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Decreased left perisylvian GABA concentration in children with autism and unaffected siblings

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Abstract

Imbalanced levels of excitation and inhibition (E/I) have been proposed to account for various behavioral and electrophysiological phenotypes in autism. Although proton magnetic resonance spectroscopy (¹H-MRS) studies have been published on various metabolite levels in autism, including glutamate, the major excitatory neurotransmitter, few ¹H-MRS studies have yet been conducted the major inhibitory neurotransmitter GABA.

Seventeen individuals with autism spectrum disorders (ASD) participated in a single-voxel, point resolved spectroscopy (PRESS) study conducted on a 3T magnet. Data were also acquired on 14 unaffected siblings of children with autism, and 17 age- and gender- matched healthy control subjects. GABA concentration was measured along with Creatine (Cr) in a single voxel aligned with the auditory cortex in the perisylvian region of the left hemisphere.

The ratio of GABA to Cr was significantly lower in the ASD group than the control subjects. Siblings also exhibited lower GABA/Cr ratios compared to controls. Cr concentration did not differ between groups. The volumes of gray matter, white matter and CSF did not differ between groups in the whole brain or within the spectroscopy voxel.

Reduced auditory GABA concentration in ASD is consistent with one previous MRS study of GABA concentration in the frontal lobe in autism, suggesting that multiple neocortical areas may be involved. Lower GABA levels are consistent with theories of ASD as a disorder involving impaired inhibitory neurotransmission and E/I imbalance. The reduction in unaffected siblings suggests that it may be a heritable biomarker, or endophenotype, of autism.

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Keywords

GABA; creatine; spectroscopy; j-editing; MEGA-PRESS; auditory cortex

Introduction

Autism spectrum disorders (ASDs), which are characterized by impairments in social interaction, communication, and restricted/stereotyped behaviors, are relatively common, with population prevalence around 1 percent (Kogan et al., 2009). While medical conditions with known etiology account for up to 10 percent of cases (e.g., Fragile X syndrome, Tuberous Sclerosis), most cases do not have clear origins (Kielinen et al., 2004; Schaefer and Lutz, 2006).

Gamma-amino-butyric-acid (GABA), the major inhibitory transmitter in the CNS, has been implicated in the pathophysiology of autism (Coghlan et al., 2012). Evidence for GABAergic inhibitory problems in ASD converges from a variety of scientific disciplines and has also been of interest for some time (e.g., Hussman, 2001). Previous studies have reported reduced GABAA-receptor binding in hippocampus, neocortex and cerebellum (Blatt et al., 2001; Fatemi et al., 2009). Reduced protein levels of several GABA receptor subunits have also been reported in frontal cortex in ASD (Fatemi et al., 2009).

Genetic evidence also implicates GABA. GABA receptor genes, most notably GABRB3, have been of significant interest in autism (e.g., Ma et al. (2005)). Cook et al. (1998) reported linkage disequilibrium between autism and a marker for GABRB3 in the 15q11-13 chromosome region, a result replicated by some studies (e.g., Buxbaum et al., 2002), but not by others (e.g., Salmon et al., 1999). Other GABA receptor subunit genes have also been identified as potential candidates for autism-related pathology (Ma et al., 2005). Partial duplication of 15q is observed in a number of cases of ASD, a region including several GABA genes (e.g., GABRB3: Buxbaum et al., 2002). Messenger RNA levels of glutamate decarboxylase (GAD), the enzyme that converts glutamate to GABA and is highly related to intraneuronal GABA, have been reported to be reduced by about 40% in cerebellar Purkinje cells in persons with autism (Yip et al., 2007) and up to 50% in parietal and/or cerebellar tissues (Fatemi et al., 2002). A region on chromosome-2 encodes GAD67, with significant linkage reported in two separate autism studies (International Molecular Genetic Study of Autism, 2001; Martin et al., 2000).

