RESEARCH ARTICLE

Reduced GABA and Altered Somatosensory Function in Children with Autism Spectrum Disorder

Nicolaas A.J. Puts, Ericka L. Wodka, Ashley D. Harris, Deana Crocetti, Mark Tommerdahl, Stewart H. Mostofsky, and Richard A.E. Edden

LAY ABSTRACT

Sensory (e.g., touch) abnormalities are common in children with autism spectrum disorder (ASD). In previous work we have shown that children with ASD have altered sensitivity to touch. This altered sensitivity is specific to tasks that involve filtering and habituation to touch. GABA is the main inhibitory neurotransmitter in the human brain, and plays a key role in encoding touch. Previous studies suggest that the GABA system is altered in ASD. Using magnetic resonance spectroscopy (MRS), it is now possible to measure GABA levels in the human brain. Recent MRS work suggests that GABA levels are reduced in ASD.

In this study we investigated whether brain GABA levels are altered in ASD and whether changes in brain GABA levels can predict differences in tactile sensitivity. Our results suggest that GABA levels are reduced in sensorimotor areas, but not in occipital visual areas. Lower GABA levels are associated with less "filtering" of touch information, and are not associated with habituation in ASD, whereas they are in typically developing children. Further research is necessary to elucidate the specific GABAergic mechanisms altered in ASD. Our results suggest that reduced brain GABA levels could underlie altered tactile function in ASD, and that altered GABA function in ASD disrupts the link between GABA and behavior. Understanding the link between brain GABA and tactile behavior is an important step in understanding brain behavior links in ASD, potentially leading to future therapies to reduce the severity of sensory symptoms.

SCIENTIFIC ABSTRACT

Background: Abnormal responses to tactile stimuli are a common feature of autism spectrum disorder (ASD). Several lines of evidence suggest that GABAergic function, which has a crucial role in tactile processing, is altered in ASD. In this study, we determine whether in vivo GABA levels are altered in children with ASD, and whether alterations in GABA levels are associated with abnormal tactile function in these children.

Methods: GABA-edited magnetic resonance spectroscopy was acquired in 37 children with Autism and 35 typically developing children (TDC) from voxels over primary sensorimotor and occipital cortices. Children performed tactile tasks previously shown to be altered in ASD, linked to inhibitory mechanisms. Detection threshold was measured with- and without the presence of a slowly increasing sub-threshold stimulus. Amplitude discrimination was measured with- and without the presence of an adapting stimulus, and frequency discrimination was measured.

Results: Sensorimotor GABA levels were significantly reduced in children with autism compared to healthy controls. Occipital GABA levels were normal. Sensorimotor GABA levels correlated with dynamic detection threshold as well as with the effect of sub-threshold stimulation. Sensorimotor GABA levels also correlated with amplitude discrimination after adaptation (an effect absent in autism) and frequency discrimination in controls, but not in children with autism.

Conclusions: GABA levels correlate with behavioral measures of inhibition. Children with autism have reduced GABA, associated with abnormalities in tactile performance. We show here that altered in vivo GABA levels might predict abnormal tactile information processing in ASD and that the GABA system may be a future target for therapies. *Autism Res* 2016, 0: 000–000. © 2016 International Society for Autism Research, Wiley Periodicals, Inc.

Keywords: autism; somatosensory; GABA; magnetic resonance spectroscopy; tactile; MRS; touch

Introduction

Autism spectrum disorder (ASD) is characterized by impairments in social interaction, disordered communication, and repetitive behaviors. Despite decades of study, the neurophysiological basis of ASD remains poorly understood. Multiple lines of evidence suggest that γ-aminobutyric acid (GABA), the main inhibitory neurotransmitter in the brain, plays a role in the pathophysiology of ASD, including evidence from mouse models [Chao et al., 2010; Fukuda, Itoh, Ichikawa, Washiyama, & Goto, 2005], expression of GABA receptor genes [Abrahams & Geschwind, 2008; DeLorey, 2005], altered GABA levels [Gaetz et al., 2014; Rojas, Maharajh, Teale, & Rogers, 2008], and altered cortical structure [Casanova et al., 2006; Fatemi, Folsom, Reutiman, & Thuras, 2009; Oblak, Gibbs, & Blatt, 2010].

