Published in final edited form as:

Neurobiol Aging. 2020 November ; 95: 240–249. doi:10.1016/j.neurobiolaging.2020.07.015.

A Quantitative Meta-Analysis of Brain Glutamate Metabolites in Aging

David R. Roalf, Ph.D.¹, Valerie J. Sydnor, B.A.¹, Madison Woods¹, David A. Wolk, M.D.², J. Cobb Scott, Ph.D.^{1,3}, Ravinder Reddy, Ph.D.⁴, Paul J. Moberg, Ph.D.^{1,2}

¹Department of Psychiatry, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA;

²Department of Neurology, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA.

³VISN4 Mental Illness Research, Education, and Clinical Center at the Corporal Michael J. Crescenz VA Medical Center, Philadelphia, Pennsylvania

⁴Center for Magnetic Resonance and Optical Imaging, Department of Radiology, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA.

Abstract

Glutamate (Glu) is a key molecule in cellular metabolism, the most abundant excitatory neurotransmitter in the brain, and the principal neurotransmitter of cortical efferents. Glutamate dysfunction, on the other hand, is common in neurodegenerative disorders, and likely contributes

*Correspondence: David R. Roalf, Ph.D., Neuropsychiatry Section, Department of Psychiatry, 5th Floor, Richards Building, 3700 Hamilton Walk, Philadelphia, PA, 19104. Telephone: (215) 662-4678. Fax: (215) 662-7903. roalf@pennmedicine.upenn.edu.

Author Credit

David R. Roalf, PhD was responsible for conceptualization of the study, data review and analysis, manuscript preparation and editing. Dr. Roalf approves of the final version of this manuscript.

Valerie J. Sydnor, BS was responsible data entry and review and manuscript editing. Ms. Sydnor approves of the final version of this manuscript.

Madison Woods was responsible for data collection (literature review), data entry. Ms. Woods approves of the final version of this manuscript.

David Wolk, MD contributed content for the manuscript and provided copy editing assistance. Dr. Wolk approves of the final version of this manuscript.

J. Cobb Scott, PhD contributed content for the manuscript, statistical analysis and provided copy editing assistance. Dr. Cobb approves of the final version of this manuscript.

Ravinder Reddy contributed content for the manuscript and provided copy editing assistance. Dr. Reddy approves of the final version of this manuscript.

Paul J. Moberg was responsible for conceptualization of the study, manuscript preparation and editing. Dr. Moberg approves of the final version of this manuscript.

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Financial Disclosures/Conflicts of Interest:

David R. Roalf, PhD reports no potential conflicts of interest or financial disclosures related to this work.

Valerie J. Sydnor, BA reports no potential conflicts of interest or financial disclosures related to this work.

Madison Woods reports no potential conflicts of interest or financial disclosures related to this work.

David Wolk, MD reports no potential conflicts of interest or financial disclosures related to this work.

J. Cobb Scott, PhD reports no potential conflicts of interest or financial disclosures related to this work.

Ravinder Reddy reports no potential conflicts of interest or financial disclosures related to this work.

Paul J. Moberg reports no potential conflicts of interest or financial disclosures related to this work.

to age-related declines in behavioral and cognitive functioning. Nonetheless, the extant literature measuring age-related changes in brain glutamate *in vivo* has yet to be comprehensively and quantitatively summarized. This meta-analysis examines proton spectroscopy (¹H MRS) measures of Glu-related brain metabolites across 589 healthy young and older adults. Glu (Cohen's $d = -0.82$) and Glu+glutamine (Cohen's $d = -0.51$) concentrations were significantly lower in older compared to younger adults, whereas the concentration of glutamine ($d = 0.43$) was significantly higher in older individuals. Notably, ¹H MRS methodological choices impacted effect sizes for age-related Glu differences. Glu metabolite change appears to be a robust marker of aging-related neurological change, however additional studies are needed to elucidate age-related trajectories of glutamatergic alterations and their relationship to cognitive phenotypes.

Keywords

aging; glutamate; spectroscopy (¹H MRS); meta-analysis; MRI

1.0 Introduction

Over the next several decades the number of Americans aged 65 and older is projected to double from 46 to 98 million (Mather et al., 2015). This increase is a direct result of meaningful advances in geriatric medicine and healthcare. Despite these advances, age-related cognitive decline remains one of the most dreaded, largely unmodifiable, and poorly understood features of aging. The age-related improvements in cognition (e.g. in memory and executive function) seen from childhood through adolescence (Zelazo et al., 2004) are mirrored by systematic, age-related declines in cognitive functions in the final decades of life (Dempster, 1992; Mayr, 2001). This decline in complex cognitive functioning arises due to a loss of integrity (e.g. a loss of synaptic connectivity) of the neural systems underlying cognition (Craik and Grady, 2002). Among the biological mechanisms proposed to contribute to such integrity loss, alterations in brain glutamate (Glu) are considered critical, given Glu's ubiquity and involvement in cellular energetics and high-order cognitive functions (Coyle, 2006; Curtis and Johnston, 1974; Erci ska et al., 1990; McEntee and Crook, 1993; Robbins and Murphy, 2006).

Glutamate is the most abundant endogenous excitatory amino acid in the brain (Cotman and Monaghan, 1988) and the primary neurotransmitter used by pyramidal cells in the cerebral cortex (Cotman and Monaghan, 1988). As such, it is not surprising that Glu has been implicated in age-related declines in functioning. Animal studies, for example, have consistently demonstrated aging-related reductions in brain Glu (McEntee and Crook, 1993), as well as a loss of NMDA-type glutamate receptors (Wenk et al., 1991). Studies of brain neurochemistry in humans have relied predominantly on post-mortem tissue (Hynd et al., 2003), yet these studies unfortunately have a number of limitations (e.g. post-mortem interval, time in storage, tissue pH) making quantification challenging. Critically, in recent years the use of high-field, non-invasive neuroimaging techniques has enabled the *in vivo* study of Glu neurometabolites in the human brain (Soares and Law, 2009), including in aging (Cleeland et al., 2019).

The examination of *in vivo* Glu can be accomplished using invasive electrodes (Huberfeld et al., 2011), positron emission tomography (PET), and proton magnetic resonance spectroscopy (¹HMRS). Unlike electrode measurements that measure mostly extracellular glutamate and PET that measures glutamate receptor availability, ¹HMRS measures total glutamate present in both intra- and extra-cellular compartments. ¹HMRS allows for the measurement of many, albeit not all, brain metabolites within specific regions of the brain (e.g. (Barker et al., 2001)). Nonetheless, the quantification of Glu by ¹HMRS presents a particular challenge, due predominantly to the overlap of Glu and glutamine (Gln) resonances. Consequently, the ¹HMRS readouts from these neurochemicals are commonly combined and referred to as “Glx” (Glu + Gln). Still, ¹HMRS has been the primary method used for *in vivo* Glu/Gln/Glx imaging in aging to date. In fact, a recent review reported that brain Glu in healthy older adults appears to be consistently lower when compared to younger individuals, but the size of this effect was not quantified, and potential moderating variables were not investigated statistically (Cleeland et al., 2019). To our knowledge there has not been a systematic, quantitative analysis of the extant Glu ¹HMRS data in aging, as has been done with other neurometabolites (see (Haga et al., 2009)).

