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AUTISM SPECTRUM (C DISSANAYAKE, SECTION EDITOR)

Magnetic Resonance Spectroscopy Studies of Glutamate and GABA in Autism: Implications for Excitation-Inhibition Imbalance Theory

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Abstract One popular major theory of neurotransmitter dysfunction is an imbalance in excitation and inhibition (EI theory).The EI imbalance theory is thought to impact widely across neural circuits mediating language, social, and cognitive functions, and could potentially explain some aspects of the autism phenotype. Evidence from genetic and molecular studies provide support for abnormal suppression of γ-aminobutyric acid (GABA) function and an overabundance of glutamatergic transmission as potential mechanisms of this hyperexcitability. Proton magnetic resonance spectroscopy (^{1}H-MRS) is a potentially exciting neuroimaging tool allowing in vivo estimation of glutamate and GABA neurotransmitters in people with autism spectrum disorder (ASD). We reviewed all available published studies of ASD reporting ^{1}H-MRS measurement of glutamate, GABA, or both neurotransmitters. Glutamate results across studies are equivocal, with nearly equal numbers of studies reporting increases or decreases in autism. However, the age of the individuals studied appears to relate to the direction of the findings, suggesting that future longitudinal studies of glutamate should be conducted. Although fewer GABA-specific studies have been published, all have reported decreases in autism. Overall, from ^{1}H-MRS studies alone, support for the glutamate side of the EI imbalance theory is tenuous, but this is an indication of serious limitations in the ^{1}H-MRS literature. For GABA dysfunction, the GABA findings to date are consistent for reduced concentration in autism; however, there are only a few published ^{1}H-MRS studies of GABA in autism, all from studies with a small number of subjects. More studies, particularly longitudinal developmental studies across both child and adult development, are needed.

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Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental condition defined by a common set of impairments in communication, social interactions, restricted interests, and behavioral inflexibility that emerges in early childhood and persists into adulthood (American Psychiatric Association [APA]; Diagnostic and Statistical Manual of Mental Disorders, 5th edition [DSM-V], http://www.dsm5.org/). Previous estimates in the US have indicated that approximately 1 % of the general population has been diagnosed with some form of an ASD [1]. While the full effect of recent revisions to the DSM-V remains to be seen, a report from the Centers for Disease Control and Prevention's Autism and Developmental Disabilities Monitoring Network indicated the new criteria will increase rates of ASD diagnosis but have little to no effect on gender disparity, with ASD rates remaining higher in males (1 in 42) than in females (1 in 189) [2]. Despite the high prevalence of ASDs, the underlying genetic and environmental factors contributing to symptomology remain largely unknown as only 10 % of cases have known origins [3, 4]. ASDs appear to be highly heritable as siblings of individuals with ASD exhibit an 18 % recurrence risk of developing ASD [5], and concordance rates have approached nearly 90 % in monozygotic twins and 31 % in dizygotic twins [6, 7].

Excitation-Inhibition Imbalance Theory

One hypothesis concerning the pathophysiology of autism is a cortical excitatory-inhibitory imbalance due to increased glutamatergic (excitatory) and/or diminished GABAergic

(inhibitory) signaling (EI theory) [8••, 9]. The literature indicates that an imbalance of excitatory and inhibitory neurotransmission produces a state of hyperexcitability in areas of the brain responsible for language, social interaction, and multisensory perception in persons with ASDs [8••, 9, 10•, 11]. Such an imbalance could also explain the increased prevalence of seizure disorders, known to result from neural hyperexcitability, in autism [12, 13].

Numerous lines of research provide evidence for cellular abnormalities that may contribute to EI imbalance in autism. Autism genetic studies report linkage and association with glutamate- and γ-aminobutyric acid (GABA)-related genes [14–16]. Fatemi et al. [17] reported that reductions and alterations to GABA-A receptor subtypes in parietal, cerebellar, and superior frontal regions may decrease GABA binding affinity in persons with ASDs. Altered GABAergic and glutamatergic neurotransmission is observed in multiple mouse models of autism [18, 19]. Postmortem studies also implicate glutamatergic and GABA dysfunction in autism, including findings of increased AMPA-type glutamate receptor messenger RNA (mRNA) [20], increased metabotropic glutamate receptor expression [21], decreased GABA-A and GABA-B protein expression [22–24], and decreased expression of glutamic acid decarboxylase, the enzyme that catalyzes the conversion of glutamate to GABA [24, 25]. Postmortem autism tissue analysis has revealed elevated glutamate and its precursor glutamine [26]. Together, these lines of research provide significant converging support for deficits in cortical EI neurotransmission in autism.

Proton Magnetic Resonance Spectroscopy

Additional support for a cortical imbalance involving glutamatergic and GABAergic neurotransmission in autism could be provided by proton magnetic resonance spectroscopy (^{1}H-MRS) studies. ^{1}H-MRS enables in vivo quantification of metabolite concentrations in defined regions of the brain by their characteristic resonance in strong magnetic fields [27]. While magnetic resonance imaging (MRI) is sensitive to the distribution of water-associated protons in tissue, ^{1}H-MRS provides the concentration of protons attached to other molecules in the brain. ^{1}H-MRS results in a spectrum whose peaks at different frequencies indicate proton nuclei in different chemical environments. The area under each of these peaks represents the relative concentration of nuclei detected for a given metabolite, and ^{1}H-MRS can detect metabolites in concentrations of 0.5–10 Nm, with a spatial resolution of approximately 1–10 cm^3. In simple terms, ^{1}H-MRS spectra represent a chemical fingerprint of brain tissue.