Difficulties in sensory (including tactile) processing are a long recognized feature of ASD [Rogers & Ozonoff, 2005]. Impairments in the response to sensory stimulation were reported in Kanner's original account of the disorder, are described as phenotypically characteristic of ASD, and have been added to DSM-5 criteria for ASD. However, the underlying cortical mechanisms remain unclear. We recently reported, using tightly controlled tactile psychophysics, that children with ASD have impairments in specific tactile tasks, as compared to TDC.

Detection threshold in TDC increases after being exposed to dynamically increasing sub-threshold stimulation, compared to a static condition. This effect is absent in children with ASD, and children with ASD also show higher static detection threshold than TDC. Sub-threshold stimulation predominantly activates GABAergic feedforward mechanisms, suppressing cortical activity [Blankenburg et al., 2003; Connors, Malenka, & Silva, 1988; Zhang & Sun, 2011] and therefore reducing the detectability of subsequent stimuli. Given that this effect is absent in children with ASD [Puts, Edden, Wodka, Mostofsky, & Tommerdahl, 2013; Puts, Wodka, Tommerdahl, Mostofsky, & Edden, 2014a], it is suggestive of abnormal feed-forward inhibitory mechanisms. Mechanisms to suppress weak stimulation, such as "sensory gating," have been implicated in filtering of sensory information, so that only relevant information is processed further [Blankenburg et al., 2003; Favorov & Kursun, 2011; Francisco, Favorov, & Tommerdahl, 2013].

In our previous study, we also showed higher-amplitude discrimination thresholds in ASD compared to TDC, and we showed that adaptation (habituation to prior sensory stimulation) is absent in ASD [Puts et al., 2014a; Tannan, Holden, Zhang, Baranek, & Tommerdahl, 2008]. As GABAergic lateral inhibitory connectivity in the cortex plays a role in separating tactile stimulus signals, as does the decrease in cortical firing rate that occurs during tactile habituation [Tommerdahl, Favorov, & Whitsel, 2010; Whitsel et al., 1989], these behavioral results are suggestive of altered inhibitory function in ASD.

Given the importance of GABA in the regulation and control of neuronal activity and in tactile processing, it is important to link specific aspects of tactile function that may be altered in ASD, to altered GABAergic function. This understanding is key in understanding the pathophysiology underlying tactile abnormalities in ASD.

It is possible to *directly* measure the concentration of GABA in vivo in the human brain using edited magnetic resonance spectroscopy (MRS) [Puts & Edden, 2012]. One study in children with ASD showed that motor cortex GABA levels were decreased in ASD, but that occipital GABA levels were normal [Gaetz et al., 2014], but little is known about the functional relevance of these changes [Ford & Crewther, 2016]. In healthy controls, MRS of GABA has been used to show functional regionally specific correlations between brain GABA levels and behavior, including tactile frequency discrimination [Puts, Edden, Evans, Mcglone, & Mcgonigle, 2011], brain activity [Donahue, Near, Blicher, & Jezzard, 2010; Muthukumaraswamy, Edden, Jones, Swettenham, & Singh, 2009], as well as learning [Floyer-Lea, Wylezinska, Kincses, & Matthews, 2006]. Abnormal GABA levels have been shown in several neurodevelopmental, psychiatric and neurological disorders (for a review see [Puts & Edden, 2012]).

To date, no studies have attempted to investigate whether brain GABA levels in ASD are associated with tactile behavioral abnormalities found in this population. The aim of this study was to investigate the relationship between GABA levels and tactile performance in ASD, as measured using a battery of vibrotactile tests linked to GABAergic function. First we hypothesize that children with ASD will have lower GABA levels in the sensorimotor cortex compared to TDC. Second, as it is suggested that sub-threshold stimulation drives GABAergic feed-forward inhibition, we hypothesize that participants with higher levels of GABA show a stronger increase in dynamic detection threshold compared to the static condition and that children with ASD show an absence of this effect [Puts et al., 2014a], which correlates with lower GABA levels. Third, as tactile adaptation leads to a decrease in firing rate through GABAergic signaling, we expect that participants with higher GABA levels show higher amplitude discrimination thresholds (more GABA would lead to a stronger decrease in firing rate which would be reflected by a stronger effect of adaptation) after adaptation compared to amplitude discrimination without adaptation, and

that children with ASD show no adaptation. Lastly, animal studies have shown that GABA plays an important role in encoding the periodicity of neuronal firing that encode stimulus frequency [McLaughlin & Juliano, 2005]; in previous work we showed that tactile frequency discrimination correlates negatively with GABA levels in both healthy adults [Puts et al., 2011] and healthy children [Puts et al., 2015]. In this study we will explore this link in children with ASD.