In-vivo GABA concentration measurement in human subjects is possible using ¹H-MRS methods, although to date there has only been a single study in ASD (Harada et al., 2010). This is likely due to the challenge inherent in spectral editing techniques necessitated by overlap of the GABA resonances with other metabolites in unedited spectra (Puts and Edden, 2012). Harada et al. (2010) reported reduced GABA concentration in the frontal lobe, but not striatum, in ASD. Some of the subjects in that study, however, were sedated with triclofos, a GABA agonist, which might complicate interpretation of the results. Although elevated, rather than reduced, plasma GABA levels have been found previously in ASD (Dhossche et al., 2002), it is unclear if there is a straightforward relationship between plasma and CNS levels of GABA, because GABA does not cross the blood-brain barrier.

The current study was designed to explore the hypothesis that decreased GABA concentration would be found in the auditory cortex and surrounding left perisylvian region in persons with ASD. Based in part on our own prior work, the auditory cortex is a location that exhibits electrophysiological deficits in gamma-band oscillations in ASD (Gandal et al., 2010; Rojas et al., 2008; Rojas et al., 2011; Wilson et al., 2007), which have been closely linked to GABAergic mechanisms and inhibitory interneurons (Bartos et al., 2007; Brunel

and Wang, 2003). In addition, structural abnormalities of the perisylvian region have been noted in ASD, including alteration in the normal asymmetry of the planum temporale (Herbert et al., 2002; Rojas et al., 2005) and pars opercularis (Herbert et al., 2002). Functional MRI studies have indicated anomalous activation of Broca's area and reductions in left-right asymmetry in frontal language regions (Knaus et al., 2010; Knaus et al., 2008). We also predicted that GABA concentration would be lower in siblings of persons with ASD, based on evidence that auditory gamma-band abnormalities are also present in first-degree relatives of individuals with ASD (Rojas et al., 2008; Rojas et al., 2011) and the high heritability of autism (Bailey et al., 1995).

Material and Methods

Participants

A total of 48 subjects underwent un-sedated MRI scans in this study. Seventeen individuals with ASD participated who met DSM-IV clinical criteria for ASD (Autistic Disorder, N = 9, Asperger's Disorder, N = 7 and PDD-NOS, N = 1), as applied by an experienced clinical psychologist (SH). In addition, ASD participants also met criteria on the Autism Diagnostic Observation Schedule (Lord et al., 2000), and either the Autism Diagnostic Interview, Revised (ADI-R: Lord et al., 1994) or the Social Communication Questionnaire (SCQ: Rutter et al., 2003). Twelve of the ASD subjects were unmedicated at the time of the MRI scan and 5 were taking medications (N = 4 on selective serotonin reuptake inhibitors (SSRIs), N = 1 on atypical antipsychotic medications).

Fourteen unaffected siblings (SIB) of persons with ASD also participated. SIB participants had one affected proband meeting the same criteria for ASD as discussed above. A third group of 17 healthy typically developing controls (TD) was included. TD subjects had no family (1st degree relatives) or personal history of neurodevelopmental disorder including ASD. All participants in the TD group were medication-free at the time of the scan.

Participants in all 3 groups had full scale IQs of 80 or higher on the Wechsler Abbreviated Scale of Intelligence (WASI: (Psychological Corporation, 1999)). Table 1 provides additional details concerning the sample. Informed consent was obtained to participate in the experiment, consistent with the local Institutional Review Board and Declaration of Helsinki. For those participants too young to consent, a process of assent was applied, along with consent from a legal guardian.

Behavioral measures

In addition to IQ assessment and diagnosis, several measures related to aspects of the autism phenotype and/or relevant to the auditory region assessed were administered. These included the Social Responsiveness Scale (SRS: Constantino and Todd, 2005) is an informant-based (spouse/partner/parent) measure of reciprocal social behaviors, with higher scores, especially above 80, highly indicative of serious social impairment. The Broad Autism Phenotype Questionnaire (BAPQ: Hurley et al., 2007) is also an informant based measure designed to tap into broader traits associated with autism and also found in unaffected first-degree relatives and includes subscales for pragmatic language ability, aloof personality and rigid personality traits. To assess gross receptive and expressive language skill, the Peabody Picture Vocabulary Test (PPVT: Dunn, 1997) and the Expressive Vocabulary Test (EVT: Williams, 1997) were given.

