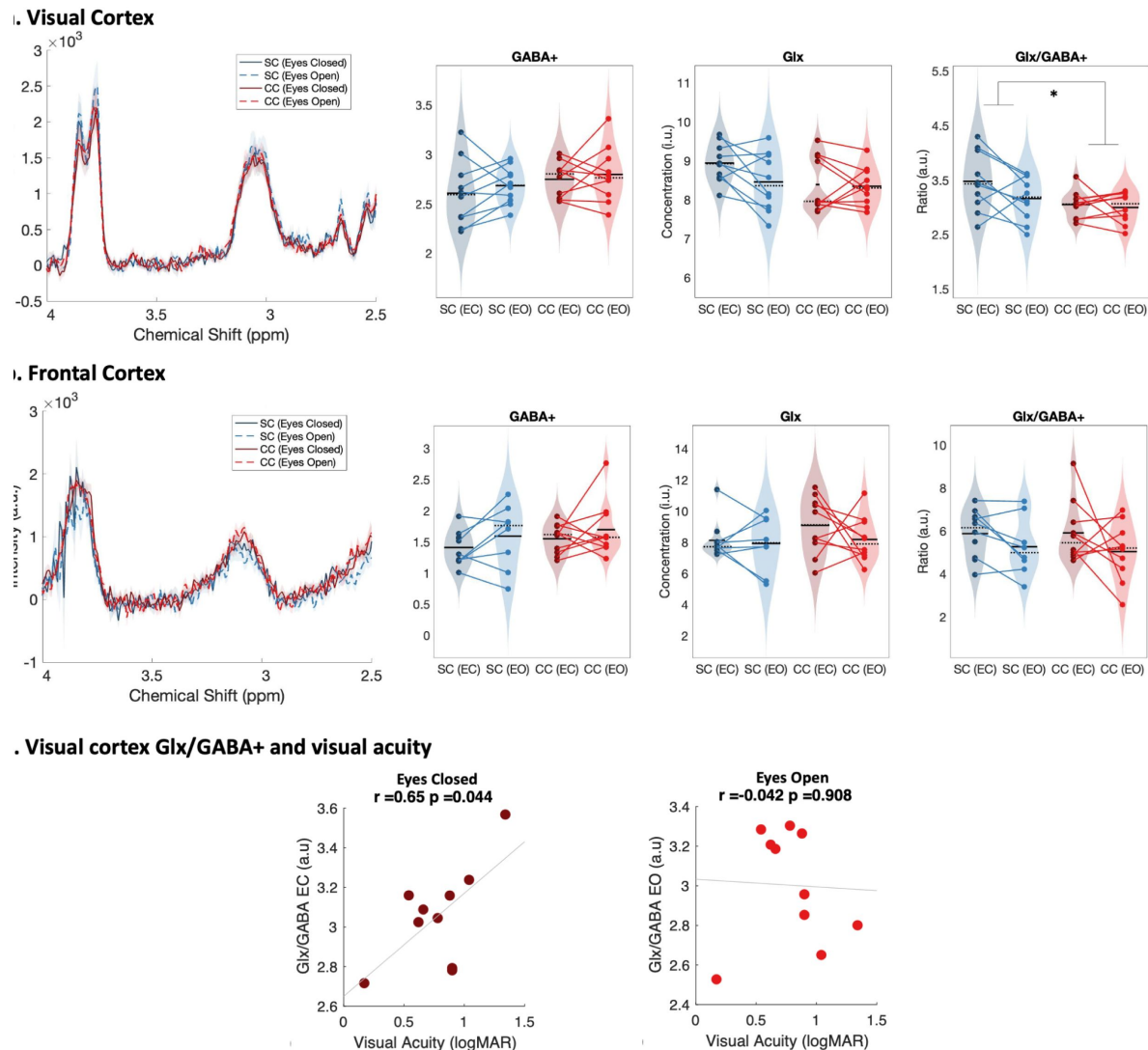
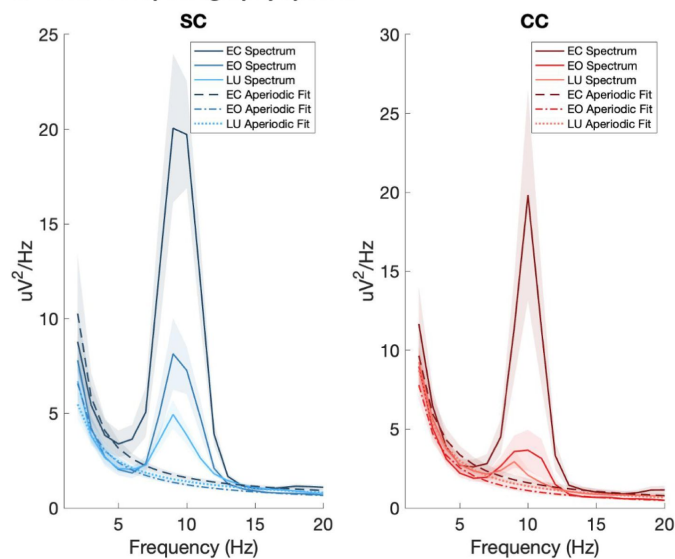


Figure 2

Edited spectra obtained from Magnetic Resonance Spectroscopy (MRS).

a. Average edited spectra showing GABA+ and edited Glx peaks in the visual cortices of normally sighted individuals (SC, green) and individuals with reversed congenital cataracts (CC, red) are shown. Edited MRS DIFF spectra are separately displayed for the eyes open (EO) and eyes closed (EC) conditions using dashed and solid lines respectively. The standard error of the mean is shaded. Water-normalized GABA+, Glx and Glx/GABA+ concentration distributions for each group and condition are depicted as violin plots on the right. The solid black lines indicate mean values and dotted lines indicate median values. The coloured lines connect values of individual participants across conditions. b. Corresponding average edited MRS spectra and water-normalized GABA+, Glx and Glx/GABA+ concentration distributions measured from the frontal cortex are displayed. c. Correlations between visual cortex Glx/GABA+ concentrations in the visual cortex of CC individuals and visual acuity in logMAR units are depicted for the eyes closed (EC, left) and eyes open (EO, right) conditions.

a. Electroencephalography spectra



b. Aperiodic intercept and slope

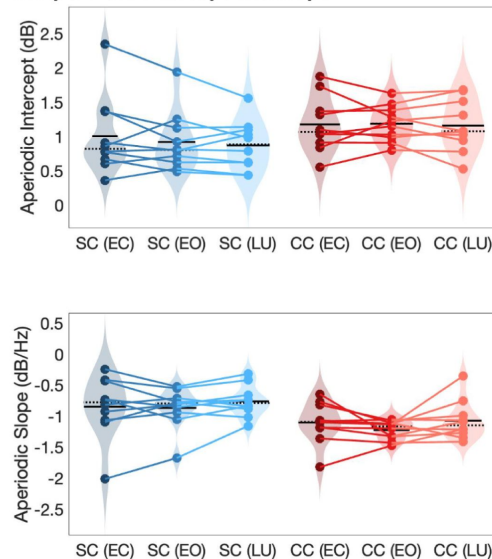


Figure 3

Full spectrum and aperiodic activity of the electroencephalogram (EEG).

a. EEG spectra across O1 and O2 with the corresponding aperiodic (1/f) fits for normally sighted individuals (SC, blue, left) and individuals with reversed congenital cataracts (CC, red, right). Spectra of EEG recordings are displayed for the eyes closed (EC) and eyes opened (EO) conditions, as well as while viewing stimuli that changed in luminance (LU). Shaded regions represent the standard error of the mean. b. Aperiodic intercept (top) and slope (bottom) value distributions for each group and condition are displayed as violin plots. Solid black lines indicate mean values, dotted black lines indicate median values. Coloured lines connect values of individual participants across conditions.

The aperiodic intercept (1-20 Hz) was compared between CC and SC individual to estimate group differences in broadband neural activity (Manning et al., 2009; Musall et al., 2014; Winawer et al., 2013) and was found to be significantly larger at occipital electrodes in CC than SC individuals (main effect of group: $F(1,59) = 5.2$, $p = 0.026$) (Figure 3). Eye closure did not affect the magnitude of the aperiodic intercept in either group (main effect of condition: $F(2,59) = 0.16$, $p = 0.848$, group-by-condition interaction: $F(2,59) = 0.11$, $p = 0.892$).

Within the CC group, visual acuity, time since surgery and duration of blindness did not significantly correlate with the aperiodic slope or the intercept (all p 's > 0.083, Supplementary Material S7). Age negatively correlated with the aperiodic intercept across CC and SC individuals, that is, a flattening of the intercept was observed with age. Similar effects of chronological age have been previously observed (Hill et al., 2022; Voytek et al., 2015) (Supplementary Material S6, Supplementary Material S9).

Glx concentration predicted the aperiodic intercept in CC individuals' visual cortices during ambient and flickering visual stimulation

We exploratorily tested the relationship between Glx, GABA+ and Glx/GABA+ measured at rest and the EEG aperiodic intercept measured at rest and during flickering visual stimulation, separately for the CC and the SC group.

Visual cortex Glx concentration in CC individuals was positively correlated with the aperiodic intercept either when participants had their eyes open during rest ($r = 0.91$, $p = 0.001$, Bonferroni corrected) or when they viewed flickering stimuli ($r = 0.90$, $p < 0.001$, Bonferroni corrected). Corresponding correlations were not significant for Glx concentrations in the eyes closed condition ($r = 0.341$, $p > 0.99$, Bonferroni corrected). By contrast, in SC individuals no significant correlation was observed between visual cortex Glx concentration and aperiodic intercept in any condition (all p 's > 0.99, Bonferroni corrected) (Figure 4).

A negative correlation between the aperiodic slope and Glx concentration in CC individuals (i.e., steeper slopes with increasing Glx concentration) was observed during visual stimulation, but did not survive correction for multiple comparisons (Supplementary Material S8). No such correlation was observed between Glx concentration and aperiodic slope in the eyes open or closed conditions. Visual cortex GABA+ concentration and Glx/GABA+ concentration ratios did not significantly correlate with the aperiodic intercept or slope in either CC or SC individuals, during any experimental condition (Supplementary Material S8).