There have been 13 independent ¹HMRS studies (two at 1.5 Tesla (Chang et al., 1996; Raininko and Mattsson, 2010), eight at 3 Tesla (Ding et al., 2016; Hädel et al., 2013; Mooney et al., 2017; Sailasuta et al., 2008; Schubert et al., 2004; Simmonite et al., 2019; Suri et al., 2017; Zahr et al., 2008), two at 4 Tesla (Boumezbeur et al., 2010; Kaiser et al., 2005) and one at 7 Tesla (Marjańska et al., 2017)) that provide age-related measures of Glu-related metabolites in healthy older and younger adults. What is clear from qualitative review of these studies is that Glu-related metabolite concentrations may indeed differ in older and younger adults. The magnitude and pattern of these differences, however, remain unquantified. As noted in a recent review, there is significant heterogeneity across studies in terms of (a) which metabolite(s) (Glu, Gln, and/or Glx) were measured, (b) which brain region(s) Glu-related metabolites were measured in, and (c) what ¹HMRS protocol was employed. Critically, these factors may affect the age-related effect sizes reported (Cleeland et al., 2019), and, by extension, our understanding of the neurobiology of aging. Consequently, a systematic analysis of all available data that incorporates diverse moderating variables is needed to better elucidate the nature of age-related changes in brain Glu.

In the current work, we conduct a comprehensive meta-analysis of existing studies examining Glu-related metabolites in healthy aging. This approach allows for results to be consolidated across studies, providing a more powerful estimate of true population differences. We furthermore seek to identify the impact of various potential moderators on previously reported results, including demographic (e.g. sex), methodological (e.g. ¹HMRS technique), and neurological (e.g. brain region) moderators. The goals of these moderator analyses are to reveal regional and tissue type-specific changes in Glu-related metabolites in aging, to gain insight into methodological sensitivity, and to provide future guidance for the field. We hypothesized that brain Glu would be lower in healthy older adults when compared to younger adults and anticipated that heterogeneity across studies would be explained by specific methodological choices. Overall, we propose that a refined appreciation of glutamate availability in the human brain throughout aging—and its relationship to

molecular, cellular, and cognitive changes—will lead to improvements in our understanding of cognitive aging and offer new targets for intervention in age-related cognitive decline.

2.0 Materials and methods

The extraction of relevant articles and article data and reporting of meta-analytic aims, methods, results, and biases followed the Meta-Analysis of Observational Studies in Epidemiology (MOOSE) reporting standards (Supplemental Figure 1) (Stroup et al., 2000). The search strategy included an initial broad search in PubMed, MEDLINE, PsychINFO, and Google Scholar databases, followed by a manual review of articles utilizing references from identified original articles and reviews. We used the following search criteria: “aging OR age”, AND “glutamate” OR “GLU” OR “Glx” OR “Glutamine” or “GLN”, AND “spectroscopy” OR “¹HMRS”, AND “brain”. The search was limited to articles published prior to September 30, 2019 that were written in English and that enrolled human participants.

Studies included in the meta-analysis met the following criteria: 1) inclusion of ¹HMRS measures of Glu, Gln, or Glx; 2) samples comprised of unrelated, healthy young and old adults with no history of major medical, neurological or psychiatric conditions; 3) a minimum age difference of 25 years between ‘young’ and ‘old’ participants as determined by group averages or the age-range provided in the primary study; and 4) data or statistical information that enabled the calculation of effect sizes. Studies reporting results from either independent samples (i.e. younger vs. older) or correlations with age were included. Two authors (MW, DRR) initially reviewed each potential publication for inclusion based on the aforementioned criteria. Consensus of the first (DRR) and senior (PJM) authors was needed to exclude an article. Three authors (VJS, DRR, PJM) were involved in extracting relevant data from publications and quality checking the extracted data for accuracy. Variables extracted for meta-analytic analysis, when provided, included ¹HMRS concentration values (absolute or ratio) and standard deviations, age-related correlations with Glu, Gln or Glx, metabolite measured (Glu/Gln/Glx), ¹HMRS sequence, MRI field strength, echo time (TE), ¹HMRS voxel type, brain region of interest (ROI) studied, old/young sample age, and sex. We identified thirteen (n = 13) independent publications for inclusion that reported age-related measures of Glu/Gln/Glx ¹HMRS, with a combined total of 44 effects (k) (Table 1). Four (n = 4) additional articles that were originally considered were ultimately excluded due to: 1) lack of quantitative Glu metabolite values provided with respect to age (Mader et al., 2002; Tisell et al., 2013; Tunc-Skarka et al., 2014) or 2) sample overlap between manuscripts (Zahr et al., 2013). Data included in the final analysis is available in the Supplemental Material and on our GitHub repository: <https://github.com/droalf/Aging-Glutamate-MetaAnalysis.git>.

2.1 Statistical Analyses

Meta-analysis was completed across all studies to determine age-related effect sizes, followed by meta-regressions and analyses of publication bias. Analyses were carried out using Comprehensive Meta-Analysis version 2.21.064 (Biostat, 2005), using standard random-effects models. Glu-related metabolite level (Glu, Gln, Glx) differences between

older and young participants were standardized using Cohen's d (effect size; quantified as the difference between the two raw mean scores divided by the pooled standard deviation (SD)). When means and SDs were not available, Cohen's d was calculated from reported univariate F-tests, t-statistics, r-values, or p-values. Confidence intervals (CI) for each effect are reported. In order to control for differences in sample size during effect size calculation, studies were weighted according to their inverse variance estimates. Prior convention has classified effect sizes as small ($d = \pm 0.2$), medium ($d = \pm 0.5$), or large ($d = \pm 0.8$) based on these methods (Cohen, 1988). Negative effect size values indicate lower brain metabolite level in older relative to younger adults. Homogeneity of effect size across studies was assessed using the Cochran Q-statistic (Hedges and Olkin, 1985).

2.1.1 Moderator Variables and Meta-regression—Meta-regression was used to investigate potential moderating variables that vary across studies (e.g., sex, MRI field strength, etc.) in order to better understand heterogeneity between studies. The following moderator variables were evaluated in follow-up analyses with meta-regression: (a) metabolite (Glu, Gln, Glx), (b) MRI field strength, (c) ^1H MRS protocol (d) ^1H MRS acquisition voxel type (single vs. multislice), (e) whether Glu values were reported as an absolute concentration or a ratio, (f) brain region, (g) TE, and (h) sex. Additional demographic and clinical characteristics of interest, including race, years of education, and cognitive functioning, were insufficiently reported across studies to be included in formal analyses.

2.1.2 Analysis of Small Study Bias—It is possible that studies with large effects are overrepresented in the extant literature. The specific concern is that studies with relatively large effects are more likely to be published than studies with small effects, even when addressing the same question, especially when results align with theory. This may lead to a small study bias in the literature that can possibly influence the results of a meta-analysis. Small study bias was therefore assessed using several convergent methods: (1) visual inspection of a funnel plot: an asymmetric scatterplot of effect size by study precision may indicate bias; (2) adjusted rank-correlation (e.g. Spearman) test according to the methods of Begg and Mazumdar (Begg and Mazumdar, 1994), Egger et al. (Egger et al., 1997), and Duval and Tweedy (Duval and Tweedie, 2000): a high correlation between study effect sizes and sampling variances is suggestive of bias; and (3) a fail-safe file drawer analysis: a probability-based metric used to determine the number of null studies needed to invalidate the reported effect (Rosenthal, 1979). Finally, to address the potential of certain studies being outliers, a trim-and-fill method was used to adjust average effect size to account for publication bias where appropriate (Duval and Tweedie, 2000).