Structural MRI and 1H-MRS

MR spectroscopic data were acquired using a 3.0T GE Signa HDx long-bore MR scanner (General Electric Healthcare, WI) and GE 8-channel phased-array head coil. Subjects

watched a movie during the exams using MR compatible goggles and headphones (Resonance Technology Inc., Northridge, CA) to aid in compliance and minimize subject motion. A T1-weighted sequence was acquired for tissue segmentation using a 3D inversion recovery fast, spoiled gradient echo (IR-SPGR) technique (matrix=256², FOV 22 cm, TR/TE/TI= 10/3/450 ms, NEX=1), resulting in 138, 1.2 mm thick axial slices with an in-plane resolution of .86 mm². The imaging protocol for localization of the spectroscopy voxels included an initial 3-plane scout, followed by a sagittal T2-weighted FSE (FOV 22 cm, TE/TR = 95/5000 ms, echo train length (ETL)= 20, slice thickness/gap = 3/0 mm, ~20 slices, matrix=512 × 256, NEX=1, flow compensation (slice), time = 45 s). This series was used to prescribe the spectroscopy voxel, such that the spectroscopy voxel was centered on the auditory cortex from left-to-right, defined operationally as the first-transverse temporal gyrus (Heschl's gyrus). Criteria for determination of Heschl's gyrus have been described previously (Rojas et al., 1997). The voxel was placed so that the posterior portion encompassed Heschl's gyrus's posterior boundary. The longer dimension of the voxel was oriented anteriorly along the perisylvian fissure and included portions of insula, parietal and frontal operculum. The voxel placement for a typical subject is shown in Figure 1.

The GABA acquisitions were performed using a “J-editing” technique implemented by in-house modification of GE's PROBE-P sequence (presscsi) with MEGA suppression (MEGAPRESS) by the addition of two spectrally selective 180 degree Gaussian pulses of 16 ms duration, centered at 1.9 ppm (Mescher et al., 1998). The J-difference method requires two acquisitions, one with the J-editing pulses on and one with the editing pulses off, with the GABA spectrum obtained by taking the difference between the two acquisitions. The sequence was written to interleave frames of data acquired with the editing pulses on and off, rather than as completely separate acquisitions, to minimize misregistration between the two acquisitions. The “edit-off” acquisition was done by centering the editing pulses at 7.5 ppm (symmetrically on the other side of the water resonance) to avoid differences in baseline artifacts rather than completely turning the editing pulses off (Bogner et al., 2010). No eddy current or artifact differences were noted between the edit-on and edit-off data. Acquisition parameters used were TR/TE= 2500/70 ms, 512 total averages (256 edit-on and 256 edit-off, voxel size ~ 30mm × 30mm × 40mm, time = 1300 s).

GE's SAGE spectroscopy processing software (off-line version DEV2005.2) was used for processing of the MEGAPRESS acquisitions. The processing steps involved included separation of the two sets of data frames, and then using the PROBE SVQ recon in SAGE, which utilizes the following steps: 1) the residual water signal in each frame is used to correct for phase, frequency, and residual eddy currents (internal water referencing); 2) a high pass filter is applied (bandwidth=20 Hz); 3) application of a 2.0 Hz Gaussian line broadening filter; 4) zero-filling once; 5) Fourier transformation into frequency-domain edit-on and edit-off spectra; 6) baseline correction using a cubic spline algorithm and the first and last points of the frequency spectrum. The edit-on spectrum is subtracted from the edit-off spectrum to produce the GABA (difference) spectra. No attempts were made to correct for co-edited macromolecular resonances, and as such we refer to the GABA signals as GABA+. The areas under the peaks in both the edit-off and difference spectra were determined using a Levenberg-Marquardt least-squares algorithm. The starting points for the fits were determined using the SAGE peak picking routine. The fits were done assuming Gaussian lineshapes, and with frequency, linewidth and amplitude as the fitted parameters. The “true” lineshape of the edited GABA+ is often referred to as a “pseudo doublet” or “pseudo-triplet”, and as such the experimental lineshape is usually poorly fit using a single Gaussian line. For this reason the GABA+ signals at ~ 3.02 ppm in the difference spectrum were fit using three Gaussians. Over-parameterization of each fit was checked by using a correlation matrix (obtained by inversion of the covariance matrix generated by the Marquardt-Levinson least-squares fit) to check that correlations between parameters were