^{1}H-MRS allows non-invasive, in vivo measurement of GABA and glutamate neurotransmitter concentrations in human subjects. As such, it should allow for an examination of the EI theory in terms of transmitter concentrations in autism. While there have been many studies employing ^{1}H-MRS in autism [reviewed in 28, 29••], few have specifically reported amino acid transmitter metabolite levels. The brevity of this literature is most likely tied to the methodological difficulties caused by the overlap between the relatively low signal-to-noise ratios of amino acid neurotransmitters within typical ^{1}H-MRS spectra, as well as the overlap between glutamate and GABA signals and other metabolite signals. As a result of this low signal-to-noise ratio, many ^{1}H-MRS studies combine the resonances of glutamate and glutamine into a signal measure known as Glx. In addition, GABA measurement in particular is challenging due to overlap between its resonance and those of creatine (Cr) and macromolecules and requires special techniques such as spectral editing sequences [30]. Despite such challenges, ^{1}H-MRS studies of glutamate and GABA in autism are increasing, motivated in large part by the EI theory.

In addition to the challenges of differentiating the glutamate and GABA signals, there are several limitations to the ^{1}H-MRS technique. ^{1}H-MRS requires significant time acquisition and is typically restricted to only a few regions of interest (ROIs). ROI analyses are also required due to the low concentrations of the metabolites measured. As a result, comparisons across studies are often difficult due to regional differences. Despite, these limitations ^{1}H-MRS is the only non-invasive method that currently enables the direct quantification of glutamate and GABA concentrations. These molecules degrade rapidly postmortem, resulting in difficulties in direct measurement of their concentration in postmortem tissue studies. ^{1}H-MRS is therefore an important technology allowing us to probe the EI imbalance in autism. All published ^{1}H-MRS studies in autism concerning either glutamate, GABA, or both are considered in this review (see Table 1 for a summary). To identify eligible studies for the review, a PubMed search was conducted combining the terms 'magnetic resonance spectroscopy', '^{1}H-MRS', 'proton spectroscopy', 'glutamate', and 'GABA' with 'autism', 'autistic disorder', 'autism spectrum disorder', 'Asperger syndrome' and 'Asperger's syndrome'. Studies that reported ^{1}H-MRS glutamate and/or GABA concentration results in an autism spectrum group were then selected.

Proton Magnetic Resonance Spectroscopy (^{1}H-MRS) Studies of Glutamate in Autism

Glutamate is measured at a single resonance (2.35 ppm) but due to concerns of low signal to noise, particularly using 1.5 T MRI systems, many ^{1}H-MRS studies use a combined metric termed Glx which combines the resonances of glutamine, glutamate, and GABA together. Where discussed below, the term 'Glx' refers to the metabolite combination, the term 'Glu' refers specifically to glutamate, and 'Gln' refers to glutamine.

Table 1 ^{1}H-MRS studies of glutamate and GABA in autism

Study, year	*N*	Overall percentage male	Mean ages	Magnet field strength (T)	ROI	Measure	ASD finding relative to comparison group	Effect size
Page et al. [34], 2006	HC: 13/19[a] ASD(h): 20/17[a]	79/78	HC: 34.3 ASD: 35.6	1.5	R hippocampus, R parietal cortex	Glx	↑Hippocampus, parietal NS	Hippcampus: −0.90 Parietal: −0.09
DeVito et al. [39], 2007	HC: 29 ASD(h): 26	100	HC: 11.1 ASD: 9.8	3.0	L/R frontal lobe, L/R temporal lobe, L/R occipital lobe, L/R cerebellum	Glx	↓ L/R frontal, occipital and cerebellum; others NS	Frontal: 0.21/0.41 Temporal: 0.54/0.04 Occipital: 0.60/0.51 Cerebellum: 0.50/0.55
Hardan et al. [36], 2008	HC: 16 ASD(h): 18	100	HC: 11.9 ASD: 11.6	1.5	L/R thalamus	Glx	NS	0.52/0.03
Harada et al. [10•], 2011	HC: 10 ASD(nr): 12	NR	HC: 5.9 ASD: 5.2	3.0	Frontal lobe and LN	Glu, GABA, GABA/Glu ratio	All measures NS for LN, ↓ frontal GABA, ↓ frontal GABA/Glu	Frontal Glu: −0.42 Frontal GABA: 1.67 LN Glu: −0.42 LN GABA: 0.00
Bejjani et al. [41], 2012[b]	HC: 10 ASD(h): 8	67	HC: 13.2 ASD: 11.2	1.5	ACC	Glx	↑	−1.03
Bejjani et al. [41], 2012[b]	HC: 16 ASD(m): 26	71	HC: 11.8 ASD: 10.2	1.5	L/R ACC	Glx	↑	−0.12/−0.55
Bernardi et al. [37], 2011	HC: 14 ASD(h): 14	86	HC: 29.7 ASD: 29.2	3.0	L/R ACC, thalamus, IPS, TPJ	Glx	↓R ACC; all others NS	ACC: 0.28/1.30 Thalamus: 0.35/0.45 TPJ: 0.26/0.23 IPS: 1.73/0.16
Joshi et al. [35], 2012	HC: 7 ASD(h): 7	100	HC: NR ASD: 14	4.0	Bilateral ACC, L/R hippocampus	Glu	↑ACC, hippocampus NS	ACC: −2.47 Hippocampus: 1.34/1.56
Brown et al. [42], 2013	HC: 15 ASD(h): 13	54	HC: 41.08 ASD: 36.89	3.0	L/R Heschl's gyrus	Glx, Glu	↑ Glu, ↑ Glx	Glx: −1.31/−1.01 Glu: −1.73/−1.02
Horder et al.[40], 2013	HC: 14 nASD(m): 15 bASD(m): 13	88	HC: 29 nASD: 27 bASD: 34	1.5	L basal ganglia, L frontal lobe, and L medial parietal lobe	Glx	↓ basal ganglia; all others NS	Basal Ganglia: 1.60 Frontal: 0.49 Parietal: 0.37
Corrigan et al. [43••], 2013	HC: 10,18,29 DD: 13,14,12 ASD(m): 45,31,29[c]	84/69/84	3–4 years, 6–7 years, 9–10 years	1.5	Gray matter and white matter	Glx	↓in white matter, 3- to 4-year age range only; all others NS	GM 3–4 years: 0.42 GM 6–7 years: 0.64 GM 9–10 years: 0.47
Hassan et al. [31••], 2013	HC: 10 ASD(nr): 10	55	HC: 11.3 ASD: 11.4	1.5	Bilateral ACC, L cerebellum, L striatum, L frontal lobe	Glu	↑ all regions tested	ACC: −1.83 Striatum: −1.93 Cerebellum: −2.28 Frontal: −1.64
Doyle-Thomas et al. [32], 2014	HC: 16 ASD(h): 20	64	HC: 12.9 ASD: 11.5	3.0	Caudate, putamen, thalamus	Glx	↑putamen; all other NS	NA
Van Elst et al. [38], 2014	HC: 29 ASD(h): 29	65	HC: 35.79 ASD: 35.31	3.0	ACC, L cerebellum	Glx, Glu, Gln	Glu and Glx: ↓ACC; all others NS	ACC Glx: 0.90 Cerebellum Glx: 0.35 ACC Glu: 0.82 Cerebellum Glu: 0.25

