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

Rashi Pant[1], Kabilan Pitchaimuthu[1,2], José Ossandón[1], Idris Shareef[3,4], Sunitha Lingareddy[5], Jürgen Finsterbusch[6], Ramesh Kekunnaya[3], Brigitte Röder[1]

[1]Department of Psychology and Human Movement Science, University of Hamburg, Hamburg, Germany
[2]Department of Medicine and Optometry, Linnaeus University, Kalmar, Sweden
[3]Child Sight Institute, Jasti V Ramanamma Children's Eye Care Centre, LV Prasad Eye Institute, Hyderabad, India
[4]Department of Psychology, University of Nevada, Reno, USA
[5]LUCID Medical Diagnostics, Hyderabad, India
[6] Institute of Systems Neuroscience, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

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.

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; Toyoizumi 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 (Heitmann et al., 2023; Raczy 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 (Raczy 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 (Raczy 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 an 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) 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; Liu et al., 2015; Steel et al., 2020; Takei et al., 2016; L. Zhang et al., 2020). 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). Additionally we calculated the aperiodic intercept, which has previously been linked to broadband neuronal firing (Manning et al., 2009; Musall et al., 2014; Winawer et al., 2013). 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) 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).

	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*

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).

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.

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.

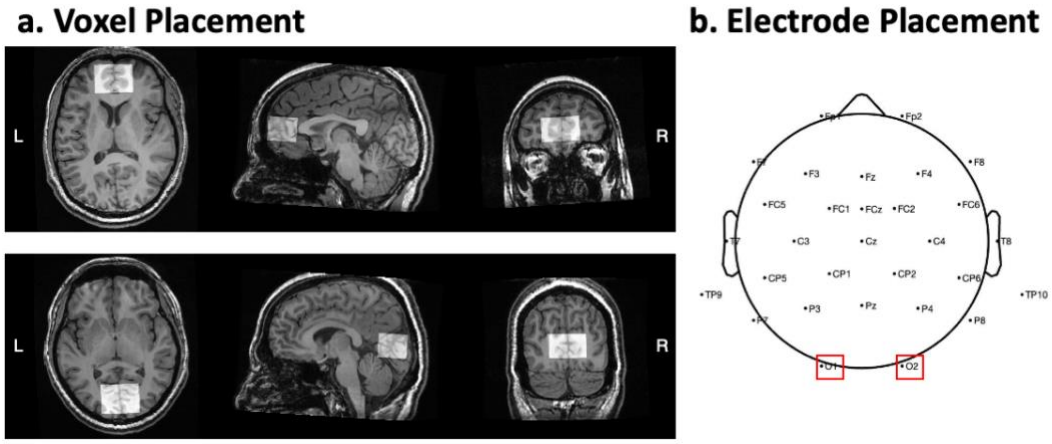


Figure 1: Voxel placement for Magnetic Resonance Spectroscopy and electrode placement for Electroencephalography. *a. Position of the frontal cortex (top) and visual cortex (bottom) voxels in a single subject. Figures output from GannetCoRegister. b. Electrode montage according to the 10/20 electrode system with marked occipital electrodes (red) preselected for analysis.*

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 mm x 30 mm x 25mm, 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,* G*annetSegment* and *GannetQuantify* functions (Edden et al., 2014). Briefly, reported concentration values were corrected for the differences in GABA concentration and relaxation times between different kinds of tissue in the voxel (grey matter, white matter and cerebrospinal fluid) (Harris et al., 2015). 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). 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). Further, the Glx/GABA+ ratio has been employed in prior studies of visual (Takei et al., 2016; L. Zhang et al., 2020), cingulate (Bezalel et al., 2019), frontal (Liu et al., 2015) and auditory cortex (Grent-'t-Jong et al., 2022).

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) and medial-temporal cortex (Träber et al., 2006) of neuro-typical individuals as well as in various pathologies across visual, frontal and temporal cortex (Paslakis et al., 2014), for example, schizophrenia (Mullins et al., 2003). 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) or permanent early blindness (Weaver et al., 2013) in humans. TARQUIN 4.3.11 was employed to analyze the OFF-spectrum data (Wilson et al., 2011) 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).

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), in conjunction with considering a given study's sample size (Mikkelsen et al., 2018). 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). Note that CRLB values above 50% are considered unreliable (Wilson et al., 2019). 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; Jenkins et al., 2019). 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) 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 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 ±120 µV 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 ±120 µV 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.

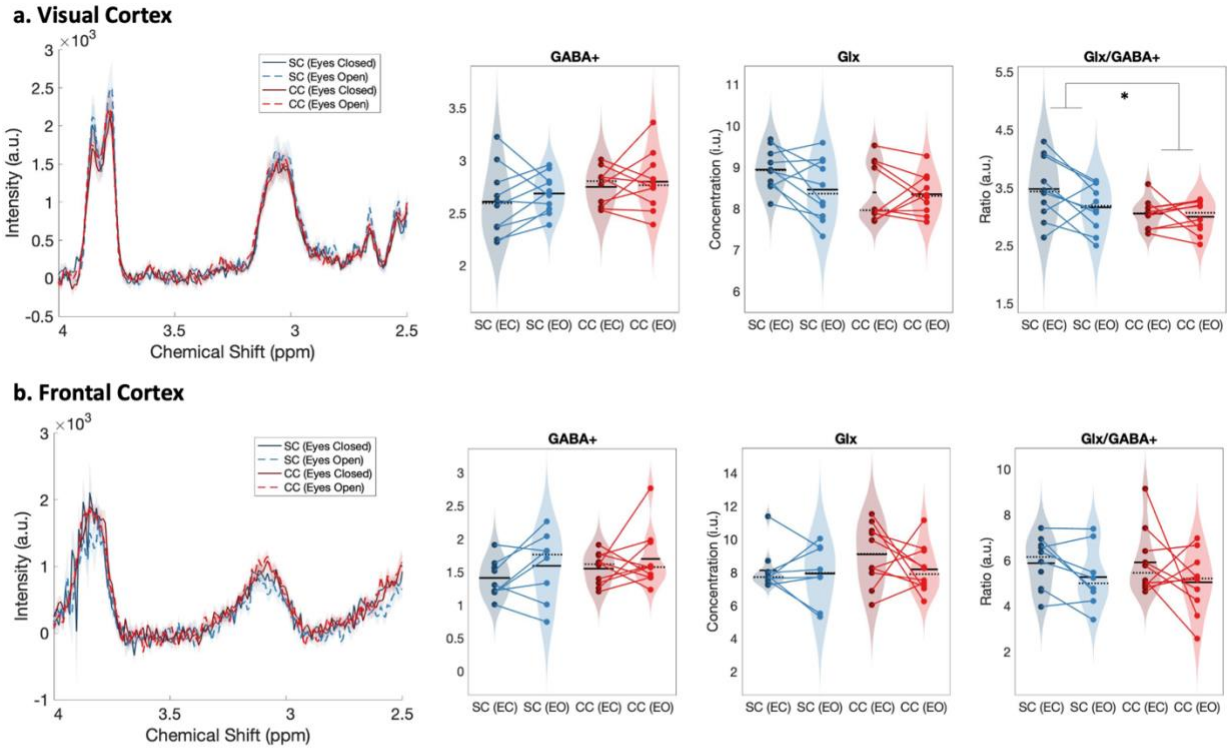
Correlation analyses between MRS and EEG measures

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).



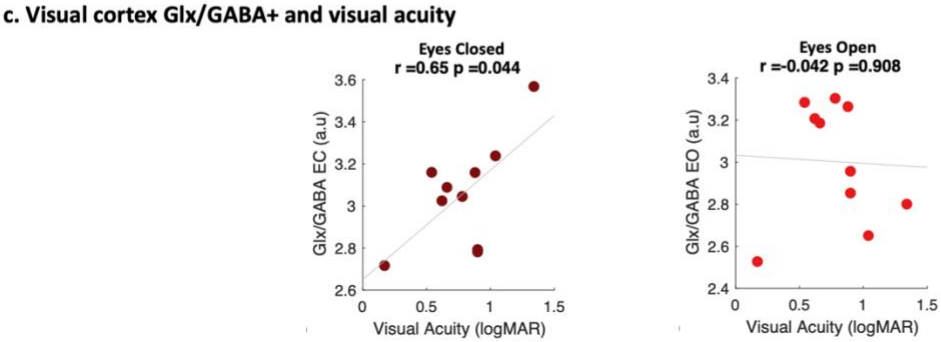


Figure 2: Edited spectra obtained from Magnetic Resonance Spectroscopy (MRS). *a. Average edited spectra showing GABA+ and edited Glx peaks in the visual cortices of normally sighted individuals (SC, green) and individuals with reversed congenital cataracts (CC, red) are shown. Edited MRS DIFF spectra 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.*

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.