3.0 Results

3.1 Overall Meta-analysis

Across all studies, the average age for older participants was 69.24 years (range 54–78) and the average age for younger participants was 22.71 years (range 19–26). Analysis of study-specific effect sizes for differences between older and younger participants across all three metabolites (Glu/Gln/Glx) revealed an overall medium effect size ($d = -0.43$, 95% CI =

$-0.69 < \delta < -0.18$, $k = 44$) that was significantly heterogeneous ($Q_B[43] = 252.42$, $p < 0.001$). Given that the variability in study-specific effect sizes between healthy older and younger groups differed more than would be expected from sampling error alone, analyses of potential moderator variables were undertaken.

3.2 Moderator Analyses

3.2.1. Metabolite Type.—Follow-up moderator analysis of metabolite type revealed significant heterogeneity across metabolites ($Q_B[2] = 23.50$, $p < 0.001$). Effect sizes for Glu ($d = -0.82$, $CI = -1.17 < \delta < -0.47$, $k = 17$) and Glx ($d = -0.51$, $CI = -0.81 < \delta < -0.21$, $k = 18$) were homogenous ($Q_B[1] = 1.78$, $p = 0.18$) in magnitude and direction (Young > Old, Figure 1). The effect size for Gln ($d = 0.43$, $CI = 0.82 < \delta < 0.03$, $k = 9$), on the other hand, was significantly different from both Glu ($Q_B[1] = 22.09$, $p < 0.001$) and Glx ($Q_B[1] = 13.84$, $p < 0.001$) and opposite in direction (Young < Old, Figure 1). Given the differences in magnitude and direction of metabolite effect sizes and the relatively small number of Gln effects ($k = 9$), additional moderator analyses are limited to Glu and Glx.

Recalculating the overall meta-analytic effect based only on studies of Glu and Glx resulted in a moderate-to-large negative effect size ($d = -0.66$, $CI = -0.90 < \delta < -0.43$, $k = 35$) indicating that Glu ($k = 17$) and Glx ($k = 18$) levels are lower in older as compared to younger adults. However, effects remained significantly heterogeneous ($Q_B[34] = 127.11$, $p < 0.001$) between studies, as can be seen in panels A and B of Figure 1.

3.2.2 MRI Field Strength.—MRI field strength can affect the ability to quantify Glu and Glx due to spectral overlap effects; quantification at higher fields is expected to be more precise. Across all studies, reported $^1\text{HMRs}$ effects for Glu and Glx were most frequent at 3T ($k = 26$), followed by 1.5T ($k = 4$), 4T ($k = 3$), and 7T ($k = 2$). Effect sizes did not significantly differ by MRI field strength (Figure 2A) ($Q_B[3] = 3.15$, $p = 0.36$). Average effect sizes were nominally largest at 4.0T ($d = -1.09$, 95% $CI = -1.93 < \delta < -0.25$), followed by 3.0T ($d = -0.68$, 95% $CI = -0.96 < \delta < -0.40$), then 1.5T ($d = -0.37$, 95% $CI = -1.07 < \delta < 0.32$) and 7T ($d = -0.34$, 95% $CI = -0.82 < \delta < 0.14$). It should be noted, however, that there has only been one 7T study, which contributed both 7T effects.

3.2.3 $^1\text{HMRs}$ Protocol.— $^1\text{HMRs}$ protocols vary in their sensitivity and specificity for brain glutamate metabolites. Unsurprisingly, there was significant variability in the $^1\text{HMRs}$ protocols employed across included studies. PRESS ($k = 12$), CT-PRESS ($k = 6$), and STEAM ($k = 4$) were the most commonly used protocols, followed by MEGA-PRESS ($k = 2$), SPECIAL ($k = 1$), and LASER-POCE ($k = 1$). One EPSI study reported nine effects ($k = 9$). Analysis revealed significant heterogeneity in effect sizes across $^1\text{HMRs}$ sequence types ($Q_B[6] = 54.91$, $p < 0.001$). Significant heterogeneity remained after excluding studies only contributing one effect. Studies implementing SPECIAL ($d = -2.17$, 95% $CI = -2.61 < \delta < -1.73$), LASER-POCE ($d = -1.70$, 95% $CI = -2.92 < \delta < -0.48$), and EPSI ($d = -0.91$, 95% $CI = -1.30 < \delta < -0.52$) sequences showed the largest effects, on average; however, for each protocol type, one study contributed all effects. The smallest average effect sizes were found for CT-PRESS ($d = -0.09$, 95% $CI = -0.82 < \delta < 0.64$) protocols and for MEGA-PRESS protocols ($d = -0.02$, 95% $CI = -0.52 < \delta < 0.48$). CT-PRESS and MEGA-PRESS summary

effects were smaller than effects for PRESS ($d = -0.68$, 95% CI = $-0.90 < \delta < -0.45$) and STEAM ($d = -0.57$, 95% CI = $-1.05 < \delta < -0.09$) sequences (Figure 2B). Pairwise comparisons across all $^1\text{HMRs}$ protocols are shown in Supplemental Table 1.

3.2.4 Voxel Type.—Single voxel spectroscopy (SVS; $k = 26$), wherein data is collected from one large ($\sim 30\text{cm}^3$) voxel in the brain, is the most commonly acquired type of $^1\text{HMRs}$ data. Across all studies included in this meta-analysis, only one study ($k = 9$) acquired multivoxel data covering the whole brain. Effect sizes appeared homogenous across voxel type (single v. multivoxel) ($Q_B[1] = 1.82$, $p = 0.17$), i.e., while the effect sizes for multivoxel data ($d = -0.90$ 95% CI = $-1.29 < \delta < -0.52$) were larger than for SVS data ($d = -0.57$ 95% CI = $-0.86 < \delta < -0.28$), this difference was not statistically significant (Figure 2C).

3.2.5 Concentration vs. Ratio.— $^1\text{HMRs}$ studies can report absolute concentration values or ratios (e.g. Glu/Creatine). Moderator analysis revealed significant heterogeneity among study-specific effect sizes for reported Glu differences between older and younger individuals ($Q_B[1] = 10.16$, $p = 0.001$) depending on the measurement approach used. Effect sizes for absolute concentration values were significantly larger ($d = -0.77$ 95% CI = $-1.02 < \delta < -0.52$) than for ratios ($d = -0.08$ 95% CI = $-0.42 < \delta < 0.25$; Figure 2D).

3.2.6 Brain Tissue Type.—The majority of studies reported metabolite effects derived from mixed tissue ($k = 18$), followed by gray matter (GM; $k = 13$), then white matter (WM; $k = 4$). Moderator analysis indicated a non-significant trend in effect sizes dependent on tissue type ($Q_B[2] = 5.80$, $p = 0.055$; Figure 3A). Effect sizes in GM were nominally larger ($d = -1.00$ 95% CI = $-1.30 < \delta < -0.70$) than effects in regions with a mixed tissue ($d = -0.44$ 95% CI = $-0.80 < \delta < -0.08$) or WM ($d = -0.60$ 95% CI = $-1.13 < \delta < -0.07$) composition.