Discussion

Research in non-human animals has provided convincing evidence that the ratio of excitation to inhibition (E/I) in visual cortex is reliant on early visual experience (Froemke, 2015; Haider et al., 2006; Hensch et al., 1998; Takesian & Hensch, 2013; Wu et al., 2022). In parallel, research in humans who were born blind due to dense bilateral cataracts, and received delayed sight restoration surgery in childhood or as adults, has found limited recovery of both basic visual and higher order visual functions (Birch et al., 2009; Röder & Kekunnaya, 2021). The present study tested whether neurotransmitter concentrations and electrophysiological markers of cortical E/I ratio depend on early visual experience in humans, and how possible changes in visual cortex E/I ratio relate to visual recovery. First, we employed Magnetic Resonance Spectroscopy (MRS) and assessed Glutamate/Glutamine (Glx) and Gamma-Aminobutyric Acid (GABA+) concentrations, as well as their ratio, in the visual cortex (Shibata et al., 2017; Steel et al., 2020; Takei et al., 2016). Second, the slope and intercept of the aperiodic spectrum of EEG

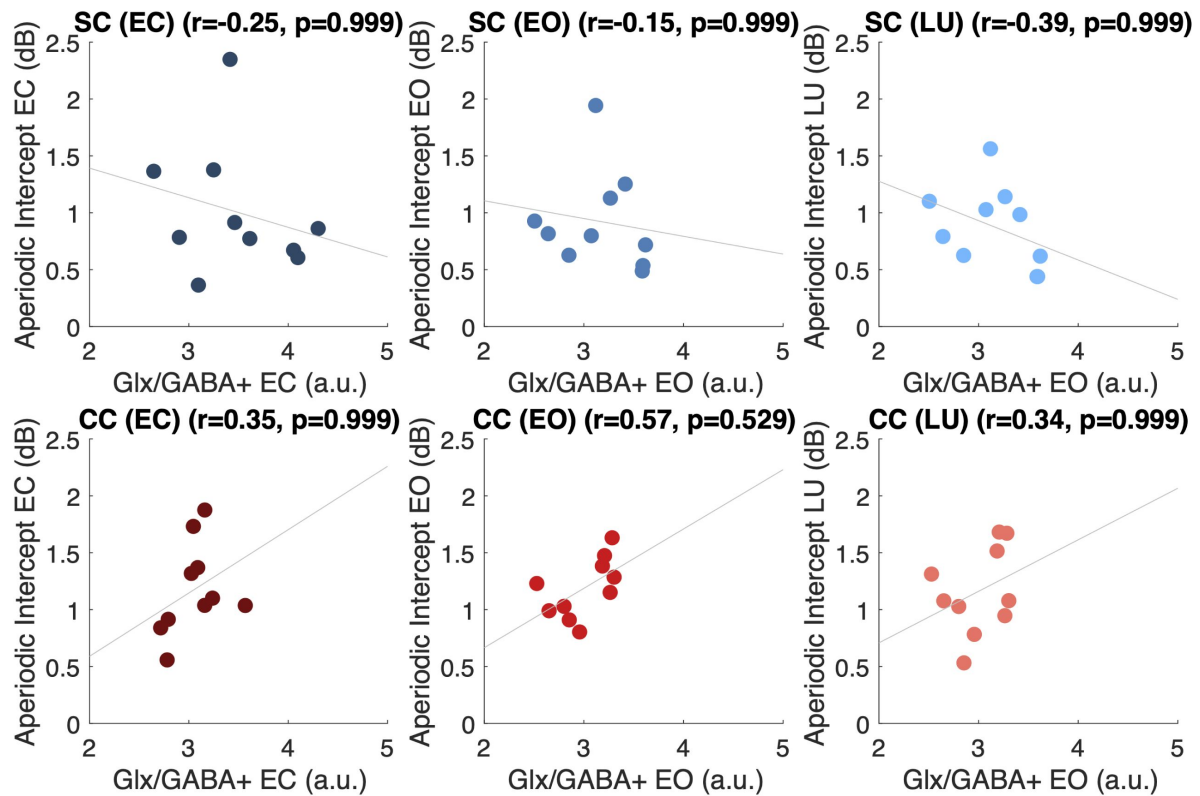


Figure 4

Exploratory correlation analyses between the aperiodic intercept (1-20 Hz) and glutamate/glutamine (Glx) concentration in the visual cortex.

Correlations between water-normalized Glx concentration and aperiodic intercept are shown for the eyes closed (EC, left), eyes open (EO, middle) and visual stimulation (LU, right) conditions for sighted controls (SC, green, top) and individuals with reversed congenital cataracts (CC, red, bottom). Displayed p values are Bonferroni corrected for multiple comparisons.

resting-state activity during eye opening and eye closure (Gao et al., 2017 [↗](#); Muthukumaraswamy & Liley, 2018 [↗](#); Ossandón et al., 2023 [↗](#)), as well as during flickering stimulation (Pant et al., 2023 [↗](#)), were measured over the occipital cortex in the same individuals. The EEG measures allowed us to relate neurotransmitter changes to neural activity changes in congenital cataract-reversal individuals.

We found a lower Glx/GABA+ concentration ratio in the visual cortex of congenital cataract-reversal (CC) individuals as compared to normally sighted controls (SC). Additionally, the slope of the aperiodic EEG power spectrum was steeper for the low frequency range (1-20 Hz), and its intercept was higher in CC than SC individuals. In the CC group, Glx concentration correlated with the intercept of the aperiodic component during ambient and flickering stimulation. Glx/GABA+ concentration ratio during eye closure predicted visual acuity of CC individuals. Together, the present results provide strong evidence for experience-dependent development of the E/I ratio in the human visual cortex, with consequences for behavior.

Altered Glx/GABA+ ratio after sight restoration in congenitally blind humans

Previous MRS studies in the visual cortex of permanently congenitally blind humans reported higher Glx concentrations (Coullon et al., 2015 [↗](#)) in five anophthalmic humans, and numerically lower GABA concentrations in congenitally blind humans (Weaver et al., 2013 [↗](#)) (n = 9), as compared to normally sighted individuals. These results were interpreted as suggesting a higher E/I ratio in the visual cortex of permanently congenitally blind humans, which would be consistent with the extant literature on higher BOLD activity in the visual cortices of the same population (Bedny, 2017 [↗](#); Röder & Kekunnaya, 2022 [↗](#)). We observed a lower Glx/GABA+ ratio in CC individuals, which suggests a lower rather than higher E/I ratio. Our results imply a change in neurotransmitter concentrations as a consequence of *restoring* vision following congenital blindness. Here, we speculate that due to limited structural plasticity after a phase of congenital blindness, the neural circuits of CC individuals, which had adapted to blindness after birth, employ available, likely predominantly physiological plasticity mechanisms (Knudsen, 1998 [↗](#); Mower et al., 1985 [↗](#); Röder et al., 2021 [↗](#)), in order to re-adapt to the newly available visual excitation following sight restoration.

Structural remodeling for typical E/I balance requires visual experience following birth (Hensch & Fagiolini, 2005 [↗](#); Takesian & Hensch, 2013 [↗](#); H. Zhang et al., 2018 [↗](#)) and is linked to a sensitive period (Desai et al., 2002 [↗](#); Hensch & Fagiolini, 2005 [↗](#)). A repeatedly documented finding in permanently congenitally blind humans is the increased thickness of visual cortex (Andelin et al., 2019 [↗](#); Guerreiro et al., 2015 [↗](#); Hölig et al., 2023 [↗](#); Jiang et al., 2009 [↗](#)), which was not observed in late-blind humans (Andelin et al., 2019 [↗](#)). These structural changes in permanently congenitally blind individuals were interpreted as a lack of experience dependent pruning of exuberant synapses and/or reduced myelination typically shifting of the grey-white matter boundary (Natu et al., 2019 [↗](#)). In parallel, it was observed that the overproduction of synapses during the initial phase of brain development in non-human primates was independent of experience, but that synaptic pruning, predominantly of excitatory synapses, depended on visual experience (Bourgeois, 1996 [↗](#); Bourgeois et al., 1989 [↗](#)). This lack of excitatory pruning has been thought to underlie the observed higher excitability of visual cortex due to congenital visual deprivation (Benevento et al., 1992 [↗](#); Huang et al., 2015 [↗](#); Morales et al., 2002 [↗](#)), which was interpreted as a homeostatic adjustment of the E/I ratio resulting from the lack of visual feedforward excitation (Huang et al., 2015 [↗](#); Turrigiano & Nelson, 2004 [↗](#)). Indeed, sight restoration in non-human primates after several months of congenital bilateral lid suture resulted in higher spontaneous firing in visual association cortex (Hyvärinen et al., 1981 [↗](#)). Further, neuronal spiking was increased in dark reared mice when exposed to ambient light (Benevento et al., 1992 [↗](#)). Crucially, increased visual cortex thickness (Feng et al., 2021 [↗](#); Guerreiro et al., 2015 [↗](#); Hölig et al., 2023 [↗](#)) and higher BOLD activity during rest with the eyes open (Raczy et al.,

2022) have been observed for CC individuals as well, suggesting incomplete recovery of cortical structure and function after sight restoration in humans. Thus, the restored feedforward drive to visual cortex after surgery might reach a visual cortex with a lower threshold for excitation, possible due to a relatively higher number of excitatory synapses (Bourgeois, 1996; Morales et al., 2002).

Studies in non-human animals have demonstrated that excitation and inhibition appear to go hand-in-hand (Froemke, 2015; Haider et al., 2006; Isaacson & Scanziani, 2011; Tao & Poo, 2005). Outside of the sensitive period, the visual cortices of CC individuals to maintain neural circuit stability (Lee & Kirkwood, 2019; Turrigiano & Nelson, 2004). An overall reduction in Glx/GABA ratio would counteract the aforementioned adaptations to congenital blindness, e.g. a lower threshold for excitation, which might come with the risk of runaway excitation in the presence of restored visually-elicited excitation. Previous MRS studies have observed a reduction of GABA concentrations in visual cortex (Lunghi et al., 2015) and an increase in the BOLD response (Binda et al., 2018) following monocular blindfolding in adulthood, which was interpreted as indicative of adult homeostatic plasticity. Further, studies in adult mice have provided support for a homeostatic adjustment of the E/I ratio following prolonged changes in neural activity (Chen et al., 2022; Goel & Lee, 2007; Keck et al., 2017; Whitt et al., 2013). For example, a long period of decreased activity following enucleation in adult mice commensurately decreased inhibitory drive (Keck et al., 2011), primarily onto excitatory neurons (Barnes et al., 2015). In line with the lowered Glx/GABA+ ratio being a compensatory measure, the observed correlation of the Glx/GABA+ ratio during eye closure and visual acuity suggests that the more successful this downregulation of the E/I ratio, the better the visual recovery.

The correlation of a lower Glx/GABA+ ratio with better visual acuity is reminiscent of a previously identified correlation in a larger group of CC individuals between decreased visual cortex thickness and better visual acuity (Hölig et al., 2023). Hence, CC individuals with more advanced structural normalization appear to have a better starting point for functional recovery mediated by physiological plasticity.

Glx correlated with the aperiodic intercept of EEG resting-state activity during visual stimulation in congenital cataract-reversal individuals

An increased intercept of the aperiodic component of EEG activity was observed in the same CC individuals who underwent MRS assessment, irrespective of eye opening or eye closure, as well as during flickering stimulation. The intercept of the aperiodic component has been linked to overall neuronal spiking activity (Manning et al., 2009; Musall et al., 2014) and fMRI BOLD activity (Winawer et al., 2013).

Moreover, the slope of the aperiodic component for the low frequency range (1-20 Hz) was steeper in CC individuals. By contrast, the slope of the higher (20-40 Hz) frequency range was flatter in CC than SC individuals (Ossandón et al., 2023). Higher frequencies (such as 20-40 Hz) have been predominantly associated with local circuit activity and feedforward signaling (Bastos et al., 2018; Van Kerkoerle et al., 2014); the increased 20-40 Hz slope may therefore signal increased spontaneous spiking activity in local networks. We speculate that the steeper slope of the aperiodic activity for the lower frequency range (1-20 Hz) in CC individuals reflects the concomitant increase in inhibition.

Interestingly, in CC individuals, the intercept of the aperiodic activity was highly correlated with the Glx concentration during rest with eyes open, and during flickering stimulation (also see Supplementary Material S11). Based on the assumption that the aperiodic intercept reflects broadband firing (Manning et al., 2009; Winawer et al., 2013), this suggests that the Glx

concentration might be related to broadband firing in CC individuals during active and passive visual stimulation. As this is the first study testing a relationship between aperiodic EEG parameters and MRS parameters in humans, we interpret this finding based on results from non-human animals. In typically developed adults, sparse neuronal coding (Toyoizumi et al., 2013) after synaptic pruning reflected decorrelated activity (Chini et al., 2022; Sompolinsky et al., 2001; Trägenap et al., 2023; Vinje & Gallant, 2000). Given that visual deprivation resulted in interrupted pruning of predominantly excitatory synapses in monkeys' visual cortices (Bourgeois, 1996, 1997), we speculate that in the absence of adequate inhibitory tuning for incoming visual input, restored feedforward drive might result in more correlated spiking activity in CC individuals. Indeed, poorly tuned visual responses were evidenced in prior work with CC individuals, including larger (population) receptive fields (Heitmann et al., 2023) and a broader scalp topography of the first cortical visual event related potential (Sourav et al., 2018), as well as higher gamma band activity (Ossandón et al., 2023), which has been linked to firing in feedforward circuits (Van Kerkoerle et al., 2014). Electrophysiological studies with CC individuals have additionally demonstrated a reduced stimulus-selectivity of visual association cortex (Le Grand et al., 2001; Röder et al., 2013; Segalowitz et al., 2017), consistent with imprecise neural representations in this population.