less than 0.5. All fits were visually inspected for deviations by generating a FID (assuming Gaussian lines) using the fitted values followed by Fourier transformation to produce a synthesized spectrum. This generated spectrum was then overlaid on the experimental spectrum to check for inaccuracies and errors in the fits. This was done for both the edit-off and difference spectra. Finally, the sum of the three fitted line areas for the GABA+ signals were divided by the fitted creatine (Cr) peak area at ~ 3.07 ppm to yield a GABA+ to Cr ratio (GABA+/Cr). The GABA+ signals were also quantitated using an integration routine in SAGE, which was used as a further check on the fitted values. Signal-to-noise ratios (SNR) and linewidths from LCModel were used as a check in statistical analyses to assure that data quality did not differ between groups. Examples of the MRS spectra are shown in Figure 2.

T1-weighted scans were processed using the VBM8 toolbox (<http://dbm.neuro.uni-jena.de/vbm/>) within SPM8 (<http://www.fil.ion.ucl.ac.uk/spm/>). Total grey matter, white matter and cerebro-spinal fluid (CSF) volumes were calculated, as well as the volumes for each within the spectroscopy voxel of interest. The separate SPM8 tissue probability maps for gray matter, white matter and CSF had a >.5 probability threshold applied and the number of voxels above that threshold in each ROI in each image were summed to produce a volume for the voxel within native space.

Results

Demographics and Behavioral data

One-way ANOVAs was used to examine demographic variables for significant differences. There were no significant differences in age between groups, $F(2,45) = .76$, $p = .48$. Age has previously been found to correlate with some MRS metabolites in some published studies, so we computed Pearson r correlation coefficients between the 2 MRS variables and age. Neither was significantly correlated with age (GABA+/Cr: $r(48) = .06$, $p = .69$; Cr: $r(48) = -.05$, $p = .71$).

A chi-square test was used to assess gender differences between groups. There were significantly more males in the ASD group than the other two groups, $\chi^2(2) = 6.30$, $p < .05$. There were not sufficient numbers of each gender to evaluate its impact on GABA+/Cr and Cr as a separate factor within ANOVA, so we analyzed gender collapsed across group separately as a potential confound in an independent Student's t -test (see below).

SRS scores were significantly different between groups, $F(2, 45) = 75.70$, $p < .001$. Post-hoc testing revealed that the ASD group had higher SRS than the TD ($p < .001$) and sibling groups ($p < .001$). The sibling group trended toward higher scores compared to TD ($p = .08$). The BAPQ total score was also significant, $F(2, 45) = 52.58$, $p < .001$, with post-hoc comparisons revealing significant elevation in the ASD group relative to both the TD ($p < .001$) and sibling group ($p < .001$). No other differences on the BAPQ total score were significant. On BAPQ subscales, the rigidity subscale was also significant, $F(2,45) = 32.50$, $p < .001$, with post-hoc tests revealing significantly higher scores for ASD compared to TD ($p < .001$) and siblings ($p < .001$). The aloof subscale was significantly different, $F(2,45) = 48.44$, $p < .001$ and post-hoc tests indicated significantly higher scores for the ASD group compared to TD ($p < .001$) and SIB ($p < .001$). The TD and SIB groups were not significantly different ($p = .36$). Scores on the pragmatic subscale were also significantly different, $F(2,45) = 36.01$, $p < .001$, with post-hoc tests indicating that the ASD group differed from both TD ($p < .001$) and SIB ($p < .001$) groups. The TD and SIB groups did not differ significantly ($p = .15$).

Wechsler full scale IQ scores were not significantly different between groups, $F(2,45)=1.45$, $p=.25$. On the PPVT scaled score, the groups were significantly different, $F(2,45)=3.48$, $p < .04$. Post-hoc testing indicated that the TD group had higher scores than the ASD group ($p = .02$), but did not differ from the SIB group ($p = .73$). The SIB group also had higher scores than the ASD group ($p = .05$). EVT scaled scores did not differ significantly between groups, $F(2,45)=1.53$, $p = .23$. Means and standard deviations for all behavioral data are presented in Table 1.