Table 1 (continued)

Study, year	*N*	Overall percentage male	Mean ages	Magnet field strength (T)	ROI	Measure	ASD finding relative to comparison group	Effect size
Rojas et al. [45•], 2014	HC: 17 ASD(h): 17	65	HC: 12.44 ASD: 14.01	3.0	L auditory cortex	GABA/Cr	↓	0.82
Gaetz et al. [44•], 2014	HC: 15/11/10 ASD(mr): 17/13/8[d]	78/79/88	HC: 12.7/ 11.1/13.3 ASD: 11.5/12.2/13	3.0	L auditory cortex, L motor cortex, L+R visual cortex	GABA/Cr	↓in auditory and motor; visual NS	Auditory: 1.78 Motor: 0.88 Visual: NA

HC healthy control, *ASD* autism spectrum disorder, *nASD* narrowly defined ASD, *bASD* broadly defined ASD, *DD* developmental disorder, *ASD(h)* high-functioning autism based on IQ, *ASD(m)* mixed higher and lower functioning based on IQ, *ASD(nr)* IQ not reported. *L* left, *R* right, *L/R* both left and right ROI; *L+R* midline ROI, *Glx* glutamate+glutamine, *Glu* glutamate, *ACC* anterior cingulate cortex, *IPS* intraparietal sulcus, *TPJ* temporoparietal junction, *NS* non-significant, *LN* lenticular nucleus, *ROI* region of interest, ↓ indicates decrease in autism group compared with controls, ↑ indicates increase in autism group compared with controls, *1 H-MRS* proton magnetic resonance spectroscopy, *GABA* γ-aminobutyric acid, *Cr* creatine, *NR* not reported, *GM* grey matter, *NA* not available

[a] First 'N/% male' is for the hippocampal voxel, while the second 'N/% male' is for the parietal cortex

[b] Studies by Bejjani et al. are described in a single published paper but are separate samples

[c] Data correspond to *N* and age group for the three age groups included in the study

[d] Data correspond to auditory, motor, and visual voxel *N* for the study

The word glutamate is used generally to refer to any of these measurements. Table 1 summarizes the metrics, voxel locations, and other key aspects for each study published on glutamate spectroscopy in autism to date. The results of these studies are discussed, by region of analysis, below.

Subcortical Regions: Basal Ganglia, Thalamus, and Hippocampus

Four studies have been published pertaining to glutamate measures in basal ganglia regions in autism. Two studies of school-age children observed significantly higher striatal Glu [31••] or striatal Glx levels [5, 32]; one study of 12 young children with autism reported no significant differences in Glu for a voxel encompassing the left putamen and globus pallidus [10•]; and one study of 28 adults with ASD also observed no significant differences in Glx obtained from the left caudate and putamen compared with control subjects [33]. Typical of the current state of autism ^{1}H-MRS studies of glutamate, the four studies differed in subject ages (younger children, older children, and adults), location specificity of the ROIs (caudate, putamen and/or globus pallidus), and glutamate measure (Glu or Glx), making generalization across studies difficult.

For hippocampal ROIs, Page et al. [34] found higher Glx concentrations in the right hippocampus in adults with autism (N= 20) relative to comparison subjects (N= 13). However, a more recent study by Joshi et al. [35] encompassing a small number of subjects did not report significant differences in glutamate in medial-temporal lobe voxels, including the left and right hippocampus in adolescents with autism (N= 7) and controls (N= 7).

