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Neurochemical changes in GABA+, Glx, and the excitatory/inhibitory ratio in the calcarine cortex with healthy aging

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Abstract

Aging has been associated with widespread alterations in neural structure and function, but the underlying biochemical changes remain less understood despite recent neurophysiological work suggesting age-related alterations in the excitatory/inhibitory (E/I) balance. In the current study, we used ^{1}H-MRS to quantify levels of excitatory (Glx: glutamate + glutamine) and inhibitory (GABA+: GABA + macromolecules) neurotransmitters in the calcarine cortex in 187 healthy adults (19–79 years-old). These neurotransmitter estimates were used to compute the E/I ratio, and all three parameters were examined for aging effects. Given our large sample, we also conducted supplementary analyses to estimate the concentration of secondary metabolites commonly implicated in aging, including total creatine (tCr), total N-acetyl aspartate (tNAA), total choline (tCho), and myo-inositol (mI). Following best practices, metabolite concentrations were estimated relative to the unsuppressed water signal and corrected for voxel tissue composition (i.e., gray matter, white matter, CSF). Our results indicated significant age-related declines in both

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CRediT authorship contribution statement

Ilenia Salsano: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Ryan J. Glesinger:** Writing – review & editing, Project administration, Investigation, Data curation. **Jason A. John:** Writing – review & editing, Project administration, Investigation, Data curation. **Grant M. Garrison:** Writing – review & editing, Project administration, Investigation, Data curation. **Lucy K. Horne:** Writing – review & editing, Project administration, Investigation, Data curation. **Grace C. Ende:** Writing – review & editing, Project administration, Investigation, Data curation. **Danielle L. Rice:** Writing – review & editing, Project administration, Investigation, Data curation. **Anna T. Coutant:** Writing – review & editing, Project administration, Investigation, Data curation. **Kennedy A. Kress:** Writing – review & editing, Project administration, Investigation, Data curation. **Aubrie J. Petts:** Writing – review & editing, Project administration, Investigation, Data curation. **Georg Oeltzschner:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Resources, Methodology, Investigation, Funding acquisition, Formal analysis. **Tony W. Wilson:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

GO is a paid consultant for Neurona Therapeutics (unrelated to this work). All other authors report no biomedical financial interests or potential conflicts of interest.

GABA+ and Glx, along with a reduction in the E/I ratio, suggesting diminished inhibitory and excitatory tone with advancing age. We also found a decline in tNAA and an increase in tCr with increasing age. In summary, we provide compelling evidence through one of the largest studies of its kind for age-related shifts in excitatory and inhibitory neurotransmitter levels in the visual cortex. These changes may be critical to well-known, age-related physiological changes, including reduced neural selectivity and processing efficiency. These findings provide novel evidence that neurochemical alterations may contribute to the functional declines in cortical processing seen in healthy aging.

Keywords

Mega-press; MRS; Calcarine cortex; Age; Glutamate; tCr; NAA

1. Introduction

The number of Americans over age 65 has increased significantly over the past few decades and is expected to dramatically rise from about 58 million in 2022 to 82 million by 2050 (US Census Bureau, n.d). These increases partially reflect the major advancements in healthcare that have been realized over the past 50 years, although with increasing age the prevalence of neurological disease also increases. Structural and functional MRI studies have consistently shown that healthy aging is associated with widespread brain changes. Structurally, aging is linked to volume loss and cortical thinning, particularly in frontal and parietal regions (Jernigan et al., 1991; Good et al., 2001; Resnick et al., 2003; Salat et al., 2004; Lemaitre et al., 2005; Walhovd et al., 2005; Fjell et al., 2009). Functionally, older adults exhibit reduced within-network connectivity, especially in the default mode network, which has been associated with poorer cognitive performance (Onoda et al., 2012; Betzel et al., 2014; Song et al., 2014; Sala-Llonch et al., 2015; Siman-Tov et al., 2017). Task-based studies corroborate and extend these connectivity findings, with decreased connectivity observed in networks supporting motor control, attention, and executive functions (Andrews-Hanna et al., 2007; Geerligs et al., 2014; Varangis et al., 2019), and widespread activation changes that are generally thought to reflect compensatory processing with increasing age (Grady et al., 1994; Cabeza, 2002; Reuter-Lorenz and Cappell, 2008; Dennis and Cabeza, 2011; Grady, 2012; Cabeza et al., 2018; Koen and Rugg, 2019).

While tremendous progress has been made in characterizing structural and functional brain changes with healthy aging, alterations in neurochemical processes with aging remain far less understood. Gamma-aminobutyric acid (GABA) and glutamate (Glu) are the principal inhibitory and excitatory neurotransmitters, respectively, in the human brain, and together maintain an excitation–inhibition balance that is widely thought to be a fundamental property of healthy brain function (Sears and Hewett, 2021). Both GABA and Glu have become central targets in recent studies leveraging proton magnetic resonance spectroscopy (^{1}H-MRS) in the context of healthy aging (Puts and Edden, 2012), with many studies probing the impact of aging on GABA. Animal work suggests age-related declines in GABAergic function, including reductions in interneuron density and inhibitory synaptic contacts (Majdi et al., 2007; Hua et al., 2008; Peters et al., 2008; Stanley et al., 2012),

as well as alterations in GABA terminals (Rubio et al., 2012; Soler et al., 2017) and GABA-synthesizing enzymes (Akbarian et al., 1995; Marín, 2012; McQuail et al., 2015). Most human ^{1}H-MRS studies have quantified GABA+, a composite signal including GABA and co-edited macromolecules, and the results have been mixed (Aufhaus et al., 2013; Gao et al. 2013; Silveri et al. 2013; Ghisleni et al. 2015; Rowland et al., 2016; Mikkelsen et al., 2017; Porges et al., 2017a; Puts et al. 2017; Marenco et al., 2018; Simmonite et al. 2019). For instance, Gao et al. (2013) measured GABA+ across the adult lifespan (20–76 years) in frontal and parietal regions and reported a gradual age-related decline of approximately 4–5 % per decade. Similar findings have been reported in the medial frontal lobe across multiple overlapping age ranges (e.g., 43–92 years: Porges et al., 2017a; 18–55 years: Marenco et al., 2018). In contrast, Ghisleni et al. (2015) reported a positive association between GABA+ levels and age in left frontal cortex in a cohort of participants between 13 and 53 years (Ghisleni et al., 2015). Additionally, Aufhaus et al. (2013) investigated GABA+ and GABA levels in the anterior cingulate cortex of 21–52 year-old participants and found a decline with age only for GABA+, which may suggest that the relationship with age differs between GABA and GABA+, although further work is needed. A recent meta-analysis suggested that GABA may increase during development, plateau in adolescence, and gradually decline with age (Porges et al., 2021), which may explain some of the discrepancies, although most studies in this area have focused exclusively on adults so the early trajectory likely had a relatively limited impact.

The origin of such inconsistencies remains under investigation and likely reflects multiple factors. First, the age ranges across studies rarely align and most studies used between-group comparisons (e.g., young vs. older adults) rather than modeling age continuously. Second, GABA levels are region-specific, with higher concentrations in sensorimotor and occipital cortices and lower levels in prefrontal and parietal regions (Puts and Edden, 2012; Mullins et al., 2014), which may explain variable findings across studies that focused on different brain regions. Third, there has been wide variation in the type of quantification used (i.e., the reference metabolite chosen to report GABA levels). GABA is often reported relative to another high-concentration metabolite, such as total creatine (tCr), total N-acetyl aspartate (tNAA), or the internal tissue water signal. Although Gao et al. (2013) observed similar age-related GABA+ reductions using either tCr or tNAA, Maes et al. (2018) found opposite results when using GABA with two different references, highlighting the instability of both tCr and tNAA in aging studies. Moreover, unsuppressed water-referenced GABA levels are sensitive to tissue composition within the voxel (Porges et al., 2017b; Maes et al., 2018). Thus, tissue correction is strongly recommended in order to avoid partial volume effects (Harris et al., 2015; Porges et al., 2017b; Peek et al., 2023). However, relatively few aging studies have applied comprehensive tissue correction methods, such as water referencing combined with full segmentation-based correction for voxel composition (e.g., Thomson et al., 2024). Lastly, sample sizes have been modest, with many studies including fewer than 50 participants which is problematic for aging (e.g., Aufhaus et al., 2013; Simmonite et al., 2019), and only one exceeding 200 (Mikkelsen et al., 2017), although this study did not directly test for aging effects.

Beyond GABA, there are a collection of studies using ^{1}H-MRS to examine age-related alterations in Glu concentration. While the evidence broadly suggests that Glu levels are

lower in older relative to younger adults, the findings in this area are also reasonably variable (for review, see Haga et al., 2009 and Cleeland et al., 2019; for a meta-analysis, see Roalf et al., 2020). While many of the same limitations identified above in aging studies of GABA also apply to aging studies of Glu, there are additional concerns, with the most concerning being that different studies focused on different metabolites in the Glu family. Essentially, estimating the concentration of Glu is particularly challenging for ^{1}H-MRS due to its spectral overlap with glutamine resonances at the most commonly used magnetic field strengths (i.e., 1.5 to 3T). Thus, some studies of aging have collapsed across Glu and glutamine resonances to form a compound measurement commonly referred to as Glx (i.e., Glu + glutamine; Chang et al., 1996; Raininko and Mattsson, 2010; Chen et al., 2013; Ding et al., 2016; Mooney et al., 2017; Simmonite et al., 2019), while others have reported their best estimate of Glu (Sailasuta et al., 2008; Zahr et al., 2013; Suri et al., 2017) or individual concentrations of Glu and glutamine (Schubert et al., 2004; Kaiser et al., 2005; Hädel et al., 2013; Marjańska et al., 2017), and this has likely contributed to the variation in age-related findings (Roalf et al., 2020).

In the current study, we aimed to overcome many of these previous limitations and quantify age-related changes in the levels of GABA+ and Glx in a large sample of healthy adults with an age range across most of the adult lifespan (i.e., n = 187, age range: 19–79 years; mean age ~ 48). Despite the considerable scientific interest in both metabolites, few ^{1}H-MRS studies have probed both simultaneously in the context of aging and the few that have examined relatively small samples (Gao et al., 2013; Ghisleni et al., 2015; Huang et al., 2017; Simmonite et al., 2019). Examining the two together is of particular interest given the recent surge of investigations focused on excitatory/inhibitory balance in the context of mental health (i.e., E/I ratio; Yizhar et al., 2011; Nelson and Valakh, 2015; Sohal and Rubenstein, 2019). Specifically, a collection of electrophysiological studies have linked aberrations in the E/I balance to multiple psychiatric and neurological conditions (Lisman, 2012; Marín, 2012; Foss-Feig et al., 2017).

To estimate both simultaneously, we leveraged the well-known MEscher-GArwood Point RESolved Spectroscopy (MEGA-PRESS; Mescher et al., 1998; Mullins et al., 2014) J-difference spectral editing sequence and modeled the association of metabolite estimates with age as a continuous variable, which should provide a more nuanced understanding of neurochemical aging profiles compared to traditional group comparisons. We angled the voxel to follow the calcarine fissure bilaterally, as this early visual region likely has the highest neuronal cell density in human and nonhuman primates (Collins et al., 2010), estimated at approximately 75,000–90,000 neurons/mm^3 in macaque and similar in humans, combined with relatively uniform tissue composition, factors known to improve signal-to-noise ratio for low-concentration metabolites like GABA (Garcia-Marin et al., 2024; Mikkelsen et al., 2018). Following best practices (Tkáč et al., 2021; Peek et al., 2023), metabolite concentrations were expressed relative to the unsuppressed water signal, a more stable reference than tCr or tNAA, and fully corrected for voxel tissue composition, which is essential for accurate GABA quantification (Harris et al., 2015). While our focus was on GABA+, Glx, and the E/I ratio, given our large sample size, we also quantified secondary metabolites of general interest to the field, including tNAA, total choline (tCho), tCr, and myo-inositol (mI). tNAA, predominantly NAA, reflects neuronal viability and mitochondrial

metabolism (Patel and Clark, 1979; Ross and Sachdev, 2004; Rae, 2014; Scavuzzo et al., 2018), while tCho includes choline-containing compounds involved in membrane turnover and signaling (Chiu et al., 2014; Harris et al., 2014; Rae, 2014). tCr is a marker of cellular energy homeostasis and acts as an osmolyte (Ross and Sachdev, 2004; Rae, 2014) and mI serves as a brain osmolyte and is implicated in glial activity and signaling (Novotny et al., 2003; Duarte et al., 2014; Hoyer et al., 2014). Our primary hypotheses were that GABA+ and Glx concentrations, as well as the E/I ratio, would decrease with age. While the other metabolites were not a focus, we generally expected age-related declines in tNAA and increases in tCho and mI based on the literature (Cleeland et al., 2019).