¹H-MRS

To examine data quality between groups, the linewidths and SNR of the spectra were examined in separate oneway ANOVAs by group. The mean linewidth for the groups were not significantly different, $F(2,45) = .37$, $p > .1$ (means $\pm$ SD; HC: .05 $\pm$.006, SIB: .05 $\pm$.008, ASD: .05 $\pm$.026). There were also no significant differences for SNR between groups, $F(2,45) = 1.39$, $p > .1$ (means $\pm$ SD; HC: 60.17 $\pm$ 1.72, SIB: 58.35 $\pm$ 1.89, ASD: 56.12 $\pm$ 1.72).

For group differences, GABA+/Cr and Cr were entered into separate oneway ANOVAs by group. To examine the potential impact of gender differences in the ASD group as a confound for group analyses on spectroscopy, a Student's t-test was computed between genders for GABA+/Cr and Cr. Neither variable exhibited significant gender differences (GABA+/Cr: $t(46)=1.04$, $p=.31$; Cr: $t(46)=-.56$, $p=.58$). Gender was not given further consideration in any subsequent analyses. Figure 3 illustrates the mean and standard deviation for all spectroscopy measures in each group.

To evaluate the primary hypotheses on GABA+/Cr, we contrast coded the main effect of group into planned comparisons to assess 1) whether the ASD group was significantly lower than the TD group and 2) whether the SIB group was significantly lower than the TD group. A 3rd contrast assessed a secondary concern over whether the ASD and SIB groups differed. A significant contrasted effect of group was significant for GABA+/Cr, $F(2, 45) = 5.35$, $p = .008$. Contrast 1 was significant ($p = .007$), indicating that the ASD group had lower GABA+/Cr ratios than the TD group, Contrast 2 was also significant ($p=.007$), indicating that the sibling group was also significantly lower than the TD group. Contrast 3 was non-significant ($p=.89$) indicating that the SIB and ASD groups were not different from each other.

Structural MRI

There were no significant differences between groups for total grey matter, total white matter, total CSF, or for gray, white or CSF within the voxel of interest (see Table 2 for means, SD and statistics).

Discussion

Our hypotheses of reduced GABA concentration in the auditory cortex of persons with ASD and unaffected siblings of persons with ASD were supported. The lower GABA+/Cr ratio in ASD is partly consistent with one previous study reporting GABA changes in the frontal lobe, but not the lenticular nuclei (Harada et al., 2010). In the Harada et al. study, GABA concentration, in addition to quantification relative to an internal water standard, was also expressed as a ratio to n-acetyl-aspartate (NAA) and also to glutamate, rather than to Cr, as is more common in the MRS literature. NAA, usually interpreted as a proxy neuronal marker, is generally reduced in ASD studies (Chugani et al., 1999; DeVito et al., 2007; Endo et al., 2007; Friedman et al., 2006; Gabis et al., 2008; Hardan et al., 2008; Hisaoka et al., 2001; Levitt et al., 2003; Otsuka et al., 1999). Similarly, glutamate, an excitatory amino acid neurotransmitter, may also be altered in ASD, although fewer studies have been reported

and results mixed, with some reporting increased glutamate (Page et al., 2006) and others reduced glutamate (Bernardi et al., 2011; DeVito et al., 2007). Cr levels as measured with MRS tend to be stable across clinical conditions, which is one reason spectroscopy studies use ratios of metabolites to Cr, such as GABA+/Cr (Soares and Law, 2009). As with both NAA and glutamate, Cr has been reported in some studies of ASD to be altered (Levitt et al., 2003; Page et al., 2006). We did not find significant effects for Cr across groups in the current study, suggesting that the reduction was due to GABA, not Cr elevation, consistent with the internal water GABA concentration finding in the Harada et al. paper. Interpretation of the results can be complicated for ratio measures such as GABA+/Cr, GABA/NAA and GABA/Glutamate without reporting statistical results for the reference, or at least one of the two measures in a ratio, separately. The Harada et al. (2010) study, taken together with our current finding, suggests reduced GABA in ASD subjects in two separate regions of neocortex.