3.2.7 Brain Region.—Metabolite levels were measured across a wide range of cortical and subcortical ROIs. Effects were grouped by brain region to facilitate meaningful comparisons across studies. Regions included: frontal lobe ($k = 9$), parietal lobe ($k = 5$), temporal lobe ($k = 2$), occipital lobe ($k = 4$), subcortical areas ($k = 6$), cerebellum ($k = 3$), brain stem ($k = 2$), and mixed cortical ($k = 4$). The meta-regression revealed significant heterogeneity among study effect sizes across these brain regions ($Q_B[7] = 33.22$, $p < 0.001$; Figure 3B). Meta-analytic average effect sizes for frontal ($d = -0.51$, 95% CI = $-0.91 < \delta < -0.12$), parietal ($d = -1.39$, 95% CI = $-2.10 < \delta < -0.68$), temporal ($d = -0.80$, 95% CI = $-1.33 < \delta < -0.27$), occipital ($d = -0.59$, 95% CI = $-1.02 < \delta < -0.17$), subcortical ($d = -0.84$, 95% CI = $-1.19 < \delta < -0.50$), and mixed cortical ($d = -0.85$, 95% CI = $-1.31 < \delta < -0.39$) regions were lower in older as compared to younger adults (Young > Old). Effects within the parietal lobe were significantly larger than those within the frontal lobe ($Q_B[1] = 4.49$, $p = 0.03$). Effects in all other cortical regions were roughly equivalent. Effects within the cerebellum ($d = 0.42$, 95% CI = $-0.07 < \delta < 0.90$) were in the opposite direction (Young < Old) and significantly differed from all other ROIs except the Brainstem ($d = 0.28$, CI = $-0.31 < \delta < 0.87$). Pairwise comparisons across brain regions are shown in Supplemental Table 2. In addition, effect sizes are colored by ROI in Figures 1A–C to allow for comparison across studies within specific brain regions.

3.2.8 Echo Time.—Glutamate metabolites are best detected in the human brain using short TEs (Wijtenburg and Knight-Scott, 2011). TEs in the included studies ranged from 8ms to 139ms. Several studies used multiple TEs during acquisition, as such the effective TE was used for analysis. Analysis of TE indicated a significant age-related difference in glutamate metabolite ($Z = 4.41$, $p < .001$) in the expected direction—studies using shorter TEs resulted in larger age-related glutamate effect sizes (Figure 3C).

3.2.9 Sex.—Analysis of sex composition of the samples revealed a significant age-related sex difference in glutamate metabolite effect sizes ($Z = 4.02$, $p < .001$). Samples with a greater percentage of females exhibited smaller age effects (Figure 3D).

3.3 Sensitivity Analysis: Comparing Univariate and Multivariate Approaches

Several studies included in this meta-analysis reported ¹HMRS effects from more than one brain region. Thus, it is possible that considering each effect size as an independent effect could bias the meta-analytic outcome. As such, we performed sensitivity analyses using mixed-effects multivariate models that account for dependency amongst effects derived from the same study (Cheung, 2019; Scott et al., 2015). We defined a two-level mixed-effects model, where Level 1 is represented by multiple effect sizes within studies, and Level 2 is represented by the different studies. This model examines the variability of effects sizes between studies (random factor) and the association between various explanatory variables (fixed factors) and effect sizes. To apply this model to meta-analytic data, we used the standardized mean effect sizes (Cohen's d) and determined the sampling variance of each effect size. The model considers the Level 1 effect-size variances as fixed/known (as calculated). The fixed- and random-effects parameters and their variances and covariance are estimated via adaptive quadrature, a robust and flexible numeric integration approach that allows for heteroscedastic Level 1 variances (Rabe-Hesketh et al., 2004, 2005). Models were fit using the program 'gllamm' of Stata Version 13 (Grilli and Rampichini, 2006; Rabe-Hesketh et al., 2004; StataCorp, 2013).

The summary effects derived using multivariate analyses aligned with our univariate approach (See Supplemental Results). Most effect sizes and comparisons between moderators were similar. The only notable difference was that the effect sizes across brain tissue type (GM, WM, Mixed), which were considered homogenous in the univariate approach ($p = 0.055$), were significantly different using the multivariate approach ($p < 0.001$). The direction of the individual comparisons was the same (GM>Mixed>WM). Thus, it appears that the univariate approach employed in this meta-analysis is robust to the inclusion of multiple effects from the same study and offers conservative effect size estimates.

3.4 Publication Bias

Investigations into potential small study publication biases for studies examining Glu and Glx revealed a symmetric funnel plot (Supplemental Figure 2) and non-significant Begg ($p = 0.36$) and Egger ($p = 0.13$) tests. To further examine biases, we estimated the number of potential outlier studies using the Duval and Tweedie (Duval and Tweedie, 2000) "trim and fill" method. This procedure indicated that one study could be considered an outlier and could be trimmed from analysis. This process generated an estimated effect size of $d = -0.72$

that was slightly larger than the original estimate. Lastly, calculation of a fail-safe N revealed that 1,037 “null” studies would need to be found and incorporated into the analysis to negate the observed effect. As such, these methods support the notion that the current meta-analytic data accurately represent the extant literature concerning glutamate across the lifespan.

4.0 Discussion

The present findings are in alignment with our hypothesis that aging results in significant alterations in glutamatergic neurometabolite concentrations. The meta-analytically derived summary effect size for Glu and Glx differences between younger and older individuals was substantial ($d = -0.66$), and most, though not all, studies reported at least a trend towards lower brain Glu/Glx in older adults. Of note, metabolite (Glu/Glx/Gln) studied, $^1\text{HMRS}$ protocol, measuring ratio versus absolute concentration values, brain region, echo time, and sex were moderating factors that significantly influenced reported age-related effect sizes. In contrast, effect sizes across studies were robust to differences in MRI field strength, brain tissue type (GM/WM/mixed), and voxel type selection (single versus multi).

Overall, the present findings indicate that age-related changes in Glu neurometabolites result in nearly global lower levels of Glu and Glx. While effect sizes for Glu and Glx did not significantly differ, it is important to note that the effect size for Glu was, on average, 60% larger than that for Glx. Smaller effects for Glx are likely the result of the mixing of Glu and Gln due to spectral overlap in $^1\text{HMRS}$ studies, particularly at lower MRI field strengths (Ramadan et al., 2013). Alterations in glutamate levels have vast implications for the aging brain. At the cellular level, age-related changes in the brain include reductions in neuron size, neuron density, dendritic arborization, and axonal length (Drachman, 2006), all of which may be related to, or account for, alterations in brain Glu. Finally, age-related reductions in Glu metabolites likely affect the balance of neural transmission, ultimately affecting cognitive functioning (e.g. learning and memory).

Interestingly, meta-analysis of Gln effects uncovered an opposite pattern: older adults consistently showed higher levels of Gln than younger individuals. The dissociation between Glu/Glx and Gln changes during aging provides insight into putative underlying biological mechanisms, given that Glu is primarily located within neurons whereas Gln localizes to glia cells. Glia—most notably astrocytes—undergo significant aging-associated changes that may influence synthesis or conversion of glutamine (Palmer and Ousman, 2018). Nonetheless, given the limited number of effects ($k = 9$) reported for Gln in aging *in vivo*, we were unable to perform a comprehensive meta-analysis of Gln. As such, additional studies are needed to elucidate the role of brain Gln in the aging process, and to assess cell-type related mechanisms. Future studies probing age-related changes should consider the magnitude and direction of expected changes in Glu, Glx and Gln when interpreting findings.

Methodological heterogeneity across studies accounted for a considerable amount of variation in Glu/Glx effect sizes. Quite importantly, $^1\text{HMRS}$ acquisition protocol (i.e. sequence) and TE accounted for significant heterogeneity. Interestingly, studies utilizing more advanced MRS techniques, including SPECIAL, LASER-POCE, and EPSI sequences,

reported more robust effects compared to those that used the more commonly employed PRESS or STEAM sequences. These advanced techniques attempt to combine the benefits of multiple 'traditional' MRS approaches (e.g. combining the benefits of STEAM-like TEs and full signal intensity of PRESS) in order to better optimize measurement of brain metabolites like Glu. It must be noted, however, that studies using these more advanced techniques were limited in number, thus these results should be considered preliminary. As expected, studies using shorter TEs reported larger effect sizes (Wijtenburg and Knight-Scott, 2011).