Three studies have examined thalamic glutamate, all of which have reported no significant group differences. Hardan et al. [14–16, 36] reported no difference in left or right thalamic Glx levels between 18 pre-adolescents with autism and 16 control subjects. Doyle-Thomas et al. [32] also recently reported no significant differences in Glx in 20 children and adolescents with autism compared with 16 typically-developing children. Finally, a study of adults with autism (N= 14) observed no significant differences relative to controls (N= 14) [37].

Cerebellum

Hassan et al. [31••] reported significantly higher Glu levels in the left cerebellum in children with autism (N= 10) compared with age- and gender-matched controls (N= 10). In that study, only the left side of the cerebellum was examined. Glu concentration was also positively correlated with levels of Glu determined by blood draw. However, a recent larger study

reported no significant differences in left cerebellar Glx in adults with autism ($N=24$) compared with control subjects ($N=24$) [38].

Frontal Lobe

DeVito et al. [39] reported reduced frontal lobe Glx in a chemical shift imaging study comparing autism ($N=26$) and control ($N=29$) groups. However, in two other studies, one involving children with autism [10•] and the other an adult sample [40], no significant differences in glutamate were observed between groups. The autism group in the DeVito et al. study had significantly lower verbal IQ than controls, a factor that was not considered in their group comparisons. In the Harada et al. [10•] study, subjects were much younger than in the Horder et al. [40] study, and slightly younger than those in the DeVito et al. study. A recent study of ten children with autism and ten controls reported increased Glu in the left frontal lobe in autism, a finding that was correlated with increased blood levels of Glu in the same sample [27, 31••].

Anterior Cingulate Cortex

With six published studies, the anterior cingulate cortex (ACC) is the most commonly examined ROI in autism ^{1}H-MRS studies of glutamate. Four studies (two reported within one published paper) have reported significantly increased glutamate concentration in ACC [35, 41]. Joshi et al. [35] reported increased Glu levels in ACC in seven adolescent males with autism, while Bejjani et al. [41] also found increased Glx in the ACC of eight similarly-aged individuals with autism in a single voxel ^{1}H-MRS pilot study. In addition, Bejjani et al. [41] reported results of a larger chemical shift imaging study in adolescents with autism ($N=26$) compared with control subjects ($N=16$), replicating the ACC Glx increase in the autism group. Finally, Hassan et al. [31••] recently reported higher ACC Glu concentration in ten children and adolescents with autism compared with ten typically-developing children.

Two other studies have reported reductions in glutamate in the ACC ROIs. Bernardi et al. [37] observed significantly lower Glx in the right ACC in a sample of 14 adults with autism. Similarly, a recent study with a larger adult sample ($N=29$ each, autism and control) found significantly reduced ACC Glx concentration in autism [38]. For the ACC region, sample age may be a significant predictor of the direction of glutamate changes in autism relative to typically-developing controls. Notably, the ACC is the only region for which glutamate has been examined using postmortem tissue from autism donors. Shimmura et al. [26] reported elevated glutamate and glutamine levels in the ACC of a sample of seven postmortem datasets. Other regions were not examined in this study but the results are supportive of elevated glutamate in the ACC, taken together with the majority of positive ^{1}H-MRS studies in the ACC.

Temporal Lobe

There have been two ^{1}H-MRS studies of temporal lobe cortical glutamate. DeVito et al. [39] used chemical shift imaging and defined a large region including all temporal lobe voxels within one slice, but not encompassing the entire temporal lobe. The authors did not find a significant group difference for Glx when comparing 26 subjects with autism and 29 controls. A recent study by Brown et al. [42] observed bilateral Glx and Glu increases in adults with autism ($N=13$) compared with controls ($N=15$) in an auditory ROI centered on Heschl's gyrus. The two studies differed in a number of respects, including participant age (adults vs. children), use of multi- versus single-voxel techniques, and size of the ROI.

Parietal Lobe

Page et al. [34] reported no significant differences between the control ($N=13$) and autism ($N=20$) groups in right parietal cortex Glx concentration. These authors did not specify the location of their voxel within the parietal lobe but a more recent study by Horder et al. [40] examined Glx in a right mid-parietal location. They studied both narrowly defined (meeting International Classification of Diseases, 10th Revision [ICD-10] and Autism Diagnostic Interview–Revised [ADI-R] criteria) and more broadly defined (did not meet ADI-R criteria but did meet ICD-10 criteria) autism groups. They found both groups ($N=28$) did not differ in parietal lobe Glx concentration compared with a control sample ($N=14$). Bernardi et al. [37] included two parietal ROIs in a study of 14 adults with autism and matched comparison subjects. In the intraparietal sulcus, there were no significant group differences but the autism group had lower Glx concentration in the left temporoparietal junction region.

Occipital Lobe

Only a single study examined glutamate levels in occipital cortex. DeVito et al. [39] reported significantly reduced levels of occipital lobe Glx in adults with autism ($N=26$) compared with controls ($N=29$).