2. Methods

2.1. Participants

One hundred eighty-seven cognitively healthy volunteers participated in the study (99 females; mean age: 47.74 [SD: 17.22], range: 19–79 years). Participants were recruited from the Omaha, Nebraska metropolitan area through flyers, social media advertisements, and from participation in other studies in our laboratory. General cognitive status was measured using the Montreal Cognitive Assessment (MoCA; Nasreddine et al., 2005). Exclusion criteria for the study included any diagnosed neurological or psychiatric disorder, any medical illness associated with CNS dysfunction, history of head trauma, substance use disorder, the presence of metallic implants that could impede magnetic resonance imaging (MRI) data acquisition, and pregnancy. All study procedures were approved by the Institutional Review Board of Boys Town National Research Hospital and all participants provided written informed consent prior to participating.

2.2. MRI and ^{1}H-MRS acquisition

MRI and ^{1}H-MRS data were acquired using a 3T Siemens Prisma scanner equipped with a 32-channel head coil. High-resolution T_1-weighted anatomical images were obtained using a 3D magnetization-prepared rapid acquisition gradient echo (MP-RAGE) sequence (TR = 2400 ms, TE = 2.05 ms, 192 slices, slice thickness = 1 mm, voxel size = 1 × 1 × 1 mm^3, FOV = 256 mm, flip angle = 8°, GRAPPA acceleration factor = 2). This anatomical scan was used for voxel placement and tissue segmentation. ^{1}H-MRS data were acquired from a 30 × 20 × 20 mm^3 voxel placed over the calcarine visual cortex (Fig. 1A) using the MEGA-PRESS sequence with these acquisition parameters: TR = 2000 ms, TE = 68 ms, 192 transients (96 edit-ON, 96 edit-OFF), with frequency-selective editing pulses (full-width at half-maximum [FWHM] bandwidth = 55 Hz) applied at 1.9 ppm (edit-ON) and 9.0 ppm (edit-OFF). Water suppression was performed using the VAriable pulse Power and Optimized Relaxation delays (VAPOR) technique (Tkáč et al., 1999). A MEGA-PRESS unsuppressed water reference scan was acquired using the same parameters (8 transients) to enable eddy-current correction of metabolite data (Klose, 1990). Additionally, a short-echo (TE = 30 ms, 4 averages) unsuppressed water scan was acquired to serve as a voxel-specific reference, allowing metabolite amplitudes to be scaled to the water signal and corrected for tissue composition and relaxation effects. This short TE-scan (30 ms) provides a more accurate estimate of the true water signal amplitude, as it is less affected by T_2-weighting

effects than the water reference acquired with the same TE (68 ms) of the MEGA-PRESS sequence used for eddy-current correction.

While Glu is relatively abundant (6.0–12.5 mM), GABA exists at much lower concentrations (~0.5–2 mM) and overlaps with high-intensity metabolite signals, making it harder to resolve (Govindaraju et al., 2000; Harris et al., 2017). Glu can also be estimated with PRESS or STEAM sequences, but accurate GABA detection at standard field strengths generally requires edited sequences such as MEGA-PRESS. MEGA-PRESS uses a J-difference editing approach to isolate GABA by applying an editing pulse at 1.9 ppm in alternating scans (edit-ON/OFF), with the resulting difference spectrum revealing the edited GABA peak. Due to a considerable signal contribution of “co-edited” macromolecules at 3 ppm that cannot be reliably separated from the ‘pure’ GABA signal, the composite measure of these two compounds is commonly reported and referred to as “GABA+” in the literature (i.e., GABA + macromolecules; Henry et al., 2001; Edden et al., 2014). Glu quantification is similarly affected by its overlap with glutamine, and their combined signal is commonly reported as “Glx” (i.e., Glu + glutamine; Mullins et al., 2014), as noted above. Importantly, Glx concentration estimates that are derived from MEGA-PRESS off-resonance spectra strongly correlate with values obtained using conventional PRESS (Öngür et al., 2011; Stan et al., 2015; Maddock et al., 2016, 2018), making MEGA-PRESS a robust method for simultaneous quantification of GABA+ and Glx (O’Gorman et al., 2011; Puts and Edden, 2012; Harris et al., 2017; Maddock et al., 2018). The MEGA-PRESS sequence used in this study was obtained through a research agreement with the Center for Magnetic Resonance Research (CMRR) at the University of Minnesota (https://www.cmrr.umn.edu/spectro/; Marjańska et al., 2013; Tremblay et al., 2014).

2.3. ^{1}H-MRS processing and analysis

Raw ^{1}H-MRS data (difference and edit-OFF spectra) were processed using Osprey (Version 2.9.0; Oeltzschner et al., 2020), an open-source ^{1}H-MRS analysis toolbox implemented in Matlab (version 2018b). Osprey provides a standardized automated pipeline comprising preprocessing, spectral modeling, voxel coregistration, tissue segmentation and metabolite quantification. Preprocessing included eddy-current correction using the unsuppressed water reference scan, followed by frequency and phase correction using robust spectral registration (Near et al., 2015; Mikkelsen et al., 2020; Oeltzschner et al., 2020). Fourier transformation and water signal removal were subsequently applied. Edit-ON and edit-OFF spectra were then averaged and subtracted to isolate GABA+ at 3.02 ppm by removing overlapping metabolite signals (creatine, phosphocreatine, N-acetylaspartate) from the difference spectrum. Spectral modeling was conducted on both edit-OFF (Fig. 1B, top) and difference spectra (Fig. 1B, bottom) using the Osprey fitting algorithm, applying a default fitting range of 0.5–4 ppm, and knot spacing of 0.4 ppm (Oeltzschner et al., 2020). Average metabolite spectra were modeled using Osprey’s basis set, which includes metabolites simulated via density matrix methods using the FID-A toolbox (Simpson et al. 2017). This basis set was specifically tailored for the Siemens scanner, MEGA-PRESS sequence, and a 55 Hz editing pulse bandwidth. Macromolecular and lipid contributions were modeled using Gaussian basis functions (Oeltzschner et al., 2020). A total of 18 metabolites were included in the basis set: ascorbic acid, aspartic acid, creatine,

CrCH2, GABA, Glu, glutamine, glycerophosphocholine, glutathione, mI, lactate, NAA, N-acetylaspartylglutamate, phosphocholine, phosphocreatine, phosphatidylethanolamine, scyllo-inositol, and taurine. Five macromolecules (MM09, MM12, MM14, MM17, MM20) and three lipids (Lip09, Lip13, Lip20) were included in the edit-OFF models. Two macromolecular components (MM09, coMM3) were included in the difference spectra models, with a constraint on their relative amplitudes to stabilize the co-edited MM model component at 3.0 ppm (Zöllner et al., 2022). Composite estimates were further calculated as tNAA (total NAA; NAA + *N*-acetylaspartylglutamate), tCr (total creatine; creatine + phosphocreatine), tCho (total choline; glycerophosphocholine + phosphocholine).

Coregistration of the ^{1}H-MRS voxel to the anatomical T_1-weighted image and image segmentation into gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF) fractions was performed using SPM12 (Wellcome Department of Cognitive Neurology). All voxel placements were visually inspected for accuracy. Metabolite concentrations were estimated relative to the unsuppressed water signal from the short TE water scan. All metabolite concentration estimates were corrected for voxel tissue composition (GM, WM, CSF fractions), with tissue-specific water content and T_1 and T_2 relaxation time literature values described previously (Gasparovic et al., 2006; Puts et al., 2013; Harris et al. 2015; Oeltzschner et al., 2020). Given the impact of GM tissue composition on metabolite estimates, we conducted follow-up analyses to assess the integrity of the correction, and these are reported in the Supplemental Materials. Concentration estimates are reported in institutional units (i.u), which approximate millimolar units. GABA+ was quantified from the difference spectra, while all other metabolites were quantified from the edit-OFF spectra. All spectra were visually inspected for quality, and quantitative quality metrics were extracted from Osprey, including creatine signal-to-noise ratio (Cr_SNR), creatine linewidth (Cr_FWHM), water linewidth (water_FWHM), frequency shift, relative residual amplitude of the edit-off spectrum (relResA), and the GABA-edited difference spectrum (relResdiff1). In this study, we focused on GABA+, Glx, and the E/I ratio, and additionally reported tNAA, tCho, mI and tCr to enrich the literature. We additionally computed the E/I ratio index as Glx/GABA+ for each participant.

2.4. Statistical analysis

Statistical analyses were performed using SPSS (version 29). We identified and excluded outliers (i.e., ±3 SD from the mean) per metabolite prior to statistical testing. Of note, the exclusion of an outlier for one metabolite did not lead to removal of the corresponding spectrum from all other metabolites. To examine the relationship between GABA+, Glx, E/I ratio, tNAA, tCho, mI, tCr and healthy aging, we conducted Pearson correlations. We also correlated GM, WM, and CSF voxel fractions with age to assess age-related tissue composition differences. In addition, GABA+ and Glx analyses were followed up with two-tailed, independent-samples *t*-tests comparing the younger and older participants to improve interpretation. Finally, to assess potential effects of cognitive status, additional confirmatory correlations between age and metabolite levels were performed after excluding participants with MoCA scores below 22.

3. Results

Across the full sample, the mean MoCA score was 26.97 (SD: 2.38; range: 19–30), with only seven participants scoring below 22, including six who scored 21 and one who scored 19. We did not exclude anyone based on MoCA scores (see below). The mean and SD of the spectra quality metrics were as follows: Cr_SNR = 110.65 ± 21.91, Cr_FWHM = 6.36 ± 0.71 Hz, water_FWHM = 6.63 ± 0.82 Hz, frequency shift = −3.38 ± 1.82 Hz, relResA = 9.94 ± 21.16, and relResdiff1 = 3.95 ± 6.86. Exploratory Pearson correlations between spectra quality metrics and age are reported in the Supplemental Materials. Although these parameters were examined to ensure overall data quality and consistency, we did not apply them as exclusionary criteria as hard thresholds for exclusion do not currently exist. Instead, metabolite outliers were excluded based on values exceeding ±3 SD from the mean (see previous section), consistent with common practices for outlier detection. Following exclusion of outliers, 184 spectra were included in the final analyses of GABA+ (97 females; mean age: 47.46 [SD: 17.20], range: 19–79 years). In addition, 186 spectra were included in the final analyses of Glx (98 females; mean age: 47.87 [SD: 17.18], range: 19–79 years), tNAA (99 females; mean age: 47.59 [SD: 17.14], range: 19–79 years) and tCr (99 females; mean age: 47.59 [SD: 17.14], range: 19–79 years). Finally, for the E/I ratio, 183 spectra were included in the final analyses (96 females; mean age: 47.58 [SD: 17.16], range: 19–79 years). As expected, the GM fraction of the ^{1}H-MRS voxel decreased with age (0.61 ± 0.06; $r = -0.543$, $p < .001$), while WM (0.28 ± 0.06; $r = 0.176$, $p = .016$) and CSF (0.11 ± 0.05; $r = 0.477$, $p < .001$) fractions increased with age. All metabolite concentration estimates were corrected for voxel tissue composition.