There are a multitude of factors that must be considered when choosing what ^1H MRS acquisition scheme to employ, including (but not limited to) scanner and sequence availability, hardware design, B_1 homogeneity, shimming capabilities, and the availability of short echo time sequences. Still, sequence parameters will affect the quantification of Glu metabolites *in vivo* (Ramadan et al., 2013), and thus must be considered in future efforts that aim to study Glu in aging. For example, PRESS may be selected over LASER as the large number of RF pulses used in LASER results in higher RF power requirement and longer TEs, neither of which are optimal for measuring brain Glu at 3T (Zhu and Barker, 2011). PRESS and STEAM sequences have been compared in detail and the most significant difference is that PRESS has twice the signal as STEAM (Zhu and Barker, 2011). STEAM sequences are often preferred when short TEs are required (e.g. at 7T), however, modified PRESS sequences can achieve short TEs of ~20ms, thus achieving TE close to that of STEAM while retaining more signal. Whereas the PRESS sequence does include chemical shift artifacts for Glu and NAA, these artifacts can be minimized by setting water acquisition spectrum excitation and refocusing pulses in resonance with the water peak at 4.7ppm. Nonetheless, overall, the 'traditional' sequences (e.g. PRESS, STEAM) still provided evidence of robust and significant age-related change in Glu metabolites.

More recently, studies have compared PRESS and MEGA-PRESS sequences for measuring glutamate metabolites (Glu, Glx, Gln). MEGA-PRESS studies are implemented to measure J-coupled metabolites, such as GABA, but are not traditionally used to report Glu, Glx, or Gln. Still, two recent studies (Maddock et al., 2018; van Veenendaal et al., 2018) directly compared PRESS and MEGA-PRESS for the measurement of Glu, Glx and Gln. Van Veenendaal et al. (2018) found only a small positive correlation between glutamate measures when using PRESS and MEGA-PRESS ($r = 0.30$), while Maddock et al. (2018) found the correlation between glutamate measured using these two types of sequences to be much larger ($r = 0.88$); notably both studies indicated that correlations were stronger for Glu as compared to Glx. There were significant methodological differences between these two studies (Maddock et al., 2018; van Veenendaal et al., 2018), including acquisition parameters (TR/TE), that could explain some of the discrepant results. Overall, while these studies suggest that MEGA-PRESS protocols provide comparable *in vivo* results as PRESS sequences for Glu and Glx, caution should be taken when directly comparing the results given the limited associations. Notably, we report larger age-related effect sizes for PRESS ($d = -0.68$) as compared to MEGA-PRESS ($d = -0.02$).

Other advanced techniques have not necessarily been directly compared to PRESS or STEAM in older samples. Notably, the use of whole brain EPSI techniques have emerged in

recent years and comparability data in older samples is lacking. This technique offers unprecedented neurometabolic information as it generates $^1\text{HMRS}$ spectra across the brain via one (~20 minute) acquisition, yielding metabolite concentrations comparable to those obtained from conventional SVS (Maghsudi et al., 2019). While standardization across the field is a challenge given the physical limitations at any given research site, we believe that the present data provide evidence in favor of more advanced approaches using shorter TEs when investigating age-related changes in brain Glu.

The majority of effects included in this meta-analysis reported absolute concentrations of metabolites; the remainder reported ratios (e.g. Glu/Creatine). Those utilizing absolute concentration produced significantly larger age-related effects than those that analyzed ratios. Although many clinical studies do indeed utilize metabolite ratios when comparing values within affected and unaffected brain tissue, techniques that quantify absolute concentrations are readily available and can be successfully applied, particularly at high field strength. Absolute concentrations may be particularly relevant to use in aging, as simultaneous decreases in Glu and creatine could occur—in this case, ratios would obscure neurobiologically-relevant decreases in Glu. Furthermore, relative quantification may introduce substantial errors, lead to erroneous metabolite values, and introduce misinterpretation of spectral data (Jansen et al., 2006). Hence, it is not surprising that most of the extant aging research has used absolute concentration values, or that these absolute effects were larger.

While the meta-analytically derived effect size was only nominally larger within gray matter than within white matter and mixed tissue, brain region analyzed varied by study and significantly affected the age-related difference in Glu metabolites observed. Regional effects were largest within the parietal lobe, followed by the subcortex and temporal, occipital, and frontal lobes. Notably, only effects within the cerebellum indicated significantly larger amounts of Glu/Glx with age, but this should be interpreted with caution as there were only two reported effects from this ROI. The regional pattern of aging-associated Glu/Glx changes observed in this meta-analysis align to some degree with reported patterns of aging-associated brain volume change (Mueller et al., 2007; Resnick et al., 2000) and aging- and disease-related change in the neuronal marker N-acetyl aspartate (NAA, a putative marker of neuronal integrity) (Angelic et al., 2001; Schuff et al., 2002). Alterations in brain Glu thus appear to overlap anatomically with decreases in brain volume. It is of interest for future studies to delineate whether age-related changes in brain glutamate precede, or are a product of, changes in regional brain volume. Longitudinal studies are particularly apposite for resolving this question convincingly. Of note, ROI selection itself was very heterogeneous across the included studies, which makes synthesis of these data challenging. Consequently, it is difficult to make conclusions about the degree of spatial specificity of glutamatergic deficits in aging (though currently, effects seem largely diffuse), or to discern interrelationships among Glu metabolite levels across diverse brain regions.

Higher MRI field strength is known to increase the spectral resolution of $^1\text{HMRS}$, especially for glutamate and glutamine (Moser et al., 2012). Greater MRI field strength was associated with larger effect sizes at lower field strengths (1.5>3.0T>4.0T), but results from 7T did not follow this pattern. $^1\text{HMRS}$ studies at 3T were by far the most numerous, and these did

evince nominally larger age-related effects than 1.5T studies. Effect sizes for 4T studies were additionally larger than for 3T studies, but effect sizes reported for the one study at 7T were the smallest. This relatively small ($n = 33$) sole 7T study measured Glu and Gln from the posterior cingulate cortex and the occipital cortex. Effect sizes for Glu were larger than for Gln, and the Glu effect size within the occipital lobe was similar to those observed in other studies at lower field strength. Clearly, it is inappropriate to make definitive conclusions about findings at 7T based upon a few effects from one study. Other studies have, however, reported improved estimates of Glu metabolites at 7T using ^1H MRS methods (Mekle et al., 2009), thus data collection at higher field strengths appears promising for improving our understanding of age-related Glu metabolite change. Still, 3T MRIs are the most widely available, and are capable of identifying robust age-related effects in Glu metabolites. It will be interesting to compare results from the present analysis to those obtained by other novel imaging methods than can specifically measure brain glutamate, such as Glutamate Chemical Exchanges Saturation Transfer (GluCEST), which can only be employed at 7T (Cai et al., 2012; Davis et al., 2015; Nanga et al., 2018; Roalf et al., 2017), but offers highly sensitive measures of brain Glu independent of Gln.