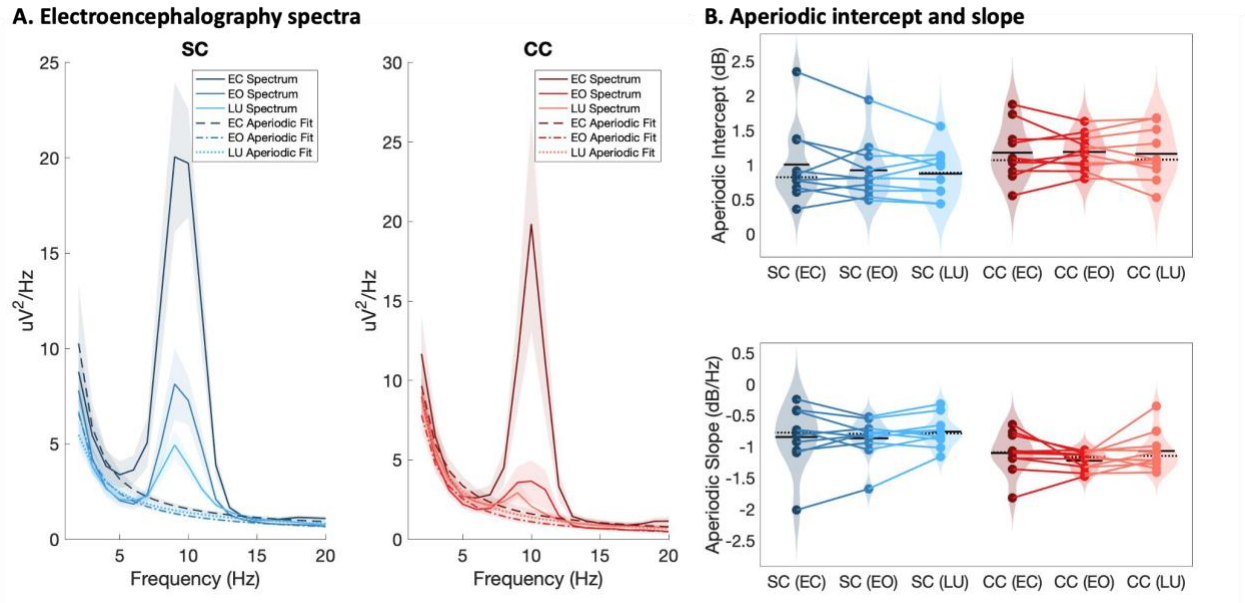


Figure 3: Full spectrum and aperiodic activity of the electroencephalogram (EEG). *a. EEG spectra across O1 and O2 with the corresponding aperiodic (1/f) fits for normally sighted individuals (SC, blue, left) and individuals with reversed congenital cataracts (CC, red, right). Spectra of EEG recordings 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 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).

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.

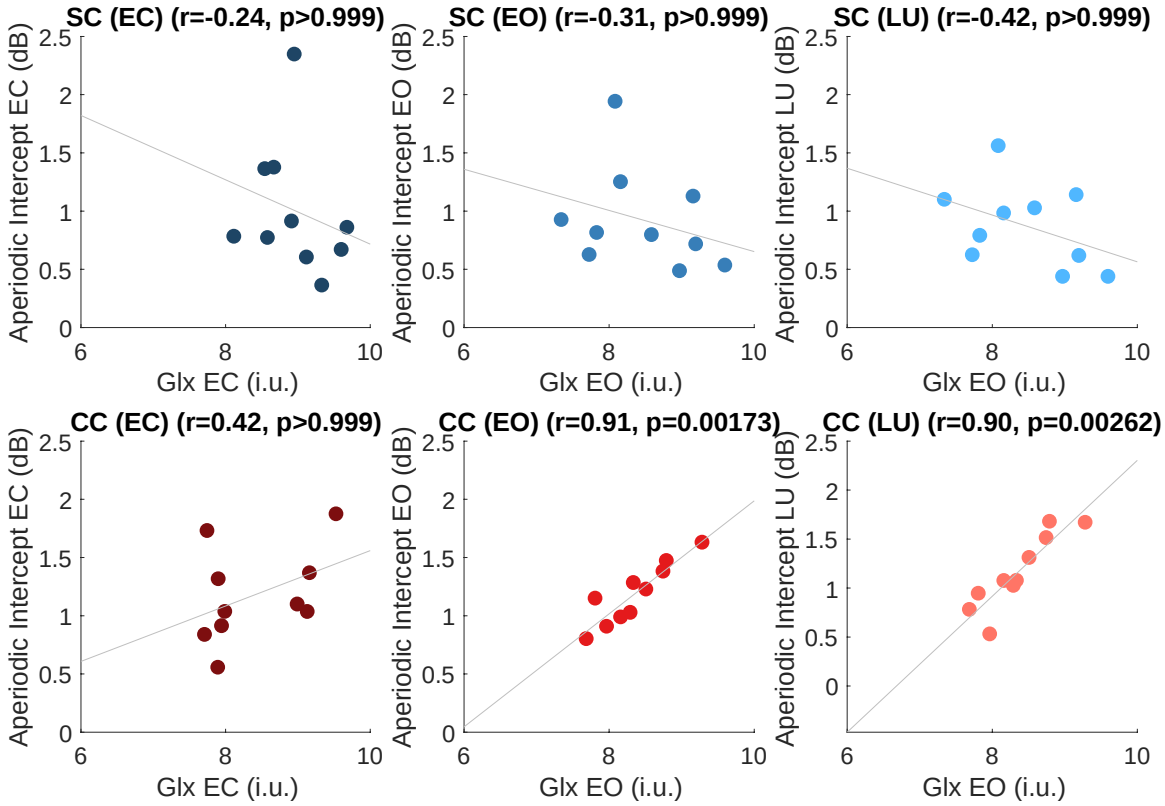


Figure 4: Exploratory correlation analyses between the aperiodic intercept (1-20 Hz) and glutamate/glutamine (Glx) concentration in the visual cortex. *Correlations between water-normalized Glx concentration and aperiodic intercept are shown for the eyes closed (EC, left), eyes open (EO, middle) and 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.*

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 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 though 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 andGABA+ 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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KEYWORDS

Brain development, Sensitive periods, Visual Cortex, congenital cataracts, sight recovery, Excitatory-Inhibitory balance.

REFERENCES

Andelin, A. K., Olavarria, J. F., Fine, I., Taber, E. N., Schwartz, D., Kroenke, C. D., & Stevens, A. A. (2019). The effect of onset age of visual deprivation on visual cortex surface area across-species. *Cerebral Cortex (New York, NY)*, *29*(10), 4321. https://doi.org/10.1093/CERCOR/BHY315

Badde, S., Ley, P., Rajendran, S. S., Shareef, I., Kekunnaya, R., & Röder, B. (2020). Sensory experience during early sensitive periods shapes cross-modal temporal biases. *eLife*. https://doi.org/10.7554/ELIFE.61238

Barnes, S. J., Sammons, R. P., Jacobsen, R. I., Mackie, J., Keller, G. B., & Keck, T. (2015). Subnetwork-specific homeostatic plasticity in mouse visual cortex in vivo. *Neuron*, *86*(5), 1290–1303. https://doi.org/10.1016/J.NEURON.2015.05.010

Baroncelli, L., Maffei, L., & Sale, A. (2011). New perspectives in amblyopia therapy on adults: A critical role for the excitatory/inhibitory balance. *Frontiers in Cellular Neuroscience*, *5*(NOVEMBER), 1–6. https://doi.org/10.3389/FNCEL.2011.00025

Bastos, A. M., Loonis, R., Kornblith, S., Lundqvist, M., & Miller, E. K. (2018). Laminar recordings in frontal cortex suggest distinct layers for maintenance and control of working memory.

Proceedings of the National Academy of Sciences of the United States of America, *115*(5), 1117–1122. https://doi.org/10.1073/PNAS.1710323115/SUPPL_FILE/PNAS.1710323115.SAPP.PDF

Batardière, A., Barone, P., Knoblauch, K., Giroud, P., Berland, M., Dumas, A. M., & Kennedy, H. (2002). Early specification of the hierarchical organization of visual cortical areas in the macaque monkey. *Cerebral Cortex (New York, N.Y. : 1991)*, *12*(5), 453–465. https://doi.org/10.1093/CERCOR/12.5.453

Bedny, M. (2017). Evidence from blindness for a cognitively pluripotent cortex. In *Trends in Cognitive Sciences*. https://doi.org/10.1016/j.tics.2017.06.003

Benevento, L. A., Bakkum, B. W., Port, J. D., & Cohen, R. S. (1992). The effects of dark-rearing on the electrophysiology of the rat visual cortex. *Brain Research*, *572*(1–2), 198–207. https://doi.org/10.1016/0006-8993(92)90470-T

Bezalel, V., Paz, R., & Tal, A. (2019). Inhibitory and excitatory mechanisms in the human cingulate-cortex support reinforcement learning: A functional Proton Magnetic Resonance Spectroscopy study. *NeuroImage*, *184*, 25–35. https://doi.org/10.1016/J.NEUROIMAGE.2018.09.016

Bielicki, G., Chassain, C., Renou, J. P., Farges, M. C., Vasson, M. P., Eschalier, A., & Durif, F. (2004). Brain GABA editing by localized in vivo 1H magnetic resonance spectroscopy. *NMR in Biomedicine*, *17*(2), 60–68. https://doi.org/10.1002/NBM.863

Binda, P., Kurzawski, J. W., Lunghi, C., Biagi, L., Tosetti, M., & Morrone, M. C. (2018). Response to short-term deprivation of the human adult visual cortex measured with 7T BOLD. *eLife*, *7*. https://doi.org/10.7554/ELIFE.40014

Birch, E. E., Cheng, C., Stager, D. R., Weakley, D. R., & Stager, D. R. (2009). The critical period for surgical treatment of dense congenital bilateral cataracts. *Journal of Aapos*, *13*(1), 67. https://doi.org/10.1016/J.JAAPOS.2008.07.010

Birch, E. E., Stager, D., Leffler, J., & Weakley, D. (1998). Early treatment of congenital unilateral cataract minimizes unequal competition. *Investigative Ophthalmology and Visual Science*.

Birch, E. E., Swanson, W. H., Stager, D. R., Woody, M., & Everett, M. (1993). Outcome after very early treatment of dense congenital unilateral cataract. *Investigative Ophthalmology and Visual Science*.