Pearson correlations indicated a significant negative correlation between GABA+ and Glx concentration estimates and age (GABA+: $r = -0.172$, $p = .02$; Glx: $r = -0.270$, $p < .001$; Fig. 2). To further examine age-related effects on GABA+ and Glx levels and improve comparability to past studies, we conducted independent samples *t*-tests on younger and older groups. First, participants who fell within ±0.5 SDs of the mean age of the full sample were excluded, and those 0.5 SD above the mean were categorized into the older group while those 0.5 SD below the mean became members of the younger group. The two groups were closely matched for GABA+ (Younger: n = 67; 37 females; mean age: 28.3 [SD: 5.71], range: 19–39 years; Older: n = 70; 36 females; mean age: 65.7 [SD: 6.53], range: 56–79 years) and Glx analyses (Younger: n = 67; 37 females; mean age: 28.6 [SD: 5.83], range: 19–39 years; Older: n = 69; 35 females; mean age: 66.3 [SD: 6.23], range: 57–79 years). Complementing the correlational analyses, the results indicated that GABA+ ($t(131) = 3.22$, $p = .0016$) and Glx levels ($t(134) = 3.80$, $p < .001$) were significantly lower in older compared to younger adults (Fig. 2). Regarding the E/I ratio, Pearson correlations indicated a significant negative correlation ($r = -0.150$, $p = .043$; Fig. 3). Finally, regarding the other metabolites, a significant negative correlation between tNAA and age ($r = -0.340$, $p < .001$) and a positive correlation between tCr and age ($r = 0.159$, $p = .03$; Fig. 4) were also observed. No significant correlations were found between age and mI or tCho.

To assess whether our findings were possibly affected by cognitive decline in this healthy aging sample, we re-computed our analyses without the seven participants who scored below 22 on the MoCA. We chose 22 as the cutoff as this is a conservative threshold that is often

used to identify early-stage mild cognitive impairment. When the seven participants with MoCA scores below 22 were excluded, the correlation between age and GABA+ slightly strengthened ($r = -0.214$, $p = .004$), and the same was observed for the age-Glx correlation ($r = -0.299$, $p < .001$). The correlation of the E/I ratio with age was not affected ($r = -0.149$, $p = .048$). These findings indicate that the observed age-related associations were not driven by participants with lower cognitive scores.

4. Discussion

In this study, we investigated age-related neurochemical changes in a large cohort of healthy adults ($N = 187$; age range: 19–79 years) using ^{1}H-MRS to quantify GABA+, Glx, and the E/I ratio in the calcarine cortex, a region chosen for its high neural density and homogenous tissue composition (Collins et al., 2010; Garcia-Marin et al., 2024), which make it ideal for quantifying metabolites such as Glx and GABA+. We found robust negative associations between age and both GABA+ and Glx levels, suggesting that healthy aging is accompanied by a gradual decline in both inhibitory and excitatory neurotransmitter pools within the occipital cortex. Notably, we also observed a significant age-related reduction in the Glx/GABA+ ratio, a proxy for E/I balance. In addition, we conducted supplementary analyses to quantify a set of secondary metabolites and found that tNAA decreased significantly with age, while tCr increased with age. No age-related associations were observed for mI or tCho. Together, these results provide a comprehensive aging profile of neurochemical changes in the visual cortex and contribute to a growing literature on metabolic and neurotransmitter concentrations in humans.

Our findings of decreased GABA+ levels with age are in agreement with some previous ^{1}H-MRS studies. For example, Simmonite et al. (2019) reported significantly lower occipital GABA+ concentrations in older adults (aged 59–87) compared to younger ones (aged 18–23). Likewise, Gao et al. (2013) demonstrated reductions in GABA+ with increasing age across frontal and parietal cortices in adults aged 20–76, and similar findings were reported in the frontal cortex in 43–92 year-olds (Porges et al., 2017a). However, others have reported the opposite pattern of increased GABA with age in the frontal cortex (Ghisleni et al., 2015), or no change in the anterior cingulate cortex (Aufhaus et al., 2013). As mentioned in the introduction, such inconsistencies may reflect the frequent use of between-group (i.e., younger vs. older groups) compared to continuous approaches, relatively small sample sizes for aging research, the focus on different brain regions in different studies, different degrees of sophistication in the tissue composition correction, and/or the type of reference compound used (i.e., relative to water or a high concentration metabolite). In the current study, we attempted to address and overcome these issues by using a large sample that extended across most of the adult lifespan, performing both between group and continuous comparisons, and quantifying GABA+ relative to unsuppressed water, while correcting for effects of tissue composition (i.e., GM, WM, CSF) on tissue water content and water relaxation times. Our between-group and continuous comparisons yielded equivalent findings and are in agreement with a recent meta-analysis focused on lifespan changes that suggested that GABA+ rises during development, plateaus in early adulthood, and declines with aging (Porges et al., 2021). Support for such age-related declines in GABA+ with aging also comes from animal models. Studies in aged cats and monkeys have shown reductions

in the number and function of GABAergic neurons in the visual cortex (Leventhal et al., 2003; Hua et al., 2008), despite preserved total neuron count, suggesting a selective vulnerability of inhibitory systems. Reductions in GABAergic interneuron density, GABA receptor expression, and enzyme levels have also been observed in rodent hippocampus and cortex (Majdi et al., 2007; Rubio et al., 2012; Stanley et al., 2012; Soler et al., 2017). Importantly, these changes often occur independently of gross neuronal loss, reflecting a functional downregulation of inhibitory signaling. These changes are consistent with our observed decrease in GABA+ levels and may reflect a functional downregulation of inhibitory tone in aging rather than structural degeneration, especially since we corrected for GM volume.

Parallel to the decline in GABA+, we also found that Glx concentrations decreased with age in the calcarine cortex. Glx, comprising Glu and glutamine, reflects the excitatory pool, and the findings focusing on Glx have been more consistent than the GABA+ literature. For instance, Marjańska et al. (2017) reported reduced levels in the occipital cortex of older (aged 70–88) compared to younger adults (aged 19–22), while Gao et al. (2013) observed significant negative correlations between Glx and age in both frontal and parietal cortices. Similar age-related decreases have been reported across other brain areas, including the anterior cingulate (Hädel et al., 2013), motor cortex (Kaiser et al., 2005), precuneus (Suri et al., 2017), striatum (Zahr et al., 2013) and basal ganglia (Ghisleni et al., 2015). The current data support and extend these findings by replicating the pattern in the largest sample to date, correcting for tissue composition (since most past studies had not), and showing the same pattern of findings using both between-groups and continuous models of aging. While our approach was rigorous and leveraged a large sample, another recent large-scale study in 102 individuals (age range: ~20s–60 s years) did not show age-related Glx changes after tissue- and relaxation correction, derived from short-TE ^{1}H-MRS measurements, in the posterior cingulate cortex and the centrum semiovale (Gong et al., 2022). However, this study used the PRESS sequence and the gray matter content in both of the target voxels was likely considerably less than the current study, which may have contributed to the observed disparities. Given that Glu is the brain's primary excitatory neurotransmitter and plays a central role in synaptic plasticity and neuroenergetic metabolism (Meldrum, 2000; Magistretti and Allaman, 2015), the reductions in Glx observed in the current study likely reflect broader age-related declines in synaptic integrity and metabolic efficiency (Mattson and Magnus, 2006; Fjell and Walhovd, 2010). Moreover, diminished excitatory tone in sensory regions like the calcarine cortex may contribute to the well-documented age-related declines in perceptual sensitivity and cortical responsivity (Schmolesky et al., 2000; Hua et al., 2006; Yu et al., 2006; Betts et al., 2007). For instance, Hua et al. (2006) reported elevated spontaneous activity and reduced orientation selectivity in visual cortical neurons of aged cats, while Yu et al. (2006) found similar functional degradation in senescent rhesus monkeys. These findings align with the notion that age-related reductions in glutamatergic tone may impair stimulus-specific neuronal responsiveness and contribute to the functional decline of sensory systems in aging. Future work should examine whether these age-related declines in Glx underlie the well-known age-related increases in spontaneous cortical activity seen in the motor cortices (Heinrichs-Graham and Wilson, 2016; Heinrichs-Graham et al., 2018) and other brain regions, and/or if conditions with weaker spontaneous neural

activity (Springer et al., 2023; Schantell et al., 2024; Webert et al., 2024) are associated with increased Glx.

Importantly, we also observed a significant age-related decline in the Glx/GABA+ ratio, a measure that is more frequently being used as a proxy for E/I balance. To our knowledge, only a few prior ^{1}H-MRS studies have reported trajectories of Glx/GABA+, primarily focusing on development through early adulthood (Thomson et al., 2024). For instance, Thomson et al. (2024) used nonlinear modeling and found a robust decline in Glx/GABA+ ratio from age five to 35 years, primarily driven by reductions in Glx. Extending these findings, our results demonstrate that this imbalance continues into older age, reflected by parallel reductions in both excitatory and inhibitory neurochemical pools. A lower E/I ratio may indicate reduced excitatory drive and/or a compensatory shift toward increased inhibition, potentially dampening cortical responsiveness and limiting neural flexibility. Animal studies provide further support for this interpretation, as aged mice are known to exhibit reduced Glu/GABA ratios in the somatosensory cortex, accompanied by impaired synaptic responsiveness and diminished plasticity (Liguz-Lecznar et al., 2015). Likewise, cognitively-impaired aged rats have been shown to exhibit a selective reduction in excitatory input without a corresponding decline in inhibition in the parietal cortex, leading to a relative increase in inhibitory tone (Wong et al., 2006). Further, work in nonhuman primates has shown age-related reductions in the frequency of spontaneous excitatory postsynaptic currents along with increased inhibitory postsynaptic currents in the prefrontal cortex, indicating a shift in synaptic signaling toward inhibition (Luebke et al., 2004). Our *in vivo* findings contribute to this literature by demonstrating similar E/I alterations within a primary sensory region, namely the visual cortex, where age-related structural decline is thought to be minimal (Leuba and Garey, 1987; Raz et al., 2004). Indeed, evidence of age-related changes in visual cortex are primarily physiological in nature, with senescent animals exhibiting broader orientation tuning, elevated baseline firing rates, and slowed temporal processing in early visual areas, patterns linked to altered intracortical inhibition and reduced GABA levels (Schmolesky et al., 2000; Mendelson and Wells, 2002; Leventhal et al., 2003; Hua et al., 2006; Wang et al., 2006). Taken together, these results suggest that neurochemical and physiological alterations play a central role in age-related declines in visual cortical function.

In addition to primary neurotransmitters, we conducted supplementary analyses to quantify secondary metabolites including tNAA, tCho, tCr, and mI. Most notably, we observed an age-related decline in tNAA, which has been consistently reported across both cortical and subcortical regions and widely interpreted as a marker of reduced neuronal integrity, dendritic atrophy, and/or diminished mitochondrial function with age (Erickson et al., 2012; K. I. 2015; Hadel et al., 2013; Marjańska et al., 2017; Suri et al., 2017). Although again the findings have not been universal, as Gong et al. (2022) did not find tNAA or NAA decreases with age in the posterior cingulate cortex or centrum semiovale. Conversely, we found a significant increase in tCr with age, consistent with previous studies (Chiu et al., 2014; Marjańska et al., 2017), including several involving large-sample studies (Charlton et al., 2007, $n = 106$; McIntyre et al., 2007, $n = 106$; Reyngoudt et al., 2012, $n = 90$; Suri et al., 2017, $n = 147$; Gong et al., 2022, $n = 102$). Age-related increases in tCr are commonly interpreted as reflecting compensatory shifts in cellular energy metabolism,

given creatine's role in ATP buffering. Finally, we did not observe significant associations between age and either mI or tCho, which are in line with several investigations reporting no age-related change in mI (Reyngoudt et al., 2012; Chiu et al., 2014; Ding et al., 2016; Eylers et al., 2016) or tCho (Reyngoudt et al., 2012; Hadel et al., 2013; Z.-Y. Yang et al., 2015), although some other studies have found age-related associations (Zahr et al., 2013; Yang et al., 2014; Z.-Y. 2015; Suri et al., 2017; Gong et al., 2022). These mixed findings highlight the methodological heterogeneity across the ^{1}H-MRS literature, including differences in voxel location, tissue correction, reference choices, and acquisition parameters. In addition to such methodological heterogeneity, questions also remain about best practices in estimating metabolites using ^{1}H-MRS. While such questions regarding best practices exist for all noninvasive imaging modalities, some of the current ones for ^{1}H-MRS are that corrections for water content as well as metabolite and water relaxation times (i.e., the Gasparovic method; Gasparovic et al., 2006) have long relied on literature reference values, which in general are sparse and do not consider the potential age dependencies of water content, water relaxation times, or metabolite relaxation times. Several recent large-scale studies suggest that these parameters may change in a region-specific manner with age (Murali-Manohar et al., 2024, 2025; Hupfeld et al., 2025; Simegn et al., 2025). Such age dependencies, as well as differences between segmentation algorithms (Archibald et al., 2025), could confound many correction approaches and affect metabolite concentration estimates, but more work is needed and currently ongoing. To this end, large-scale datasets like that generated here will be a valuable resource for those interested in clarifying the complex interplay between age-related changes of anatomy, water content, relaxation behavior, and metabolite concentration estimation.