Why might an enhanced inhibitory drive, as indicated by the lower Glx/GABA ratio and, possibly, the 1-20 Hz range of aperiodic EEG activity, not allow for complete recovery of neural tuning? As mentioned above, E/I balance is reflective of multiple interacting mechanisms, and typically maintained by both feedforward inhibition and feedback-mediated inhibition (Froemke, 2015; Isaacson & Scanziani, 2011; Keck et al., 2017; Wu et al., 2022). Feedback connectivity typically emerges later than feedforward connectivity (Batardière et al., 2002; Burkhalter, 1993), and thus is likely more susceptible to damage from early sensory deprivation (Magrou et al., 2018; Yusuf et al., 2022). The markedly reduced alpha oscillatory activity during rest (Ossandón et al., 2023) and in response to white-noise stimulation (Pant et al., 2023) has been interpreted as indirect evidence for deficits in recurrent connectivity within the visual system of CC individuals. Moreover, deficits in the higher harmonic responses and intermodulation frequency responses in steady state visual-evoked responses (Pitchaimuthu et al., 2021), both indicative of bidirectional processing in visual cortex (Kim et al., 2011), have been reported as additional evidence for altered recurrent circuitry in CC individuals. Therefore, the downregulated E/I ratio in CC individuals reflected in the present study likely differs from fine-tuned inhibition within recurrent networks necessary for typical visual processing.

Aperiodic EEG activity correlated with chronological age in sight recovery individuals and normally sighted controls

In both the CC and SC group, the intercept of the aperiodic component of EEG activity decreased with chronological age, irrespective of condition. This chronological age effect replicates corresponding earlier reports in healthy populations (Hill et al., 2022; Voytek et al., 2015). The effect of chronological age in the present study is, again, similar to a correspondingly preserved effect of chronological age on the cortical thickness in CC individuals in cross-sectionally assessed MRI data (Hölig et al., 2023). The reduction of visual cortex thickness during childhood, after an initial increase, is a well-documented trend in brain development (Gilmore et al., 2020; Natu et al., 2019). Thus, we speculate that some typical developmental changes emerge despite aberrant visual experience.

Limitations

To the best of our knowledge, the present study is the first assessment of neurotransmitter concentrations within the visual cortex of sight recovery individuals with a history of congenital blindness by employing non-invasive MRS.

We are aware that MRS has a low spatial specificity. Moreover, MRS measures do not allow us to distinguish between presynaptic, postsynaptic and vesicular neurotransmitter concentrations. However, previous work has validated the link between MRS measures of GABA and Glutamate and the activity of inhibitory and excitatory neuronal assemblies, respectively, both in rodents and non-human primates (Bielicki et al., 2004 [↗](#); Takado et al., 2022 [↗](#)). Further, our phantom testing showed high correlations between the experimentally varied metabolite concentrations and the extracted GABA and Glx concentrations, validating the employed assessment and analysis pipelines (Figure S3). Finally, all reported group differences in MRS parameters were specific to visual cortex, and were not found for the frontal control voxel.

The sample size of the present study is relatively high for the rare population, but undoubtedly, overall, rather small. We nevertheless think that our results are valid. Our findings neurochemically (Glx and GABA+ concentration), and anatomically (visual cortex) specific. The MRS parameters varied with parameters of the aperiodic EEG activity and visual acuity. The group differences for the EEG assessments corresponded to those of a larger sample of CC individuals (n=38) (Ossandón et al., 2023 [↗](#)), and effects of chronological age were as expected from the literature.

Conclusion

The present study in sight recovery individuals with a history of congenital blindness indicates that E/I balance is a result of early experience, and crucial for human behavior. We provide evidence that E/I balance in sight recovery individuals is altered even years after surgery, which might result from the previous adaptation to congenital blindness.

Conflict of interest

Dr. Sunitha Lingareddy is the Managing Director Radiology at Lucid Medical Diagnostics, Hyderabad, India. All other authors have no conflicts to declare.

Author contributions

RP, KP, JO, JF and BR conceptualized the study. RP, KP, IS and PR collected the data. IS, PR and RK diagnosed, recruited and provided clinical assessments of participants. RP, JO and KP analyzed the data. BR and JF supervised data analysis and methodological decisions. BR, RK and SL provided infrastructure, resources and funding. RP and BR wrote the original draft of the manuscript, and all authors provided edits and reviews on the final draft of the manuscript.

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Reviewer #1 (Public Review):

Summary:

In this human neuroimaging and electrophysiology study, the authors aimed to characterize the effects of a period of visual deprivation in the sensitive period on excitatory and inhibitory balance in the visual cortex. They attempted to do so by comparing neurochemistry conditions ('eyes open', 'eyes closed') and resting state, and visually evoked EEG activity between ten congenital cataract patients with recovered sight (CC), and ten age-matched control participants (SC) with normal sight.

First, they used magnetic resonance spectroscopy to measure in vivo neurochemistry from two locations, the primary location of interest in the visual cortex, and a control location in the frontal cortex. Such voxels are used to provide a control for the spatial specificity of any effects because the single-voxel MRS method provides a single sampling location. Using MR-visible proxies of excitatory and inhibitory neurotransmission, Glx and GABA+ respectively, the authors report no group effects in GABA+ or Glx, no difference in the functional conditions 'eyes closed' and 'eyes open'. They found an effect of the group in the ratio of Glx/GABA+ and no similar effect in the control voxel location. They then performed multiple exploratory correlations between MRS measures and visual acuity, and reported a weak positive correlation between the 'eyes open' condition and visual acuity in CC participants.

The same participants then took part in an EEG experiment. The authors selected only two electrodes placed in the visual cortex for analysis and reported a group difference in an EEG

index of neural activity, the aperiodic intercept, as well as the aperiodic slope, considered a proxy for cortical inhibition. They report an exploratory correlation between the aperiodic intercept and Glx in one out of three EEG conditions.

The authors report the difference in E/I ratio, and interpret the lower E/I ratio as representing an adaptation to visual deprivation, which would have initially caused a higher E/I ratio. Although intriguing, the strength of evidence in support of this view is not strong. Amongst the limitations are the low sample size, a critical control cohort that could provide evidence for a higher E/I ratio in CC patients without recovered sight for example, and lower data quality in the control voxel.

Strengths of study:

How sensitive period experience shapes the developing brain is an enduring and important question in neuroscience. This question has been particularly difficult to investigate in humans. The authors recruited a small number of sight-recovered participants with bilateral congenital cataracts to investigate the effect of sensitive period deprivation on the balance of excitation and inhibition in the visual brain using measures of brain chemistry and brain electrophysiology. The research is novel, and the paper was interesting and well-written.

Limitations:

- Low sample size. Ten for CC and ten for SC, and a further two SC participants were rejected due to a lack of frontal control voxel data. The sample size limits the statistical power of the dataset and increases the likelihood of effect inflation.
- Lack of specific control cohort. The control cohort has normal vision. The control cohort is not specific enough to distinguish between people with sight loss due to different causes and patients with congenital cataracts with co-morbidities. Further data from more specific populations, such as patients whose cataracts have not been removed, with developmental cataracts, or congenitally blind participants, would greatly improve the interpretability of the main finding. The lack of a more specific control cohort is a major caveat that limits a conclusive interpretation of the results.
- MRS data quality differences. Data quality in the control voxel appears worse than in the visual cortex voxel. The frontal cortex MRS spectrum shows far broader linewidth than the visual cortex (Supplementary Figures). Compared to the visual voxel, the frontal cortex voxel has less defined Glx and GABA+ peaks; lower GABA+ and Glx concentrations, lower NAA SNR values; lower NAA concentrations. If the data quality is a lot worse in the FC, then small effects may not be detectable.
- Because of the direction of the difference in E/I, the authors interpret their findings as representing signatures of sight improvement after surgery without further evidence, either within the study or from the literature. However, the literature suggests that plasticity and visual deprivation drive the E/I index up rather than down. Decreasing GABA+ is thought to facilitate experience-dependent remodelling. What evidence is there that cortical inhibition increases in response to a visual cortex that is over-sensitised due to congenital cataracts? Without further experimental or literature support this interpretation remains very speculative.
- Heterogeneity in the patient group. Congenital cataract (CC) patients experienced a variety of duration of visual impairment and were of different ages. They presented with co-morbidities (absorbed lens, strabismus, nystagmus). Strabismus has been associated with abnormalities in GABAergic inhibition in the visual cortex. The possible interactions with residual vision and confounds of co-morbidities are not experimentally controlled for in the correlations, and not discussed.

- Multiple exploratory correlations were performed to relate MRS measures to visual acuity (shown in Supplementary Materials), and only specific ones were shown in the main document. The authors describe the analysis as exploratory in the 'Methods' section. Furthermore, the correlation between visual acuity and E/I metric is weak, and not corrected for multiple comparisons. The results should be presented as preliminary, as no strong conclusions can be made from them. They can provide a hypothesis to test in a future study.

- P.16 Given the correlation of the aperiodic intercept with age ("Age negatively correlated with the aperiodic intercept across CC and SC individuals, that is, a flattening of the intercept was observed with age"), age needs to be controlled for in the correlation between neurochemistry and the aperiodic intercept. Glx has also been shown to negatively correlate with age.

- Multiple exploratory correlations were performed to relate MRS to EEG measures (shown in Supplementary Materials), and only specific ones were shown in the main document. Given the multiple measures from the MRS, the correlations with the EEG measures were exploratory, as stated in the text, p.16, and in Figure 4. Yet the introduction said that there was a prior hypothesis "We further hypothesized that neurotransmitter changes would relate to changes in the slope and intercept of the EEG aperiodic activity in the same subjects." It would be great if the text could be revised for consistency and the analysis described as exploratory.

- The analysis for the EEG needs to take more advantage of the available data. As far as I understand, only two electrodes were used, yet far more were available as seen in their previous study (Ossandon et al., 2023). The spatial specificity is not established. The authors could use the frontal cortex electrode (FP1, FP2) signals as a control for spatial specificity in the group effects, or even better, all available electrodes and correct for multiple comparisons. Furthermore, they could use the aperiodic intercept vs Glx in SC to evaluate the specificity of the correlation to CC.

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Reviewer #2 (Public Review):

Summary:

The manuscript reports non-invasive measures of activity and neurochemical profiles of the visual cortex in congenitally blind patients who recovered vision through the surgical removal of bilateral dense cataracts. The declared aim of the study is to find out how restoring visual function after several months or years of complete blindness impacts the balance between excitation and inhibition in the visual cortex.

Strengths:

The findings are undoubtedly useful for the community, as they contribute towards characterising the many ways this special population differs from normally sighted individuals. The combination of MRS and EEG measures is a promising strategy to estimate a fundamental physiological parameter - the balance between excitation and inhibition in the visual cortex, which animal studies show to be heavily dependent upon early visual experience. Thus, the reported results pave the way for further studies, which may use a similar approach to evaluate more patients and control groups.