Bottari, D., Kekunnaya, R., Hense, M., Troje, N. F., Sourav, S., & Röder, B. (2018). Motion processing after sight restoration: No competition between visual recovery and auditory compensation. *NeuroImage*. https://doi.org/10.1016/j.neuroimage.2017.11.050

Bottari, D., Troje, N. F., Ley, P., Hense, M., Kekunnaya, R., & Röder, B. (2016). Sight restoration after congenital blindness does not reinstate alpha oscillatory activity in humans. *Scientific Reports*. https://doi.org/10.1038/srep24683

Bourgeois, J. P. (1996). Synaptogenesis in the occipital cortex of macaque monkey devoid of retinal input from early embryonic stages. *The European Journal of Neuroscience*, *8*(5), 942–950. https://doi.org/10.1111/J.1460-9568.1996.TB01581.X

Bourgeois, J. P. (1997). Synaptogenesis, heterochrony and epigenesis in the mammalian neocortex. *Acta Paediatrica, International Journal of Paediatrics, Supplement*, *86*(422), 27–33. https://doi.org/10.1111/j.1651-2227.1997.tb18340.x

Bourgeois, J. P., Jastreboff, P. J., & Rakic, P. (1989). Synaptogenesis in visual cortex of normal and preterm monkeys: Evidence for intrinsic regulation of synaptic overproduction. *Proceedings of the National Academy of Sciences of the United States of America*, *86*(11), 4297–4301. https://doi.org/10.1073/PNAS.86.11.4297

Brainard, D. H. (1997). The Psychophysics Toolbox. *Spatial Vision*, *10*(4). https://doi.org/10.1163/156856897X00357

Brooks, W. M., Friedman, S. D., & Stidley, C. A. (1999). Reproducibility of 1 H-MRS In Vivo. *Reson Med*, *41*, 193–197. https://doi.org/10.1002/(SICI)1522-2594(199901)41:1

Burkhalter, A. (1993). Development of forward and feedback connections between areas v1 and v2 of human visual cortex. *Cerebral Cortex*. https://doi.org/10.1093/cercor/3.5.476

Cavassila, S., Deval, S., Huegen, C., Van Ormondt, D., & Graveron-Demilly, D. (2001). Cramér-Rao bounds: An evaluation tool for quantitation. *NMR in Biomedicine*, *14*(4), 278–283. https://doi.org/10.1002/NBM.701

Chattopadhyaya, B., Di Cristo, G., Higashiyama, H., Knott, G. W., Kuhlman, S. J., Welker, E., & Huang, Z. J. (2004). Experience and activity-dependent maturation of perisomatic GABAergic innervation in primary visual cortex during a postnatal critical period. *Journal of Neuroscience*, *24*(43), 9598–9611. https://doi.org/10.1523/JNEUROSCI.1851-04.2004

Chen, L., Li, X., Tjia, M., & Thapliyal, S. (2022). Homeostatic plasticity and excitation-inhibition balance: The good, the bad and the ugly. *Current Opinion in Neurobiology*, *75*, 102553. https://doi.org/10.1016/J.CONB.2022.102553

Chini, M., Pfeffer, T., & Hanganu-Opatz, I. (2022). An increase of inhibition drives the developmental decorrelation of neural activity. *eLife*, *11*. https://doi.org/10.7554/ELIFE.78811

Coullon, G. S. L., Emir, U. E., Fine, I., Watkins, K. E., & Bridge, H. (2015). Neurochemical changes in the pericalcarine cortex in congenital blindness attributable to bilateral anophthalmia. *Journal of Neurophysiology*. https://doi.org/10.1152/jn.00567.2015

Delorme, A., & Makeig, S. (2004). EEGLAB: An open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. *Journal of Neuroscience Methods*, *134*(1). https://doi.org/10.1016/j.jneumeth.2003.10.009

Desai, N. S., Cudmore, R. H., Nelson, S. B., & Turrigiano, G. G. (2002). Critical periods for experience-dependent synaptic scaling in visual cortex. *Nature Neuroscience 2002 5:8*, *5*(8), 783–789. https://doi.org/10.1038/nn878

Donoghue, T., Dominguez, J., & Voytek, B. (2020). Electrophysiological frequency band ratio measures conflate periodic and aperiodic neural activity. *eNeuro*, *7*(6). https://doi.org/10.1523/ENEURO.0192-20.2020

Donoghue, T., Haller, M., Peterson, E. J., Varma, P., Sebastian, P., Gao, R., Noto, T., Lara, A. H., Wallis, J. D., Knight, R. T., Shestyuk, A., & Voytek, B. (2020). Parameterizing neural power spectra into periodic and aperiodic components. *Nature Neuroscience*. https://doi.org/10.1038/s41593-020-00744-x

Edden, R. A. E., Puts, N. A. J., Harris, A. D., Barker, P. B., & Evans, C. J. (2014). Gannet: A batch-processing tool for the quantitative analysis of gamma-aminobutyric acid-edited MR

spectroscopy spectra. *Journal of Magnetic Resonance Imaging*. https://doi.org/10.1002/jmri.24478

Ehrlich, L. H. (1948). Spontaneous absorption of congenital cataract following maternal rubella. *Archives of Ophthalmology*, *39*(2), 205–209. https://doi.org/10.1001/ARCHOPHT.1948.00900020210007

Elliott, D. B. (2016). The good (logMAR), the bad (Snellen) and the ugly (BCVA, number of letters read) of visual acuity measurement. *Ophthalmic and Physiological Optics*, *36*(4), 355–358. https://doi.org/10.1111/OPO.12310

Favaro, J., Colombo, M. A., Mikulan, E., Sartori, S., Nosadini, M., Pelizza, M. F., Rosanova, M., Sarasso, S., Massimini, M., & Toldo, I. (2023). The maturation of aperiodic EEG activity across development reveals a progressive differentiation of wakefulness from sleep. *NeuroImage*, *277*. https://doi.org/10.1016/J.NEUROIMAGE.2023.120264

Feng, Y., Collignon, O., Maurer, D., Yao, K., & Gao, X. (2021). Brief postnatal visual deprivation triggers long-lasting interactive structural and functional reorganization of the human cortex. *Frontiers in Medicine*, *8*, 752021. https://doi.org/10.3389/FMED.2021.752021/BIBTEX

Froemke, R. C. (2015). Plasticity of cortical excitatory-inhibitory balance. *Annual Review of Neuroscience*, *38*, 195–219. https://doi.org/10.1146/annurev-neuro-071714-034002

Gandhi, S. P., Yanagawa, Y., & Stryker, M. P. (2008). Delayed plasticity of inhibitory neurons in developing visual cortex. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(43), 16797–16802. https://doi.org/10.1073/pnas.0806159105

Gao, R., Peterson, E. J., & Voytek, B. (2017). Inferring synaptic excitation/inhibition balance from field potentials. *NeuroImage*, *158*(March), 70–78. https://doi.org/10.1016/j.neuroimage.2017.06.078

Gianfranceschi, L., Siciliano, R., Walls, J., Morales, B., Kirkwood, A., Huang, Z. J., Tonegawa, S., & Maffei, L. (2003). Visual cortex is rescued from the effects of dark rearing by overexpression of BDNF. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(21), 12486–12491. https://doi.org/10.1073/PNAS.1934836100/ASSET/30EA89DF-8245-4DE1-BCC3-03302D84B3D9/ASSETS/GRAPHIC/PQ1934836005.JPEG

Gilmore, J. H., Langworthy, B., Girault, J. B., Fine, J., Jha, S. C., Kim, S. H., Cornea, E., & Styner, M. (2020). Individual variation of human cortical structure is established in the first year of life. *Biological Psychiatry. Cognitive Neuroscience and Neuroimaging*, *5*(10), 971. https://doi.org/10.1016/J.BPSC.2020.05.012

Goel, A., & Lee, H. K. (2007). Persistence of experience-induced homeostatic synaptic plasticity through adulthood in superficial layers of mouse visual cortex. *Journal of Neuroscience*, *27*(25), 6692–6700. https://doi.org/10.1523/JNEUROSCI.5038-06.2007

Grent-’t-Jong, T., Gajwani, R., Gross, J., Gumley, A. I., Lawrie, S. M., Schwannauer, M., Schultze-Lutter, F., Williams, S. R., & Uhlhaas, P. J. (2022). MR-Spectroscopy of GABA and glutamate/glutamine concentrations in auditory cortex in clinical high-risk for psychosis individuals. *Frontiers in Psychiatry*, *13*(March), 1–10. https://doi.org/10.3389/fpsyt.2022.859322

Guerreiro, M. J. S., Erfort, M. V., Henssler, J., Putzar, L., & Röder, B. (2015). Increased visual cortical thickness in sight-recovery individuals. *Human Brain Mapping*. https://doi.org/10.1002/hbm.23009

Gyurkovics, M., Clements, G. M., Low, K. A., Fabiani, M., & Gratton, G. (2021). The impact of 1/f activity and baseline correction on the results and interpretation of time-frequency analyses of EEG/MEG data: A cautionary tale. *NeuroImage*, *237*. https://doi.org/10.1016/j.neuroimage.2021.118192

Hadad, B. S., Maurer, D., & Lewis, T. L. (2012). Sparing of sensitivity to biological motion but not of global motion after early visual deprivation. *Developmental Science*. https://doi.org/10.1111/j.1467-7687.2012.01145.x

Haider, B., Duque, A., Hasenstaub, A. R., & McCormick, D. A. (2006). Neocortical network activity in vivo is generated through a dynamic balance of excitation and inhibition. *Journal of Neuroscience*. https://doi.org/10.1523/JNEUROSCI.5297-05.2006

Haller, M., Donoghue, T., Peterson, E., Varma, P., Sebastian, P., Gao, R., Noto, T., Knight, R. T., Shestyuk, A., & Voytek, B. (2018). Parameterizing neural power spectra. *bioRxiv*, 299859.