Gray Matter and White Matter Studies of Glutamate

Two studies examined glutamate concentration over a range of gray and white matter voxels using chemical shift imaging methodology [39, 43••]. DeVito et al. [39] examined Glx across a number of ROIs defined within two thick oblique axial slices, including subregions within the frontal, temporal, and occipital lobes, as well as the cerebellum (lobar and cerebellar findings reviewed above). Reporting no differences between left and right hemisphere Glx, DeVito et al. pooled the hemispheres and lobar regions into a larger examination of gray matter versus white matter regions in children and adolescents with autism (N= 26) and matched controls (N= 29). The authors reported significant reductions in Glx concentration across gray matter voxels in autism. A significant diagnosis by tissue type interaction indicated that the findings were specific to gray matter rather than white matter. The more recent of the two studies examining gray and white matter is also the only longitudinal ^{1}H-MRS study of glutamate in autism [43••]. This report examined data from three cohorts (ages 3–4, 6–7 and 9–10 years) in children with autism and developmental delays (DD) compared with cross-sectional samples of typically-developing children within the same age ranges. Glx gray matter concentration did not differ between groups for any of the age ranges examined. However, white matter levels of Glx were significantly lower in the autism group compared with controls in the 3- to 4-year-old age group. In the two older age ranges, there were no differences between controls and autism subjects but the DD group exhibited lower white matter Glx levels than controls at those two age ranges. Glx concentration in gray and white matter was inversely correlated with age, although slopes did not differ between groups for either tissue type. The Corrigan et al. study [43••] is also noteworthy because it has the largest sample size for the autism group (N= 45, 35, and 29 for the three age ranges, respectively) reported in the literature to date.

Glutamate spectroscopy studies to date have not reported consistent findings but there is wide variation in the ROIs and age range of the participants examined. Although there are not yet enough studies to ascertain if there is systematic regional glutamate variation in autism, age appears to play a significant role in glutamate findings and is considered in more detail below for both glutamate and GABA. Next, we consider GABA findings in autism.

^{1}H-MRS Studies of GABA in Autism

Three studies utilized ^{1}H-MRS to investigate GABA concentrations in children with autism [10•, 44•, 45•], all using the MEGA-PRESS sequence J-editing technique for better isolation of the GABA signal from overlapping resonances from other species. These studies have chosen various ROIs (reviewed below) but despite differences in the selected ROIs, they have primarily reported reductions in GABA concentrations in autism relative to control subjects.

Auditory Cortex

Rojas et al. [45•] reported decreased ratios of GABA/Cr in both autism (N= 17) and in the unaffected siblings of autism (N= 14) relative to control subjects (N= 17) in the perisylvian region of the left hemisphere. Similarly, Gaetz et al. [44•] reported reduced GABA/Cr ratios in autism (N= 13) relative to control subjects (N= 11) in the left hemispheric superior temporal gyrus. Gaetz et al. also investigated GABA concentrations in motor and visual ROIs, with the most significant reductions found in the auditory cortex suggesting that this region is particularly impacted by abnormalities in GABA neurotransmission. Furthermore, findings of diminished GABA in the unaffected siblings of children with autism are of particular interest since they suggest that reductions in neural GABA concentrations may be an endophenotype of autism.

Visual Cortex

In addition to diminished ratios of GABA to Cr in the auditory cortex, Gaetz et al. [44•] examined GABA/Cr ratios in the visual cortex in autism (N= 8) and controls (N= 10), and observed no group differences in this region. Given the small sample size in this portion of the study, these results should be considered preliminary. However, they may suggest a degree of cortical specificity to deficient GABA signaling in autism, given the significant findings from the same study for the auditory and motor cortices.

Frontal Lobe

The first ^{1}H-MRS study to examine GABA concentration in autism included a left hemisphere frontal ROI placed above the body of the lateral ventricles with the posterior margin ahead of the central sulcus. In this study, Harada et al. [10•] observed reduced GABA concentration as well as diminished ratios of GABA to N-acetylaspartate (NAA) and GABA to glutamate in autism (N= 12) relative to controls (N= 10). This study did not examine GABA/Cr ratios, making direct comparisons to the other two studies more difficult. It also highlights the complexity of direct comparisons between ^{1}H-MRS studies that report different ratios and/or absolute concentration of metabolites. Gaetz et al. [44•] also investigated GABA/Cr ratios in a motor cortex ROI that included the left central

sulcus, posterior to the Harada et al. voxel. They reported reduced GABA concentrations in autism (N= 17) relative to controls (N= 15) from this ROI.

Lenticular Nucleus/Basal Ganglia

Harada et al. [10•] examined GABA concentrations in the left lenticular nucleus in the same group of autism (N= 12) and control (N= 10) subjects and reported no significant group differences in GABA concentrations within this ROI. In addition to the relative small sample size, a limiting factor in this study was that the majority of subjects (ten autism, nine controls) were sedated with triclofos, a GABA agonist that might complicate the results. In the two other GABA studies [44•, 45•], the majority of subjects were unmedicated, with the exception of selective serotonin reuptake inhibitors (SSRIs) in both studies (four subjects in the study by Rojas et al. [45•] and two subjects in the study by Gaetz et al. [44•]), one subject in each study on atypical antipsychotic medications, and one participant taking an unspecified medication to treat mood disorder in the study by Gaetz et al. While medication use is a potential confound in many studies of autism, including ^{1}H-MRS studies, there is evidence that SSRIs may increase GABA concentration, inconsistent with the observed diminished GABA [46, 47]. Replication in unmedicated samples, as well as studies investigating the impact of medication use on GABA levels specifically in autism samples, are areas warranting further research.

Serum Studies of Glutamate and GABA in Autism

Glutamate and GABA concentration may be obtained relatively non-invasively from blood draws, and there have been several studies of this type of measurement in autism. Results from these studies may provide converging validation from another measurement approach. Although the intent of this review is to summarize evidence from ^{1}H-MRS studies, we provide a brief commentary here and refer interested readers to other reviews for more in-depth discussion [48, 49].