Finally, sex composition of the study moderated the overall effect, with a larger percentage of females in the study sample resulting in a smaller age-related glutamate effect size. The majority of effects ($k = 31$, average Cohen's $d = -0.40$) were from studies with well-balanced samples (50–57% female) and only a handful ($k = 9$; average Cohen's $d = -0.62$) were from studies ($k = 9$) that include a greater number of males than females. This meta-analytic result may indicate that there are sex differences in the aging processes that affect brain Glu, further suggesting that sex should be considered a relevant biological factor in future studies. Continued examination of sex effects will help to clarify the impact of sex on brain aging as well as reconcile inconsistencies between studies reporting sex differences in Glu metabolites in young adults (O'Gorman et al., 2011) and across the lifespan (Hädel et al., 2013; Marjańska et al., 2017; Sailasuta et al., 2008; Zahr et al., 2013), versus those finding no sex differences (Ding et al., 2016; Kaiser et al., 2005; Raininko and Mattsson, 2010). Yet, the predominance of studies reviewed here did not directly measure sex effects (Boumezbeur et al., 2010; Chang et al., 2009; Kumar et al., 2018; Mooney et al., 2017; Schubert et al., 2004; Suri et al., 2017; Zahr et al., 2008).

4.1 Limitations and Future Directions

The present meta-analysis should be considered in context with its limitations. First, this field is still relatively small, and this meta-analysis includes only 13 studies reporting effects of Glu-related metabolites in healthy aging. Larger studies of glutamate in aging populations are needed.

As noted above there is little consistency in the acquisition of ^1H MRS data, and, unsurprisingly, some methodological choices affect the measurement of brain Glu. Variability across the methodological space may have made the present synthesis across studies less precise. Findings under certain methodological conditions (e.g. 7T MRI) should be considered tentative and future studies are warranted.

Moderator and meta-regression analyses were performed only on those studies where relevant data was reported. Many studies did not report other data that may be relevant to the Glu changes observed in the meta-analysis, including medication status, cognitive function, or general clinical symptoms. Amazingly, only three studies measured the relationship between brain Glu and aging-related cognitive changes (Simmonite et al., 2019; Zahr et al., 2008; Zahr et al., 2013). All three studies suggest that lower Glu is associated with poorer cognitive performance in older adults, but again these samples are small. As such, future studies should explore age-related Glu change in relation to clinical symptoms and cognitive performance. We echo previous calls (Haga et al., 2009) for large-scale studies of middle aged and older adults to provide more robust data on age-related Glu changes. This would allow a more nuanced view of brain glutamate changes in healthy aging, enable better understanding of how non-linear age-related trajectories of structural brain change (Vinke et al., 2018) are associated with glutamate, and offer more insight into disease-related changes. It is our contention that researchers should continue to investigate Glu metabolite change in aging and neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease, while using consistent and comparable methodologies and reporting sufficient phenotypic data. This will allow for study outcomes to be better synthesized in the future.

In conclusion, the present meta-analytic review is the first to quantitatively synthesize data on glutamatergic metabolite change in the aging brain. The results of this meta-analysis demonstrate clear alterations in glutamate metabolite levels (Glu/Glx) in older individuals. Future studies examining glutamate-related metabolite concentrations in conditions such as mild cognitive impairment are warranted; this has been an area of limited investigation despite initial work suggesting that reduced glutamate may be a biomarker of aging-related cognitive decline (Zeydan et al., 2017). Investigations linking age-related glutamate change to clinical and cognitive changes, and studies of the underlying genetic and neurobiological mechanisms contributing to this age-related decline, will improve our understanding of the aging brain.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Funding Sources:

This work was supported by the National Institute of Mental Health grants R01MH119185 to DRR & PJM and R01MH120174 to DRR. Support for Dr. J. Cobb Scott was from VA Office of Research & Development I01RX002699 and the Life Span Brain Institute (LiBI)—a collaboration between the University of Pennsylvania School of Medicine and Children's Hospital of Philadelphia. This work was also supported by a NARSAD Young Investigator Grant from the Brain & Behavior Research Foundation (DRR), and NBIB under Grant No. P41 EB015893 (RR).

The funding sources were not directly involved in study design, collection, data analysis or interpretation, nor manuscript writing.

References

- Angelic E, Bonmartin A, Boudraa A, Gonnaud P-M, Mallet J-J, Sappey-Marinié D, 2001 Regional differences and metabolic changes in normal aging of the human brain: proton MR spectroscopic imaging study. *American Journal of Neuroradiology* 22(1), 119–127. [PubMed: 11158897]

- Barker PB, Hearshen DO, Boska MD, 2001 Single-voxel proton MRS of the human brain at 1.5T and 3.0T. *Magn. Reson. Med* 45(5), 765–769. [PubMed: 11323802]
- Begg CB, Mazumdar M, 1994 Operating characteristics of a rank correlation test for publication bias. *Biometrics*, 1088–1101. [PubMed: 7786990]
- Boumezbeur F, Mason GF, De Graaf RA, Behar KL, Cline GW, Shulman GI, Rothman DL, Petersen KF, 2010 Altered brain mitochondrial metabolism in healthy aging as assessed by in vivo magnetic resonance spectroscopy. *J. Cereb. Blood Flow Metab* 30(1), 211–221. [PubMed: 19794401]
- Cai K, Haris M, Singh A, Kogan F, Greenberg JH, Hariharan H, Detre JA, Reddy R, 2012 Magnetic resonance imaging of glutamate. *Nat. Med* 18(2), 302–306. [PubMed: 22270722]
- 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. [PubMed: 8637436]
- Chang L, Jiang CS, Ernst T, 2009 Effects of age and sex on brain glutamate and other metabolites. *Magn. Reson. Imaging* 27(1), 142–145. [PubMed: 18687554]
- Cheung MW-L, 2019 A guide to conducting a meta-analysis with non-independent effect sizes. *Neuropsychol. Rev.*, 1–10. [PubMed: 31030409]
- Cleeland C, Pipingas A, Scholey A, White D, 2019 Neurochemical changes in the aging brain: A systematic review. *Neurosci. Biobehav. Rev*
- Cohen J, 1988 *Statistical Power Analysis for the Behavioral Sciences*, 2nd ed Erlbaum, Hillsdale, New Jersey.
- Cotman CW, Monaghan DT, 1988 Excitatory amino acid neurotransmission: NMDA receptors and Hebb-type synaptic plasticity. *Annu. Rev. Neurosci* 11(1), 61–80. [PubMed: 2452598]
- Coyle JT, 2006 Glutamate and schizophrenia: beyond the dopamine hypothesis. *Cell. Mol. Neurobiol* 26(4), 363–382.
- Craik FI, Grady CL, 2002 Aging, memory, and frontal lobe functioning
- Curtis DR, Johnston GA, 1974 Amino acid transmitters in the mammalian central nervous system, *Ergebnisse der Physiologie Reviews of Physiology*, Volume 69 Springer, pp. 97–188.
- Davis KA, Nanga RPR, Das S, Chen SH, Hadar PN, Pollard JR, Lucas TH, Shinohara RT, Litt B, Hariharan H, 2015 Glutamate imaging (GluCEST) lateralizes epileptic foci in nonlesional temporal lobe epilepsy. *Sci. Transl. Med* 7(309), 309ra161–309ra161.
- Dempster FN, 1992 The rise and fall of the inhibitory mechanism: Toward a unified theory of cognitive development and aging. *Dev. Rev* 12(1), 45–75.
- Ding X-Q, Maudsley AA, Sabati M, Sherif 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. [PubMed: 27164326]
- Drachman DA, 2006 Aging of the brain, entropy, and Alzheimer disease. *Neurology* 67(8), 1340–1352. [PubMed: 17060558]
- Duval S, Tweedie R, 2000 Trim and fill: a simple funnel-plot-based method of testing and adjusting for publication bias in meta-analysis. *Biometrics* 56(2), 455–463. [PubMed: 10877304]
- Egger M, Smith GD, Schneider M, Minder C, 1997 Bias in meta-analysis detected by a simple, graphical test. *BMJ* 315(7109), 629–634. [PubMed: 9310563]
- Erecska M, Zaleska MM, Nelson D, Nissim I, Yudkoff M, 1990 Neuronal glutamine utilization: glutamine/glutamate homeostasis in synaptosomes. *J. Neurochem* 54(6), 2057–2069. [PubMed: 1971010]
- Grilli L, Rampichini C, 2006 A review of random effects modelling using gllamm in Stata Department of Statistics, University of Florence.
- 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. *J. Magn. Reson. Imaging* 38(6), 1480–1487. [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. [PubMed: 17719145]
- Hedges LV, Olkin I, 1985 *Statistical methods for meta-analysis* Academic press.