Harris, A. D., Puts, N. A. J., & Edden, R. A. E. (2015). Tissue correction for GABA-edited MRS: considerations of voxel composition, tissue segmentation and tissue relaxations. *Journal of Magnetic Resonance Imaging : JMRI*, *42*(5), 1431. https://doi.org/10.1002/JMRI.24903

Heitmann, C., Zhan, M., Linke, M., Hölig, C., Kekunnaya, R., Hoof, R. van, Goebel, R., & Röder, B. (2023). Early visual experience refines the retinotopic organization within and across visual cortical regions. *Current Biology*, *0*(0). https://doi.org/10.1016/J.CUB.2023.10.010

Henry, M. E., Lauriat, T. L., Shanahan, M., Renshaw, P. F., & Jensen, J. E. (2011). Accuracy and stability of measuring GABA, glutamate, and glutamine by proton magnetic resonance spectroscopy: A phantom study at 4 Tesla. *Journal of Magnetic Resonance*. https://doi.org/10.1016/j.jmr.2010.11.003

Hensch, T. K. (2005). Critical period plasticity in local cortical circuits. *Nature Reviews Neuroscience*, *6*(11), 877–888. https://doi.org/10.1038/nrn1787

Hensch, T. K., & Bilimoria, P. M. (2012). Re-opening windows: Manipulating critical periods for brain development. *Cerebrum: The Dana Forum on Brain Science*.

Hensch, T. K., & Fagiolini, M. (2004). Excitatory-inhibitory balance and critical period plasticity in developing visual cortex. *Progress in Brain Research*, *147*(SPEC. ISS.), 115–124. https://doi.org/10.1016/S0079-6123(04)47009-5

Hensch, T. K., & Fagiolini, M. (2005). Excitatory-inhibitory balance and critical period plasticity in developing visual cortex. *Progress in Brain Research*, *147*(SPEC. ISS.), 115–124. https://doi.org/10.1016/S0079-6123(04)47009-5

Hensch, T. K., Fagiolini, M., Mataga, N., Stryker, M. P., Baekkeskov, S., & Kash, S. F. (1998). Local GABA circuit control of experience-dependent plasticity in developing visual cortex. *Science*. https://doi.org/10.1126/science.282.5393.1504

Hill, A. T., Clark, G. M., Bigelow, F. J., Lum, J. A. G., & Enticott, P. G. (2022). Periodic and aperiodic neural activity displays age-dependent changes across early-to-middle childhood. *Developmental Cognitive Neuroscience*, *54*, 101076. https://doi.org/10.1016/J.DCN.2022.101076

Hölig, C., Guerreiro, M. J. S., Lingareddy, S., Kekunnaya, R., & Röder, B. (2023). Sight restoration in congenitally blind humans does not restore visual brain structure. *Cerebral Cortex*, *33*(5), 2152–2161. https://doi.org/10.1093/CERCOR/BHAC197

Homan, R. W., Herman, J., & Purdy, P. (1987). Cerebral location of international 10-20 system electrode placement. *Electroencephalography and Clinical Neurophysiology*, *66*(4). https://doi.org/10.1016/0013-4694(87)90206-9

Huang, S., Hokenson, K., Bandyopadhyay, S., Russek, S. J., & Kirkwood, A. (2015). Brief dark exposure reduces tonic inhibition in visual cortex. *Journal of Neuroscience*, *35*(48), 15916–15920. https://doi.org/10.1523/JNEUROSCI.1813-15.2015

Hyvärinen, J., Carlson, S., & Hyvärinen, L. (1981). Early visual deprivation alters modality of neuronal responses in area 19 of monkey cortex. *Neuroscience Letters*, *26*(3), 239–243. https://doi.org/10.1016/0304-3940(81)90139-7

Isaacson, J. S., & Scanziani, M. (2011). How inhibition shapes cortical activity. *Neuron*, *72*(2), 231–243. https://doi.org/10.1016/J.NEURON.2011.09.027

Jenkins, C., Chandler, M., Langbein, F. C., & Shermer, S. M. (2019). Seeking ground truth for GABA quantification by edited magnetic resonance spectroscopy: Comparative analysis of TARQUIN, LCModel, JMRUI and GANNET. In *arXiv*.

Jensen, O., & Mazaheri, A. (2010). Shaping functional architecture by oscillatory alpha activity: Gating by inhibition. *Frontiers in Human Neuroscience*. https://doi.org/10.3389/fnhum.2010.00186

Jiang, J., Zhu, W., Shi, F., Liu, Y., Li, J., Qin, W., Li, K., Yu, C., & Jiang, T. (2009). Thick visual cortex in the early blind. *Journal of Neuroscience*. https://doi.org/10.1523/JNEUROSCI.5451-08.2009

Kalia, A., Lesmes, L. A., Dorr, M., Gandhi, T., Chatterjee, G., Ganesh, S., Bex, P. J., & Sinha, P. (2014). Development of pattern vision following early and extended blindness. *Proceedings of the National Academy of Sciences*, *111*(5), 2035–2039. https://doi.org/10.1073/pnas.1311041111

Keck, T., Hübener, M., & Bonhoeffer, T. (2017). Interactions between synaptic homeostatic mechanisms: An attempt to reconcile BCM theory, synaptic scaling, and changing excitation/inhibition balance. *Current Opinion in Neurobiology*, *43*, 87–93. https://doi.org/10.1016/J.CONB.2017.02.003

Keck, T., Scheuss, V., Jacobsen, R. I., Wierenga, C. J., Eysel, U. T., Bonhoeffer, T., & Hübener, M. (2011). Loss of sensory input causes rapid structural changes of inhibitory neurons in adult mouse visual cortex. *Neuron*, *71*(5), 869–882. https://doi.org/10.1016/J.NEURON.2011.06.034

Khanna, R., Foster, A., Krishnaiah, S., Mehta, M., & Gogate, P. (2013). Visual outcomes of bilateral congenital and developmental cataracts in young children in south India and causes of poor outcome. *Indian Journal of Ophthalmology*, *61*(2), 65–70. https://doi.org/10.4103/0301-4738.107194

Kim, Y. J., Grabowecky, M., Paller, K. A., & Suzuki, S. (2011). Differential roles of frequency-following and frequency-doubling visual responses revealed by evoked neural

harmonics. *Journal of Cognitive Neuroscience*, *23*(8), 1875. https://doi.org/10.1162/JOCN.2010.21536

Kleiner, M., Brainard, D. H., Pelli, D. G., Broussard, C., Wolf, T., & Niehorster, D. (2007). What's new in Psychtoolbox-3? *Perception*, *36*.

Knudsen, E. I. (1998). Capacity for plasticity in the adult owl auditory system expanded by juvenile experience. *Science*, *279*(5356), 1531–1533. https://doi.org/10.1126/SCIENCE.279.5356.1531

Knudsen, E. I. (2004). Sensitive periods in the development of the brain and behavior. *Journal of Cognitive Neuroscience*. https://doi.org/10.1162/0898929042304796

Le Grand, R., Mondloch, C. J., Maurer, D., & Brent, H. P. (2001). Early visual experience and face processing. *Nature 2001 410:6831*, *410*(6831), 890–890. https://doi.org/10.1038/35073749

Lee, H.-K., & Kirkwood, A. (2019). Mechanisms of homeostatic synaptic plasticity in vivo. *Frontiers in Cellular Neuroscience*, *13*, 520. https://doi.org/10.3389/FNCEL.2019.00520

Levelt, C. N., & Hübener, M. (2012). Critical-Period plasticity in the visual cortex. *Annual Review of Neuroscience*. https://doi.org/10.1146/annurev-neuro-061010-113813

Lewis, T. L., & Maurer, D. (2005). Multiple sensitive periods in human visual development: Evidence from visually deprived children. *Developmental Psychobiology*, *46*(3), 163–183. https://doi.org/10.1002/dev.20055

Lewis, T. L., & Maurer, D. (2009). Effects of early pattern deprivation on visual development. *Optometry and Vision Science : Official Publication of the American Academy of Optometry*, *86*(6), 640–646. https://doi.org/10.1097/OPX.0B013E3181A7296B

Liu, B., Wang, G., Gao, D., Gao, F., Zhao, B., Qiao, M., Yang, H., Yu, Y., Ren, F., Yang, P., Chen, W., & Rae, C. D. (2015). Alterations of GABA and glutamate-glutamine levels in premenstrual dysphoric disorder: A 3T proton magnetic resonance spectroscopy study. *Psychiatry Research - Neuroimaging*, *231*(1), 64–70. https://doi.org/10.1016/J.PSCYCHRESNS.2014.10.020

Lombardi, F., Herrmann, H. J., & de Arcangelis, L. (2017). Balance of excitation and inhibition determines 1/f power spectrum in neuronal networks. *Chaos: An Interdisciplinary Journal of Nonlinear Science*, *27*(4), 047402. https://doi.org/10.1063/1.4979043

Lozano-Soldevilla, D. (2018). On the physiological modulation and potential mechanisms underlying parieto-occipital alpha oscillations. *Frontiers in Computational Neuroscience*, *12*, 23. https://doi.org/10.3389/FNCOM.2018.00023/BIBTEX

Lozano-Soldevilla, D., Ter Huurne, N., Cools, R., & Jensen, O. (2014). GABAergic modulation of visual gamma and alpha oscillations and its consequences for working memory performance. *Current Biology*. https://doi.org/10.1016/j.cub.2014.10.017