Methods and Materials
Population

In total, this study was performed on 37 children with ASD (10.69 ± 1.4 years, six female) and 35 TDC (average age 10.09 ± 1.25 years; eight female) with all children between 8 and 12 years old. Of these, 16 children with ASD and 16 children with ASD children were included in previous behavioral studies [Puts et al., 2014a]; GABA MRS data from 16 TDC were also presented in [Puts et al., 2015]. Informed consent was obtained from a parent of each child (who also assented to testing), with the approval of Kennedy Krieger Institute and Johns Hopkins School of Medicine Institutional Review Boards.

Participants in the ASD cohort met the Diagnostic and Statistical Manual of Mental Disorders Fourth Edition (DSM-IV) criteria for ASD and this was confirmed with the Autism Diagnostic Observation Schedule-Generic (ADOS-G) [Lord et al., 2000] and Autism Diagnostic Interview-Revised (ADI-R) [Lord, Rutter, & Le Couteur, 1994]. Children with identifiable causes of autism (e.g., Fragile X syndrome) and neurological disorders including epilepsy were excluded. Standard intellectual functioning was assessed using the Wechsler Intelligence Scale for Children-Fourth Edition (WISC-IV) [Wechsler, 2003]. Children with full-scale IQ scores below 80 were excluded from participation in all studies unless there was a 12 point or greater index discrepancy, in which case either the Verbal Comprehension Index or perceptual reasoning index (PRI) was required to be ≥80 and the lower of the two was required to be ≥65. To avoid effects on cognitive and behavioral measures, stimulant medications were discontinued the day prior to and the day of testing. Participants were, however, allowed to continue treatment with other psychotropic medications that would normally require a longer washout period, for both ethical and practical reasons. All children in the TDC cohort were free of criteria for psychiatric disorders as assessed using the Diagnostic Interview for Children and Adolescents-Fourth Edition (DICA-IV). None of the children in the TDC cohort were prescribed psychoactive medications.

Handedness was evaluated using the Edinburgh Handedness Inventory [Oldfield, 1971].

MRI Acquisition

All GABA-edited MRS data were acquired using a Philips 3T Achieva MRI scanner (Best, the Netherlands; 32-channel head coil for receive and body coil for transmit). For 16 children with ASD and 16 TDC, MEGA-PRESS [Mescher, Merkle, Kirsch, Garwood, & Gruetter, 1998] data were acquired from $(3 \text{ cm})^3$ voxels placed on the right sensorimotor cortex (SM1; as tactile stimulation was performed on the left hand, Fig. 1A) and midline occipital cortex (OCC; Fig. 1C) using the following parameters for each voxel acquisition: 40 blocks of 8-step phase cycles in a ~10 min acquisition. A separate water scan was acquired for quantification. In a further 21 children with ASD and 19 TDC, MEGA-PRESS data were also acquired in a $(3 \text{cm})^3$ voxel over sensorimotor cortex using a sequence where editing on and off scans were interleaved at the level of each 16-step phase cycle, acquiring for a total of 320 scans, with an unsuppressed water reference acquired as part of the sequence rather than separate. While there were no differences in data quality between the two acquisitions, interleaving at the level of each 16 step phase cycle allows for better post-hoc frequency correction and therefore reduce the potential effect of subject motion and scanner drift. The following parameters were common between the two acquisitions: TE 68, 14 ms editing pulses at 7.46 ppm (edit-OFF) and 1.9 ppm (edit-ON) ordered OFF-first, TR 2s, 2048 datapoints, 2 kHz spectral width, VAPOR water suppression [Tkac, Starcuk, Choi, & Gruetter, 1999]. The SM1 voxel was centered on the central sulcus, posterior to the hand-knob [Yousry et al., 1997] in the axial plane (Fig. 1A) and rotated to align with the cortical surface (previously described [Puts et al., 2011]). The OCC voxel (only acquired in 16 TDC and 16 ASD) was centered on the midline and aligned with the cerebellar tentorium (Fig. 1C). Prior to voxel placement, a 1 mm^3 isotropic T1-weighted image (MP-RAGE) was acquired for voxel localization and segmentation (TR = 7.99 ms, TE = 3.76 ms, Flip angle = 8°) for each participant. To motivate participant compliance during scanning, children were allowed to watch a movie and "points" were given for lying still that could be exchanged for prizes.