Weaknesses:

The main issue is the lack of an appropriate comparison group or condition to delineate the effect of sight recovery (as opposed to the effect of congenital blindness). Few previous studies suggested an increased excitation/Inhibition ratio in the visual cortex of congenitally blind patients; the present study reports a decreased E/I ratio instead. The authors claim that this implies a change of E/I ratio following sight recovery. However, supporting this claim would require showing a shift of E/I after vs. before the sight-recovery surgery, or at least it would require comparing patients who did and did not undergo the sight-recovery surgery (as common in the field).

MR Spectroscopy shows a reduced GLX/GABA ratio in patients vs. sighted controls; however, this finding remains rather isolated, not corroborated by other observations. The difference between patients and controls only emerges for the GLX/GABA ratio, but there is no accompanying difference in either the GLX or the GABA concentrations. There is an attempt to relate the MRS data with acuity measurements and electrophysiological indices, but the explorative correlational analyses do not help to build a coherent picture. A bland correlation between GLX/GABA and visual impairment is reported, but this is specific to the patients' group (N=10) and would not hold across groups (the correlation is positive, predicting the lowest GLX/GABA ratio values for the sighted controls - the opposite of what is found). There is also a strong correlation between GLX concentrations and the EEG power at the lowest temporal frequencies. Although this relation is intriguing, it only holds for a very specific combination of parameters (of the many tested): only with eyes open, only in the patient group.

For these reasons, the reported findings do not allow us to draw firm conclusions on the relation between EEG parameters and E/I ratio or on the impact of early (vs. late) visual experience on the excitation/inhibition ratio of the human visual cortex.

<https://doi.org/10.7554/eLife.98143.1.sa1>

Reviewer #3 (Public Review):

This manuscript examines the impact of congenital visual deprivation on the excitatory/inhibitory (E/I) ratio in the visual cortex using Magnetic Resonance Spectroscopy (MRS) and electroencephalography (EEG) in individuals whose sight was restored. Ten individuals with reversed congenital cataracts were compared to age-matched, normally sighted controls, assessing the cortical E/I balance and its interrelationship to visual acuity. The study reveals that the Glx/GABA ratio in the visual cortex and the intercept and aperiodic signal are significantly altered in those with a history of early visual deprivation, suggesting persistent neurophysiological changes despite visual restoration.

My expertise is in EEG (particularly in the decomposition of periodic and aperiodic activity) and statistical methods. I have several major concerns in terms of methodological and statistical approaches along with the (over)interpretation of the results. These major concerns are detailed below.

(1) Variability in visual deprivation:

- The document states a large variability in the duration of visual deprivation (probably also the age at restoration), with significant implications for the sensitivity period's impact on visual circuit development. The variability and its potential effects on the outcomes need thorough exploration and discussion.

(2) Sample size:

- The small sample size is a major concern as it may not provide sufficient power to detect subtle effects and/or overestimate significant effects, which then tend not to generalize to new data. One of the biggest drivers of the replication crisis in neuroscience.
- The main problem with the correlation analyses between MRS and EEG measures is that the sample size is simply too small to conduct such an analysis. Moreover, it is unclear from the methods section that this analysis was only conducted in the patient group (which the reviewer assumed from the plots), and not explained why this was done only in the patient group. I would highly recommend removing these correlation analyses.

(3) Statistical concerns:

- The statistical analyses, particularly the correlations drawn from a small sample, may not provide reliable estimates (see <https://www.sciencedirect.com/science/article/pii/S0092656613000858>, which clearly describes this problem).
- Statistical analyses for the MRS: The authors should consider some additional permutation statistics, which are more suitable for small sample sizes. The current statistical model (2x2) design ANOVA is not ideal for such small sample sizes. Moreover, it is unclear why the condition (EO & EC) was chosen as a predictor and not the brain region (visual & frontal) or neurochemicals. Finally, the authors did not provide any information on the alpha level nor any information on correction for multiple comparisons (in the methods section). Finally, even if the groups are matched w.r.t. age, the time between surgery and measurement, the duration of visual deprivation, (and sex?), these should be included as covariates as it has been shown that these are highly related to the measurements of interest (especially for the EEG measurements) and the age range of the current study is large.
- EEG statistical analyses: The same critique as for the MRS statistical analyses applies to the EEG analysis. In addition: was the 2x3 ANOVA conducted for EO and EC independently? This seems to be inconsistent with the approach in the MRS analyses, in which the authors chose EO & EC as predictors in their 2x2 ANOVA.
- Figure 4: The authors report a p-value of >0.999 with a correlation coefficient of -0.42 with a sample size of 10 subjects. This can't be correct (it should be around: $p = 0.22$). All statistical analyses should be checked.
- Figure 2c. Eyes closed condition: The highest score of the *Glx/GABA ratio seems to be ~3.6. In subplot 2a, there seem to be 3 subjects that show a Glx/GABA ratio score > 3.6. How can this be explained? There is also a discrepancy for the eyes-closed condition.

(4) Interpretation of aperiodic signal:

- Several recent papers demonstrated that the aperiodic signal measured in EEG or ECoG is related to various important aspects such as age, skull thickness, electrode impedance, as well as cognition. Thus, currently, very little is known about the underlying effects which influence the aperiodic intercept and slope. The entire interpretation of the aperiodic slope as a proxy for E/I is based on a computational model and simulation (as described in the Gao et al. paper).
- Especially the aperiodic intercept is a very sensitive measure to many influences (e.g. skull thickness, electrode impedance...). As crucial results (correlation aperiodic intercept and MRS measures) are facing this problem, this needs to be reevaluated. It is safer to make statements on the aperiodic slope than intercept. In theory, some of the potentially confounding measures are available to the authors (e.g. skull thickness can be computed from T1w images; electrode impedances are usually acquired alongside the EEG data) and could be therefore controlled.

- The authors wrote: "Higher frequencies (such as 20-40 Hz) have been predominantly associated with local circuit activity and feedforward signaling (Bastos et al., 2018; Van Kerkoerle et al., 2014); the increased 20-40 Hz slope may therefore signal increased spontaneous spiking activity in local networks. We speculate that the steeper slope of the aperiodic activity for the lower frequency range (1-20 Hz) in CC individuals reflects the concomitant increase in inhibition." The authors confuse the interpretation of periodic and aperiodic signals. This section refers to the interpretation of the periodic signal (higher frequencies). This interpretation can not simply be translated to the aperiodic signal (slope).

- The authors further wrote: We used the slope of the aperiodic (1/f) component of the EEG spectrum as an estimate of E/I ratio (Gao et al., 2017; Medel et al., 2020; Muthukumaraswamy & Liley, 2018). This is a highly speculative interpretation with very little empirical evidence. These papers were conducted with ECoG data (mostly in animals) and mostly under anesthesia. Thus, these studies only allow an indirect interpretation by what the 1/f slope in EEG measurements is actually influenced.

(5) Problems with EEG preprocessing and analysis:

- It seems that the authors did not identify bad channels nor address the line noise issue (even a problem if a low pass filter of below-the-line noise was applied).

- What was the percentage of segments that needed to be rejected due to the 120 μ V criteria? This should be reported specifically for EO & EC and controls and patients.

- The authors downsampled the data to 60Hz to "to match the stimulation rate". What is the intention of this? Because the subsequent spectral analyses are conflated by this choice (see Nyquist theorem).

- "Subsequently, baseline removal was conducted by subtracting the mean activity across the length of an epoch from every data point." The actual baseline time segment should be specified.

- "We excluded the alpha range (8-14 Hz) for this fit to avoid biasing the results due to documented differences in alpha activity between CC and SC individuals (Bottari et al., 2016; Ossandón et al., 2023; Pant et al., 2023)." This does not really make sense, as the FOOOF algorithm first fits the 1/f slope, for which the alpha activity is not relevant.

- The model fits of the 1/f fitting for EO, EC, and both participant groups should be reported.

(6) Validity of GABA measurements and results:

- According to a newer study by the authors of the Gannet toolbox (<https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/abs/10.1002/nbm.5076>), the reliability and reproducibility of the gamma-aminobutyric acid (GABA) measurement can vary significantly depending on acquisition and modeling parameter. Thus, did the author address these challenges? Furthermore, the authors wrote: "We confirmed the within-subject stability of metabolite quantification by testing a subset of the sighted controls (n=6) 2-4 weeks apart. Looking at the supplementary Figure 5 (which would be rather plotted as ICC or Blant-Altman plots), the within-subject stability compared to between-subject variability seems not to be great. Furthermore, I don't think such a small sample size qualifies for a rigorous assessment of stability."

- "Why might an enhanced inhibitory drive, as indicated by the lower Glx/GABA ratio" Is this interpretation really warranted, as the results of the group differences in the Glx/GABA ratio seem to be rather driven by a decreased Glx concentration in CC rather than an increased GABA (see Figure 2).

- Glx concentration predicted the aperiodic intercept in CC individuals' visual cortices during ambient and flickering visual stimulation. Why specifically investigate the Glx concentration, when the paper is about E/I ratio?

(7) Interpretation of the correlation between MRS measurements and EEG aperiodic signal:

- The authors wrote: "The intercept of the aperiodic activity was highly correlated with the Glx concentration during rest with eyes open and during flickering stimulation (also see Supplementary Material S11). Based on the assumption that the aperiodic intercept reflects broadband firing (Manning et al., 2009; Winawer et al., 2013), this suggests that the Glx concentration might be related to broadband firing in CC individuals during active and passive visual stimulation." These results should not be interpreted (or with very caution) for several reasons (see also problem with influences on aperiodic intercept and small sample size). This is a result of the exploratory analyses of correlating every EEG parameter with every MRS parameter. This requires well-powered replication before any interpretation can be provided. Furthermore and importantly: why should this be specifically only in CC patients, but not in the SC control group?

(8) Language and presentation:

- The manuscript requires language improvements and correction of numerous typos. Oversimplifications and unclear statements are present, which could mislead or confuse readers (see also interpretation of aperiodic signal).

- The authors state that "Together, the present results provide strong evidence for experience-dependent development of the E/I ratio in the human visual cortex, with consequences for behavior." The results of the study do not provide any strong evidence, because of the small sample size and exploratory analyses approach and not accounting for possible confounding factors.

- "Our results imply a change in neurotransmitter concentrations as a consequence of *restoring* vision following congenital blindness." This is a speculative statement to infer a causal relationship on cross-sectional data.

- In the limitation section, the authors wrote: "The sample size of the present study is relatively high for the rare population, but undoubtedly, overall, rather small." This sentence should be rewritten, as the study is plain underpowered. The further justification "We nevertheless think that our results are valid. Our findings neurochemically (Glx and GABA+ concentration), and anatomically (visual cortex) specific. The MRS parameters varied with parameters of the aperiodic EEG activity and visual acuity. The group differences for the EEG assessments corresponded to those of a larger sample of CC individuals (n=38) (Ossandón et al., 2023), and effects of chronological age were as expected from the literature." These statements do not provide any validation or justification of small samples. Furthermore, the current data set is a subset of an earlier published paper by the same authors "The EEG data sets reported here were part of data published earlier (Ossandón et al., 2023; Pant et al., 2023)." Thus, the statement "The group differences for the EEG assessments corresponded to those of a larger sample of CC individuals (n=38)" is a circular argument and should be avoided.

<https://doi.org/10.7554/eLife.98143.1.sa0>

Author response:

eLife assessment

This potentially useful study involves neuro-imaging and electrophysiology in a small cohort of congenital cataract patients after sight recovery and age-matched control participants with normal sight. It aims to characterize the effects of early visual deprivation on excitatory and inhibitory balance in the visual cortex. While the findings are taken to suggest the existence of persistent alterations in Glx/GABA ratio and aperiodic EEG signals, the evidence supporting these claims is incomplete. Specifically, small sample sizes, lack of a specific control cohort, and other methodological limitations will likely restrict the usefulness of the work, with relevance limited to scientists working in this particular subfield.