Lunghi, C., Emir, U. E., Morrone, M. C., & Bridge, H. (2015). Short-Term monocular deprivation alters GABA in the adult human visual cortex. *Current Biology*. https://doi.org/10.1016/j.cub.2015.04.021

Maffei, A., Nelson, S. B., & Turrigiano, G. G. (2004). Selective reconfiguration of layer 4 visual cortical circuitry by visual deprivation. *Nature Neuroscience 2004 7:12*, *7*(12), 1353–1359. https://doi.org/10.1038/nn1351

Magrou, L., Barone, P., Markov, N. T., Killackey, H. P., Giroud, P., Berland, M., Knoblauch, K., Dehay, C., & Kennedy, H. (2018). How areal specification shapes the local and interareal

circuits in a macaque model of congenital blindness. *Cerebral Cortex*, *28*(8), 3017–3034. https://doi.org/10.1093/CERCOR/BHY125

Manning, J. R., Jacobs, J., Fried, I., & Kahana, M. J. (2009). Broadband shifts in local field potential power spectra are correlated with single-neuron spiking in humans. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *29*(43), 13613–13620. https://doi.org/10.1523/JNEUROSCI.2041-09.2009

Maurer, D., & Hensch, T. K. (2012). Amblyopia: Background to the special issue on stroke recovery. *Developmental Psychobiology*, *54*(3), 224–238. https://doi.org/10.1002/dev.21022

Maurer, D., & Lewis, T. L. (2017). Visual Systems. In *The Neurobiology of Brain and Behavioral Development*. https://doi.org/10.1016/B978-0-12-804036-2.00008-X

McKyton, A., Ben-Zion, I., Doron, R., & Zohary, E. (2015). The limits of shape recognition following late emergence from blindness. *Current Biology*. https://doi.org/10.1016/j.cub.2015.06.040

Medel, V., Irani, M., Ossandón, T., & Boncompte, G. (2020). Complexity and 1/f slope jointly reflect cortical states across different E/I balances. *bioRxiv*, 2020.09.15.298497. https://doi.org/10.1101/2020.09.15.298497

Mescher, M., Merkle, H., Kirsch, J., Garwood, M., & Gruetter, R. (1998). *Simultaneous in vivo spectral editing and water suppression*. https://doi.org/10.1002/(SICI)1099-1492(199810)11:6

Mikkelsen, M., Loo, R. S., Puts, N. A. J., Edden, R. A. E., & Harris, A. D. (2018). Designing GABA-edited Magnetic Resonance Spectroscopy studies: Considerations of scan duration,

Signal-To-Noise ratio and sample size. *Journal of Neuroscience Methods*, *303*, 86. https://doi.org/10.1016/J.JNEUMETH.2018.02.012

Miller, K. J. (2010). Broadband spectral change: Evidence for a macroscale correlate of population firing rate? *Journal of Neuroscience*, *30*(19), 6477–6479. https://doi.org/10.1523/JNEUROSCI.6401-09.2010

Morales, B., Choi, S. Y., & Kirkwood, A. (2002). Dark rearing alters the development of GABAergic transmission in visual cortex. *Journal of Neuroscience*, *22*(18), 8084–8090. https://doi.org/10.1523/JNEUROSCI.22-18-08084.2002

Mower, G. D., Caplan, C. J., Christen, W. G., & Duffy, F. H. (1985). Dark rearing prolongs physiological but not anatomical plasticity of the cat visual cortex. *The Journal of Comparative Neurology*, *235*(4), 448–466. https://doi.org/10.1002/CNE.902350404

Mullins, P. G., McGonigle, D. J., O'Gorman, R. L., Puts, N. A. J., Vidyasagar, R., Evans, C. J., Edden, R. A. E., Brookes, M. J., Garcia, A., Foerster, B. R., Petrou, M., Price, D., Solanky, B. S., Violante, I. R., Williams, S., & Wilson, M. (2014). Current practice in the use of MEGA-PRESS spectroscopy for the detection of GABA. In *NeuroImage*. https://doi.org/10.1016/j.neuroimage.2012.12.004

Mullins, P. G., Rowland, L., Bustillo, J., Bedrick, E. J., Lauriello, J., & Brooks, W. M. (2003). Reproducibility of 1H-MRS measurements in schizophrenic patients. *Magnetic Resonance in Medicine*, *50*(4), 704–707. https://doi.org/10.1002/MRM.10598

Musall, S., Von Pföstl, V., Rauch, A., Logothetis, N. K., & Whittingstall, K. (2014). Effects of neural synchrony on surface EEG. *Cerebral Cortex*, *24*(4), 1045–1053. https://doi.org/10.1093/CERCOR/BHS389

Muthukumaraswamy, S. D., & Liley, D. T. (2018). 1/F electrophysiological spectra in resting and drug-induced states can be explained by the dynamics of multiple oscillatory relaxation processes. *NeuroImage*, *179*(November 2017), 582–595. https://doi.org/10.1016/j.neuroimage.2018.06.068

Nanda, A., Johnson, G. W., Mu, Y., Ahrens, M. B., Chang, C., Englot, D. J., Breakspear, M., & Rubinov, M. (2023). Time-resolved correlation of distributed brain activity tracks E-I balance and accounts for diverse scale-free phenomena. *Cell Reports*, *42*(4), 112254. https://doi.org/10.1016/J.CELREP.2023.112254

Natu, V. S., Gomez, J., Barnett, M., Jeska, B., Kirilina, E., Jaeger, C., Zhen, Z., Cox, S., Weiner, K. S., Weiskopf, N., & Grill-Spector, K. (2019). Apparent thinning of human visual cortex during childhood is associated with myelination. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(41), 20750–20759. https://doi.org/10.1073/PNAS.1904931116/SUPPL_FILE/PNAS.1904931116.SAPP.PDF

Ossandón, J. P., Stange, L., Gudi-Mindermann, H., Rimmele, J. M., Sourav, S., Bottari, D., Kekunnaya, R., & Röder, B. (2023). The development of oscillatory and aperiodic resting state activity is linked to a sensitive period in humans. *NeuroImage*, *275*, 120171. https://doi.org/10.1016/J.NEUROIMAGE.2023.120171

Pant, R., Ossandón, J., Stange, L., Shareef, I., Kekunnaya, R., & Röder, B. (2023). Stimulus-evoked and resting-state alpha oscillations show a linked dependence on patterned visual experience for development. *NeuroImage: Clinical*, 103375. https://doi.org/10.1016/J.NICL.2023.103375

Paslakis, G., Träber, F., Roberz, J., Block, W., & Jessen, F. (2014). N-acetyl-aspartate (NAA) as a correlate of pharmacological treatment in psychiatric disorders: A systematic review. *European Neuropsychopharmacology*, *24*(10), 1659–1675. https://doi.org/10.1016/J.EURONEURO.2014.06.004

Pedersini, C. A., Miller, N. P., Gandhi, T. K., Gilad-Gutnick, S., Mahajan, V., Sinha, P., & Rokers, B. (2023). White matter plasticity following cataract surgery in congenitally blind patients. *Proceedings of the National Academy of Sciences*, *120*(19), e2207025120. https://doi.org/10.1073/PNAS.2207025120

Penny, W., Friston, K., Ashburner, J., Kiebel, S., & Nichols, T. (2007). Statistical Parametric Mapping: The analysis of functional brain images. *Statistical Parametric Mapping: The Analysis of Functional Brain Images*.

Pitchaimuthu, K., Dormal, G., Sourav, S., Shareef, I., Rajendran, S. S., Ossandón, J. P., Kekunnaya, R., & Röder, B. (2021). Steady state evoked potentials indicate changes in nonlinear neural mechanisms of vision in sight recovery individuals. *Cortex*, *144*, 15–28. https://doi.org/10.1016/J.CORTEX.2021.08.001

Plöchl, M., Ossandón, J. P., & König, P. (2012). Combining EEG and eye tracking: Identification, characterization, and correction of eye movement artifacts in electroencephalographic data. *Frontiers in Human Neuroscience*, *6*(OCTOBER 2012), 1–23. https://doi.org/10.3389/fnhum.2012.00278

Putzar, L., Hötting, K., & Röder, B. (2010). Early visual deprivation affects the development of face recognition and of audio-visual speech perception. *Restorative Neurology and Neuroscience*. https://doi.org/10.3233/RNN-2010-0526

Putzar, L., Hötting, K., Rösler, F., & Röder, B. (2007). The development of visual feature binding processes after visual deprivation in early infancy. *Vision Research*. https://doi.org/10.1016/j.visres.2007.07.002

Raczy, K., Holig, C., Guerreiro, M. J. S., Lingareddy, S., Kekunnaya, R., & Roder, B. (2022). Typical resting-state activity of the brain requires visual input during an early sensitive period. *Brain Communications*, *4*(4). https://doi.org/10.1093/BRAINCOMMS/FCAC146

Rajendran, S. S., Bottari, D., Shareef, I., Pitchaimuthu, K., Sourav, S., Troje, N. F., Kekunnaya, R., & Röder, B. (2020). Biological action identification does not require early visual input for development. *eNeuro*, *7*(5).

