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Short communication: Sex-linked differences in gamma-aminobutyric acid (GABA) are related to social functioning in autism spectrum disorder

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ABSTRACT

Magnetic resonance spectroscopy (MRS) was utilized to investigate sex differences in gamma-aminobutyric acid (GABA) between adults with autism spectrum disorder (ASD) and neurotypical (NT) controls. GABA at the right superior temporal sulcus (STS) is reported for 12 ASD and 14 NT participants. The results show no group differences in GABA. There was, however, a significant positive association between GABA at the STS and autism-related social impairments in females with ASD. These findings provide preliminary support for sex differences in GABAergic distribution and processes that contribute to social functioning in ASD.

1. Introduction

Gamma-aminobutyric acid (GABA), the brain's primary inhibitory neurotransmitter, is often implicated in autism spectrum disorder (ASD). GABA is thought to play a crucial role in dysregulated cortical excitation/inhibition, a commonly cited theory regarding the neuropathophysiology of ASD (Rubenstein and Merzenich, 2003). An increasing number of studies have investigated GABA in ASD using magnetic resonance spectroscopy (MRS). Reduced GABA has been reported in frontal (Harada et al., 2011; Kubas et al., 2012), motor/sensorimotor (Gaetz et al., 2014; Puts et al., 2017), temporal/auditory (Gaetz et al., 2014; Rojas et al., 2014) and occipital (Drenthen et al., 2016) regions in ASD. Other sites, however, show no GABA differences (Brix et al., 2015; Cochran et al., 2015; Gaetz et al., 2014; Harada et al., 2011; Robertson et al., 2016). Importantly, even in the absence of GABA differences at voxels of interest, recent investigations indicate a relationship between reduced GABA and autistic symptomatology (Brix et al., 2015), including social impairment (Cochran et al., 2015). Furthermore, during tasks related to cortical and/or behavioral inhibition, GABA has been shown to correlate with behavioral outcomes for controls, but not individuals with ASD (Puts et al., 2017; Robertson et al., 2016), further indicating dysfunctional GABAergic mechanisms in ASD.

One factor largely overlooked in the ASD literature is that of biological sex. Although it is reasonably well established that neurobiological sex differences exist in ASD (Kirkovski et al., 2013), to our knowledge there has been no prior investigation of the role of biological sex on neurochemical (specifically, GABA) profiles in ASD.

GABA, measured from the right dorsolateral prefrontal cortex (DLPFC) and superior temporal sulcus (STS), was compared between adults with ASD and neurotypical (NT) controls. These sites are involved in social processing (Kalbe et al., 2010; Schurz et al., 2014), and are also implicated in ASD (Castelli et al., 2002; Kirkovski et al., 2016a). It was hypothesized that GABA would be reduced in the ASD compared to control group. The role of biological sex, and the relationship with autistic traits, were also explored.

2. Methods

2.1. Participants

Data for 26 participants (12 ASD [5 male, 7 female], 14 NT [9 male, 5 female]) is presented. All ASD participants were previously diagnosed by a clinician (psychologist, psychiatrist, or pediatrician) external to the study, and did not differ on age or IQ (Supplementary Table S1). The Autism Spectrum Quotient (AQ; Baron-Cohen et al., 2001) and Ritvo Autism and Asperger's Diagnostic Scale-Revised (RAADS-R; Ritvo et al., 2011) were administered to characterize symptoms (Supplementary Table S2). No participants in this sample reported taking medications at the time of the study.

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2.2. Materials and Procedures

2.2.1. Neuroimaging data acquisition and analysis

Data were acquired on a Siemens Tim Trio 3 T MRI scanner, following diffusion tensor imaging (DTI) and functional MRI (fMRI) scans. High resolution T1-weighted structural images were acquired sagittally: protocol = MPRAGE, repetition time (TR) = 1900 ms, echo time (TE) = 2.52 ms, inversion time = 900 ms, spatial resolution = $1 \times 1 \times 1\ mm^3$. A MEGA-PRESS sequence; (TR = 1500, TE = 68, NS = 240 averages [120 ON, 120 OFF]), was conducted at two regions 1) right DLPFC ($2 \times 2 \times 2$ cm), 2) right pSTS ($2 \times 2 \times 2$ cm). Each acquisition followed by a water-unsuppressed scan at the same location (TR = 1500, TE = 68, averages = 24). The voxels of interest were placed visually, and checked by a researcher and the radiographer for consistency. The standard basis-set provided by LCmodel (Version 6.3-1H) was used to fit the data and estimate GABA against water (GABA/Water) in arbitrary unit (a.u.). Only data that met the following criteria: %SD (GABA) = < 20% (Cramér-Rao lower bounds), FWHM = < .1, S/N = > 10, were included. There were no group differences in data quality (all $p > .05$). Because so few participants achieved data restriction criteria for DLPFC, these data are not presented.

An SPM8 based in-house method was used to reconstruct MRS voxels in each participant's native space, in order to assess group differences in tissue volume. After segmentation, the proportion of gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF) were established. An example of voxel placement, segmentation, and extracted spectra is presented in Fig. 1A. GM (U = 70, $p > .05$), WM (U = 77, $p > .05$), or CSF (U = 58, $p > .05$) proportions did not differ between groups (Supplementary Table S3). As the GABA level is mainly contributed in gray matter, the partial volume corrected GABA level was calculated using the equation below:

$$GABA' = \frac{GABA}{water} X \frac{1}{GM}$$

All analyses presented are based on corrected GABA.