Plasma and serum studies of glutamate levels suggest a more consistent picture than ^{1}H-MRS studies. Most studies of plasma or serum glutamate levels report significant increases in autism [31••, 50–53], while, to our knowledge, only a single paper has reported a negative finding [54]. The only study to combine both ^{1}H-MRS measurement of glutamate and blood measurements reported both measures were higher in autism and were highly correlated [31••].

For GABA, the blood plasma picture is murkier. Dhossche et al. [55] reported higher GABA levels in plasma in autism subjects, but decreased platelet levels of GABA have also been reported [54]. Amino acid neurotransmitters do not easily cross the blood-brain barrier, therefore it is difficult to know precisely how peripheral blood markers such as these relate to brain levels, if at all.

Age Effects on Glutamate and GABA

As mentioned, sample ages appeared to exert some influence on the direction of glutamate findings for at least some structures. To gain a preliminary sense for whether this is a systematic effect in glutamate spectroscopy studies in autism, we examined this further by regressing the effect-size measure (Cohen's d) against sample age, taking all studied structures and treating multiple structures within a study as a separate study (Fig. 1). Because measures were variable (Glx, Glu), we combined all measures in the analysis using the standardized effect size (Cohen's d) between the autism and control groups. Overall, there was no effect of age, taking into consideration all studies [$r(43)=0.12$, $R^2=0.01$, $p>0.05$]. However, we observed that between the mean sample ages of 15 and 25 years there have been no published glutamate data (see Table 1). Therefore, we computed a separate regression for the older samples and found a significant effect of sample age on Cohen's d [$r(17)=-0.69$, $R^2=0.47$, $p<0.003$]. In younger adults, the group effect appears to favor higher glutamate concentration in the control group, while for older adults, the reverse appears to be true. However, the findings for the ACC, as reviewed above, appear to be the reverse of this overall age relationship, showing a positive relationship between effect size and age [$r(8)=0.75$, $R^2=0.56$, $p<0.04$].

Age-related changes have been reported previously, with two adult studies reporting decreased glutamate concentration with age in samples over 20 years of age [56, 57]. Among the studies publishing data on glutamate, a significant age-associated increase in concentration was reported in adolescence and early adulthood in a cross-sectional study; however, in that study, Glx was normalized to Cr, and separate absolute concentration data were not reported [32]. In the only longitudinal study of Glx and autism, Corrigan et al. [43••] observed age-related reductions in absolute Glx concentration across the ages of 3–10 years, with no significant differences in slope between the autism and control groups.

In contrast to the glutamate data, which have been obtained in a larger number of studies across a wider age range, the three GABA studies have been conducted in child samples only. We observed a potential age-related trend in the GABA data, such that effect sizes tended to be positive with higher ages, indicating lower GABA in the autism group relative to controls (Fig. 2; $R^2=0.71$). We did not assess that regression for significance due to the low number of study points (N= 5), even collapsing across all studied regions.