Tactile Psychophysics

All children performed a battery of tactile tasks outside the scanner. The procedures have been described in detail elsewhere [Puts et al., 2013]. This battery has been shown to be suitable for children and was acquired in 30–40 minutes, with a break half-way. A CM4 four-digit tactile stimulator (Cortical Metrics,
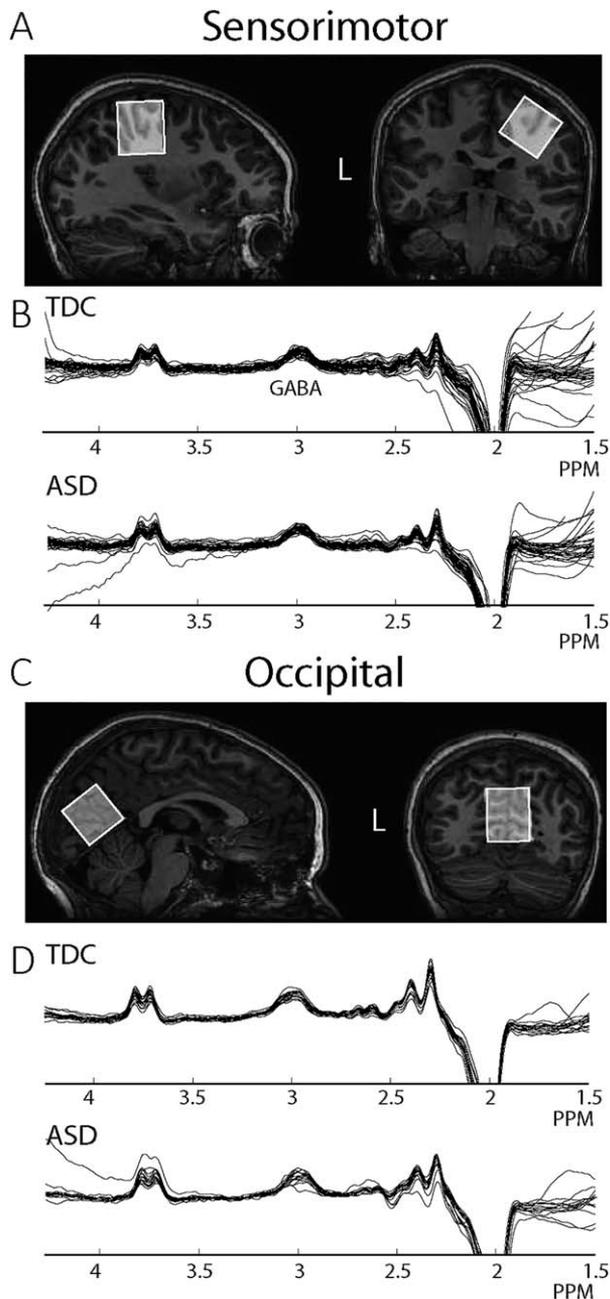
Figure 1. (A) Sensorimotor (SM1) MRS voxel location. The voxel was placed on the hand area of the right primary motor cortex and rotated to align with the edge of the brain. (B) Good quality spectra from all participants retained (six ASD and three TDC excluded). (C) Occipital voxel location. The voxel was placed on the midline and the bottom edge aligned with the cerebellar tentorium. (D) Good quality spectra were acquired in 16 children with ASD and 16 TDC.

North Carolina) was used for vibrotactile stimulation [Holden et al., 2012]. Stimuli were delivered to the glabrous skin of left digits 2 (LD2) and 3 (LD3) and all stimuli were presented within the flutter range (25–50 Hz) using sinusoidal stimuli. Visual feedback, task

responses, and data collection was performed on an Acer Onebook Netbook computer, running Cortical Metrics software [Holden et al., 2012]. All tasks used stepwise tracking for threshold determination and stimulus order was pseudo-randomized. Children performed the following tasks:

1. Static and dynamic detection threshold (Fig. 2A). In the static task, children were asked to detect a weak stimulus and indicate on which finger they felt the stimulus (starting amplitude 25 μm, 25 Hz, 500 ms; Inter Trial Interval = 5 s; 24 trials). In the dynamic task, stimulus amplitude started at zero after a variable delay (0–2500 ms) and increased with 2 microns per second (ITI 10 s; 7 trials). Participants were asked to indicate on which finger the stimulus was perceived.
2. Amplitude discrimination without adaptation (un-adapted) and with single-site adaptation (Fig. 2B). In the un-adapted condition, participants were asked to judge which of two simultaneously applied stimuli was more intense (both 25 Hz; 500 ms; Standard stimulus amplitude: 100 μm; initial comparison stimulus amplitude: 200 μm; ITI 5 s; 20 trials). In the adapted task, one of the two test stimuli was preceded by a 1 second long "adapting" stimulus [Puts et al., 2013; Tannan et al., 2008] (also 25 Hz; amplitude 100 μm) which participants were told to ignore. The effect of adaption was calculated as the percentage difference between the threshold in the single-site adaptation task and the no-adaptation task (adapted–unadapted)/unadapted * 100).
3. Sequential frequency discrimination (Fig. 2C). Participants were asked to indicate which of two stimuli (with the same amplitude but varying frequency; both 500 ms; 200 μm; standard stimulus frequency 30 Hz; comparison stimulus starting frequency 40 Hz) had higher frequency.

MRS Analysis

Using Gannet 2.0 [Edden, Puts, Harris, Barker, & Evans, 2014], GABA data were frequency- and phase-corrected using spectral registration [Near et al., 2015] and filtered with a 3 Hz exponential line broadening and zero-filled to 32768 points. GABA concentration was estimated using a five-parameter Gaussian model, fitting between 2.79 and 3.55 ppm. GABA levels were calculated relative to the unsuppressed water signal from the same voxel. For each subject, GABA levels were tissue-corrected, assuming the concentration of GABA in grey matter (GM) is twice the concentration of GABA in while matter (WM) or alpha = 0.5, as per Harris, Puts, & Edden, 2015b, then, also as per Harris et al 2015b, group normalization for mean tissue fractions was performed across all data. GM, WM and CSF
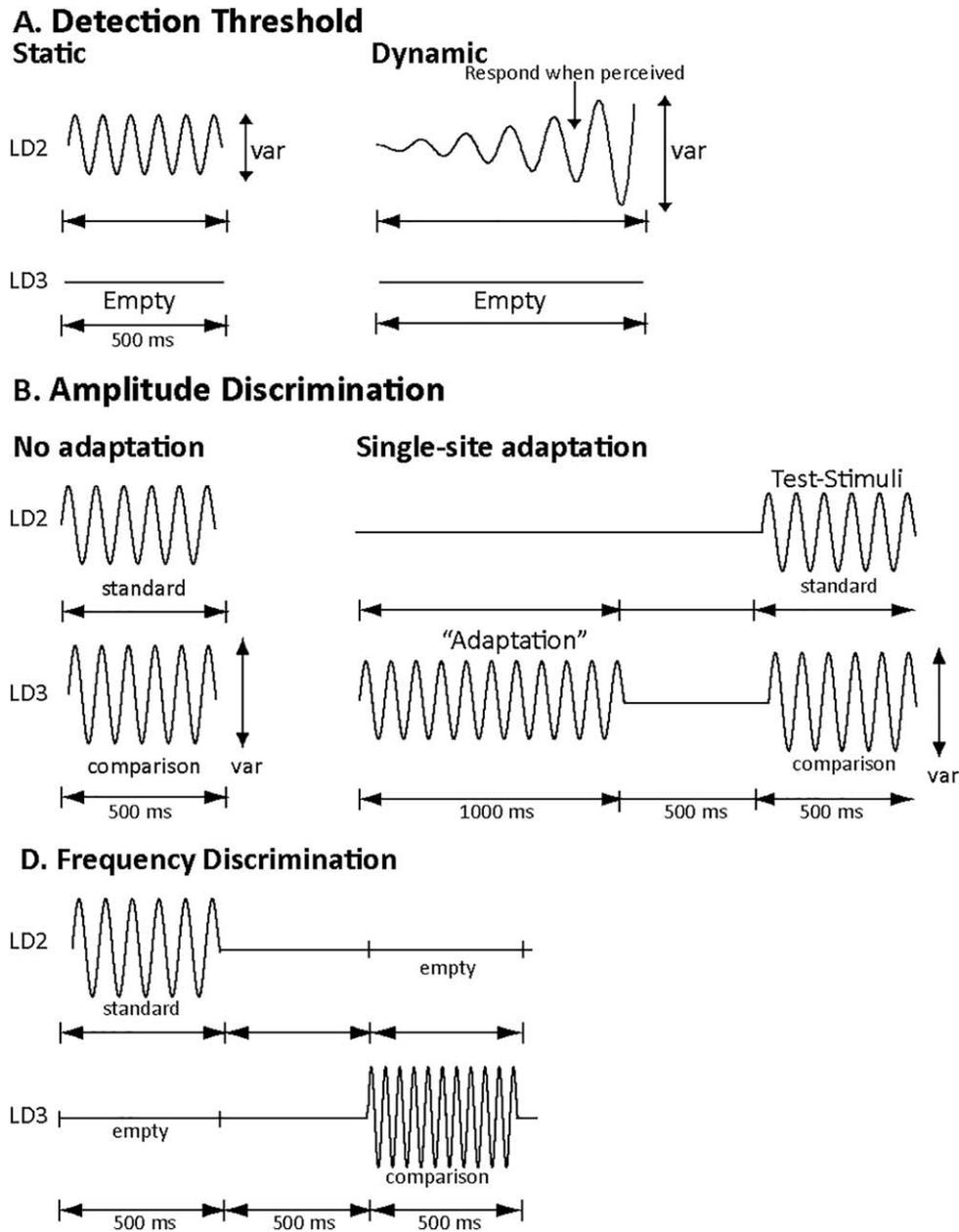
Figure 2. Psychophysical Tasks. (A) Static and dynamic detection threshold. Participants were asked to detect a weak stimulus that was either Static, or Dynamic (increasing in amplitude) and determine stimulus location. (B) Amplitude discrimination with- and without single site adaptation. In each trial, participants were asked to judge which of two simultaneously applied test stimuli was most intense, trials were preceded by 1s adapting stimulus in the adaptation condition. (C) Sequential frequency discrimination. Participants were asked to judge which of two sequentially applied stimuli had the highest frequency.