3. Results

Non-parametric inferential tests were used given the small sample size. Comparisons were run between groups, and then stratified by sex. Mann-Whitney U tests revealed no significant differences in GABA' between groups (see Supplementary Table S4).

Spearman's rank order correlations were conducted between clinical measures and GABA'. Correlations were conducted for the whole sample, at a group level, and stratified by sex. Accounting for this, as well as DLPFC data having been analyzed (though not reported), a more conservative threshold of $\alpha = .0005$ was used based on a Bonferroni correction.

A positive relationship between STS GABA' and the RAADS-R "social relatedness" subscale was identified in females with ASD, $r_s = .94$, $p = .0005$. Though not surviving correction for multiple comparisons, there was an indication of positive relationships between STS GABA' and AQ "total", $r_s = -.89$, $p = .007$, AQ "communication" $r_s = .81$, $p = .007$, and AQ "imagine" $r_s = .93$, $p = .003$ also in this sub-group.

4. Discussion

Contrary to expectation, we did not identify any group differences in GABA'. Similar findings have been reported previously (Brix et al., 2015; Cochran et al., 2015; Gaetz et al., 2014; Harada et al., 2011). Indeed two studies, to our knowledge, that compared older adolescent/adult samples similarly found no differences in GABA measures (Port et al., 2017; Robertson et al., 2016), and also at a temporal site (Port et al., 2017).

Within a NT population, Greenhouse et al. (2016) demonstrate that GABA, while stable within voxels across time, varies across brain areas. Given the conflicting findings concerning GABA abnormalities in ASD, it is plausible that GABAergic impairment is not a global phenomenon in ASD, but rather, that specific brain regions are affected to varying degrees. Another possibility is that GABAergic impairment may not be seen universally across the autism spectrum, but rather might be mediated by ASD severity (e.g. Enticott et al., 2010).

Further, there were no differences in GABA' when data were stratified by sex. As this is the first study to report such analyses, it is difficult to speculate in this regard. Our previous research reporting a related sample, however, demonstrates similar findings (Kirkovski et al., 2016a, 2015, 2016b). Another consideration is that there is evidence of age related decline in GABA concentration among healthy populations, and this decline appears to be greater among females (Gao et al., 2013). The degree to which this pattern applies to clinical populations, such as ASD, is unclear and warrants further longitudinal investigation of sex differences in GABA.

Clinically, this is not the first study to show a relationship between GABA and ASD traits. The direction of the relationship(s) identified in females with ASD is contradictory to previous reports, which indicate relationships between reduced GABA and increased symptomatology (Brix et al., 2015; Cochran et al., 2015). Thus, while neurobiological abnormalities exist within ASD, the directionality of these abnormalities is heterogeneous, and biological sex might contribute to this variability. It has been previously demonstrated that unexpected effects emerge when data are stratified by sex, which might be otherwise concealed (Holt et al., 2014; Kirkovski et al., 2016a, 2016b).

There are several limitations to this study that must be considered. Firstly, the sample size is small, particularly when stratified by sex, and only involved adults with IQ scores > 70. Accordingly, results cannot be generalized to younger samples or those with varying clinical profiles. Moreover, given the nature of the statistical analyses conducted, these variables were not able to be included as covariates and their impact further assessed. As participants had been diagnosed externally to the study, potential inconsistency between clinical professional might introduce additional variability into our sample which cannot be accounted for. Secondly, hormonal influences may affect GABA levels in healthy women (Cosgrove et al., 2007), this too was not accounted for in this study. Methodologically, there have been concerns raised surrounding the impact of gradient heating and frequency drift on MRS data (Mikkelsen et al., 2017). Unfortunately, the necessary data were not available to assess this in the reported sample. A follow up scan was conducted however, using the same parameters as the present data set, in order to assess the potential impact. We found that the amount of drift of this scan was acceptable, and hence are confident that the potential impact of gradient heating on these data is minimal. Despite these limitations, however, this preliminary study provides strong impetus for future research to investigate sex differences in ASD at a neurochemical level. Finally, although not necessarily a limitation of our study, it is also important to acknowledge the different approaches to calculating GABA, whether GABA concentration is reported alone or as a ratio to other metabolites (e.g. Cr, NAA, or GLX), which may affect the ability to directly compare our findings to the aforementioned studies.

The findings of this preliminary investigation warrant further investigation as improved understanding of GABAergic mechanisms in ASD may prove fruitful for future research. For example, there is growing interest in GABA as a potential mechanism of action for biomedical intervention for ASD (Cellot and Cherubini, 2014; Oberman, 2012). Indeed, drug (Erickson et al., 2014; Lemonnier et al., 2012) and device (Enticott et al., 2014; Oberman et al., 2015) interventions, some of which have demonstrated GABAergic effects (Stagg et al., 2009), are currently being investigated.

In summary, the present investigation did not reveal any differences in GABA between ASD and NT adults at the right STS. Biological sex, however, appeared to influence the relationship between STS GABA and autism related traits. Though preliminary, this study highlights the