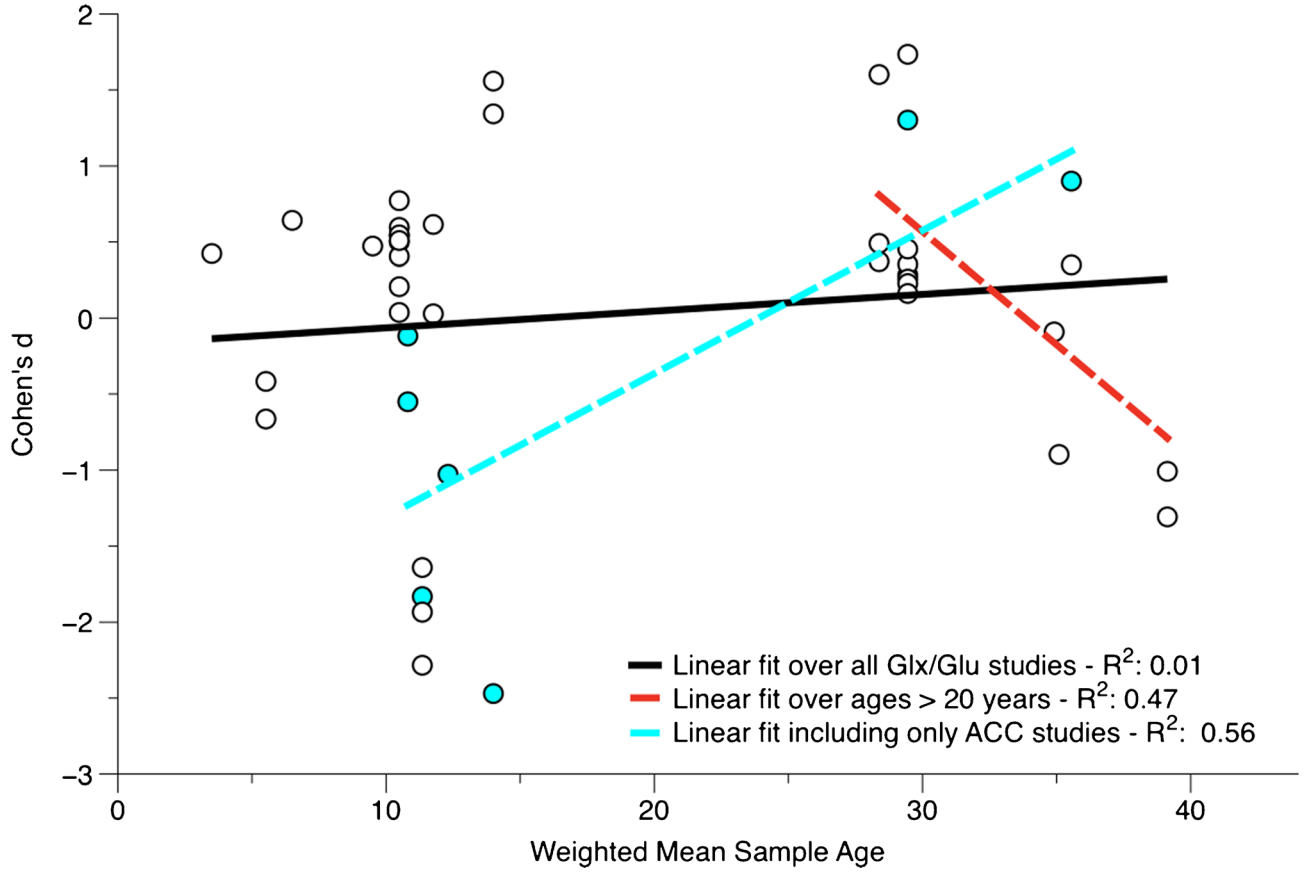

Fig. 1 Glutamate study effect sizes by sample age. Effect size (Cohen's d) was calculated by subtraction of the autism group from the control group, so positive effect sizes indicate higher concentration in controls, while negative effect sizes indicate higher concentration in autism subjects. *Cyan-filled circles* indicate studies of the ACC region. Linear regression (best-fit line and R^2) results from age as a predictor of Glx/Glu effect size are shown for all studies (*solid black line*), ACC region studies only (*dashed cyan line*) and studies of adults over 20 years of age (*dashed red line*). *ACC* anterior cingulate cortex, *Glx* glutamate and glutamine, *Glu* glutamate

Summary and Limitations

Across all studies reporting Glu or Glx measures in autism, seven reported increases in at least one structure, five reported decreases in at least one structure, and one found no significant group difference in all structures. No studies observed significant differences in both directions (increased and decreased glutamate) among different structures assessed. Therefore, for glutamate there is not compelling evidence in support of either increased or decreased concentration levels in autism. However, participant age may play a significant role in moderating the effect of diagnosis on glutamate. In the ACC in particular, we noted that older groups with autism tended towards increased glutamate levels, while younger samples tended to have reduced glutamate or no changes. There may also be regional effects and region by age interactions, although, with the exception of the ACC, there have been very few studies with similar ROI definitions to assess these relationships directly. The evidence for increased glutamate levels is strongest for the ACC region, the most studied region in autism Glu/Glx studies. Generally, most studies report only Glu or Glx rather than both, complicating any formal attempt to meta-analyze the glutamate literature for regional or other effects.

There are fewer published studies of autism reporting GABA concentration. However, unlike glutamate, the methods are somewhat more consistent in that all three studies used 3 T magnets and employed the MEGA-PRESS spectral editing technique. Two of the three studies reported GABA/Cr ratios [44•, 45•]. Ages were also less variable than in glutamate studies. All three studies reported GABA/Cr concentration reduction in autism [10•, 44•, 45•], although in one of the studies, one region—the visual cortex—did not exhibit any significant difference between groups [44•].

Overall, although it may be tempting to speculate on changes in EI imbalance in autism based on ^{1}H-MRS data, it is clear that the literature is far from mature. Taking evidence from multiple techniques (e.g. glutamate concentration from serum, GABA receptor binding in positron emission tomography [PET] studies, etc.), together with the ^{1}H-MRS literature, there appears to be considerable support for the EI