Before closing, it is important to consider several limitations. First, our results are limited to the calcarine cortex, a region chosen for its favorable properties for ^{1}H-MRS acquisition (i.e., high neural density and relatively homogeneous tissue composition). Since both GABA+ and Glx levels may vary differently with age across the cortex, caution is warranted in generalizing these findings to other brain regions. Future studies should aim to simultaneously investigate GABA+ and Glx levels across multiple regions (e.g., prefrontal and parietal cortices) to determine whether similar age-related neurochemical patterns emerge. Second, while MEGA-PRESS is specifically optimized to detect GABA+, the signal includes contributions from co-edited macromolecules. Although this convention is widely adopted, it remains a limitation of the method, and future work could consider approaches that more selectively quantify GABA. Along the same lines, a key benefit of MEGA-PRESS is that it also provides reliable Glx quantification from the edit-OFF spectrum, making it a robust method for simultaneously measuring GABA+ and Glx (O'Gorman et al., 2011; Puts and Edden, 2012; Harris et al., 2017; Maddock et al., 2018). Nonetheless, future studies could benefit from using PRESS or sLASER sequences, which are more tailored to an optimal detection of Glx and other metabolites (e.g., tNAA, tCr, tCho and mI), in order to confirm whether our age-related effects hold using different acquisition methods. Third, as discussed above, age-related changes in water and metabolite relaxation times may influence concentration estimates. While our quantification approach included standard tissue and relaxation corrections based on literature values, future studies should incorporate age-specific relaxation parameters, such as those recently made available

in atlas-based frameworks (Simegn et al., 2025) to improve the accuracy of metabolite quantification across the adult lifespan. Finally, our sample was comprised of healthy aging adults and future work should examine patients with neurological disorders to determine whether these accentuate the decline in neurotransmitter levels with aging.

Despite these limitations, the present study offers several important strengths that advance our understanding of key neurotransmitter changes in the context of healthy aging. By leveraging a large cross-sectional sample (n = 187) spanning most of the adult lifespan (19–79 years) and applying rigorous methods, we were able to assess GABA+, Glx, and the E/I ratio simultaneously, alongside secondary metabolites such as tNAA, tCr, tCho and mI, and model the effects of aging. Our use of water-referenced metabolite quantification and full tissue composition correction aligns with current best practices and enhances the reliability, validity, and generalizability of our findings. In addition, targeting the calcarine cortex enabled optimized signal-to-noise ratios for detecting subtle age-related differences in the low-concentration metabolites. Collectively, these methodological strengths position our study as a robust contribution to the growing literature on neurochemical markers of healthy aging and provide a strong foundation for future longitudinal and multi-regional investigations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data availability statement:

The data used in this article will be made publicly available through the COINS framework at the completion of the study (https://coins.trendscenter.org/).

References

Akbarian S, Kim JJ, Potkin SG, Hagman JO, Tafazzoli A, Bunney WE Jr, Jones EG, 1995. Gene expression for glutamic acid decarboxylase is reduced without loss of neurons in prefrontal cortex of schizophrenics. Arch. Gen. Psychiatry 52 (4), 258–266. 10.1001/archpsyc.1995.03950160008002. [PubMed: 7702443]

Andrews-Hanna JR, Snyder AZ, Vincent JL, Lustig C, Head D, Raichle ME, Buckner RL, 2007. Disruption of large-scale brain systems in advanced aging. Neuron 56 (5), 924–935. 10.1016/j.neuron.2007.10.038. [PubMed: 18054866]

Archibald J, Igwe KC, Kaiser A, Landheer K, Lee J, Kramer JLK, Zölch N, Gudmundson A, Zöllner HJ, Fleischer CC, Oeltzschner G, Near J, Mikkelsen M, 2025. Benchmarking the impact of anatomical segmentation on in vivo magnetic resonance spectroscopy. bioRxiv. 10.1101/2025.07.15.664931.

Aufhaus E, Weber-F0ahr W, Sack M, Tunc-Skarka N, Oberthuer G, Hoerst M, Meyer-Lindenberg A, Boettcher U, Ende G, 2013. Absence of changes in GABA concentrations with age and gender in the human anterior cingulate cortex: a MEGA-PRESS study with symmetric editing pulse frequencies for macromolecule suppression: GABA MEGA-PRESS with macromolecule suppression. Magn. Reson. Med 69 (2), 317–320. 10.1002/mrm.24257. [PubMed: 22488982]

Betts LR, Sekuler AB, Bennett PJ, 2007. The effects of aging on orientation discrimination. Vision. Res 47 (13), 1769–1780. 10.1016/j.visres.2007.02.016. [PubMed: 17466355]

Betzel RF, Byrge L, He Y, Goñi J, Zuo X-N, Sporns O, 2014. Changes in structural and functional connectivity among resting-state networks across the human lifespan. Neuroimage 102 (Pt 2), 345–357. 10.1016/j.neuroimage.2014.07.067. [PubMed: 25109530]

Cabeza R, Albert M, Belleville S, Craik F, Duarte A, Grady C, Lindenberger U, Nyberg L, Park D, Reuter-Lorenz PA, Rugg MD, 2018. Cognitive neuroscience of healthy aging: maintenance, reserve, and compensation. Neuroscience 19 (11).

Cabeza R., 2002. Hemispheric asymmetry reduction in older adults: the HAROLD model. Psychol. Aging 17 (1), 85–100. 10.1037//0882-7974.17.1.85. [PubMed: 11931290]

Chang L, Ernst T, Poland RE, Jenden DJ, 1996. In vivo proton magnetic resonance spectroscopy of the normal aging human brain. Life Sci. 58 (22), 2049–2056. 10.1016/0024-3205(96)00197-x. [PubMed: 8637436]

Charlton RA, McIntyre DJO, Howe FA, Morris RG, Markus HS, 2007. The relationship between white matter brain metabolites and cognition in normal aging: the GENIE study. Brain Res. 1164, 108–116. 10.1016/j.brainres.2007.06.027. [PubMed: 17632090]

Chen X, Liang Y, Deng Y, Li J, Chen S, Wang C, Luo P, 2013. Age-associated reduction of asymmetry in human central auditory function: a 1H-magnetic resonance spectroscopy study. Neural Plast. 2013, 735290. 10.1155/2013/735290. [PubMed: 24222864]

Chiu P-W, Mak HK-F, Yau KK-W, Chan Q, Chang RC-C, Chu L-W, 2014. Metabolic changes in the anterior and posterior cingulate cortices of the normal aging brain: proton magnetic resonance spectroscopy study at 3 T. Age 36 (1), 251–264. 10.1007/s11357-013-9545-8. [PubMed: 23709317]

Cleeland C, Pipingas A, Scholey A, White D, 2019. Neurochemical changes in the aging brain: a systematic review. Neurosci. Biobehav. Rev 98, 306–319. 10.1016/j.neubiorev.2019.01.003. [PubMed: 30625337]

Collins CE, Airey DC, Young NA, Leitch DB, Kaas JH, 2010. Neuron densities vary across and within cortical areas in primates. In: Proceedings of the National Academy of Sciences of the United States of America, 107, pp. 15927–15932. 10.1073/pnas.1010356107. [PubMed: 20798050]

Dennis NA, Cabeza R, 2011. Neuroimaging of healthy cognitive aging. In: The Handbook of Aging and Cognition. Psychology Press, pp. 1–54.

Ding X-Q, Maudsley AA, Sabati M, Sheriff S, Schmitz B, Schütze M, Bronzlik P, Kahl KG, Lanfermann H, 2016. Physiological neuronal decline in healthy aging human brain — An in vivo study with MRI and short echo-time whole-brain 1H MR spectroscopic imaging. Neuroimage 137, 45–51. 10.1016/j.neuroimage.2016.05.014. [PubMed: 27164326]

Duarte JMN, Do KQ, Gruetter R, 2014. Longitudinal neurochemical modifications in the aging mouse brain measured in vivo by 1H magnetic resonance spectroscopy. Neurobiol. Aging 35 (7), 1660–1668. 10.1016/j.neurobiolaging.2014.01.135. [PubMed: 24560998]

Edden RAE, Puts NAJ, Harris AD, Barker PB, Evans CJ, 2014. Gannet: a batch-processing tool for the quantitative analysis of gamma-aminobutyric acid–edited MR spectroscopy spectra: gannet: GABA Analysis Toolkit. J. Magn. Reson. Imaging 40 (6), 1445–1452. 10.1002/jmri.24478. [PubMed: 25548816]

Erickson KI, Leckie RL, Weinstein AM, Radchenkova P, Sutton BP, Prakash RS, Voss MW, Chaddock-Heyman L, McAuley E, Kramer AF, 2015. Education mitigates age-related decline in N-acetylaspartate levels. Brain Behav. 5 (3), e00311. 10.1002/brb3.311. [PubMed: 25798329]

Erickson KI, Weinstein AM, Sutton BP, Prakash RS, Voss MW, Chaddock L, Szabo AN, Mailey EL, White SM, Wojcicki TR, McAuley E, Kramer AF, 2012. Beyond vascularization: aerobic fitness is associated with N-acetylaspartate and working memory: NAA and fitness. Brain Behav. 2 (1), 32–41. 10.1002/brb3.30. [PubMed: 22574272]

Eylers VV, Maudsley AA, Bronzlik P, Dellani PR, Lanfermann H, Ding X-Q, 2016. Detection of normal aging effects on human brain metabolite concentrations and microstructure with whole-brain MR spectroscopic imaging and quantitative MR imaging. AJNR Am. J. Neuroradiol 37 (3), 447–454. 10.3174/ajnr.A4557. [PubMed: 26564440]

Fjell AM, Walhovd KB, 2010. Structural brain changes in aging: courses, causes and cognitive consequences. Rev. Neurosci 21 (3), 187–221. 10.1515/revneuro.2010.21.3.187. [PubMed: 20879692]

Fjell AM, Westlye LT, Amlien I, Espeseth T, Reinvang I, Raz N, Agartz I, Salat DH, Greve DN, Fischl B, Dale AM, Walhovd KB, 2009. High consistency of regional cortical thinning in aging across multiple samples. Cereb. Cortex 1991 19 (9), 2001–2012. 10.1093/cercor/bhn232.

Foss-Feig JH, Adkinson BD, Ji JL, Yang G, Srihari VH, McPartland JC, Krystal JH, Murray JD, Anticevic A, 2017. Searching for cross-diagnostic convergence: neural mechanisms governing excitation and inhibition balance in schizophrenia and autism spectrum disorders. Biol. Psychiatry 81 (10), 848–861. 10.1016/j.biopsych.2017.03.005. [PubMed: 28434615]

Gao F, Edden RAE, Li M, Puts NAJ, Wang G, Liu C, Zhao B, Wang H, Bai X, Zhao C, Wang X, Barker PB, 2013. Edited magnetic resonance spectroscopy detects an age-related decline in brain GABA levels. Neuroimage 78, 75–82. 10.1016/j.neuroimage.2013.04.012. [PubMed: 23587685]

Garcia-Marin V, Kelly JG, Hawken MJ, 2024. Neuronal composition of processing modules in human V1: laminar density for neuronal and non-neuronal populations and a comparison with macaque. Cereb. Cortex 1991 34 (2). 10.1093/cercor/bhad512.