Röder, B., & Kekunnaya, R. (2021). Visual experience dependent plasticity in humans. In *Current Opinion in Neurobiology* (Vol. 67, pp. 155–162). Elsevier Ltd. https://doi.org/10.1016/j.conb.2020.11.011

Röder, B., & Kekunnaya, R. (2022). Effects of early visual deprivation. *Oxford Research Encyclopedia of Psychology*. https://doi.org/10.1093/ACREFORE/9780190236557.013.839

Röder, B., Kekunnaya, R., & Guerreiro, M. J. S. (2021). Neural mechanisms of visual sensitive periods in humans. *Neuroscience and Biobehavioral Reviews*, *120*, 86–99. https://doi.org/10.1016/J.NEUBIOREV.2020.10.030

Röder, B., Ley, P., Shenoy, B. H., Kekunnaya, R., & Bottari, D. (2013). Sensitive periods for the functional specialization of the neural system for human face processing. *Proceedings of the National Academy of Sciences of the United States of America*. https://doi.org/10.1073/pnas.1309963110

Schaworonkow, N., & Voytek, B. (2021). Longitudinal changes in aperiodic and periodic activity in electrophysiological recordings in the first seven months of life. *Developmental Cognitive Neuroscience*, *47*. https://doi.org/10.1016/j.dcn.2020.100895

Segalowitz, S. J., Sternin, A., Lewis, T. L., Dywan, J., & Maurer, D. (2017). Electrophysiological evidence of altered visual processing in adults who experienced visual deprivation during infancy. *Developmental Psychobiology*. https://doi.org/10.1002/dev.21502

Shibata, K., Sasaki, Y., Bang, J. W., Walsh, E. G., Machizawa, M. G., Tamaki, M., Chang, L. H., & Watanabe, T. (2017). Overlearning hyperstabilizes a skill by rapidly making neurochemical processing inhibitory-dominant. *Nature Neuroscience 2017 20:3*, *20*(3), 470–475. https://doi.org/10.1038/nn.4490

Simpson, R., Devenyi, G. A., Jezzard, P., Hennessy, T. J., & Near, J. (2017). Advanced processing and simulation of MRS data using the FID appliance (FID-A)—An open source, MATLAB-based toolkit. *Magnetic Resonance in Medicine*, *77*(1), 23–33. https://doi.org/10.1002/MRM.26091

Singh, A. K., Phillips, F., Merabet, L. B., & Sinha, P. (2018). Why does the cortex reorganize after sensory loss? In *Trends in Cognitive Sciences*. https://doi.org/10.1016/j.tics.2018.04.004

Sompolinsky, H., Yoon, H., Kang, K., & Shamir, M. (2001). Population coding in neuronal systems with correlated noise. *Physical Review E*, *64*(5), 051904. https://doi.org/10.1103/PhysRevE.64.051904

Sourav, S., Bottari, D., Kekunnaya, R., & Röder, B. (2018). Evidence of a retinotopic organization of early visual cortex but impaired extrastriate processing in sight recovery individuals. *Journal of Vision*. https://doi.org/10.1167/18.3.22

Sourav, S., Bottari, D., Shareef, I., Kekunnaya, R., & Röder, B. (2020). An electrophysiological biomarker for the classification of cataract-reversal patients: A case-control study. *EClinicalMedicine*, *27*. https://doi.org/10.1016/J.ECLINM.2020.100559

Steel, A., Mikkelsen, M., Edden, R. A. E., & Robertson, C. E. (2020). Regional balance between glutamate+glutamine and GABA+ in the resting human brain. *NeuroImage*, *220*. https://doi.org/10.1016/J.NEUROIMAGE.2020.117112

Takado, Y., Takuwa, H., Sampei, K., Urushihata, T., Takahashi, M., Shimojo, M., Uchida, S., Nitta, N., Shibata, S., Nagashima, K., Ochi, Y., Ono, M., Maeda, J., Tomita, Y., Sahara, N., Near, J., Aoki, I., Shibata, K., & Higuchi, M. (2022). MRS-measured glutamate versus GABA reflects excitatory versus inhibitory neural activities in awake mice. *Journal of Cerebral Blood Flow & Metabolism*, *42*(1), 197. https://doi.org/10.1177/0271678X211045449

Takei, Y., Fujihara, K., Tagawa, M., Hironaga, N., Near, J., Kasagi, M., Takahashi, Y., Motegi, T., Suzuki, Y., Aoyama, Y., Sakurai, N., Yamaguchi, M., Tobimatsu, S., Ujita, K., Tsushima, Y., Narita, K., & Fukuda, M. (2016). The inhibition/excitation ratio related to task-induced oscillatory modulations during a working memory task: A multtimodal-imaging study using MEG and MRS. *NeuroImage*, *128*, 302–315. https://doi.org/10.1016/J.NEUROIMAGE.2015.12.057

Takesian, A. E., & Hensch, T. K. (2013). Balancing plasticity/stability across brain development. In *Progress in Brain Research*. https://doi.org/10.1016/B978-0-444-63327-9.00001-1

Tao, H. W., & Poo, M. M. (2005). Activity-dependent matching of excitatory and inhibitory inputs during refinement of visual receptive fields. *Neuron*, *45*(6), 829–836. https://doi.org/10.1016/J.NEURON.2005.01.046

Toyoizumi, T., Miyamoto, H., Yazaki-Sugiyama, Y., Atapour, N., Hensch, T. K., & Miller, K. D. (2013). A theory of the transition to critical period plasticity: Inhibition selectively suppresses spontaneous activity. *Neuron*, *80*(1), 51–63. https://doi.org/10.1016/J.NEURON.2013.07.022

Träber, F., Block, W., Freymann, N., Gür, O., Kucinski, T., Hammen, T., Ende, G., Pilatus, U., Hampel, H., Schild, H. H., Heun, R., & Jessen, F. (2006). A multicenter reproducibility study of single-voxel 1H-MRS of the medial temporal lobe. *European Radiology*, *16*(5), 1096–1103. https://doi.org/10.1007/S00330-005-0108-Y/TABLES/3

Trägenap, S., Whitney, D. E., Fitzpatrick, D., & Kaschube, M. (2023). The nature-nurture transform underlying the emergence of reliable cortical representations. *bioRxiv*, 2022.11.14.516507. https://doi.org/10.1101/2022.11.14.516507

Turrigiano, G. G., & Nelson, S. B. (2004). Homeostatic plasticity in the developing nervous system. *Nature Reviews. Neuroscience*, *5*(2), 97–107. https://doi.org/10.1038/NRN1327

Tytla, M. E., Lewis, T. L., Maurer, D., & Brent, H. P. (1993). Stereopsis after congenital cataract. *Investigative Ophthalmology and Visual Science*.

Van Kerkoerle, T., Self, M. W., Dagnino, B., Gariel-Mathis, M. A., Poort, J., Van Der Togt, C., & Roelfsema, P. R. (2014). Alpha and gamma oscillations characterize feedback and feedforward processing in monkey visual cortex. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(40), 14332–14341. https://doi.org/10.1073/pnas.1402773111

Van Vreeswijk, C., & Sompolinsky, H. (1996). Chaos in neuronal networks with balanced excitatory and inhibitory activity. *Science*, *274*(5293), 1724–1726. https://doi.org/10.1126/SCIENCE.274.5293.1724

Vinje, W. E., & Gallant, J. L. (2000). Sparse coding and decorrelation in primary visual cortex during natural vision. *Science*, *287*(5456), 1273–1276. https://doi.org/10.1126/SCIENCE.287.5456.1273/ASSET/EEAED050-142C-4A76-A242-62162F400944/ASSETS/GRAPHIC/SE0508266004.JPEG

Voytek, B., Kramer, M. A., Case, J., Lepage, K. Q., Tempesta, Z. R., Knight, R. T., & Gazzaley, A. (2015). Age-related changes in 1/f neural electrophysiological noise. *Journal of Neuroscience*, *35*(38). https://doi.org/10.1523/JNEUROSCI.2332-14.2015

Weaver, K. E., Richards, T. L., Saenz, M., Petropoulos, H., & Fine, I. (2013). Neurochemical changes within human early blind occipital cortex. *Neuroscience*. https://doi.org/10.1016/j.neuroscience.2013.08.004

Whitt, J. L., Petrus, E., & Lee, H. K. (2013). Experience-dependent homeostatic synaptic plasticity in neocortex. *Neuropharmacology*, *78*(C), 45–54. https://doi.org/10.1016/J.NEUROPHARM.2013.02.016

Wilson, M., Andronesi, O., Barker, P. B., Bartha, R., Bizzi, A., Bolan, P. J., Brindle, K. M., Choi, I. Y., Cudalbu, C., Dydak, U., Emir, U. E., Gonzalez, R. G., Gruber, S., Gruetter, R., Gupta, R. K., Heerschap, A., Henning, A., Hetherington, H. P., Huppi, P. S., … Howe, F. A. (2019). Methodological consensus on clinical proton MRS of the brain: Review and recommendations. *Magnetic Resonance in Medicine*, *82*(2), 527–550. https://doi.org/10.1002/MRM.27742

Wilson, M., Reynolds, G., Kauppinen, R. A., Arvanitis, T. N., & Peet, A. C. (2011). A constrained least-squares approach to the automated quantitation of in vivo 1H magnetic resonance spectroscopy data. *Magnetic Resonance in Medicine : Official Journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine*. https://doi.org/10.1002/mrm.22579

Winawer, J., Kay, K. N., Foster, B. L., Rauschecker, A. M., Parvizi, J., & Wandell, B. A. (2013). Asynchronous broadband signals are the principal source of the BOLD response in human visual cortex. *Current Biology : CB*, *23*(13), 1145–1153. https://doi.org/10.1016/J.CUB.2013.05.001

Wong-Riley, M. T. T. (2021). The critical period: Neurochemical and synaptic mechanisms shared by the visual cortex and the brain stem respiratory system. *Proceedings of the Royal Society B*, *288*(1958). https://doi.org/10.1098/RSPB.2021.1025