fractions were calculated using the SPM "new segment" routine implemented within Gannet 2.0. Given the limited selectivity of the 14 ms editing pulses, all reported GABA values refer to GABA + co-edited macro-molecules [Harris et al., 2015b; Mullins et al., 2014]. Movement during the scans, which may lead to underestimation of the GABA concentration [Harris et al., 2014a], was assessed from the standard deviation of the offset of the water signal across the scan [Harris et al., 2014a] and GABA signal fit errors [Evans et al., 2013], as calculated by Gannet 2.0, common metrics of data quality. Scans where no clear GABA signal could be identified, or where Gannet fitting failed to converge on a model, were excluded.

Statistical Methods

All statistical analyses were performed using SPSS (IBM SPSS Version 17.0). Behavioral tasks were analyzed

using univariate statistical models including diagnosis and task-type (static/dynamic or adapted/unadapted) as fixed factors and outcome (threshold) as the dependent variables, to confirm previous behavioral findings in a larger cohort [Puts et al., 2014a]. To confirm further previous findings of group differences in patterns of behavior [Puts et al., 2014a], Student's t-tests were used. Student's t-test was also used to test for group differences in tissue-corrected GABA levels and scan quality differences. A univariate model with "sequence" as covariate was used to test whether methodological differences impacted results. Pearson correlations were used to examine whether GABA concentration was correlated with behavioral performance. Robustness of these correlation was confirmed using Jackknife permutations. Differences between correlations in TDC and ASD were tested using Fisher r-to-z transformations.

Six children with ASD and three TDC had either poor GABA MRS data or poor T1-weighted images, and were therefore excluded from the analysis. Not all children were able to perform all vibrotactile tasks; five children with ASD and one TDC were excluded for the detection threshold tasks, four children with ASD and two TDC were excluded from the amplitude discrimination tasks, and six children with ASD were excluded for the frequency discrimination tasks.