Gasparovic C, Song T, Devier D, Bockholt HJ, Caprihan A, Mullins PG, Posse S, Jung RE, Morrison LA, 2006. Use of tissue water as a concentration reference for proton spectroscopic imaging. Magn. Reson. Med 55 (6), 1219–1226. 10.1002/mrm.20901. [PubMed: 16688703]

Geerligs L, Maurits NM, Renken RJ, Lorist MM, 2014. Reduced specificity of functional connectivity in the aging brain during task performance: functional Connectivity in the aging brain. Hum. Brain Mapp 35 (1), 319–330. 10.1002/hbm.22175. [PubMed: 22915491]

Ghisleni C, Bollmann S, Poil S-S, Brandeis D, Martin E, Michels L, O'Gorman RL, Klaver P, 2015. Subcortical glutamate mediates the reduction of short-range functional connectivity with age in a developmental cohort. J. Neurosci.: Off. J. Soc. Neurosci 35 (22), 8433–8441. 10.1523/JNEUROSCI.4375-14.2015.

Gong T, Hui SCN, Zöllner HJ, Britton M, Song Y, Chen Y, Gudmundson AT, Hupfeld KE, Davies-Jenkins CW, Murali-Manohar S, Porges EC, Oeltzschner G, Chen W, Wang G, Edden RAE, 2022. Neurometabolic timecourse of healthy aging. Neuroimage 264 (119740), 119740. 10.1016/j.neuroimage.2022.119740. [PubMed: 36356822]

Good CD, Johnsrude IS, Ashburner J, Henson RN, Friston KJ, Frackowiak RS, 2001. A voxel-based morphometric study of ageing in 465 normal adult human brains. Neuroimage 14 (1), 21–36. 10.1006/nimg.2001.0786, 1 Pt. [PubMed: 11525331]

Govindaraju V, Young K, Maudsley AA, 2000. Proton NMR chemical shifts and coupling constants for brain metabolites. NMR Biomed. 13 (3), 129–153. 10.1002/1099-1492(200005)13:3<129::aid-bm619>3.0.co;2-v. [PubMed: 10861994]

Grady C., 2012. Trends in neurocognitive aging. Nat. Rev. Neurosci 13, 491–505. [PubMed: 22714020]

Grady CL, Maisog JM, Horwitz B, Ungerleider LG, Mentis MJ, Salerno JA, Pietrini P, Wagner E, Haxby JV, 1994. Age-related changes in cortical blood flow activation during visual processing of faces and location. J. Neurosci.: Off. J. Soc. Neurosci 14 (3), 1450–1462. 10.1523/jneurosci.14-03-01450.1994.

Hädel S, Wirth C, Rapp M, Gallinat J, Schubert F, 2013. Effects of age and sex on the concentrations of glutamate and glutamine in the human brain: brain glutamate and glutamine with age and sex. J. Magn. Reson. Imaging 38 (6), 1480–1487. 10.1002/jmri.24123. [PubMed: 23564615]

Haga KK, Khor YP, Farrall A, Wardlaw JM, 2009. A systematic review of brain metabolite changes, measured with 1H magnetic resonance spectroscopy, in healthy aging. Neurobiol. Aging 30 (3), 353–363. 10.1016/j.neurobiolaging.2007.07.005. [PubMed: 17719145]

Harris AD, Puts NAJ, Edden RAE, 2015. Tissue correction for GABA-edited MRS: considerations of voxel composition, tissue segmentation, and tissue relaxations: tissue correction for GABA-edited MRS. J. Magn. Reson. Imaging 42 (5), 1431–1440. 10.1002/jmri.24903. [PubMed: 26172043]

Harris AD, Saleh MG, Edden RAE, 2017. Edited 1 H magnetic resonance spectroscopy in vivo: methods and metabolites. Magn. Reson. Med 77 (4), 1377–1389. 10.1002/mrm.26619. [PubMed: 28150876]

Harris JL, Yeh H-W, Swerdlow RH, Choi I-Y, Lee P, Brooks WM, 2014. High-field proton magnetic resonance spectroscopy reveals metabolic effects of normal brain aging. Neurobiol. Aging 35 (7), 1686–1694. 10.1016/j.neurobiolaging.2014.01.018. [PubMed: 24559659]

Heinrichs-Graham E, McDermott TJ, Mills MS, Wiesman AI, Wang Y-P, Stephen JM, Calhoun VD, Wilson TW, 2018. The lifespan trajectory of neural oscillatory activity in the motor system. Dev. Cogn. Neurosci 30, 159–168. 10.1016/j.dcn.2018.02.013. [PubMed: 29525417]

Heinrichs-Graham E, Wilson TW, 2016. Is an absolute level of cortical beta suppression required for proper movement? Magnetoencephalographic evidence from healthy aging. Neuroimage 134, 514–521. 10.1016/j.neuroimage.2016.04.032. [PubMed: 27090351]

Henry PG, Dautry C, Hantraye P, Bloch G, 2001. Brain GABA editing without macromolecule contamination. Magn. Reson. Med 45 (3), 517–520. 10.1002/1522-2594(200103)45:3<517::aid-rm1068>3.0.co;2-6. [PubMed: 11241712]

Hoyer C, Gass N, Weber-Fahr W, Sartorius A, 2014. Advantages and challenges of small animal magnetic resonance imaging as a translational tool. Neuropsychobiology. 69 (4), 187–201. 10.1159/000360859. [PubMed: 24863537]

Hua T, Kao C, Sun Q, Li X, Zhou Y, 2008. Decreased proportion of GABA neurons accompanies age-related degradation of neuronal function in cat striate cortex. Brain Res. Bull 75 (1), 119–125. 10.1016/j.brainresbull.2007.08.001. [PubMed: 18158105]

Hua T, Li X, He L, Zhou Y, Wang Y, Leventhal AG, 2006. Functional degradation of visual cortical cells in old cats. Neurobiol. Aging 27 (1), 155–162. 10.1016/j.neurobiolaging.2004.11.012. [PubMed: 16298251]

Huang D, Liu D, Yin J, Qian T, Shrestha S, Ni H, 2017. Glutamate-glutamine and GABA in brain of normal aged and patients with cognitive impairment. Eur. Radiol 27 (7), 2698–2705. 10.1007/s00330-016-4669-8. [PubMed: 27966041]

Hupfeld KE, Murali-Manohar S, Zöllner HJ, Song Y, Davies-Jenkins CW, Gudmundson AT, Simicic D, Lamesgin Simegn G, Carter EE, Hui SCN, Yedavalli V, Oeltzschner G, Porges EC, Edden RAE, 2025. Metabolite T2 relaxation times decrease across the adult lifespan in a large multi-site cohort. Magn. Reson. Med 93 (3), 916–929. 10.1002/mrm.30340. [PubMed: 39444343]

Jernigan TL, Archibald SL, Berhow MT, Sowell ER, Foster DS, Hesselink JR, 1991. Cerebral structure on MRI, part I: localization of age-related changes. Biol. Psychiatry 29 (1), 55–67. 10.1016/0006-3223(91)90210-d. [PubMed: 2001446]

Kaiser LG, Schuff N, Cashdollar N, Weiner MW, 2005. Age-related glutamate and glutamine concentration changes in normal human brain: 1H MR spectroscopy study at 4 T. Neurobiol. Aging 26 (5), 665–672. 10.1016/j.neurobiolaging.2004.07.001. [PubMed: 15708441]

Klose U., 1990. In vivo proton spectroscopy in presence of eddy currents. Magn. Reson. Med 14 (1), 26–30. 10.1002/mrm.1910140104. [PubMed: 2161984]

Koen JD, Rugg MD, 2019. Neural dedifferentiation in the aging brain. Trends Cogn. Sci. Regul. Ed 23 (7), 547–559. 10.1016/j.tics.2019.04.012.

Lemaître H, Crivello F, Grassiot B, Alpérovitch A, Tzourio C, Mazoyer B, 2005. Age- and sex-related effects on the neuroanatomy of healthy elderly. Neuroimage 26 (3), 900–911. 10.1016/j.neuroimage.2005.02.042. [PubMed: 15955500]

Leuba G, Garey LJ, 1987. Evolution of neuronal numerical density in the developing and aging human visual cortex. Hum. Neurobiol 6 (1), 11–18. [PubMed: 3583841]

Leventhal AG, Wang Y, Pu M, Zhou Y, Ma Y, 2003. GABA and its agonists improved visual cortical function in senescent monkeys. Science (1979) 300 (5620), 812–815. 10.1126/science.1082874.

Liguz-Lecznar M, Lehner M, Kaliszewska A, Zakrzewska R, Sobolewska A, Kossut M, 2015. Altered glutamate/GABA equilibrium in aged mice cortex influences cortical plasticity. Brain Struct. Funct 220 (3), 1681–1693. 10.1007/s00429-014-0752-6. [PubMed: 24659256]

Lisman J., 2012. Excitation, inhibition, local oscillations, or large-scale loops: what causes the symptoms of schizophrenia? Curr. Opin. Neurobiol 22 (3), 537–544. 10.1016/j.conb.2011.10.018. [PubMed: 22079494]

Luebke JI, Chang Y-M, Moore TL, Rosene DL, 2004. Normal aging results in decreased synaptic excitation and increased synaptic inhibition of layer 2/3 pyramidal cells in the monkey prefrontal cortex. Neuroscience 125 (1), 277–288. 10.1016/j.neuroscience.2004.01.035. [PubMed: 15051166]

Maddock RJ, Casazza GA, Fernandez DH, Maddock MI, 2016. Acute modulation of cortical glutamate and GABA content by physical activity. J. Neurosci.: Off. J. Soc. Neurosci 36 (8), 2449–2457. 10.1523/JNEUROSCI.3455-15.2016.

Maddock RJ, Caton MD, Ragland JD, 2018. Estimating glutamate and glx from GABA-optimized MEGA-PRESS: off-resonance but not difference spectra values correspond to PRESS values. Psychiatry Res.. Neuroimaging 279, 22–30. 10.1016/j.pscychresns.2018.07.003. [PubMed: 30081290]

Maes C, Hermans L, Pauwels L, Chalavi S, Leunissen I, Levin O, Cuypers K, Peeters R, Sunaert S, Mantini D, Puts NAJ, Edden RAE, Swinnen SP, 2018. Age-related differences in GABA levels are driven by bulk tissue changes. Hum. Brain Mapp 39 (9), 3652–3662. 10.1002/hbm.24201. [PubMed: 29722142]

Magistretti PJ, Allaman I, 2015. A cellular perspective on brain energy metabolism and functional imaging. Neuron 86 (4), 883–901. 10.1016/j.neuron.2015.03.035. [PubMed: 25996133]

Majdi M, Ribeiro-da-Silva A, Cuello AC, 2007. Cognitive impairment and transmitter-specific pre- and postsynaptic changes in the rat cerebral cortex during ageing: synaptic changes in the ageing rat cerebral cortex. Eur. J. Neurosci 26 (12), 3583–3596. 10.1111/j.1460-9568.2007.05966.x. [PubMed: 18088281]

Marenco S, Meyer C, van der Veen JW, Zhang Y, Kelly R, Shen J, Weinberger DR, Dickinson D, Berman KF, 2018. Role of gamma-amino-butyric acid in the dorsal anterior cingulate in age-associated changes in cognition. Neuropsychopharmacol.: Off. Publ. Am. Coll. Neuropsychopharmacol 43 (11), 2285–2291. 10.1038/s41386-018-0134-5.

Marín O., 2012. Interneuron dysfunction in psychiatric disorders. Nat. Rev. Neurosci 13 (2), 107–120. 10.1038/nrn3155. [PubMed: 22251963]

Marjańska M, Lehéricy S, Valabrègue R, Popa T, Worbe Y, Russo M, Auerbach EJ, Grabli D, Bonnet C, Gallea C, Coudert M, Yahia-Cherif L, Vidailhet M, Meunier S, 2013. Brain dynamic neurochemical changes in dystonic patients: a magnetic resonance spectroscopy study: MRS Study in dystonic patients after TMS. Mov. Disord.: Off. J. Mov. Disord. Soc 28 (2), 201–209. 10.1002/mds.25279.