As pointed out in the public reviews, there are only very few human models which allow for assessing the role of early experience on neural circuit development. While the prevalent research in permanent congenital blindness reveals the response and adaptation of the developing brain to an atypical situation (blindness), research in sight restoration addresses the question of whether and how atypical development can be remediated if typical experience (vision) is restored. The literature on the role of visual experience in the development of E/I balance in humans, assessed via Magnetic Resonance Spectroscopy (MRS), has been limited to a few studies on congenital permanent blindness. Thus, we assessed sight recovery individuals with a history of congenital blindness, as limited evidence from other researchers indicated that the visual cortex E/I ratio might differ compared to normally sighted controls.

Individuals with total bilateral congenital cataracts who remained untreated until later in life are extremely rare, particularly if only carefully diagnosed patients are included in a study sample. A sample size of 10 patients is, at the very least, typical of past studies in this population, even for exclusively behavioral assessments. In the present study, in addition to behavioral assessment as an indirect measure of sensitive periods, we investigated participants with two neuroimaging methods (Magnetic Resonance Spectroscopy and electroencephalography) to directly assess the neural correlates of sensitive periods in humans. The electroencephalography data allowed us to link the results of our small sample to findings documented in large cohorts of both, sight recovery individuals and permanently congenitally blind individuals. As pointed out in a recent editorial recommending an “exploration-then-estimation procedure,” (“Consideration of Sample Size in Neuroscience Studies,” 2020), exploratory studies like ours provide crucial direction and specific hypotheses for future work.

We included an age-matched sighted control group recruited from the same community, measured in the same scanner and laboratory, to assess whether early experience is necessary for a typical excitatory/inhibitory (E/I) ratio to emerge in adulthood. The present findings indicate that this is indeed the case. Based on these results, a possible question to answer in future work, with individuals who had developmental cataracts, is whether later visual deprivation causes similar effects. Note that even if visual deprivation at a later stage in life caused similar effects, the current results would not be invalidated; by contrast, they are essential to understand future work on late (permanent or transient) blindness.

Thus, we think that the present manuscript has far reaching implications for our understanding of the conditions under which E/I balance, a crucial characteristic of brain functioning, emerges in humans.

Finally, our manuscript is one of the first few studies which relates MRS neurotransmitter concentrations to parameters of EEG aperiodic activity. Since present research has been using aperiodic activity as a correlate of the E/I ratio, and partially of higher cognitive functions, we

think that our manuscript additionally contributes to a better understanding of what might be measured with aperiodic neurophysiological activity.

Public Reviews:

Reviewer #1 (Public Review):

Summary:

In this human neuroimaging and electrophysiology study, the authors aimed to characterize the effects of a period of visual deprivation in the sensitive period on excitatory and inhibitory balance in the visual cortex. They attempted to do so by comparing neurochemistry conditions ('eyes open', 'eyes closed') and resting state, and visually evoked EEG activity between ten congenital cataract patients with recovered sight (CC), and ten age-matched control participants (SC) with normal sight.

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Strengths of study:

How sensitive period experience shapes the developing brain is an enduring and important question in neuroscience. This question has been particularly difficult to investigate in humans. The authors recruited a small number of sight-recovered participants with bilateral congenital cataracts to investigate the effect of sensitive period deprivation on the balance of excitation and inhibition in the visual brain using measures of brain chemistry and brain electrophysiology. The research is novel, and the paper was interesting and well-written.

Limitations:

(1.1) Low sample size. Ten for CC and ten for SC, and a further two SC participants were rejected due to a lack of frontal control voxel data. The sample size limits the statistical power of the dataset and increases the likelihood of effect inflation.

Applying strict criteria, we only included individuals who were born with no patterned vision in the CC group. The population of individuals who have remained untreated past infancy is small in India, despite a higher prevalence of childhood cataract than Germany. Indeed, from the original 11 CC and 11 SC participants tested, one participant each from the CC and SC group had to be rejected, as their data had been corrupted, resulting in 10 participants in each group.

It was a challenge to recruit participants from this rare group with no history of neurological diagnosis/intake of neuromodulatory medications, who were able and willing to undergo both MRS and EEG. For this study, data collection took more than 1.5 years.

We took care of the validity of our results with two measures; first, assessed not just MRS, but additionally, EEG measures of E/I ratio. The latter allowed us to link results to a larger population of CC individuals, that is, we replicated the results of a larger group of 38 individuals (Ossandón et al., 2023) in our sub-group.

Second, we included a control voxel. As predicted, all group effects were restricted to the occipital voxel.

(1.2) Lack of specific control cohort. The control cohort has normal vision. The control cohort is not specific enough to distinguish between people with sight loss due to different causes and patients with congenital cataracts with co-morbidities. Further data from more specific populations, such as patients whose cataracts have not been removed, with developmental cataracts, or congenitally blind participants, would greatly improve the interpretability of the main finding. The lack of a more specific control cohort is a major caveat that limits a conclusive interpretation of the results.

The existing work on visual deprivation and neurochemical changes, as assessed with MRS, has been limited to permanent congenital blindness. In fact, most of the studies on permanent blindness included only congenitally blind or early blind humans (Coullon et al., 2015; Weaver et al., 2013), or, in separate studies, only late-blind individuals (Bernabeu et al., 2009). Thus, accordingly, we started with the most “extreme” visual deprivation model, sight recovery after congenital blindness. If we had not observed any group difference compared to normally sighted controls, investigating other groups might have been trivial. Based on our results, subsequent studies in late blind individuals, and then individuals with developmental cataracts, can be planned with clear hypotheses.

(1.3) MRS data quality differences. Data quality in the control voxel appears worse than in the visual cortex voxel. The frontal cortex MRS spectrum shows far broader linewidth than the visual cortex (Supplementary Figures). Compared to the visual voxel, the frontal cortex voxel has less defined Glx and GABA+ peaks; lower GABA+ and Glx concentrations, lower NAA SNR values; lower NAA concentrations. If the data quality is a lot worse in the FC, then small effects may not be detectable.

Worse data quality in the frontal than the visual cortex has been repeatedly observed in the MRS literature, attributable to magnetic field distortions (Juchem & Graaf, 2017) resulting from the proximity of the region to the sinuses (recent example: (Rideaux et al., 2022)). Nevertheless, we chose the frontal control region rather than a parietal voxel, given the potential neurochemical changes in multisensory regions of the parietal cortex due to blindness. Such reorganization would be less likely in frontal areas associated with higher cognitive functions. Further, prior MRS studies of the visual cortex have used the frontal cortex as a control region as well (Pitchaimuthu et al., 2017; Rideaux et al., 2022).

In the present study, we checked that the frontal cortex datasets for Glx and GABA+ concentrations were of sufficient quality: the fit error was below 8.31% in both groups

(Supplementary Material S3). For reference, Mikkelsen et al. reported a mean GABA+ fit error of 6.24 +/- 1.95% from a posterior cingulate cortex voxel across 8 GE scanners, using the Gannet pipeline. No absolute cutoffs have been proposed for fit errors. However, MRS studies in special populations (I/E ratio assessed in narcolepsy (Gao et al., 2024), GABA concentration assessed in Autism Spectrum Disorder (Maier et al., 2022)) have used frontal cortex data with a fit error of <10% to identify differences between cohorts (Gao et al., 2024; Pitchaimuthu et al., 2017). Based on the literature, MRS data from the frontal voxel of the present study would have been of sufficient quality to uncover group differences.

In the revised manuscript, we will add the recently published MRS quality assessment form to the supplementary materials. Additionally, we would like to allude to our apriori prediction of group differences for the visual cortex, but not for the frontal cortex voxel.

(1.4) Because of the direction of the difference in E/I, the authors interpret their findings as representing signatures of sight improvement after surgery without further evidence, either within the study or from the literature. However, the literature suggests that plasticity and visual deprivation drive the E/I index up rather than down. Decreasing GABA+ is thought to facilitate experience-dependent remodelling. What evidence is there that cortical inhibition increases in response to a visual cortex that is over-sensitised due to congenital cataracts? Without further experimental or literature support this interpretation remains very speculative.

Indeed, higher inhibition was not predicted, which we attempt to reconcile in our discussion section. We base our discussion mainly on the non-human animal literature, which has shown evidence of homeostatic changes after prolonged visual deprivation in the adult brain (Barnes et al., 2015). It is also interesting to note that after monocular deprivation in adult humans, resting GABA+ levels decreased in the visual cortex (Lunghi et al., 2015). Assuming that after delayed sight restoration, adult neuroplasticity mechanisms must be employed, these studies would predict a “balancing” of the increased excitatory drive following sight restoration by a commensurate increase in inhibition (Keck et al., 2017). Additionally, the EEG results of the present study allowed for speculation regarding the underlying neural mechanisms of an altered E/I ratio. The aperiodic EEG activity suggested higher spontaneous spiking (increased intercept) and increased inhibition (steeper aperiodic slope between 1-20 Hz) in CC vs SC individuals (Ossandón et al., 2023).

In the revised manuscript, we will more clearly indicate that these speculations are based primarily on non-human animal work, due to the lack of human studies on the subject.

(1.5) Heterogeneity in the patient group. Congenital cataract (CC) patients experienced a variety of duration of visual impairment and were of different ages. They presented with co-morbidities (absorbed lens, strabismus, nystagmus). Strabismus has been associated with abnormalities in GABAergic inhibition in the visual cortex. The possible interactions with residual vision and confounds of co-morbidities are not experimentally controlled for in the correlations, and not discussed.

The goal of the present study was to assess whether we would observe changes in E/I ratio after restoring vision at all. We would not have included patients *without* nystagmus in the CC group of the present study, since it would have been unlikely that they experienced congenital patterned visual deprivation. Amongst diagnosticians, nystagmus or strabismus might not be considered genuine “comorbidities” that emerge in people with congenital cataracts. Rather, these are consequences of congenital visual deprivation, which we employed as diagnostic criteria. Similarly, absorbed lenses are clear signs that cataracts were congenital. As in other models of experience dependent brain development (e.g. the extant literature on congenital permanent blindness, including anophthalmic individuals (Coullon et al., 2015; Weaver et al., 2013), some uncertainty remains regarding whether the (remaining,

in our case) abnormalities of the eye, or the blindness they caused, are the factors driving neural changes. In case of people with reversed congenital cataracts, at least the retina is considered to be intact, as they would otherwise not receive cataract removal surgery.

However, we consider it unlikely that strabismus caused the group differences, because the present study shows group differences in the Glx/GABA+ ratio at rest, regardless of eye opening or eye closure, for which strabismus would have caused distinct effects. By contrast, the link between GABA concentration and, for example, interocular suppression in strabismus, have so far been documented during visual stimulation (Mukerji et al., 2022; Sengpiel et al., 2006), and differed in direction depending on the amblyopic vs. non-amblyopic eye. Further, one MRS study did not find group differences in GABA concentration between the visual cortices of 16 amblyopic individuals and sighted controls (Mukerji et al., 2022), supporting that the differences in Glx/GABA+ concentration which we observed were driven by congenital deprivation, and not amblyopia-associated visual acuity or eye movement differences.

In the revised manuscript, we will discuss the inclusion criteria in more detail, and the aforementioned reasons why our data remains interpretable.