Results
Sample Characteristics and Demographics

There were no group differences in age or sex (Table 1). While there was a group difference in full-scale IQ (FSIQ TD: 115.91 ± 10.62; FSIQ ASD: 103.21 ± 15.41, df = 67, $t = -3.997$, $P < 0.001$), there were no significant group differences on the PRI, which is thought to be a more valid measure of intellectual functioning in children with ASD (TDC: 111.94 ± 11.05; ASD: 107.82 ± 12.83, df = 65, $t = -1.397$, $P = 0.168$). Average ADOS score for the ASD cohort was 13.15 ± 3.57. Eighteen children with ASD had comorbidity for attention-deficit/hyperactive disorder (ADHD) combined-type, one for ADHD inattentive-type, three for obsessive compulsive disorder, and one for oppositional defiance disorder. Participants in the ASD group on prescribed psychotropic medications at the time of assessment, were as follows: 15 children were on stimulant medications (methylphenidate, dexmethylphenidate, dextroamphetamine, lisdexamfetamine, quillivant, and were removed from these medications on the day of and before testing). One child was on antidepressants (fluoxetine), one child on other medication (atomoxetine). None of the children with ASD were on anticonvulsants or benzodiazepines. None of the TDC were on prescribed psychotropic medications. Two ASD and five TDC were left-handed. Six children

with ASD and three TDC had either poor GABA MRS data or poor T1-weighted images, and were therefore excluded from the analysis. Not all children were able to perform all vibrotactile tasks; five children with ASD and one TDC were excluded for the detection threshold tasks, four children with ASD and two TDC were excluded from the amplitude discrimination tasks, and six children with ASD were excluded for the frequency discrimination tasks.

MR Spectroscopy

SM1 GABA levels were significantly lower in children with ASD compared to TDC ($t = -2.472$, $P = 0.016$, Table 1) but not occipital GABA ($t = -0.032$, $P > 0.98$). The difference in SM1 GABA values remained significant when covarying for sequence used ($F = 4.690$, $P = 0.034$). Water offset frequency standard deviation (which reflects motion, ASD: 1.92 ± 1.71 Hz; TDC: 2.48 ± 2.04 Hz, $t = 1.177$, $P = 0.24$) and fit error of the GABA peak (ASD: 7.23 ± 2.4; TDC: 7.07 ± 1.97, $t = 0.706$, $P = 0.45$) did not differ significantly between groups indicating no significant differences in data quality between ASD and TDC. GABA did not correlate with age in either cohort or in both combined (R values between -0.068 and 0.04, P values > 0.7) or with full scale IQ (R values between -0.22 and -0.14, P values > 0.2). Given the prevalence of children with comorbid ADHD, we tested whether GABA levels differed in sub-cohorts with- and without comorbid ADHD, but found no differences in GABA concentration (1-way ANOVA; $P > 0.5$). There were no significant differences in SM1 grey matter, white matter, or CSF volumes between the two cohorts (Table 1), consistent with previous findings [Haar, Berman, Behrmann, & Dinstein, 2014]. There were no differences in fit error ($P = 0.54$) and water drift ($P = 0.49$) between the two acquisitions (40 blocks of 8 vs. 2 NSA of 160 scans). GABA levels did not correlate between sensorimotor and occipital regions as previously shown in healthy adults [Puts et al., 2011].

Behavioral Psychophysics

There was a significant effect of task (df = 1, $F = 9.2$, $P = 0.003$), and a one sided effect of diagnosis (df = 1, $F = 3.61$, $P = 0.06$) on detection threshold and no significant interaction between task and diagnosis (df = 1, $F = 9.66$, $P = 0.33$; Table 1). Post-hoc testing to investigate differences in the pattern of performance show that static detection threshold was significantly higher in ASD compared to TDC (df = 63, $t = 2.220$, $P = 0.03$), but dynamic detection threshold was not significantly different between cohorts (df = 61, $t = 0.59$, $P = 0.55$). Dynamic detection threshold was significantly higher than static detection in TDC (df = 31, $t = -3.808$, $P = 0.001$), but not in ASD (df = 29, $t = -1.62$, $P = 0.12$).