Marjańska M, McCarten JR, Hodges J, Hemmy LS, Grant A, Deelchand DK, Terpstra M, 2017. Region-specific aging of the human brain as evidenced by neurochemical profiles measured noninvasively in the posterior cingulate cortex and the occipital lobe using 1H magnetic resonance spectroscopy at 7 T. Neuroscience 354, 168–177. 10.1016/j.neuroscience.2017.04.035. [PubMed: 28476320]

Mattson MP, Magnus T, 2006. Ageing and neuronal vulnerability. Nat. Rev. Neurosci 7 (4), 278–294. 10.1038/nrn1886. [PubMed: 16552414]

McIntyre DJO, Charlton RA, Markus HS, Howe FA, 2007. Long and short echo time proton magnetic resonance spectroscopic imaging of the healthy aging brain. J. Magn. Reson. Imaging 26 (6), 1596–1606. 10.1002/jmri.21198. [PubMed: 17968966]

McQuail JA, Frazier CJ, Bizon JL, 2015. Molecular aspects of age-related cognitive decline: the role of GABA signaling. Trends. Mol. Med 21 (7), 450–460. 10.1016/j.molmed.2015.05.002. [PubMed: 26070271]

Meldrum BS, 2000. Glutamate as a neurotransmitter in the brain: review of physiology and pathology. J. Nutr 130 (4), 1007S–1015S. 10.1093/jn/130.4.1007s. [PubMed: 10736372]

Mendelson JR, Wells EF, 2002. Age-related changes in the visual cortex. Vision. Res 42 (6), 695–703. 10.1016/s0042-6989(01)00307-8. [PubMed: 11888535]

Mescher M, Merkle H, Kirsch J, Garwood M, Gruetter R, 1998. Simultaneous in vivo spectral editing and water suppression. NMR Biomed. 11 (6), 266–272. 10.1002/(sici)1099-1492(199810)11:6<266::aid-bm530>3.3.co;2-a. [PubMed: 9802468]

Mikkelsen M, Barker PB, Bhattacharyya PK, Brix MK, Buur PF, Cecil KM, Chan KL, Chen DY-T, Craven AR, Cuypers K, Dacko M, Duncan NW, Dydak U, Edmondson DA, Ende G, Ersland L, Gao F, Greenhouse I, Harris AD, Edden RAE, 2017. Big GABA: edited MR spectroscopy at 24 research sites. Neuroimage 159, 32–45. 10.1016/j.neuroimage.2017.07.021. [PubMed: 28716717]

Mikkelsen M, Saleh MG, Near J, Chan KL, Gong T, Harris AD, Oeltzschner G, Puts NAJ, Cecil KM, Wilkinson ID, Edden RAE, 2018. Frequency and phase correction for multiplexed edited MRS of GABA and glutathione. Magn. Reson. Med 80 (1), 21–28. 10.1002/mrm.27027. [PubMed: 29215137]

Mikkelsen M, Tapper S, Near J, Mostofsky SH, Puts NAJ, Edden RAE, 2020. Correcting frequency and phase offsets in MRS data using robust spectral registration. NMR Biomed. 33 (10), e4368. 10.1002/nbm.4368. [PubMed: 32656879]

Mooney RA, Cirillo J, Byblow WD, 2017. GABA and primary motor cortex inhibition in young and older adults: a multimodal reliability study. J. Neurophysiol 118 (1), 425–433. 10.1152/jn.00199.2017. [PubMed: 28424294]

Mullins PG, McGonigle DJ, O'Gorman RL, Puts NAJ, Vidyasagar R, Evans CJ, Cardiff Symposium on MRS of GABA, & Edden RAE, 2014. Current practice in the use of MEGA-PRESS spectroscopy for the detection of GABA. Neuroimage 86, 43–52. 10.1016/j.neuroimage.2012.12.004. [PubMed: 23246994]

Murali-Manohar S, Gudmundson AT, Hupfeld KE, Zöllner HJ, Hui SCN, Song Y, Simicic D, Davies-Jenkins CW, Gong T, Wang G, Oeltzschner G, Edden RAE, 2024. Metabolite T1 relaxation times decrease across the adult lifespan. NMR Biomed. 37 (9), e5152. 10.1002/nbm.5152. [PubMed: 38565525]

Murali-Manohar S, Zöllner HJ, Hupfeld KE, Song Y, Carter EE, Yedavalli V, Hui SCN, Simicic D, Gudmundson AT, Simegn GL, Davies-Jenkins CW, Oeltzschner G, Porges EC, Edden RAE, 2025. Age dependency of neurometabolite T1 relaxation times. Magn. Reson. Med 94 (2), 508–520. 10.1002/mrm.30507. [PubMed: 40228052]

Nasreddine ZS, Phillips NA, Bédirian V, Charbonneau S, Whitehead V, Collin I, Cummings JL, Chertkow H, 2005. The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment: moca: a brief screening tool for MCI. J. Am. Geriatr. Soc 53 (4), 695–699. 10.1111/j.1532-5415.2005.53221.x. [PubMed: 15817019]

Near J, Edden R, Evans CJ, Paquin R, Harris A, Jezzard P, 2015. Frequency and phase drift correction of magnetic resonance spectroscopy data by spectral registration in the time domain. Magn. Reson. Med 73 (1), 44–50. 10.1002/mrm.25094. [PubMed: 24436292]

Nelson SB, Valakh V, 2015. Excitatory/inhibitory balance and circuit homeostasis in autism spectrum disorders. Neuron 87 (4), 684–698. 10.1016/j.neuron.2015.07.033. [PubMed: 26291155]

Novotny EJ Jr, Fulbright RK, Pearl PL, Gibson KM, Rothman DL, 2003. Magnetic resonance spectroscopy of neurotransmitters in human brain. Ann. Neurol 54 (6), S25–S31. 10.1002/ana.10697. *Suppl*(S6). [PubMed: 12891651]

Oeltzschner G, Zöllner HJ, Hui SCN, Mikkelsen M, Saleh MG, Tapper S, Edden RAE, 2020. Osprey: open-source processing, reconstruction & estimation of magnetic resonance spectroscopy data. J. Neurosci. Methods 343 (108827), 108827. 10.1016/j.jneumeth.2020.108827. [PubMed: 32603810]

O'Gorman RL, Michels L, Edden RA, Murdoch JB, Martin E, 2011. In vivo detection of GABA and glutamate with MEGA-PRESS: reproducibility and gender effects. J. Magn. Reson. Imaging 33 (5), 1262–1267. 10.1002/jmri.22520. [PubMed: 21509888]

Öngür D, Haddad S, Prescot AP, Jensen JE, Siburian R, Cohen BM, Renshaw PF, Smoller JW, 2011. Relationship between genetic variation in the glutaminase gene GLS1 and brain glutamine/glutamate ratio measured in vivo. Biol. Psychiatry 70 (2), 169–174. 10.1016/j.biopsych.2011.01.033. [PubMed: 21457947]

Onoda K, Ishihara M, Yamaguchi S, 2012. Decreased functional connectivity by aging is associated with cognitive decline. J. Cogn. Neurosci 24 (11), 2186–2198. 10.1162/jocn_a_00269. [PubMed: 22784277]

Patel TB, Clark JB, 1979. Synthesis of N-acetyl-L-aspartate by rat brain mitochondria and its involvement in mitochondrial/cytosolic carbon transport. Biochem. J 184 (3), 539–546. 10.1042/bj1840539. [PubMed: 540047]

Peek AL, Rebbeck TJ, Leaver AM, Foster SL, Refshauge KM, Puts NA, Oeltzschner G, MRS Expert Panel, 2023. A comprehensive guide to MEGA-PRESS for GABA measurement. Anal. Biochem 669 (115113), 115113. 10.1016/j.ab.2023.115113. [PubMed: 36958511]

Peters A, Sethares C, Luebke JI, 2008. Synapses are lost during aging in the primate prefrontal cortex. Neuroscience 152 (4), 970–981. 10.1016/j.neuroscience.2007.07.014. [PubMed: 18329176]

Porges EC, Jensen G, Foster B, Edden RA, Puts NA, 2021. The trajectory of cortical GABA across the lifespan, an individual participant data meta-analysis of edited MRS studies. Elife 10. 10.7554/eLife.62575.

Porges EC, Woods AJ, Edden RAE, Puts NAJ, Harris AD, Chen H, Garcia AM, Seider TR, Lamb DG, Williamson JB, Cohen RA, 2017a. Frontal gamma-aminobutyric acid concentrations are associated with cognitive performance in older adults. Biol. Psychiatry: Cogn. Neurosci. Neuroimaging 2 (1), 38–44. 10.1016/j.bpsc.2016.06.004. [PubMed: 28217759]

Porges EC, Woods AJ, Lamb DG, Williamson JB, Cohen RA, Edden RAE, Harris AD, 2017b. Impact of tissue correction strategy on GABA-edited MRS findings. Neuroimage 162, 249–256. 10.1016/j.neuroimage.2017.08.073. [PubMed: 28882635]

Puts NAJ, Barker PB, Edden RAE, 2013. Measuring the longitudinal relaxation time of GABA in vivo at 3 tesla. J. Magn. Reson. Imaging 37 (4), 999–1003. 10.1002/jmri.23817. [PubMed: 23001644]

Puts NAJ, Edden RAE, 2012. In vivo magnetic resonance spectroscopy of GABA: a methodological review. Prog. Nucl. Magn. Reson. Spectrosc 60, 29–41. 10.1016/j.pnmrs.2011.06.001. [PubMed: 22293397]

Puts NAJ, Wodka EL, Harris AD, Crocetti D, Tommerdahl M, Mostofsky SH, Edden RAE, 2017. Reduced GABA and altered somatosensory function in children with autism spectrum disorder. Autism Res.: Off. J. Int. Soc. Autism Res 10 (4), 608–619. 10.1002/aur.1691.

Rae CD, 2014. A guide to the metabolic pathways and function of metabolites observed in human brain 1H magnetic resonance spectra. Neurochem. Res 39 (1), 1–36. 10.1007/s11064-013-1199-5. [PubMed: 24258018]

Raininko R, Mattsson P, 2010. Metabolite concentrations in supraventricular white matter from teenage to early old age: a short echo time 1H magnetic resonance spectroscopy (MRS) study. Acta Radiol 1987 51 (3), 309–315. 10.3109/02841850903476564.

Raz N, Gunning-Dixon F, Head D, Rodrigue KM, Williamson A, Acker JD, 2004. Aging, sexual dimorphism, and hemispheric asymmetry of the cerebral cortex: replicability of regional differences in volume. Neurobiol. Aging 25 (3), 377–396. 10.1016/S0197-4580(03)00118-0. [PubMed: 15123343]

Resnick SM, Pham DL, Kraut MA, Zonderman AB, Davatzikos C, 2003. Longitudinal magnetic resonance imaging studies of older adults: a shrinking brain. J. Neurosci.: Off. J. Soc. Neurosci 23 (8), 3295–3301. 10.1523/jneurosci.23-08-03295.2003.

Reuter-Lorenz PA, Cappell KA, 2008. Neurocognitive aging and the compensation hypothesis. Curr. Dir. Psychol. Sci 17 (3), 177–182. 10.1111/j.1467-8721.2008.00570.x.

Reyngoudt H, Claeys T, Vlerick L, Verleden S, Acou M, Deblaere K, De Deene Y, Audenaert K, Goethals I, Achten E, 2012. Age-related differences in metabolites in the posterior cingulate cortex and hippocampus of normal ageing brain: a 1H-MRS study. Eur. J. Radiol 81 (3), e223–e231. 10.1016/j.ejrad.2011.01.106. [PubMed: 21345628]

Roalf DR, Sydnor VJ, Woods M, Wolk DA, Scott JC, Reddy R, Moberg PJ, 2020. A quantitative meta-analysis of brain glutamate metabolites in aging. Neurobiol. Aging 95, 240–249. 10.1016/j.neurobiolaging.2020.07.015. [PubMed: 32866885]

Ross AJ, Sachdev PS, 2004. Magnetic resonance spectroscopy in cognitive research. Brain Res. Brain Res. Rev 44 (2–3), 83–102. 10.1016/j.brainresrev.2003.11.001. [PubMed: 15003387]

Rowland LM, Krause BW, Wijtenburg SA, McMahon RP, Chiappelli J, Nugent KL, Nisonger SJ, Korenic SA, Kochunov P, Hong LE, 2016. Medial frontal GABA is lower in older schizophrenia: a MEGA-PRESS with macromolecule suppression study. Mol. Psychiatry 21 (2), 198–204. 10.1038/mp.2015.34. [PubMed: 25824298]

Rubio SE, Vega-Flores G, Martínez A, Bosch C, Pérez-Mediavilla A, del Río J, Gruart A, Delgado-García JM, Soriano E, Pascual M, 2012. Accelerated aging of the GABAergic septohippocampal pathway and decreased hippocampal rhythms in a mouse model of Alzheimer's disease. FASEB J.: Off. Publ. Fed. Am. Soc. Exp. Biol 26 (11), 4458–4467. 10.1096/fj.12-208413.