- Huberfeld G, de La Prida LM, Pallud J, Cohen I, Le Van Quyen M, Adam C, Clemenceau S, Baulac M, Miles R, 2011 Glutamatergic pre-ictal discharges emerge at the transition to seizure in human epilepsy. *Nat. Neurosci* 14(5), 627. [PubMed: 21460834]
- Hynd MR, Lewohl JM, Scott HL, Dodd PR, 2003 Biochemical and molecular studies using human autopsy brain tissue. *J. Neurochem* 85(3), 543–562. [PubMed: 12694381]
- Jansen JF, Backes WH, Nicolay K, Kooi ME, 2006 ¹H MR spectroscopy of the brain: absolute quantification of metabolites. *Radiology* 240(2), 318–332. [PubMed: 16864664]
- 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. [PubMed: 15708441]
- Kumar J, Liddle EB, Fernandes CC, Palaniyappan L, Hall EL, Robson SE, Simmonite M, Fiesal J, Katshu MZ, Qureshi A, 2018 Glutathione and glutamate in schizophrenia: a 7T MRS study. *Mol. Psychiatry*, 1–10.
- 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 Research: Neuroimaging* 279, 22–30. [PubMed: 30081290]
- Mader I, Seeger U, Karitzky J, Erb M, Schick F, Klose U, 2002 Proton magnetic resonance spectroscopy with metabolite nulling reveals regional differences of macromolecules in normal human brain. *Journal of Magnetic Resonance Imaging: An Official Journal of the International Society for Magnetic Resonance in Medicine* 16(5), 538–546.
- Magsudi H, Schmitz B, Maudsley AA, Sherif S, Bronzlik P, Schütze M, Lanfermann H, Ding X, 2019 Regional metabolite concentrations in aging human brain: Comparison of short-TE whole brain MR spectroscopic imaging and single voxel spectroscopy at 3T. *Clin. Neuroradiol*, 1–11. [PubMed: 30762071]
- Marja ska 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. [PubMed: 28476320]
- Mather M, Jacobson L, Pollard K, 2015 Population Bulletin: Aging in the United States, Population Reference Bureau Washington, D.C.
- Mayr U, 2001 Age differences in the selection of mental sets: the role of inhibition, stimulus ambiguity, and response-set overlap. *Psychol. Aging* 16(1), 96. [PubMed: 11302371]
- McEntee WJ, Crook TH, 1993 Glutamate: its role in learning, memory, and the aging brain. *Psychopharmacology (Berl.)* 111(4), 391–401. [PubMed: 7870979]
- Mekle R, Mlynárik V, Gambarota G, Hergt M, Krueger G, Gruetter R, 2009 MR spectroscopy of the human brain with enhanced signal intensity at ultrashort echo times on a clinical platform at 3T and 7T. *Magnetic Resonance in Medicine: An Official Journal of the International Society for Magnetic Resonance in Medicine* 61(6), 1279–1285.
- 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. [PubMed: 28424294]
- Moser E, Stahlberg F, Ladd ME, Trattinig S, 2012 7-T MR--from research to clinical applications? *NMR Biomed* 25(5), 695–716. [PubMed: 22102481]
- Mueller S, Stables L, Du A, Schuff N, Truran D, Cashdollar N, Weiner M, 2007 Measurement of hippocampal subfields and age-related changes with high resolution MRI at 4 T. *Neurobiol. Aging* 28(5), 719–726. [PubMed: 16713659]
- Nanga RPR, DeBrosse C, Kumar D, Roalf D, McGeehan B, D'Aquila K, Borthakur A, Hariharan H, Reddy D, Elliott M, 2018 Reproducibility of 2D GluCEST in healthy human volunteers at 7 T. *Magn. Reson. Med*
- 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. [PubMed: 21509888]
- Palmer AL, Ousman SS, 2018 Astrocytes and aging. *Front. Aging Neurosci* 10.
- Rabe-Hesketh S, Skrondal A, Pickles A, 2004 GLLAMM manual

- Rabe-Hesketh S, Skrondal A, Pickles A, 2005 Maximum likelihood estimation of limited and discrete dependent variable models with nested random effects. *Journal of Econometrics* 128(2), 301–323.
- 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* 51(3), 309–315. [PubMed: 20170295]
- Ramadan S, Lin A, Stanwell P, 2013 Glutamate and glutamine: a review of in vivo MRS in the human brain. *NMR Biomed* 26(12), 1630–1646. [PubMed: 24123328]
- Resnick SM, Goldszal AF, Davatzikos C, Golski S, Kraut MA, Metter EJ, Bryan RN, Zonderman AB, 2000 One-year age changes in MRI brain volumes in older adults. *Cereb. Cortex* 10(5), 464–472. [PubMed: 10847596]
- Roalf D, Nanga R, Rupert P, Hariharan H, Quarmley M, Calkins M, Dress E, Prabhakaran K, Elliott M, Moberg P, 2017 Glutamate imaging (GluCEST) reveals lower brain GluCEST contrast in patients on the psychosis spectrum. *Mol. Psychiatry*
- Robbins TW, Murphy ER, 2006 Behavioural pharmacology: 40+ years of progress, with a focus on glutamate receptors and cognition. *Trends Pharmacol. Sci* 27(3), 141–148. [PubMed: 16490260]
- Rosenthal R, 1979 The file drawer problem and tolerance for null results. *Psychol. Bull* 86(3), 638.
- 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. [PubMed: 17692491]
- 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. [PubMed: 15050596]
- Schuff N, Capizzano A, Du A, Amend D, O’neill J, Norman D, Kramer J, Jagust W, Miller B, Wolkowitz O, 2002 Selective reduction of N-acetylaspartate in medial temporal and parietal lobes in AD. *Neurology* 58(6), 928–935. [PubMed: 11914410]
- Scott JC, Matt GE, Wrocklage KM, Crnich C, Jordan J, Southwick SM, Krystal JH, Schweinsburg BC, 2015 A quantitative meta-analysis of neurocognitive functioning in posttraumatic stress disorder. *Psychol. Bull* 141(1), 105. [PubMed: 25365762]
- 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. [PubMed: 30327163]
- Soares D, Law M, 2009 Magnetic resonance spectroscopy of the brain: review of metabolites and clinical applications. *Clin. Radiol* 64(1), 12–21. [PubMed: 19070693]
- StataCorp L, 2013 Stata Statistical Software: Release 13 College Station Texas.
- Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, Moher D, Becker BJ, Sipe TA, Thacker SB, 2000 Meta-analysis of observational studies in epidemiology: a proposal for reporting. *JAMA* 283(15), 2008–2012. [PubMed: 10789670]
- Suri S, Emir U, Staggs CJ, Near J, Mekle R, Schubert F, Zsoldos E, Mahmood A, Singh-Manoux A, Kivimäki M, 2017 Effect of age and the APOE gene on metabolite concentrations in the posterior cingulate cortex. *Neuroimage* 152, 509–516. [PubMed: 28323160]
- Tisell A, Leinhard OD, Warntjes JBM, Lundberg P, 2013 Procedure for quantitative 1H magnetic resonance spectroscopy and tissue characterization of human brain tissue based on the use of quantitative magnetic resonance imaging. *Magn. Reson. Med* 70(4), 905–915. [PubMed: 23169203]
- Tunc-Skarka N, Meier S, Demirakca T, Sack M, Weber-Fahr W, Brusniak W, Wolf I, Matthäus F, Schulze TG, Diener C, 2014 Effects of normal aging and SCN1A risk-gene expression on brain metabolites: evidence for an association between SCN1A and myo-inositol. *NMR Biomed* 27(2), 228–234. [PubMed: 24357141]
- van Veenendaal TM, Backes WH, van Bussel FC, Edden RA, Puts NA, Aldenkamp AP, Jansen JF, 2018 Glutamate quantification by PRESS or MEGA-PRESS: Validation, repeatability, and concordance. *Magn. Reson. Imaging* 48, 107–114. [PubMed: 29306050]
- Vinke EJ, De Groot M, Venkatraghavan V, Klein S, Niessen WJ, Ikram MA, Vernooij MW, 2018 Trajectories of imaging markers in brain aging: the Rotterdam Study. *Neurobiol. Aging* 71, 32–40. [PubMed: 30077040]