Wu, Y. K., Miehl, C., & Gjorgjieva, J. (2022). Regulation of circuit organization and function through inhibitory synaptic plasticity. *Trends in Neurosciences*, *45*(12), 884–898. https://doi.org/10.1016/J.TINS.2022.10.006

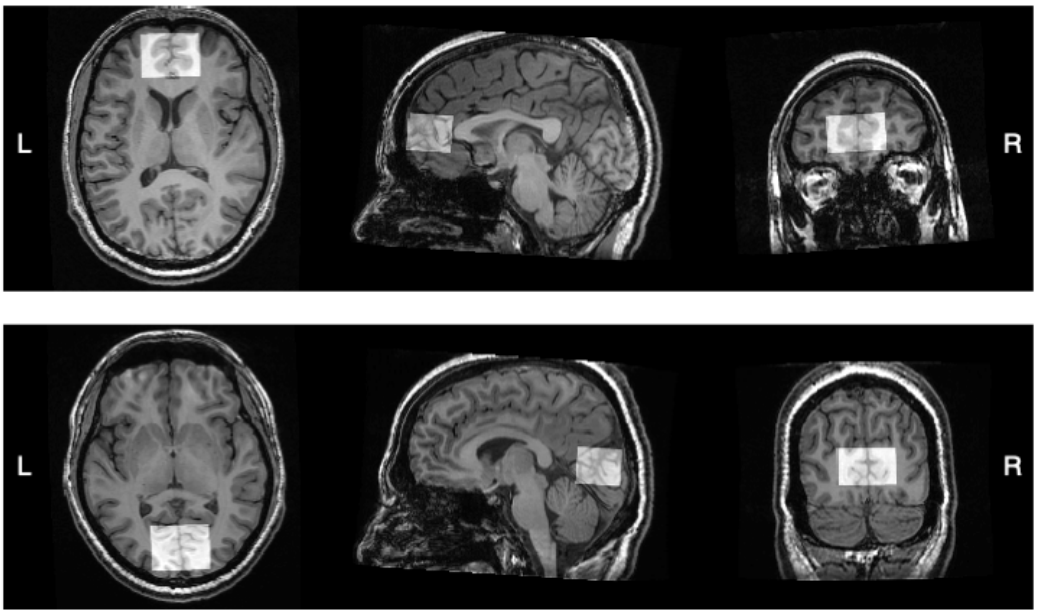
Yusuf, P. A., Lamuri, A., Hubka, P., Tillein, J., Vinck, M., & Kral, A. (2022). Deficient recurrent cortical processing in congenital deafness. *Frontiers in Systems Neuroscience*, *16*(February). https://doi.org/10.3389/fnsys.2022.806142

Zhang, H., Mu, L., Wang, D., Xia, D., Salmon, A., Liu, Q., & Wong-Riley, M. T. T. (2018). Uncovering a critical period of synaptic imbalance during postnatal development of the rat visual cortex: Role of brain-derived neurotrophic factor. *The Journal of Physiology*, *596*(18), 4511–4536. https://doi.org/10.1113/JP275814

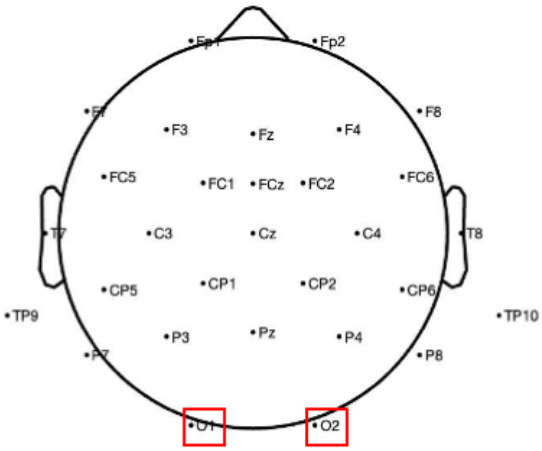
Zhang, L., Huang, C. C., Dai, Y., Luo, Q., Ji, Y., Wang, K., Deng, S., Yu, J., Xu, M., Du, X., Tang, Y., Shen, C., Feng, J., Sahakian, B. J., Lin, C. P., & Li, F. (2020). Symptom improvement in children with autism spectrum disorder following bumetanide administration is associated with decreased GABA/glutamate ratios. *Translational Psychiatry 2020 10:1*, *10*(1), 1–12. https://doi.org/10.1038/s41398-020-0692-2

Zöllner, H. J., Oeltzschner, G., Schnitzler, A., & Wittsack, H. J. (2021). In silico GABA+ MEGA-PRESS: Effects of signal-to-noise ratio and linewidth on modeling the 3 ppm GABA+ resonance. *NMR in Biomedicine*, *34*(1), e4410. https://doi.org/10.1002/NBM.4410