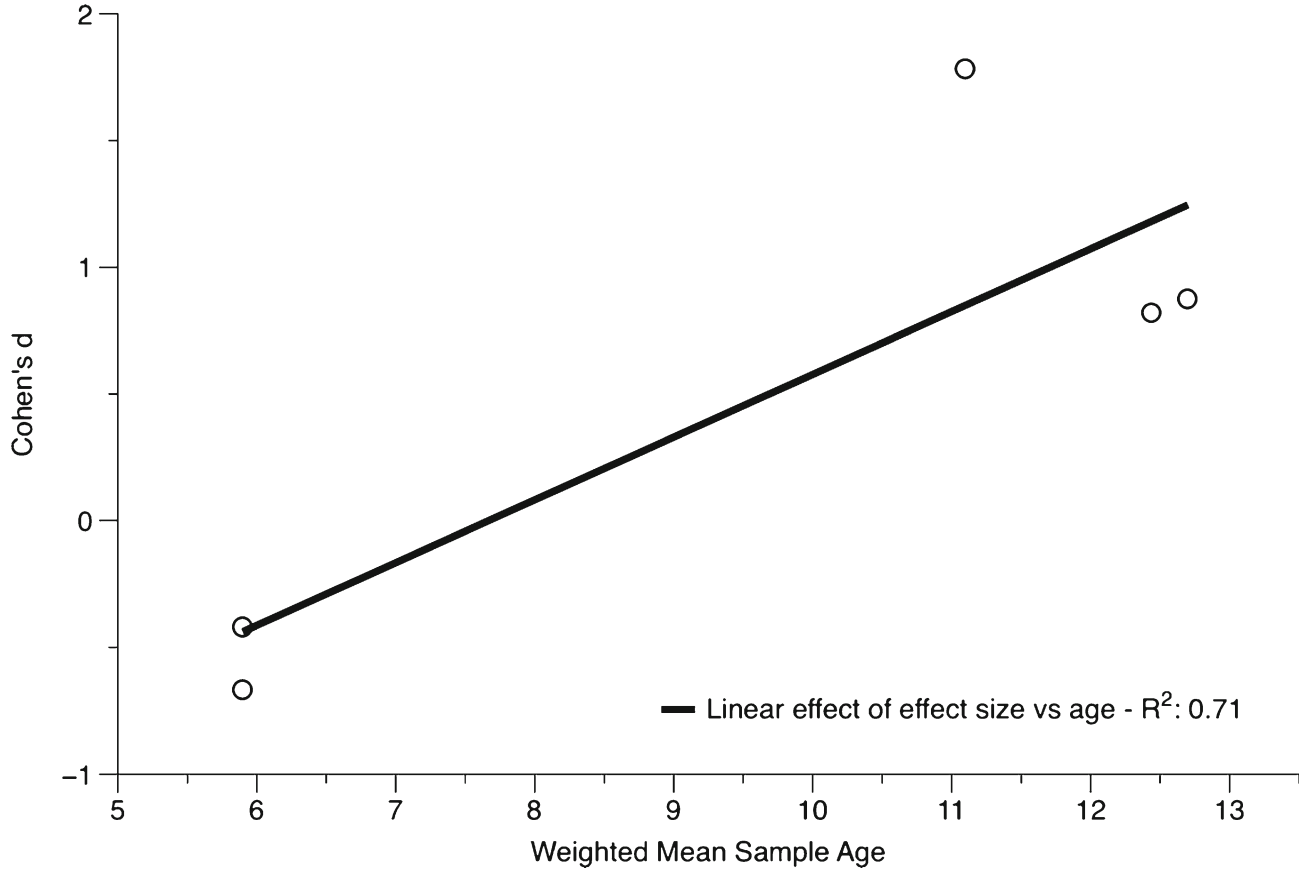

Fig. 2 GABA study effect sizes by sample age. Effect sizes calculated as in Fig. 1 are shown for GABA concentration, with the best-fitting regression line for age as a predictor of Cohen's d. *GABA* γ-aminobutyric acid

imbalance construct. However, currently, ^{1}H-MRS data do not clearly support EI dysfunction in autism.

^{1}H-MRS studies have significant limitations, as noted in the Introduction section. The technique typically requires long acquisition times, large voxels, and in single-voxel spectroscopy is normally limited to a few ROIs. Perhaps due in part to these factors, sample size for the reported studies has tended to be quite low. For example, 11 of the 13 published studies of glutamate and GABA have reported sample sizes less than $N = 20$ for their autism groups.

Another limitation on ^{1}H-MRS studies is that, to optimize signal-to-noise ratios, most studies assessing glutamate and all GABA studies opt for single-voxel acquisition methods using large ROIs. Technology developments such as moving to higher field magnets (e.g. 4 and 7 T systems) may help with the implementation of multi-voxel methods so that regional differences in amino acid neurotransmitter concentrations can be properly evaluated. Such developments should also tend to minimize differences between studies in how ROIs are defined. Although we considered ROIs within a given region (e.g. frontal lobe) to be largely equivalent in terms of this review, there are differences between studies in how the single voxels were placed or defined within the muli-voxel acquisition.

A further research gap that will need to be addressed is the examination of neural metabolite concentrations in more homogeneous subgroups within the spectrum. The ASDs represent a heterogeneous group of neurodevelopmental disorders. This heterogeneity and the complexity of the behavioral phenotype in autism is a significant challenge to research that has hampered our understanding of the disorder by exacerbating inconsistencies due to the inclusion of disparate populations both within and across studies. It may be that the inconsistent ^{1}H-MRS glutamate findings in autism have been particularly impacted by the inclusion of disparate populations within the samples.

Spectroscopic imaging has also been used to assess changes in glutamate and GABA concentration in patients with conditions associated with autism. These include studies reporting increases in glutamate in epilepsy, major depressive disorder, obsessive-compulsive disorder, and social anxiety disorder [58–61]. Similarly, reductions have also been reported for GABA in depression and attention-deficit hyperactivity disorder [62, 63]. Drugs used to treat depression, such as SSRI medications and antiepileptic drugs, have also been shown to increase GABA concentrations [47, 64]. Further characterization of symptom history dimensions in autism patients, as well as exclusion or analysis by medications often encountered in the disorder, is therefore also recommended.

Future Directions

There are quite a few studies employing ^{1}H-MRS techniques in autism that do not report Glx or Glu levels. As Aoki et al. [29••] indicate, their meta-analysis excluded glutamate due to the low number of studies; this is most likely attributable to the large number of studies at lower field strengths (e.g. 1.5 T). Future studies should therefore be conducted on 3 T and higher magnets. Such studies would also be able to separate the Glx signal into Glu and Gln for separate quantification. Together with techniques such as MEGA-PRESS, there should be an opportunity over the next several years to obtain simultaneous concentration data for Glu, Gln, and GABA.

The ^{1}H-MRS literature on glutamate does not clearly support an increase in its concentration, except perhaps in the ACC region. However, consistent with the EI hypothesis, studies of GABA concentration reduction in autism do support reduced inhibition. However, it must be kept in mind that the total number of published ^{1}H-MRS datasets in autism across those three published studies is from 46 participants with autism. More studies, particularly with large samples, and a longitudinal focus, will be needed before ^{1}H-MRS studies can contribute substantially to the EI hypothesis debate.

Compliance with Ethics Guidelines

Conflict of Interest Donald C. Rojas, Katherine M. Becker, and Lisa B. Wilson declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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