(1.6) Multiple exploratory correlations were performed to relate MRS measures to visual acuity (shown in Supplementary Materials), and only specific ones were shown in the main document. The authors describe the analysis as exploratory in the 'Methods' section. Furthermore, the correlation between visual acuity and E/I metric is weak, and not corrected for multiple comparisons. The results should be presented as preliminary, as no strong conclusions can be made from them. They can provide a hypothesis to test in a future study.

In the revised manuscript, we will clearly indicate that the exploratory correlation analyses are reported to put forth hypotheses for future studies.

(1.7) P.16 Given the correlation of the aperiodic intercept with age ("Age negatively correlated with the aperiodic intercept across CC and SC individuals, that is, a flattening of the intercept was observed with age"), age needs to be controlled for in the correlation between neurochemistry and the aperiodic intercept. Glx has also been shown to negatively correlate with age.

The correlation between chronological age and aperiodic intercept was observed across groups, but the correlation between Glx and the intercept of the aperiodic EEG activity was seen only in the CC group, even though the SC group was matched for age. Thus, such a correlation was very unlikely to be predominantly driven by an effect of chronological age.

In the revised manuscript, we will add the linear regressions with age as a covariate included below, for the relationship between aperiodic intercept and Glx concentration in the CC group.

a. A linear regression was conducted within the CC group to predict the intercept during visual stimulation, based on age and visual cortex Glx concentration. The results of the regression analysis indicated that the model explained a significant proportion of the variance in the aperiodic intercept, $R^2=0.82$, $t(2,7)=16.1$, $p=0.0024$. Note that the coefficient for age was not significant, $\beta=0.007$, $t(7)=0.82$, $p=0.439$. The regression coefficients and their respective statistics are presented in Author response table 1.

Author response table 1.

Regression Analysis Summary for Predicting Aperiodic Intercept (Visual Stimulation) in the CC group

Predictor	Estimate	SE	t	p
Model Intercept	-5.75	1.71	-3.36	0.012
Age	0.007	0.008	0.82	0.439
Glx	0.81	0.19	4.36	0.003

b. A linear regression was conducted to predict the intercept during eye opening at rest, based on age and visual cortex Glx concentration. The results of the regression analysis indicated that the model explained a significant proportion of the variance in the aperiodic intercept, $R^2=0.842$, $t_{(2,7)}=18.6$, $p=0.00159$. Note that the coefficient for age was not significant, $\beta=-0.005$, $t(7)=-0.90$, $p=0.400$. The regression coefficients and their respective statistics are presented in Author response table 2.

Author response table 2.

Regression Analysis Summary for Predicting Aperiodic Intercept (Eyes Open) in the CC group

Predictor	Estimate	SE	t	p
Model Intercept	-2.07	1.11	-1.86	0.106
Age	-0.005	0.005	-0.90	0.400
Glx	0.40	0.12	3.35	0.012

c. Given that the Glx coefficient is significant in both models and age does not significantly predict either outcome, it can be concluded that Glx independently predicts the intercept of the aperiodic intercept.

(1.8) Multiple exploratory correlations were performed to relate MRS to EEG measures (shown in Supplementary Materials), and only specific ones were shown in the main document. Given the multiple measures from the MRS, the correlations with the EEG measures were exploratory, as stated in the text, p.16, and in Figure 4. Yet the introduction said that there was a prior hypothesis "We further hypothesized that neurotransmitter changes would relate to changes in the slope and intercept of the EEG aperiodic activity in the same subjects." It would be great if the text could be revised for consistency and the analysis described as exploratory.

In the revised manuscript, we will improve the phrasing. We consider the correlation analyses as exploratory due to our sample size and the absence of prior work. However, we did hypothesize that both MRS and EEG markers would concurrently be altered in CC vs SC individuals.

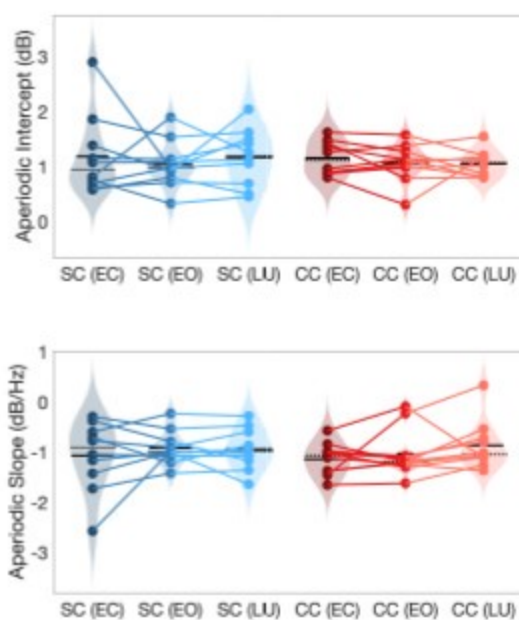
(1.9) The analysis for the EEG needs to take more advantage of the available data. As far as I understand, only two electrodes were used, yet far more were available as seen in

their previous study (Ossandon et al., 2023). The spatial specificity is not established. The authors could use the frontal cortex electrode (FP1, FP2) signals as a control for spatial specificity in the group effects, or even better, all available electrodes and correct for multiple comparisons. Furthermore, they could use the aperiodic intercept vs Glx in SC to evaluate the specificity of the correlation to CC.

The aperiodic intercept and slope did not differ between CC and SC individuals for Fp1 and Fp2, suggesting the spatial specificity of the results. In the revised manuscript, we will add this analysis to the supplementary material.

Author response image 1.

Aperiodic intercept (top) and slope (bottom) for congenital cataract-reversal (CC, red) and age-matched normally sighted control (SC, blue) individuals. Distributions of these parameters are displayed as violin plots for three conditions; at rest with eyes closed (EC), at rest with eyes open (EO) and during visual stimulation (LU). Aperiodic parameters were calculated across electrodes Fp1 and Fp2. Solid black lines indicate mean values, dotted black lines indicate median values. Coloured lines connect values of individual participants across conditions.



Further, Glx concentration in the visual cortex did not correlate with the aperiodic intercept in the SC group (Figure 4), suggesting that this relationship was indeed specific to the CC group.

The data from all electrodes has been analyzed and published in other studies as well (Pant et al., 2023; Ossandón et al., 2023).

Reviewer #2 (Public Review):

Summary:

The manuscript reports non-invasive measures of activity and neurochemical profiles of the visual cortex in congenitally blind patients who recovered vision through the surgical removal of bilateral dense cataracts. The declared aim of the study is to find out how restoring visual function after several months or years of complete blindness impacts the balance between excitation and inhibition in the visual cortex.

Strengths:

The findings are undoubtedly useful for the community, as they contribute towards characterising the many ways this special population differs from normally sighted individuals. The combination of MRS and EEG measures is a promising strategy to estimate a fundamental physiological parameter - the balance between excitation and inhibition in the visual cortex, which animal studies show to be heavily dependent upon early visual experience. Thus, the reported results pave the way for further studies, which may use a similar approach to evaluate more patients and control groups.

Weaknesses:

(2.1) The main issue is the lack of an appropriate comparison group or condition to delineate the effect of sight recovery (as opposed to the effect of congenital blindness). Few previous studies suggested an increased excitation/Inhibition ratio in the visual cortex of congenitally blind patients; the present study reports a decreased E/I ratio instead. The authors claim that this implies a change of E/I ratio following sight recovery. However, supporting this claim would require showing a shift of E/I after vs. before the sight-recovery surgery, or at least it would require comparing patients who did and did not undergo the sight-recovery surgery (as common in the field).

Longitudinal studies would indeed be the best way to test the hypothesis that the lower E/I ratio in the CC group observed by the present study is a consequence of sight restoration. However, longitudinal studies involving neuroimaging are an effortful challenge, particularly in research conducted outside of major developed countries and dedicated neuroimaging research facilities. Crucially, however, had CC and SC individuals, as well as permanently congenitally blind vs SC individuals (Coullon et al., 2015; Weaver et al., 2013), not differed on any neurochemical markers, such a longitudinal study might have been trivial. Thus, in order to justify and better tailor longitudinal studies, cross-sectional studies are an initial step.

(2.2) MR Spectroscopy shows a reduced GLX/GABA ratio in patients vs. sighted controls; however, this finding remains rather isolated, not corroborated by other observations. The difference between patients and controls only emerges for the GLX/GABA ratio, but there is no accompanying difference in either the GLX or the GABA concentrations. There is an attempt to relate the MRS data with acuity measurements and electrophysiological indices, but the explorative correlational analyses do not help to build a coherent picture. A bland correlation between GLX/GABA and visual impairment is reported, but this is specific to the patients' group (N=10) and would not hold across groups (the correlation is positive, predicting the lowest GLX/GABA ratio values for the sighted controls - the opposite of what is found). There is also a strong correlation between GLX concentrations and the EEG power at the lowest temporal frequencies. Although this relation is intriguing, it only holds for a very specific combination of parameters (of the many tested): only with eyes open, only in the patient group.

We interpret these findings differently, that is, in the context of experiments from non-human animals and the larger MRS literature.

Homeostatic control of E/I balance assumes that the ratio of excitation (reflected here by Glx) and inhibition (reflected here by GABA+) is regulated. Like prior work (Gao et al., 2024, 2024;

Narayan et al., 2022; Perica et al., 2022; Steel et al., 2020; Takado et al., 2022; Takei et al., 2016), we assumed that the ratio of Glx/GABA+ is indicative of E/I balance rather than solely the individual neurotransmitter levels. One of the motivations for assessing the ratio vs the absolute concentration is that as per the underlying E/I balance hypothesis, a change in excitation would cause a concomitant change in inhibition, and vice versa, which has been shown in non-human animal work (Fang et al., 2021; Haider et al., 2006; Tao & Poo, 2005) and modeling research (Vreeswijk & Sompolinsky, 1996; Wu et al., 2022). Importantly, our interpretation of the lower E/I ratio is not just from the Glx/GABA+ ratio, but additionally, based on the steeper EEG aperiodic slope (1-20 Hz).

As in the discussion section and response 1.4, we did not expect to see a lower Glx/GABA+ ratio in CC individuals. We discuss the possible reasons for the direction of the correlation with visual acuity and aperiodic offset during passive visual stimulation, and offer interpretations and (testable) hypotheses.

We interpret the direction of the Glx/GABA+ correlation with visual acuity to imply that patients with highest (compensatory) balancing of the consequences of congenital blindness (hyperexcitation), in light of visual stimulation, are those who recover best. Note, the sighted control group was selected based on their “normal” vision. Thus, clinical visual acuity measures are not expected to sufficiently vary, nor have the resolution to show strong correlations with neurophysiological measures. By contrast, the CC group comprised patients highly varying in visual outcomes, and thus were ideal to investigate such correlations.

This holds for the correlation between Glx and the aperiodic intercept, as well. Previous work has suggested that the intercept of the aperiodic activity is associated with broadband spiking activity in neural circuits (Manning et al., 2009). Thus, an atypical increase of spiking activity during visual stimulation, as indirectly suggested by “old” non-human primate work on visual deprivation (Hyvärinen et al., 1981) might drive a correlation not observed in healthy populations.

In the revised manuscript, we will more clearly indicate in the discussion that these are possible post-hoc interpretations. We argue that given the lack of such studies in humans, it is all the more important that extant data be presented completely, even if the direction of the effects are not as expected.

(2.3) For these reasons, the reported findings do not allow us to draw firm conclusions on the relation between EEG parameters and E/I ratio or on the impact of early (vs. late) visual experience on the excitation/inhibition ratio of the human visual cortex.