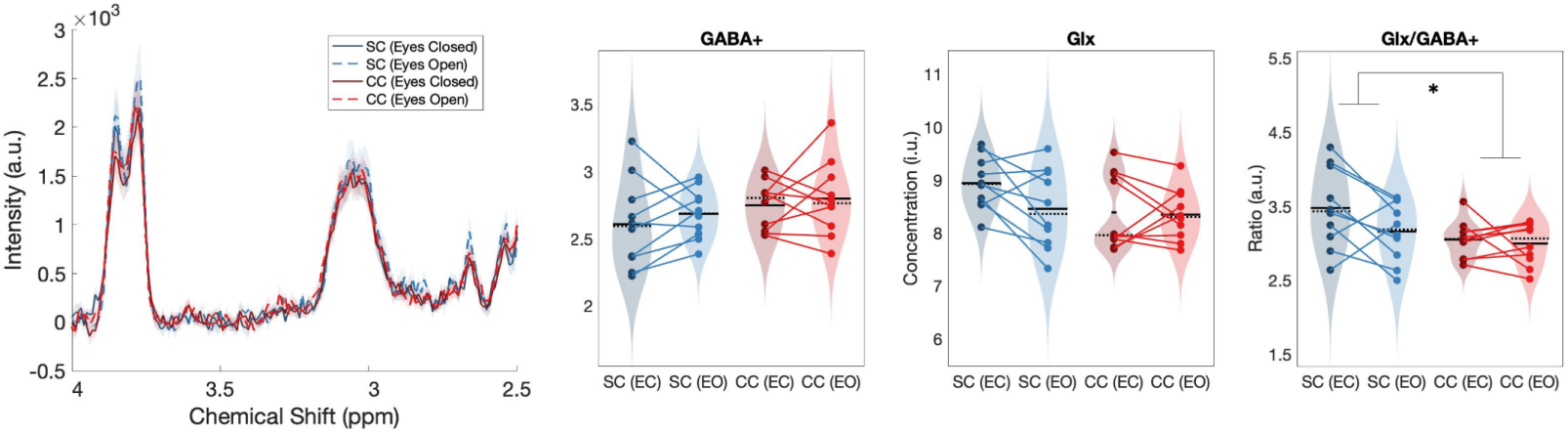
a. Voxel Placement



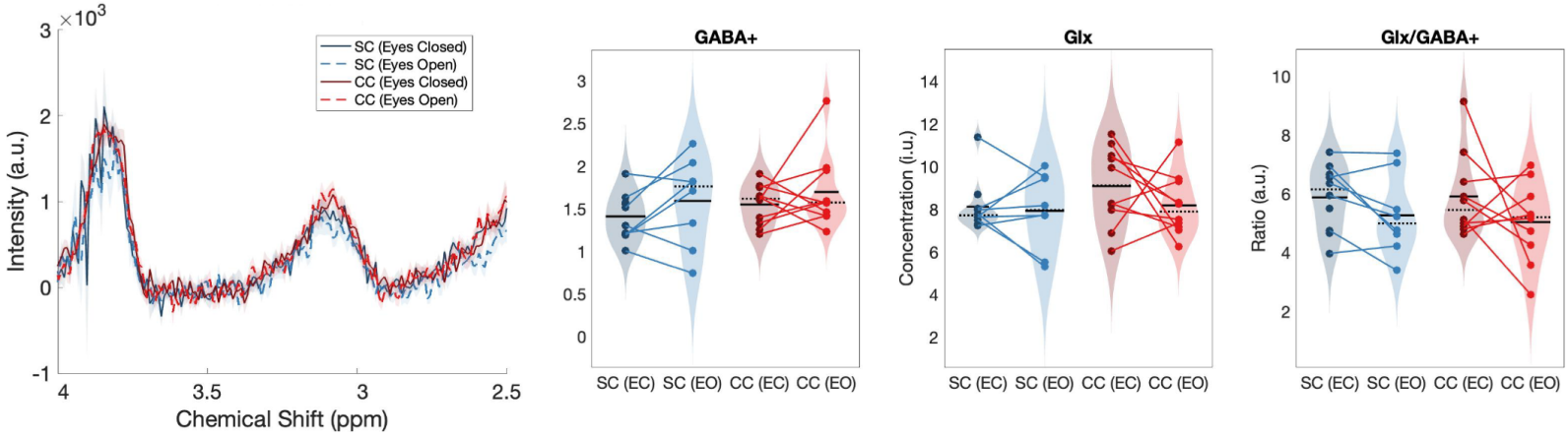
b. Electrode Placement



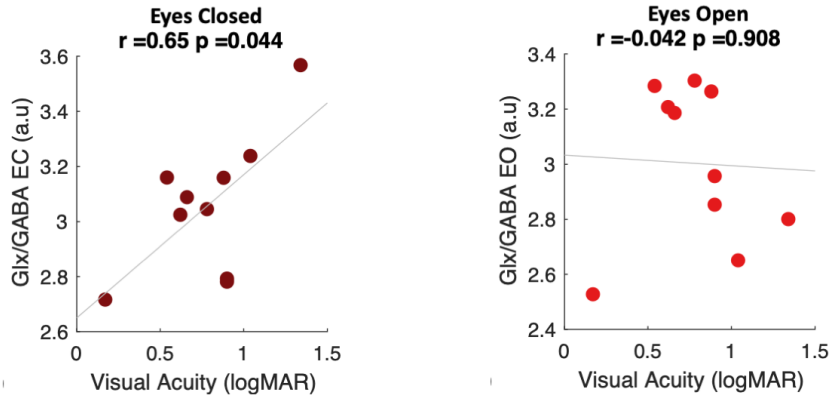
a. Visual Cortex

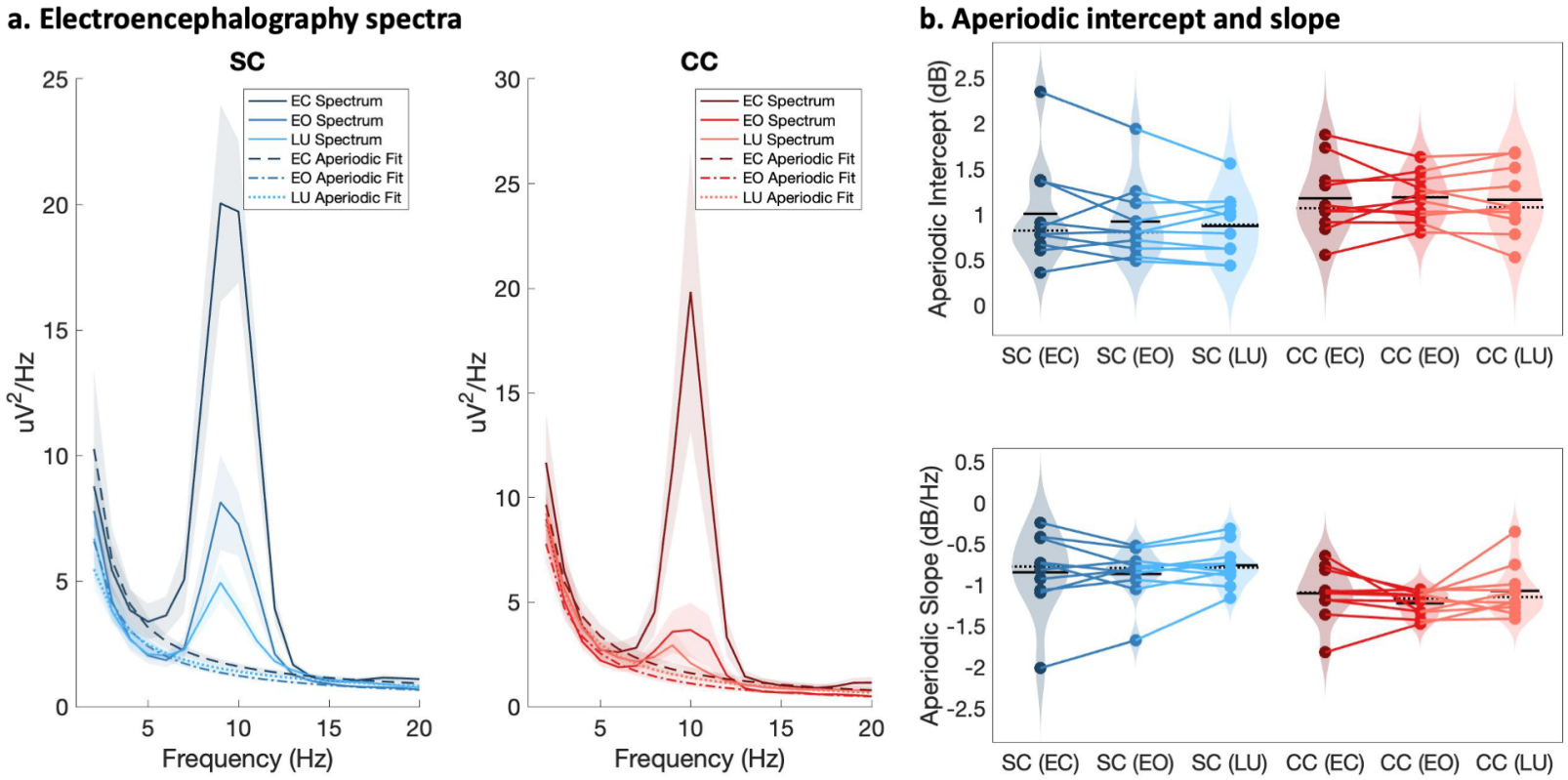


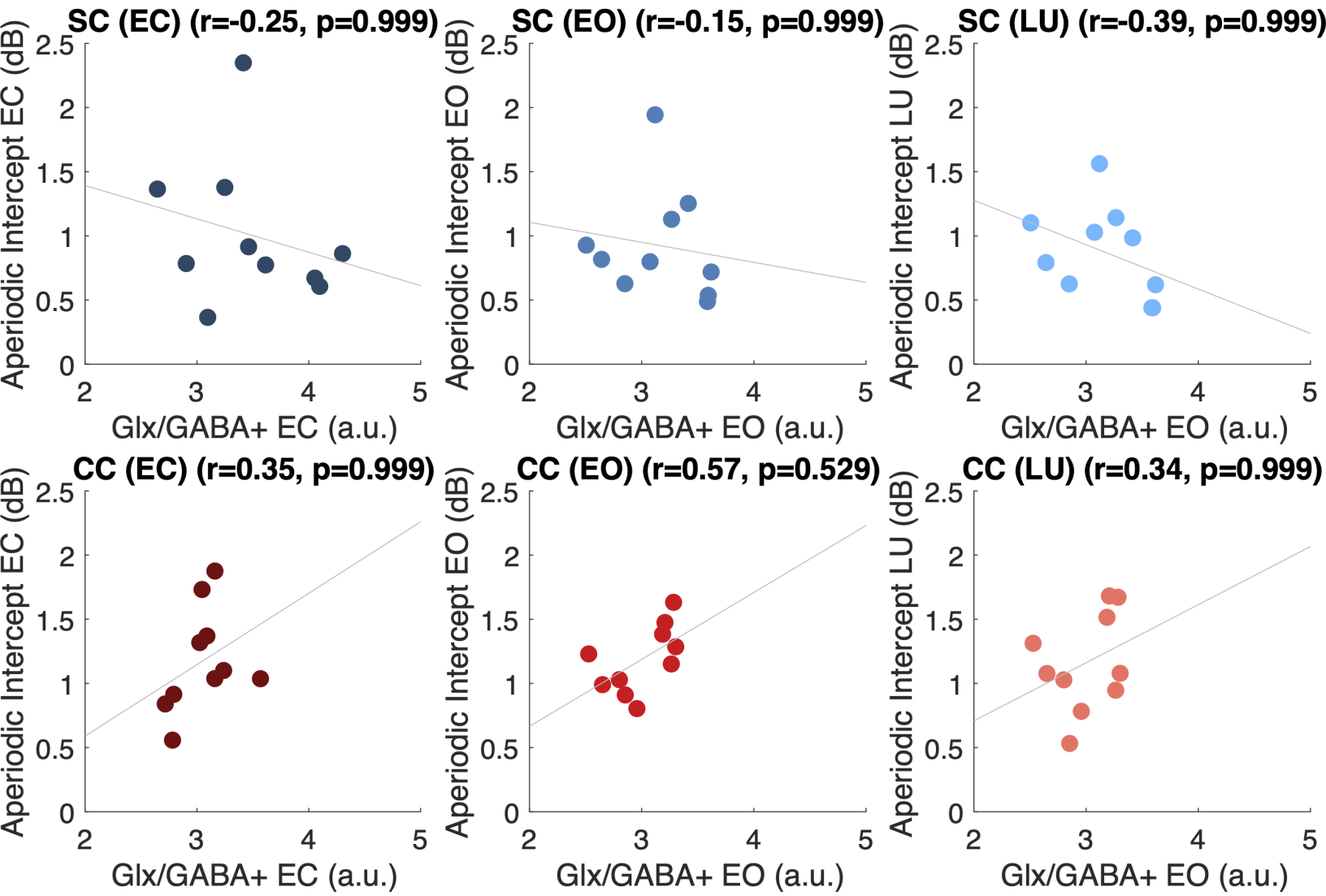
b. Frontal Cortex



c. Visual cortex Glx/GABA+ and visual acuity




a. Electroencephalography spectra
SC
CC
EC Spectrum
EO Spectrum
LU Spectrum
EC Aperiodic Fit
EO Aperiodic Fit
LU Aperiodic Fit
uV²/Hz
Frequency (Hz)
b. Aperiodic intercept and slope
Aperiodic Intercept (dB)
Aperiodic Slope (dB/Hz)
SC (EC)
SC (EO)
SC (LU)
CC (EC)
CC (EO)
CC (LU)


SC (EC) (r=-0.25, p=0.999)
SC (EO) (r=-0.15, p=0.999)
SC (LU) (r=-0.39, p=0.999)
CC (EC) (r=0.35, p=0.999)
CC (EO) (r=0.57, p=0.529)
CC (LU) (r=0.34, p=0.999)
Aperiodic Intercept EC (dB)
Aperiodic Intercept EO (dB)
Aperiodic Intercept LU (dB)
Glx/GABA+ EC (a.u.)
Glx/GABA+ EO (a.u.)
0
0.5
1
1.5
2
2.5
2
3
4
5