Sailasuta N, Ernst T, Chang L, 2008. Regional variations and the effects of age and gender on glutamate concentrations in the human brain. Magn. Reson. ImAging 26 (5), 667–675. 10.1016/j.mri.2007.06.007. [PubMed: 17692491]

Sala-Llonch R, Bartrés-Faz D, Junqué C, 2015. Reorganization of brain networks in aging: a review of functional connectivity studies. Front. Psychol 6, 663. 10.3389/fpsyg.2015.00663. [PubMed: 26052298]

Salat DH, Buckner RL, Snyder AZ, Greve DN, Desikan RSR, Busa E, Morris JC, Dale AM, Fischl B, 2004. Thinning of the cerebral cortex in aging. Cereb. Cortex 1991 14 (7), 721–730. 10.1093/cercor/bhh032.

Scavuzzo CJ, Moulton CJ, Larsen RJ, 2018. The use of magnetic resonance spectroscopy for assessing the effect of diet on cognition. Nutr. Neurosci 21 (1), 1–15. 10.1080/1028415X.2016.1218191. [PubMed: 27571302]

Schantell M, John JA, Coutant AT, Okelberry HJ, Horne LK, Glesinger R, Springer SD, Mansouri A, May-Weeks PE, Wilson TW, 2024. Chronic cannabis use alters the spontaneous and oscillatory gamma dynamics serving cognitive control. Hum. Brain Mapp 45 (11), e26787. 10.1002/hbm.26787. [PubMed: 39023178]

Schmolesky MT, Wang Y, Pu M, Leventhal AG, 2000. Degradation of stimulus selectivity of visual cortical cells in senescent rhesus monkeys. Nat. Neurosci 3 (4), 384–390. 10.1038/73957. [PubMed: 10725929]

Schubert F, Gallinat J, Seifert F, Rinneberg H, 2004. Glutamate concentrations in human brain using single voxel proton magnetic resonance spectroscopy at 3 tesla. Neuroimage 21 (4), 1762–1771. 10.1016/j.neuroimage.2003.11.014. [PubMed: 15050596]

Sears SM, Hewett SJ, 2021. Influence of glutamate and GABA transport on brain excitatory/inhibitory balance. Exp. Biol. Med 246 (9), 1069–1083. 10.1177/1535370221989263.

Silveri MM, Sneider JT, Crowley DJ, Covell MJ, Acharya D, Rosso IM, Jensen JE, 2013. Frontal lobe gaminobutyric acid levels during adolescence: associations with impulsivity and response inhibition. Biol. Psychiatry 74, 296–304. [PubMed: 23498139]

Siman-Tov T, Bosak N, Sprecher E, Paz R, Eran A, Aharon-Peretz J, Kahn I, 2017. Early age-related functional connectivity decline in high-order cognitive networks. Front. Aging Neurosci 8, 330. 10.3389/fnagi.2016.00330. [PubMed: 28119599]

Simegn GL, Song Y, Murali-Manohar S, Zöllner HJ, Davies-Jenkins CW, Simicic D, Hupfeld KE, Gudmundson AT, Muska E, Carter E, Hui SC, 2025. A water relaxation atlas for age-and region-specific metabolite concentration correction at 3 T. NMR Biomed. 38 (1). 10.1002/nbm.5300.

Simmonite M, Carp J, Foerster BR, Ossher L, Petrou M, Weissman DH, Polk TA, 2019. Age-related declines in occipital GABA are associated with reduced fluid processing ability. Acad. Radiol 26 (8), 1053–1061. 10.1016/j.acra.2018.07.024. [PubMed: 30327163]

Simpson R, Devenyi GA, Jezzard P, Hennessy TJ, Near J, 2017. Advanced processing and simulation of MRS data using the FID appliance (FID-A)-an open source, MATLAB-based toolkit. Magn. Reson. Med 77 (1), 23–33. 10.1002/mrm.26091. [PubMed: 26715192]

Sohal VS, Rubenstein JLR, 2019. Excitation-inhibition balance as a framework for investigating mechanisms in neuropsychiatric disorders. Mol. Psychiatry 24 (9), 1248–1257. 10.1038/s41380-019-0426-0. [PubMed: 31089192]

Soler H, Dorca-Arévalo J, González M, Rubio SE, Ávila J, Soriano E, Pascual M, 2017. The GABAergic septohippocampal connection is impaired in a mouse model of tauopathy. Neurobiol. Aging 49, 40–51. 10.1016/j.neurobiolaging.2016.09.006. [PubMed: 27743524]

Song J, Birn RM, Boly M, Meier TB, Nair VA, Meyerand ME, Prabhakaran V, 2014. Age-related reorganizational changes in modularity and functional connectivity of human brain networks. Brain Connect. 4 (9), 662–676. 10.1089/brain.2014.0286. [PubMed: 25183440]

Springer SD, Spooner RK, Schantell M, Arif Y, Frenzel MR, Eastman JA, Wilson TW, 2023. Regular recreational Cannabis users exhibit altered neural oscillatory dynamics during attention

reorientation. Psychol. Med 53 (4), 1205–1214. 10.1017/S0033291721002671. [PubMed: 34889178]

Stan AD, Ghose S, Zhao C, Hulsey K, Mihalakos P, Yanagi M, Morris SU, Bartko JJ, Choi C, Tamminga CA, 2015. Magnetic resonance spectroscopy and tissue protein concentrations together suggest lower glutamate signaling in dentate gyrus in schizophrenia. Mol. Psychiatry 20 (4), 433–439. 10.1038/mp.2014.54. [PubMed: 24912493]

Stanley EM, Fadel JR, Mott DD, 2012. Interneuron loss reduces dendritic inhibition and GABA release in hippocampus of aged rats. Neurobiol. Aging 33 (2), 431. 10.1016/j.neurobiolaging.2010.12.014. .e1-13.

Suri S, Emir U, Stagg CJ, Near J, Mekle R, Schubert F, Zsoldos E, Mahmood A, Singh-Manoux A, Kivimäki M, Ebmeier KP, Mackay CE, Filippini N, 2017. Effect of age and the APOE gene on metabolite concentrations in the posterior cingulate cortex. Neuroimage 152, 509–516. 10.1016/j.neuroimage.2017.03.031. [PubMed: 28323160]

Thomson AR, Hwa H, Pasanta D, Hopwood B, Powell HJ, Lawrence R, Tabuenca ZG, Arichi T, Edden RAE, Chai X, Puts NA, 2024. The developmental trajectory of 1H-MRS brain metabolites from childhood to adulthood. Cereb. Cortex 1991 34 (3). 10.1093/cercor/bhae046.

Tkáč I, Deelchand D, Dreher W, Hetherington H, Kreis R, Kumaragamage C, Považan M, Spielman DM, Strasser B, de Graaf RA, 2021. Water and lipid suppression techniques for advanced 1 H MRS and MRSI of the human brain: experts' consensus recommendations. NMR Biomed. 34 (5), e4459. 10.1002/nbm.4459. [PubMed: 33327042]

Tkác I, Starcuk Z, Choi IY, Gruetter R, 1999. In vivo 1H NMR spectroscopy of rat brain at 1 ms echo time. Magn. Reson. Med 41 (4), 649–656. 10.1002/(sici)1522-2594(199904)41:4<649::aid-rm2>3.0.co;2-g. [PubMed: 10332839]

Tremblay S, Beaulé V, Proulx S, Lafleur L-P, Doyon J, Marjańska M, Théoret H, 2014. The use of magnetic resonance spectroscopy as a tool for the measurement of bi-hemispheric transcranial electric stimulation effects on primary motor cortex metabolism. J. Vis. Exp.: JoVE 93, e51631. 10.3791/51631.

US Census Bureau. (n.d.). 2023 National Population Projections tables: Main series. Census.gov. Retrieved July 29, 2025, from https://www.census.gov/data/tables/2023/demo/popproj/2023-summary-tables.html.

Varangis E, Razlighi Q, Habeck CG, Fisher Z, Stern Y, 2019. Between-network functional connectivity is modified by age and cognitive task domain. J. Cogn. Neurosci 31 (4), 607–622. 10.1162/jocn_a_01368. [PubMed: 30605005]

Walhovd KB, Fjell AM, Reinvang I, Lundervold A, Dale AM, Eilertsen DE, Quinn BT, Salat D, Makris N, Fischl B, 2005. Effects of age on volumes of cortex, white matter and subcortical structures. Neurobiol. Aging 26 (9), 1261–1270. 10.1016/j.neurobiolaging.2005.05.020 discussion 1275-8. [PubMed: 16005549]

Wang H, Xie X, Li X, Chen B, Zhou Y, 2006. Functional degradation of visual cortical cells in aged rats. Brain Res. 1122 (1), 93–98. 10.1016/j.brainres.2006.09.010. [PubMed: 17010946]

Webert LK, Schantell M, John JA, Coutant AT, Okelberry HJ, Horne LK, Sandal ME, Mansouri A, Wilson TW, 2024. Regular cannabis use modulates gamma activity in brain regions serving motor control. J. Psychopharmacol 38 (11), 949–960. 10.1177/02698811241268876. [PubMed: 39140179]

Wong TP, Marchese G, Casu MA, Ribeiro-da-Silva A, Cuello AC, De Koninck Y, 2006. Imbalance towards inhibition as a substrate of aging-associated cognitive impairment. Neurosci. Lett 397 (1–2), 64–68. 10.1016/j.neulet.2005.11.055. [PubMed: 16378682]

Yang A, Xiao X.h., Wang Z.l., 2014. Evaluation of normal changes in pons metabolites due to aging using turbo spectroscopic imaging. AJNR Am. J. Neuroradiol 35 (11), 2099–2105. 10.3174/ajnr.A4031. [PubMed: 24994832]

Yang Z-Y, Yue Q, Xing H-Y, Tan Q-Y, Sun H-Q, Gong Q-Y, Tan Z-J, Quan H, 2015. A quantitative analysis of 1H-MR spectroscopy at 3.0 T of three brain regions from childhood to middle age. Br. J. Radiol 88 (1052), 20140693. 10.1259/bjr.20140693. [PubMed: 26081448]

Yizhar O, Fenno LE, Prigge M, Schneider F, Davidson TJ, O'Shea DJ, Sohal VS, Goshen I, Finkelstein J, Paz JT, Stehfest K, Fudim R, Ramakrishnan C, Huguenard JR, Hegemann P,

Deisseroth K, 2011. Neocortical excitation/inhibition balance in information processing and social dysfunction. Nature 477 (7363), 171–178. 10.1038/nature10360. [PubMed: 21796121]

Yu S, Wang Y, Li X, Zhou Y, Leventhal AG, 2006. Functional degradation of extrastriate visual cortex in senescent rhesus monkeys. Neuroscience 140 (3), 1023–1029. 10.1016/j.neuroscience.2006.01.015. [PubMed: 16678974]

Zahr NM, Mayer D, Rohlfing T, Chanraud S, Gu M, Sullivan EV, Pfefferbaum A, 2013. In vivo glutamate measured with magnetic resonance spectroscopy: behavioral correlates in aging. Neurobiol. Aging 34 (4), 1265–1276. 10.1016/j.neurobiolaging.2012.09.014. [PubMed: 23116877]

Zöllner HJ, Tapper S, Hui SCN, Barker PB, Edden RAE, Oeltzschner G, 2022. Comparison of linear combination modeling strategies for edited magnetic resonance spectroscopy at 3 T. NMR Biomed. 35 (1), e4618. 10.1002/nbm.4618. [PubMed: 34558129]