Indeed, the correlations we have tested between the E/I ratio and EEG parameters were exploratory, and have been reported as such. The goal of our study was not to compare the effects of early vs. late visual experience. The goal was to study whether early visual experience is necessary for a typical E/I ratio in visual neural circuits. We provided clear evidence in favor of this hypothesis. Thus, the present results suggest the necessity of investigating the effects of late visual deprivation. In fact, such research is missing in permanent blindness as well.

Reviewer #3 (Public Review):

This manuscript examines the impact of congenital visual deprivation on the excitatory/inhibitory (E/I) ratio in the visual cortex using Magnetic Resonance Spectroscopy (MRS) and electroencephalography (EEG) in individuals whose sight was restored. Ten individuals with reversed congenital cataracts were compared to age-matched, normally sighted controls, assessing the cortical E/I balance and its interrelationship to visual acuity. The study reveals that the Glx/GABA ratio in the visual cortex and the intercept and aperiodic signal are significantly altered in those with a

history of early visual deprivation, suggesting persistent neurophysiological changes despite visual restoration.

My expertise is in EEG (particularly in the decomposition of periodic and aperiodic activity) and statistical methods. I have several major concerns in terms of methodological and statistical approaches along with the (over)interpretation of the results. These major concerns are detailed below.

(3.1) Variability in visual deprivation:

- The document states a large variability in the duration of visual deprivation (probably also the age at restoration), with significant implications for the sensitivity period's impact on visual circuit development. The variability and its potential effects on the outcomes need thorough exploration and discussion.

We work with a rare, unique patient population, which makes it difficult to systematically assess the effects of different visual histories while maintaining stringent inclusion criteria such as complete patterned visual deprivation at birth. Regardless, we considered the large variance in age at surgery and time since surgery as supportive of our interpretation: group differences were found despite the large variance in duration of visual deprivation. Moreover, the existing variance was used to explore possible associations between behavior and neural measures, as well as neurochemical and EEG measures.

In the revised manuscript, we will detail the advantages and disadvantages of our CC sample, with respect to duration of congenital visual deprivation.

(3.2) Sample size:

- The small sample size is a major concern as it may not provide sufficient power to detect subtle effects and/or overestimate significant effects, which then tend not to generalize to new data. One of the biggest drivers of the replication crisis in neuroscience.

We address the small sample size in our discussion, and make clear that small sample sizes were due to the nature of investigations in special populations. It is worth noting that our EEG results fully align with those of a larger sample of CC individuals (Ossandón et al., 2023), providing us confidence about their validity and reproducibility. Moreover, our MRS results and correlations of those with EEG parameters were spatially specific to occipital cortex measures, as predicted.

The main problem with the correlation analyses between MRS and EEG measures is that the sample size is simply too small to conduct such an analysis. Moreover, it is unclear from the methods section that this analysis was only conducted in the patient group (which the reviewer assumed from the plots), and not explained why this was done only in the patient group. I would highly recommend removing these correlation analyses.

We marked the correlation analyses as exploratory; note that we do not base most of our discussion on the results of these analyses. As indicated by Reviewer 1, reporting them allows for deriving more precise hypothesis for future studies. It has to be noted that we investigate an extremely rare population, tested outside of major developed economies and dedicated neuroimaging research facilities. In addition to being a rare patient group, these individuals come from poor communities. Therefore, we consider it justified to report these correlations as exploratory, providing direction for future research.

(3.3) Statistical concerns:

- The statistical analyses, particularly the correlations drawn from a small sample, may not provide reliable estimates (see <https://www.sciencedirect.com/science/article/pii/S0092656613000858>, which clearly describes this problem).

It would undoubtedly be better to have a larger sample size. We nonetheless think it is of value to the research community to publish this dataset, since 10 multimodal data sets from a carefully diagnosed, rare population, representing a human model for the effects of early experience on brain development, are quite a lot. Sample sizes in prior neuroimaging studies in transient blindness have most often ranged from $n = 1$ to $n = 10$. They nevertheless provided valuable direction for future research, and integration of results across multiple studies provides scientific insights.

Identifying possible group differences was the goal of our study, with the correlations being an exploratory analysis, which we have clearly indicated in the methods, results and discussion.

- Statistical analyses for the MRS: The authors should consider some additional permutation statistics, which are more suitable for small sample sizes. The current statistical model (2x2) design ANOVA is not ideal for such small sample sizes. Moreover, it is unclear why the condition (EO & EC) was chosen as a predictor and not the brain region (visual & frontal) or neurochemicals. Finally, the authors did not provide any information on the alpha level nor any information on correction for multiple comparisons (in the methods section). Finally, even if the groups are matched w.r.t. age, the time between surgery and measurement, the duration of visual deprivation, (and sex?), these should be included as covariates as it has been shown that these are highly related to the measurements of interest (especially for the EEG measurements) and the age range of the current study is large.

In our ANOVA models, the neurochemicals were the outcome variables, and the conditions were chosen as predictors based on prior work suggesting that Glx/GABA+ might vary with eye closure (Kurcyus et al., 2018). The study was designed based on a hypothesis of group differences localized to the occipital cortex, due to visual deprivation. The frontal cortex voxel was chosen to indicate whether these differences were spatially specific. Therefore, we conducted separate ANOVAs based on this study design.

In the revised manuscript, we will add permutation analyses for our outcomes, as well as multiple regression models investigating whether the variance in visual history might have driven these results. Note that in the supplementary materials (S6, S7), we have reported the correlations between visual history metrics and MRS/EEG outcomes.

The alpha level used for the ANOVA models specified in the methods section was 0.05. The alpha level for the exploratory analyses reported in the main manuscript was 0.008, after correcting for (6) multiple comparisons using the Bonferroni correction, also specified in the methods. Note that the p-values following correction are expressed as multiplied by 6, due to most readers assuming an alpha level of 0.05 (see response regarding large p-values).

We used a control group matched for age and sex. Moreover, the controls were recruited and tested in the same institutes, using the same setup. We feel that we followed the gold standards for recruiting a healthy control group for a patient group.

- EEG statistical analyses: The same critique as for the MRS statistical analyses applies to the EEG analysis. In addition: was the 2x3 ANOVA conducted for EO and EC independently? This seems to be inconsistent with the approach in the MRS analyses, in which the authors chose EO & EC as predictors in their 2x2 ANOVA.

The 2x3 ANOVA was not conducted independently for the eyes open/eyes closed condition, the ANOVA conducted on the EEG metrics was 2x3 because it had group (CC, SC) and condition (eyes open (EO), eyes closed (EC) and visual stimulation (LU)) as predictors.

- Figure 4: The authors report a p -value of >0.999 with a correlation coefficient of -0.42 with a sample size of 10 subjects. This can't be correct (it should be around: $p = 0.22$). All statistical analyses should be checked.

As specified in the methods and figure legend, the reported p values in Figure 4 have been corrected using the Bonferroni correction, and therefore multiplied by the number of comparisons, leading to the seemingly large values.

Additionally, to check all statistical analyses, we put the manuscript through an independent Statistics Check (Nuijten & Polanin, 2020) (<https://michelenuijten.shinyapps.io/statcheck-web/>) and will upload the consistency report with the revised supplementary material.

- Figure 2c. Eyes closed condition: The highest score of the *Glx/GABA ratio seems to be ~ 3.6 . In subplot 2a, there seem to be 3 subjects that show a Glx/GABA ratio score > 3.6 . How can this be explained? There is also a discrepancy for the eyes-closed condition.

The three subjects that show the Glx/GABA+ ratio > 3.6 in subplot 2a are in the SC group, whereas the correlations plotted in figure 2c are only for the CC group, where the highest score is indeed ~ 3.6 .

(3.4) Interpretation of aperiodic signal:

- Several recent papers demonstrated that the aperiodic signal measured in EEG or ECoG is related to various important aspects such as age, skull thickness, electrode impedance, as well as cognition. Thus, currently, very little is known about the underlying effects which influence the aperiodic intercept and slope. The entire interpretation of the aperiodic slope as a proxy for E/I is based on a computational model and simulation (as described in the Gao et al. paper).

Apart from the modeling work from Gao et al., multiple papers which have also been cited which used ECoG, EEG and MEG and showed concomitant changes in aperiodic activity with pharmacological manipulation of the E/I ratio (Colombo et al., 2019; Molina et al., 2020; Muthukumaraswamy & Liley, 2018). Further, several prior studies have interpreted changes in the aperiodic slope as reflective of changes in the E/I ratio, including studies of developmental groups (Favaro et al., 2023; Hill et al., 2022; McSweeney et al., 2023; Schaworonkow & Voytek, 2021) as well as patient groups (Molina et al., 2020; Ostlund et al., 2021).

In the revised manuscript, we will cite those studies not already included in the introduction.

- Especially the aperiodic intercept is a very sensitive measure to many influences (e.g. skull thickness, electrode impedance...). As crucial results (correlation aperiodic intercept and MRS measures) are facing this problem, this needs to be reevaluated. It is safer to make statements on the aperiodic slope than intercept. In theory, some of the potentially confounding measures are available to the authors (e.g. skull thickness can be computed from T1w images; electrode impedances are usually acquired alongside the EEG data) and could be therefore controlled.

All electrophysiological measures indeed depend on parameters such as skull thickness and electrode impedance. As in the extant literature using neurophysiological measures to

compare brain function between patient and control groups, we used a control group matched in age/ sex, recruited in the same region, tested with the same devices, and analyzed with the same analysis pipeline. For example, impedance was kept below 10 kOhm for all subjects. There is no evidence available suggesting that congenital cataracts are associated with changes in skull thickness that would cause the observed pattern of group results. Moreover, we cannot think of how any of the exploratory correlations between neurophysiological measures and MRS measures could be accounted for by a difference e.g. in skull thickness.

- The authors wrote: "Higher frequencies (such as 20-40 Hz) have been predominantly associated with local circuit activity and feedforward signaling (Bastos et al., 2018; Van Kerkoerle et al., 2014); the increased 20-40 Hz slope may therefore signal increased spontaneous spiking activity in local networks. We speculate that the steeper slope of the aperiodic activity for the lower frequency range (1-20 Hz) in CC individuals reflects the concomitant increase in inhibition." The authors confuse the interpretation of periodic and aperiodic signals. This section refers to the interpretation of the periodic signal (higher frequencies). This interpretation cannot simply be translated to the aperiodic signal (slope).

Prior work has not always separated the aperiodic and periodic components, making it unclear what might have driven these effects in our data. The interpretation of the higher frequency range was intended to contrast with the interpretations of lower frequency range, in order to speculate as to why the two aperiodic fits might go in differing directions. We will clarify our interpretation in the revised manuscript. Note that Ossandon et al. reported highly similar results (group differences for CC individuals and for permanently congenitally blind humans) for the aperiodic activity between 20-40 Hz and oscillatory activity in the gamma range. We will allude to these findings in the revised manuscript.

- The authors further wrote: We used the slope of the aperiodic (1/f) component of the EEG spectrum as an estimate of E/I ratio (Gao et al., 2017; Medel et al., 2020; Muthukumaraswamy & Liley, 2018). This is a highly speculative interpretation with very little empirical evidence. These papers were conducted with ECoG data (mostly in animals) and mostly under anesthesia. Thus, these studies only allow an indirect interpretation by what the 1/f slope in EEG measurements is actually influenced.

Note that Muthukumaraswamy et al. (2018) used different types of pharmacological manipulations and analyzed periodic and aperiodic MEG activity in addition to monkey ECoG (Medel et al., 2020) (now published as (Medel et al., 2023)) compared EEG activity in addition to ECoG data after propofol administration. The interpretation of our results are in line with a number of recent studies in developing (Hill et al., 2022; Schaworonkow & Voytek, 2021) and special populations using EEG. As mentioned above, several prior studies have used the slope of the 1/f component/aperiodic activity as an indirect measure of the E/I ratio (Favaro et al., 2023; Hill et al., 2022; McSweeney et al., 2023; Molina et al., 2020; Ostlund et al., 2021; Schaworonkow & Voytek, 2021), including studies using scalp-recorded EEG. We will make more clear in the introduction of the revised manuscript that this metric is indirect.