We did not find gender differences in the current sample. Previous studies have been equivocal about gender differences in GABA concentration. O’Gorman et al. (2011) reported that GABA concentrations were higher in males than females in a dorsolateral prefrontal cortex voxel. Another group reported lower GABA concentration in males compared to females in the anterior cingulate cortex (Sheffield and Noseworthy, 2010). One study raised the possibility that a female difference in GABA, if any, is related to the menstrual phase of the participants, suggesting that women may have reduced GABA levels in the follicular phase of the cycle (Epperson et al., 2002). The female participants in this study were significantly younger than in all of these prior studies. Another possibility that concerns this study is that we did not have a sufficient sample size to examine a potential group by gender interaction, so if the gender difference were group specific, this study would have been underpowered to detect that difference.

A potential problem with large voxels employed to increase signal in spectroscopy studies is that the voxels are necessarily comprised of multiple tissue classes, including gray matter, white matter and cerebro-spinal fluid (CSF). GABA concentration varies between tissue classes, being higher in gray than in white matter (Bhattacharyya et al., 2011; Petroff et al., 1988), and group differences in the tissue composition of the voxel could lead to GABA differences driven by the voxel’s relative volume of gray matter. Volumes of gray and especially white matter in ASD may vary from typically developing children, an effect that is most pronounced early in life (Hazlett et al., 2011). We analyzed our T1 data for such effects, both at the whole brain level and within the voxel of interest, and found no differences in gray, white or CSF volume between groups. This suggests that the GABA findings were not simply driven by volumetric differences in tissues with the highest density of GABA within the voxel of interest.

The finding that the SIB group exhibited reduced GABA+/Cr compared to TD suggests that this finding may be a heritable ASD biomarker, or endophenotype (Gottesman and Gould, 2003). Given that ASD is highly heritable as estimated from the behavioral level (Bailey et al., 1995), it is likely that there are many neurobiological level variables that exhibit heritability as well. Phenotypic heterogeneity in ASD is recognized as a both a substantial barrier and an opportunity to progress in identifying key genetic and molecular contributions to the disorder (Abrahams and Geschwind, 2008). It is likely that ASD represents multiple distinct etiologies with some convergence of behavioral phenotype, rather than a single disorder. Approaches to the heterogeneity problem include careful phenotyping as well as the study of endophenotypes, which can also be considered genetic risk factors. Quantitative risk factors provide greater power than categorical variables such as diagnosis in genetics studies (Glahn et al., 2007). There are a number of such potential endophenotypes in ASD (Abramson et al., 1989; Koczat et al., 2002; Mosconi et al., 2010; Rojas et al., 2008; Rojas

et al., 2004). One potential advantage of GABA concentration as such an endophenotype is that it relates to molecular pathways that are already considered to be important factors in ASD (Coghlan et al., 2012).

A caveat in interpretation of the current results is that some participants in the ASD group were medicated, while none of the other participants in the SIB and TD groups were. Antipsychotic medication (N = 1 participant) is not associated with effects on GABA concentration (Goto et al., 2010). SSRI treatment (N = 4 participants), however, is relatively common in ASD and may have an effect on GABA concentration. Two studies have reported the affects of SSRI administration on human GABA concentration in the visual cortex. Bhagwagar et al. (2004) found that acute administration of 10 mg intravenous citalopram in a blinded, placebo-controlled study resulted in a significant increase in occipital GABA+/Cr ratios after 30 minutes in healthy individuals with no history of Axis I mental illness. Sanacora et al. (2002) reported a similar significant increase after a 5-week open-label treatment trial with fluoxetine or citalopram in 11 persons with major depressive disorder. Although it appears that SSRI treatment may increase GABA concentration, we note that ASD group GABA+/Cr ratios were significantly lower compared to TD, not higher (see Figure 3, SSRI subjects marked). Additionally, the presence of the same finding in the SIB group, which was un-medicated, argues against a medication effect driving the result. Nonetheless, as GABA concentration is modulated by SSRIs, replication in a larger un-medicated ASD sample is recommended.