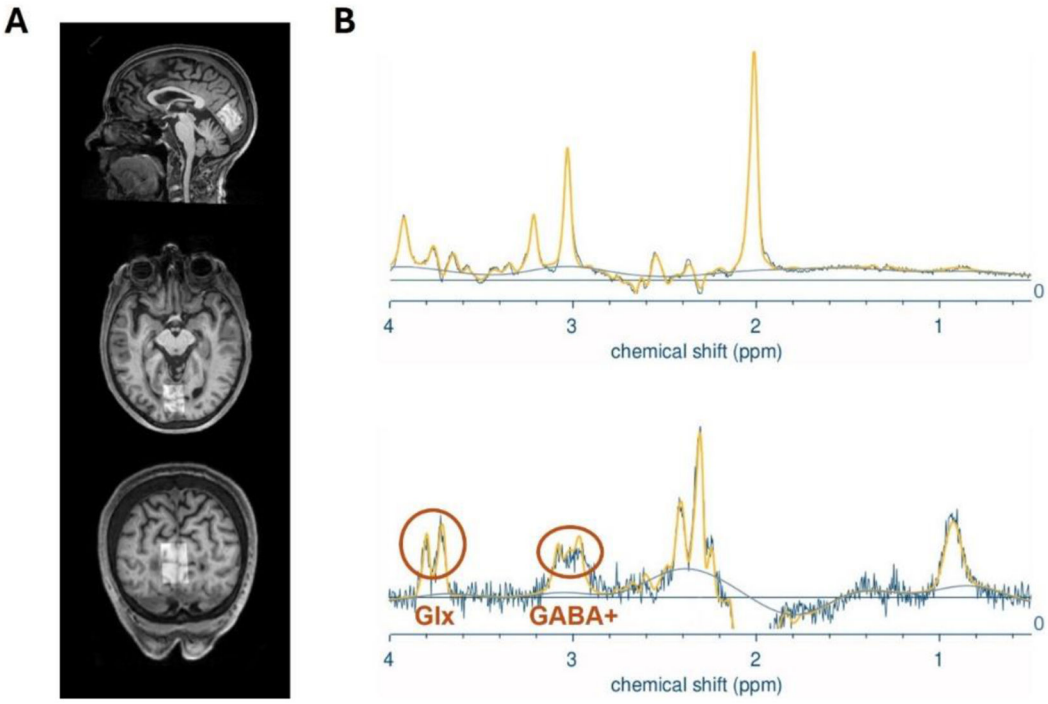

Fig. 1. Voxel placement and edit-OFF and difference spectra.

A) Example of ^{1}H-MRS voxel placement, centered on the calcarine visual cortex. **B)** Example of model fit (yellow line) for the edit-OFF (top) and difference (bottom) spectra.

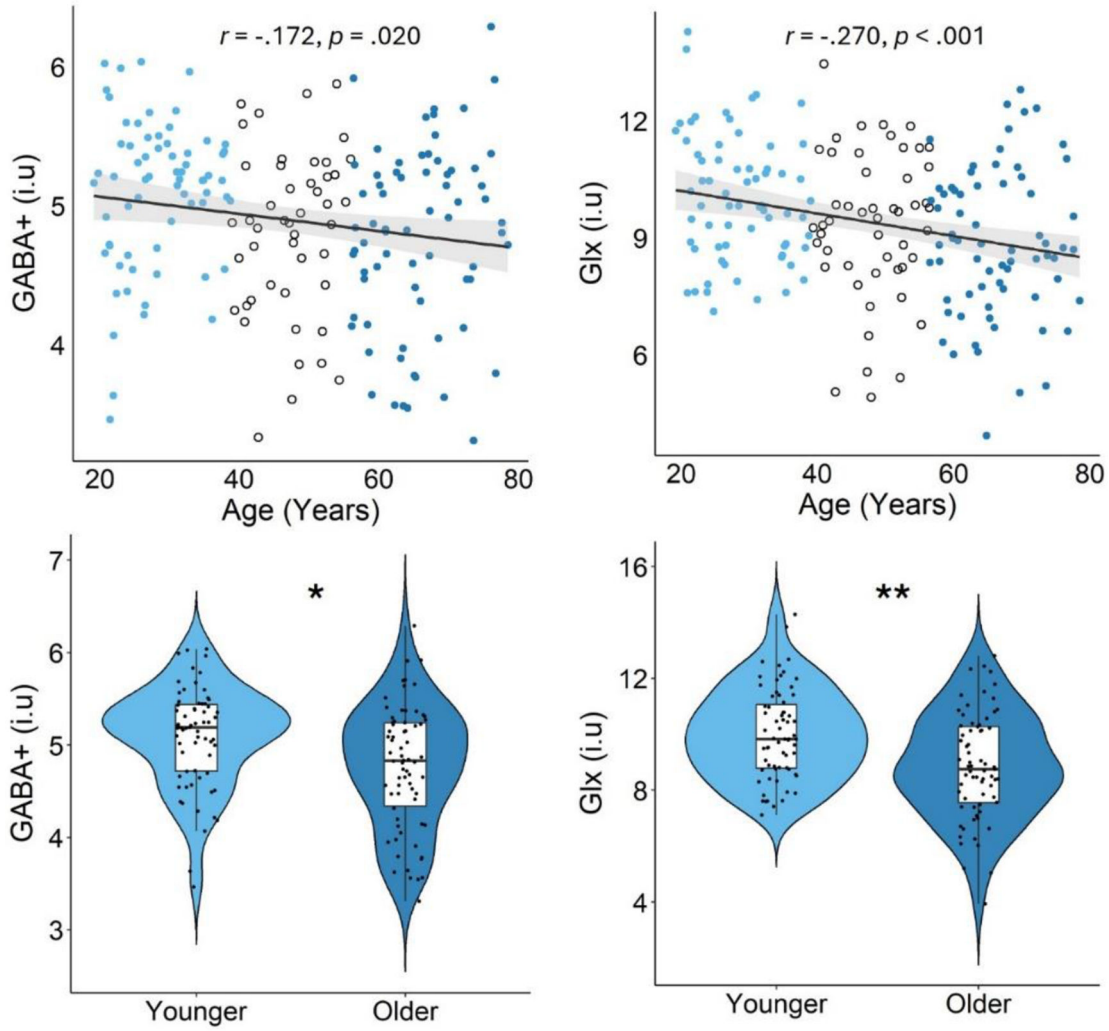

Fig. 2. Age-related decreases in GABA+ and Glx.

(**Top):** Age was negatively correlated with GABA+ (top left) and Glx (top right) across all participants. To improve comparability with past work, we also divided the sample into age groups based on ±0.5 SDs of the full group's mean age, such that participants 0.5 SDs above the mean became members of an older group (dark blue), and those 0.5 SDs below the mean became part of the younger group (light blue). Participants within ±0.5 SD were not included in this aspect of the analysis (white dots). (**Bottom):** Two-sample *t*-tests revealed significantly lower GABA+ (bottom left) and Glx (bottom right) levels in the older group compared to their younger counterparts; * $p = .0016$; ** $p < .001$.

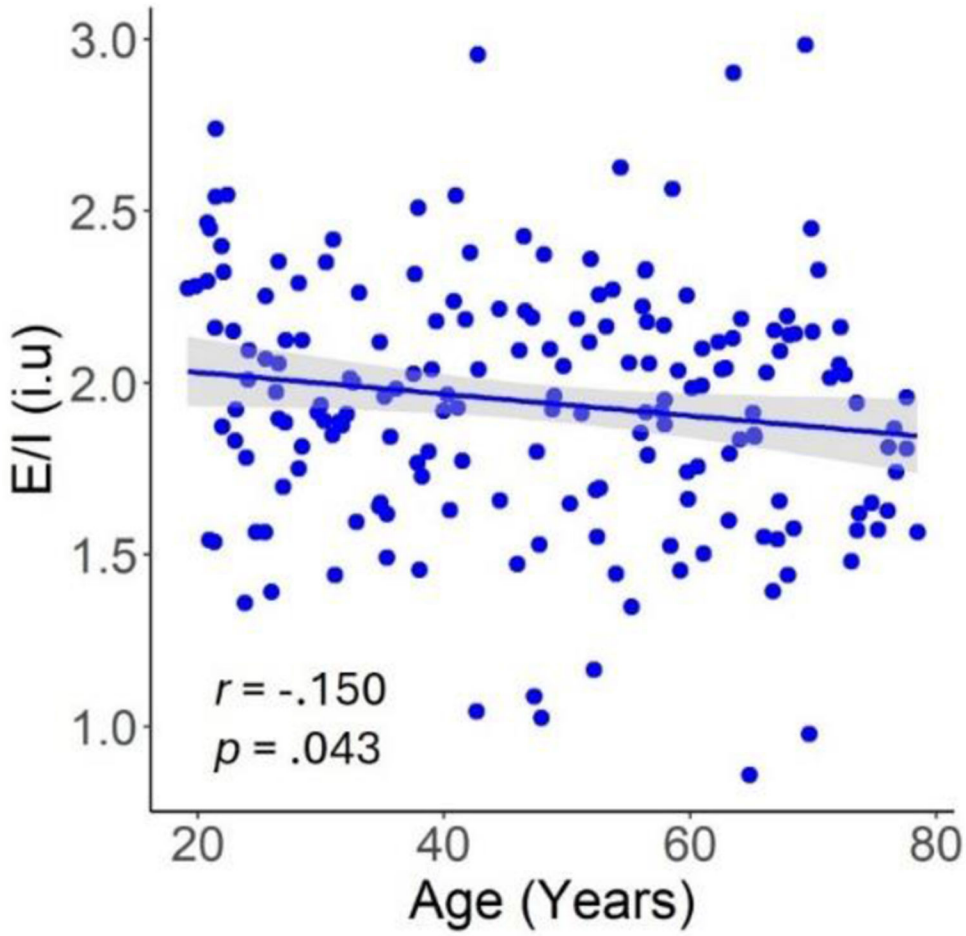

Fig. 3. Age-related decreases in the excitatory/inhibitory (E/I) ratio.
Age was negatively correlated with the E/I ratio across all participants.

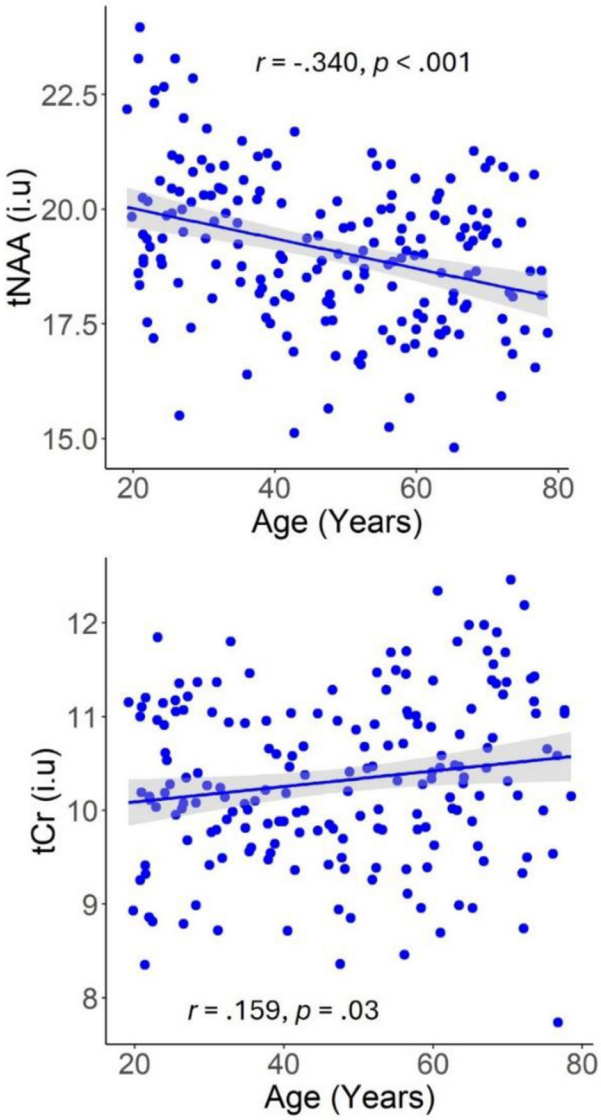

Fig. 4. Age-related decreases in tNAA and increases in tCr.

Age was negatively correlated with tNAA (top) and positively correlated with tCr (bottom) across all participants.