While a full understanding of aperiodic activity needs to be provided, some convergent ideas have emerged . We think that our results contribute to this enterprise, since our study is, to the best of our knowledge, the first which assessed MRS measured neurotransmitter levels and EEG aperiodic activity.

(3.5) Problems with EEG preprocessing and analysis:

- It seems that the authors did not identify bad channels nor address the line noise issue (even a problem if a low pass filter of below-the-line noise was applied).

As pointed out in the methods and Figure 1, we only analyzed data from two channels, O1 and O2, neither of which were rejected for any participant. Channel rejection was performed for the larger dataset, published elsewhere (Ossandón et al., 2023; Pant et al., 2023).

In both published works, we did not consider frequency ranges above 40 Hz to avoid any possible contamination with line noise. Here, we focused on activity between 0 and 20 Hz, definitely excluding line noise contaminations. The low pass filter (FIR, 1-45 Hz) guaranteed that any spill-over effects of line noise would be restricted to frequencies just below the upper cutoff frequency.

Additionally, a prior version of the analysis used the `cleanline.m` function to remove line noise before filtering, and the group differences remained stable. We will report this analysis in the supplementary version of the revised manuscript. Further, both groups were measured in the same lab, making line noise as an account for the observed group effects highly unlikely. Finally, any of the exploratory MRS-EEG correlations would be hard to explain if the EEG parameters would be contaminated with line noise.

- What was the percentage of segments that needed to be rejected due to the 120 μ V criteria? This should be reported specifically for EO & EC and controls and patients.

The mean percentage of 1 second segments rejected for each resting state condition is below. Mean percentage of 6.25 long segments rejected in each group for the visual stimulation condition are also included, and will be added to the revised manuscript:

Author response table 3.

Group	Eyes Open	Eyes Closed	Visual Stimulation
CC	13%	3.1%	0.2%
SC	18.37%	2%	0.1%

- The authors downsampled the data to 60Hz to "to match the stimulation rate". What is the intention of this? Because the subsequent spectral analyses are conflated by this choice (see Nyquist theorem).

This data were collected as part of a study designed to evoke alpha activity with visual white-noise, which ranged in luminance with equal power at all frequencies from 1-60 Hz, restricted by the refresh rate of the monitor on which stimuli were presented (Pant et al., 2023). This paradigm and method was developed by VanRullen and colleagues (Schwenk et al., 2020; Vanrullen & MacDonald, 2012), wherein the analysis requires the same sampling rate between the presented frequencies and the EEG data. The downsampling function used here automatically applies an anti-aliasing filter (EEGLAB 2019) .

- "Subsequently, baseline removal was conducted by subtracting the mean activity across the length of an epoch from every data point." The actual baseline time segment should be specified.

The time segment was the length of the epoch, that is, 1 second for the resting state conditions and 6.25 seconds for the visual stimulation conditions. This will be explicitly stated in the revised manuscript.

- "We excluded the alpha range (8-14 Hz) for this fit to avoid biasing the results due to documented differences in alpha activity between CC and SC individuals (Bottari et al.,

2016; Ossandón et al., 2023; Pant et al., 2023)." This does not really make sense, as the FOOOF algorithm first fits the 1/f slope, for which the alpha activity is not relevant.

We did not use the FOOOF algorithm/toolbox in this manuscript. As stated in the methods, we used a 1/f fit to the 1-20 Hz spectrum in the log-log space, and subtracted this fit from the original spectrum to obtain the corrected spectrum. Given the pronounced difference in alpha power between groups (Bottari et al., 2016; Ossandón et al., 2023; Pant et al., 2023), we were concerned it might drive differences in the exponent values. Our analysis pipeline had been adapted from previous publications of our group and other labs (Ossandón et al., 2023; Voytek et al., 2015; Waschke et al., 2017).

We have conducted the analysis with and without the exclusion of the alpha range, as well as using the FOOOF toolbox both in the 1-20 Hz and 20-40 Hz ranges (Ossandón et al., 2023); The findings of a steeper slope in the 1-20 Hz range as well as lower alpha power in CC vs SC individuals remained stable. In Ossandón et al., the comparison between the piecewise fits and FOOOF fits led the authors to use the former as it outperformed the FOOOF algorithm for their data.

- The model fits of the 1/f fitting for EO, EC, and both participant groups should be reported.

In Figure 3 of the manuscript, we depicted the mean spectra and 1/f fits for each group. We will add the fit quality metrics and show individual subjects' fits in the revised manuscript.

(3.6) Validity of GABA measurements and results:

- According to a newer study by the authors of the Gannet toolbox (<https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/abs/10.1002/nbm.5076>), the reliability and reproducibility of the gamma-aminobutyric acid (GABA) measurement can vary significantly depending on acquisition and modeling parameter. Thus, did the author address these challenges?

We took care of data quality while acquiring MRS data by ensuring appropriate voxel placement and linewidth prior to scanning. Acquisition as well as modeling parameters were constant for both groups, so they cannot have driven group differences.

The linked article compares the reproducibility of GABA measurement using Osprey, which was released in 2020 and uses linear combination modeling to fit the peak as opposed to Gannet's simple peak fitting (Hupfeld et al., 2024). The study finds better test-retest reliability for Osprey compared to Gannet's method.

As the present work was conceptualized in 2018, we used Gannet 3.0, which was the state-of-the-art edited spectral analysis toolbox at the time, and still is widely used. In the revised manuscript, we will include a supplementary section reanalyzing the main findings with Osprey.

- Furthermore, the authors wrote: "We confirmed the within-subject stability of metabolite quantification by testing a subset of the sighted controls (n=6) 2-4 weeks apart. Looking at the supplementary Figure 5 (which would be rather plotted as ICC or Blant-Altman plots), the within-subject stability compared to between-subject variability seems not to be great. Furthermore, I don't think such a small sample size qualifies for a rigorous assessment of stability."

Indeed, we did not intend to provide a rigorous assessment of within-subject stability. Rather, we aimed to confirm that data quality/concentration ratios did not systematically differ

between the same subjects tested longitudinally; driven, for example, by scanner heating or time of day. As with the phantom testing, we attempted to give readers an idea of the quality of the data, as they were collected from a primarily clinical rather than a research site.

In the revised manuscript we will remove the statement regarding stability, and add the Blant-Altman plot.

- *"Why might an enhanced inhibitory drive, as indicated by the lower Glx/GABA ratio" Is this interpretation really warranted, as the results of the group differences in the Glx/GABA ratio seem to be rather driven by a decreased Glx concentration in CC rather than an increased GABA (see Figure 2).*

We used the Glx/GABA+ ratio as a measure, rather than individual Glx or GABA+ concentration, which did not significantly differ between groups. As detailed in Response 2.2, we think this metric aligns better with an underlying E/I balance hypothesis and has been used in many previous studies (Gao et al., 2024; Liu et al., 2015; Narayan et al., 2022; Perica et al., 2022).

Our interpretation of an enhanced inhibitory drive additionally comes from the combination of aperiodic EEG (1-20 Hz) and MRS measures, which, when considered together, are consistent with a decreased E/I ratio.

In the revised manuscript, we will rephrase this sentence accordingly.

- *Glx concentration predicted the aperiodic intercept in CC individuals' visual cortices during ambient and flickering visual stimulation. Why specifically investigate the Glx concentration, when the paper is about E/I ratio?*

As stated in the methods, we exploratorily assessed the relationship between all MRS parameters (Glx, GABA+ and Glx/GABA+ ratio) with the aperiodic parameters (slope, offset), and corrected for multiple comparisons accordingly. We think this is a worthwhile analysis considering the rarity of the dataset/population (see 1.2, 1.6, 2.1 and reviewer 1's comments about future hypotheses). We only report the Glx – aperiodic intercept correlation in the main manuscript as it survived correction for multiple comparisons.

(3.7) *Interpretation of the correlation between MRS measurements and EEG aperiodic signal:*

- *The authors wrote: "The intercept of the aperiodic activity was highly correlated with the Glx concentration during rest with eyes open and during flickering stimulation (also see Supplementary Material S11). Based on the assumption that the aperiodic intercept reflects broadband firing (Manning et al., 2009; Winawer et al., 2013), this suggests that the Glx concentration might be related to broadband firing in CC individuals during active and passive visual stimulation." These results should not be interpreted (or with very caution) for several reasons (see also problem with influences on aperiodic intercept and small sample size). This is a result of the exploratory analyses of correlating every EEG parameter with every MRS parameter. This requires well-powered replication before any interpretation can be provided. Furthermore and importantly: why should this be specifically only in CC patients, but not in the SC control group?*

We indicate clearly in all parts of the manuscript that these correlations are presented as exploratory. Further, we interpret the Glx-aperiodic offset correlation, and none of the others, as it survived the Bonferroni correction for multiple comparisons. We offer a hypothesis in the discussion section as to why such a correlation might exist in the CC but not the SC group (see response 2.2), and do not speculate further.

(3.8) Language and presentation:

- The manuscript requires language improvements and correction of numerous typos. Over-simplifications and unclear statements are present, which could mislead or confuse readers (see also interpretation of aperiodic signal).

In the revision, we will check that speculations are clearly marked and typos are removed.

- The authors state that "Together, the present results provide strong evidence for experience-dependent development of the E/I ratio in the human visual cortex, with consequences for behavior." The results of the study do not provide any strong evidence, because of the small sample size and exploratory analyses approach and not accounting for possible confounding factors.

We disagree with this statement and allude to convergent evidence of both MRS and neurophysiological measures. The latter link to corresponding results observed in a larger sample of CC individuals (Ossandón et al., 2023).

- "Our results imply a change in neurotransmitter concentrations as a consequence of *restoring* vision following congenital blindness." This is a speculative statement to infer a causal relationship on cross-sectional data.

As mentioned under 2.1, we conducted a cross-sectional study which might justify future longitudinal work. In order to advance science, new testable hypotheses were put forward at the end of a manuscript.

In the revised manuscript we will add "might imply" to better indicate the hypothetical character of this idea.

- In the limitation section, the authors wrote: "The sample size of the present study is relatively high for the rare population, but undoubtedly, overall, rather small." This sentence should be rewritten, as the study is plain underpowered. The further justification "We nevertheless think that our results are valid. Our findings neurochemically (Glx and GABA+ concentration), and anatomically (visual cortex) specific. The MRS parameters varied with parameters of the aperiodic EEG activity and visual acuity. The group differences for the EEG assessments corresponded to those of a larger sample of CC individuals (n=38) (Ossandón et al., 2023), and effects of chronological age were as expected from the literature." These statements do not provide any validation or justification of small samples. Furthermore, the current data set is a subset of an earlier published paper by the same authors "The EEG data sets reported here were part of data published earlier (Ossandón et al., 2023; Pant et al., 2023)." Thus, the statement "The group differences for the EEG assessments corresponded to those of a larger sample of CC individuals (n=38)" is a circular argument and should be avoided.

Our intention was not to justify having a small sample, but to justify why we think the results might be valid as they align with/replicate existing literature.

In the revised manuscript, we will add a figure showing that the EEG results of the 10 subjects considered here correspond to those of the 28 other subjects of Ossandón et al. We will adapt the text accordingly, clearly stating that the pattern of EEG results of the ten subjects reported here replicate those of the 28 additional subjects of Ossandón et al. (2023).

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