Conclusions

GABA+/Cr ratios were significantly lower in individuals with ASD and in unaffected siblings of persons with ASD, consistent with predictions of impaired inhibitory neurotransmission in the disorder and with the E/I imbalance theory of ASD (Rubenstein and Merzenich, 2003). The presence of the finding in siblings suggests that reduced GABA may be a heritable biomarker. Although none of the participants in the current study had a history of seizure disorder, the prevalence of epilepsy in ASD is as high as 25 percent. Impaired inhibitory neurotransmission is also implicated in epilepsy, and may be a common risk factor for both disorders (Kang and Barnes, 2012).

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Highlights

- GABA concentration was assessed in subjects with autism, unaffected siblings and controls.
- Concentration was assessed in the left perisylvian region using MEGAPRESS.
- The autism and sibling groups exhibited significantly reduced GABA.
- Results are consistent with a heritable deficit in cortical inhibition in autism.

Figure 1.

^1H -MRS voxel placement and segmentation of T1-weighted MRI from single subject. Voxel overlay is shown in red in the top row and segmentation of gray (red), white (blue) and csf (green) are shown in the bottom row.

Figure 2. Example subtraction spectra from MEGAPRESS sequence. One example from each group is shown. TD = Typically Developing, SIB = Sibling, ASD = Autism Spectrum Disorder. The GABA and NAA peaks are labeled.

Figure 3. Mean +/- SEM GABA+/Cr. TD = Typically Developing, SIB = Sibling, ASD = Autism Spectrum Disorder. Circles indicate data points for subjects in ASD group taking SSRI medications at time of scan.

Table 1

Sample Characteristics

	TD (N=17)	SIB (N=14)	ASD (N=17)
Age (y)	12.44 (5.20)	11.70 (5.94)	14.01 (5.18)
Gender (M/F) *	8/9	6/8	14/3
FSIQ	113.06 (12.12)	109.83 (11.26)	105.13 (15.46)
PPVT*	118.76 (14.10)	116.43 (11.80)	102.94 (25.55)
EVT	105.59 (11.07)	106.64 (15.47)	96.19 (25.68)
SRS *	14.35 (10.82)	26.57 (21.70)	91.31 (23.09)
BAPQ total *	2.05 (.46)	2.33 (.65)	3.92 (.55)
BAPQ rigid *	2.34 (.67)	2.63 (.71)	4.07 (.58)
BAPQ aloof*	1.85 (.37)	2.05 (.79)	3.74 (.60)
BAPQ pragmatic *	1.95 (.54)	2.31 (.78)	3.92 (.79)

Numbers in parentheses are standard deviations. * $p < .05$.

FSIQ = Full scale IQ. SRS = Social Responsiveness Scale. BAPQ = Broad Autism Phenotype Questionnaire.

* $p < .05$, see Results.

Table 2**Whole Brain and Voxel Tissue Composition**

	TD (N=17)	SIB (N=14)	ASD (N=17)	F (p), df = 2,45
Total GM (ml)	760.32 (55.60)	764.39 (84.48)	760.59 (93.31)	.01 (.99)
Total WM (ml)	485.60 (80.05)	494.15 (47.33)	510.04 (51.29)	.67 (.51)
Total CSF (ml)	213.47 (45.43)	251.58 (82.08)	223.35 (82.19)	1.29 (.28)
Voxel GM (ml)	16.02 (2.41)	16.31 (2.79)	15.19 (3.45)	.63 (.53)
Voxel WM (ml)	7.07 (1.68)	7.29 (1.74)	7.74 (2.15)	.57 (.56)
Voxel CSF (ml)	4.00 (1.39)	3.45 (1.24)	3.93 (2.01)	.64 (.53)
Voxel Gray %	59.16 (5.95)	60.07 (8.69)	56.51 (11.98)	NA
Voxel White %	26.07 (5.84)	27.18 (7.74)	28.99 (8.60)	NA
Voxel CSF %	14.62 (4.51)	12.71 (4.43)	14.31 (5.66)	NA

note: no significant differences were found between groups for any of the total or voxel-wise tissue volumes.

NA = not assessed separately from voxel volume.