- Wenk GL, Walker LC, Price DL, Cork LC, 1991 Loss of NMDA, but not GABA-A, binding in the brains of aged rats and monkeys. *Neurobiol. Aging* 12(2), 93–98. [PubMed: 1646968]
- Wijtenburg SA, Knight-Scott J, 2011 Very short echo time improves the precision of glutamate detection at 3T in 1H magnetic resonance spectroscopy. *J. Magn. Reson. Imaging* 34(3), 645–652. [PubMed: 21761460]
- Zahr NM, Mayer D, Pfefferbaum A, Sullivan EV, 2008 Low striatal glutamate levels underlie cognitive decline in the elderly: evidence from in vivo molecular spectroscopy. *Cereb. Cortex* 18(10), 2241–2250. [PubMed: 18234683]
- 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. [PubMed: 23116877]
- Zelazo PD, Craik FI, Booth L, 2004 Executive function across the life span. *Acta Psychol. (Amst.)* 115(2–3), 167–183. [PubMed: 14962399]
- Zeydan B, Deelchand DK, Tosakulwong N, Lesnick TG, Kantarci OH, Machulda MM, Knopman DS, Lowe VJ, Jack CR Jr, Petersen RC, 2017 Decreased glutamate levels in patients with amnesic mild cognitive impairment: an sLASER proton MR spectroscopy and PiB-PET study. *J. Neuroimaging* 27(6), 630–636. [PubMed: 28661060]
- Zhu H, Barker PB, 2011 MR spectroscopy and spectroscopic imaging of the brain, *Magnetic resonance neuroimaging* Springer, pp. 203–226.

- Meta-analysis demonstrates reductions in glutamate metabolite levels in older adults.
- Methodological heterogeneity across study accounts for sizable variation of effect size.
- Brain glutamate may serve as a biomarker for delineating typical and atypical aging

Figure 1. Individual study and average effect sizes displayed by ¹H MRS metabolite type. A-C: Effects sizes are shown separately for glutamate (A), glutamate+glutamine (B), and glutamine (C). Effects are shown as mean Cohen's d (effect size) with corresponding 95% confidence intervals. (D). The meta-analytic derived average effect size ((+/- 95% CI) for each of the three metabolite types. Effects shown in A-C are ordered and colored by brain region. In all panels, negative effect sizes indicate lower metabolite levels in older compared to younger adults. *indicates significantly different than glutamine. ACC=Anterior Cingulate Cortex; PCC=Posterior Cingulate Cortex.

Figure 2. Meta-analytic average effect sizes by methodological moderator variable.

Average effect sizes (+/- 95% CI) shown for each variable were calculated from all available Glu and Glx effects. Effect sizes did not significantly differ by MRI Field Strength (A) or Voxel Type (C). Effect sizes for ¹HMRS Protocol (B) and Quantification Method (D) were significantly heterogeneous.

Figure 3. Meta-analytic average effect sizes by neurological moderator variable.

Average effect sizes (+/- 95% CI) shown for each variable were calculated from all available Glu and Glx effects. Effect sizes did not differ by (A) Brain Tissue Type but did differ by (B) Brain Region of Interest (*indicates significantly different from Cerebellum. ^indicates significantly different from Brain Stem). Meta-regression of age-related glutamate effect size on (C) echo time (TE) and (D) sex were significant.

Table 1:

Summary table of ¹H-MRS studies reporting Glu, Gln or Glx effects in healthy older and younger adults.

Table 1 Study Year	Age # Young	Age # Old	Sample Age * Range	Sample Size (Total/ Young/Old)	ROI	Sex (%F)	MRS Sequence	Field Strength	Voxel Type	Glu Type	Echo Time (TE)msec
Boumezbeur 2010	26	76	-	14/7/7	Midline Occipito-Parietal Lobe	33	LASER- POCE	4.0 T	Single	Glu	42
Chang 1996	-	-	19–78	33/-/-	Mid-Frontal Gray Matter; Right-Frontal White Matter		PRESS	1.5 T	Single	Glx	30
Ding 2016	25	65	20–70	30/17/13	Bilateral Frontal and Parietal lobes	57	EPSI	3.0 T	Multi	Glx	17.6
Hädel 2013	-	-	20–56	118/-/-	Left Hippocampus; Anterior Cingulate Cortex	50	PRESS	3.0 T	Single	Glu, Gln	80
Kaiser 2005	26	54	24–68	24/11/13	Corona Radiata; Mesial Motor Cortex	54	STEAM	4.0 T	Single	Glu, Gln	15
Marja ska 2017	21	78	19–88	33/17/16	Posterior Cingulate Cortex; Occipital Cortex	52	STEAM	7.0 T	Multi	Glu, Gln	8
Mooney 2017	24.6	70.3	20–83	31/15/16	Primary Motor Cortex	35	MEGA- PRESS	3.0 T	Single	Glx	68
Raiminko 2010	32	62	13–72	34/15/19	Supraventricular White Matter	44	PRESS	1.5 T	Single	Glx	22
Sailasuta 2008	-	-	21–71	22/16/6	Frontal White Matter; Parietal Gray Matter; Basal Ganglia	40	PRESS	3.0 T	Single	Glu	82 [^]
Schubert 2004	-	-	20–60	40/-/-	Anterior Cingulate Cortex; Hippocampus	50	PRESS	3.0 T	Single	Glu, Gln	80 [^]
Simmonite 2019	20.7	76.5	18–87	39/20/19	Occipital Cortex	56	PRESS	3.0 T	Single	Glx	35
Suri 2017	23.9	68.9	20–85	147/30/117	Posterior Cingulate Cortex	26	SPECIAL	3.0 T	Single	Glu, Gln	8.5
Zahr 2008	25.5	77.7	19–84	24/12/12	Striatum;Cerebellum; Pons	50	CT-PRESS	3.0 T	Single	Glu, Glx	139 [^]

ROI = region of interest; %F = percent female; Glu = glutamate; Gln = glutamine; Glx = glutamate+glutamine;

average age reported for cohort studies;

* minimum and maximum age included in the study;

[^] indicates average TE.