Table 1. Demographic, Behavioral and Imaging Comparisons

	TDC		ASD		P-value – between groups
Participants	37 (6F)		35 (8F)		
Age (years)	10.09 ± 1.25		10.69 ± 1.4		>0.2
Full IQ score	103.21 ± 15.41		115.91 ± 10.62		<0.001*
Perceptual reasoning score	111.94 ± 11.05		107.82 ± 12.83		>0.15
ADOS score	na		13.15 ± 3.57		
SM1 GABA conc. (tissue corrected)	2.40 ± 0.25		2.20 ± 0.44		=0.016*
Average Grey Matter fraction (SM1)	0.41		0.41		>0.5
Average White Matter fraction (SM1)	0.51		0.5		>0.3
Average CSF fraction (SM1)	0.075		0.086		>0.2
Static detection threshold	6.81 ± 2.66	=0.001*	9.01 ± 4.96	=0.12	=0.03*
Dynamic detection threshold	9.72 ± 4.71	within group	10.38 ± 3.98	within group	=0.55
Amplitude discrimination	46.1 ± 29.61	=0.02*	63.07 ± 37.01	=0.43	=0.05*
Amplitude discrimination – adaptation	67.88 ± 31.78	within group	68.03 ± 46.10	within group	>0.9
Frequency discrimination	7.52 ± 3.48		8.4 ± 3.99		>0.36

There was a significant effect of task (df = 1, $F = 5.856$, $P = 0.017$) but not of diagnosis df = 1, $F = 0.798$, $P = 0.373$) or task x diagnosis interaction ($F = 2.753$, $P = 0.1$) on amplitude discrimination threshold. Post-hoc analysis to test for differences in patterns of performance show that amplitude discrimination threshold was worse in ASD compared to TDC without adaptation at $P = 0.05$ (df = 61, $t = 1.99$, $P = 0.05$), but not when a single-site adapting stimulus was present (df = 60, $t = 0.004$, $P > 0.9$). Amplitude discrimination with adaptation was significantly worse than without adaptation in TDC (df = 28, $t = -2.306$, $P = 0.02$) but not in ASD (df = 28, $t = -0.8$, $P = 0.43$), reflecting the absence of adaptation in ASD. There were no significant differences in frequency discrimination performance (df = 62, $t = 0.907$, $P = 0.36$), although eight children with ASD were not able to perform this talk successfully. Analysis did not show differences in behavioral performance related to ADHD co-morbidity.

Correlative Analysis

As shown in Fig. 3A, GABA levels across both cohorts correlate positively with dynamic detection threshold, with higher GABA levels correlating with a higher detection threshold ($R = 0.29$, $P = 0.029$). This correlation is significant for the ASD cohort alone ($R = 0.54$, $P < 0.005$, jackknife 0.54 ± 0.0148, R-values between 0.46 and 0.60, all significant at $P = 0.05$), but not for TDC alone ($R = 0.06$, $P = 0.74$, jackknife 0.052 ± 0.011, R-values between −0.23 and 0.19, none significant at $P = 0.05$). This correlation is significantly different between TDC and ASD ($z = 2.02$, $P = 0.04$). Furthermore, the individual difference between the static and dynamic detection threshold (Fig. 3B) is significantly correlated with GABA levels for both groups ($R = 0.39$, $P < 0.005$) as well as for the ASD group alone ($R = 0.55$, $P < 0.006$, jackknife 0.54 ± 0.0058, R-values between

0.43 and 0.61, all significant at $P = 0.05$), but not for TDC alone ($R = 0.06$, $P = 0.74$, jackknife 0.051 ± 0.0202, R-values between −0.084 and 0.11, none significant at $P = 0.05$) . This correlation is significantly different between TDC and ASD ($z = 2.01$, $P = 0.04$). As shown in Fig. 3C, amplitude discrimination performance after single-site adaptation correlates with GABA levels in TDC ($R = 0.44$, $P = 0.019$, jackknife 0.44 ± 0.0059, R-values between 0.36 and 0.55, P-values between 0.07 and 0.003) but not in ASD ($R = -0.21$, $P = 0.3$, jackknife -0.21 ± 0.0051, R-values between −0.33 and −0.11, none significant at $p = 0.05$) or when grouped. These correlations were significantly different between TDC and ASD ($z = 2.39$, $P = 0.017$). GABA did not correlate with the difference between adaptation and amplitude discrimination without adaptation. Finally, Fig. 3D shows a strong negative correlation between GABA levels and tactile frequency discrimination in TDC, a replication [Puts et al., 2011; Puts et al., 2015] ($R = -0.41$, $P = 0.025$, jackknife 0.41 ± 0.0049, R-values between −0.32 and −0.49, P-values between 0.08 and 0.003); this correlation is absent in ASD ($R = 0.19$, $P = $ n.s, jackknife 0.20 ± 0.0076, R-values between 0.11 and 0.27, none significant at $P = 0.05$), and these correlations were significantly different ($z = 2.19$, $P = 0.03$). GABA values did not correlate with ADOS score or IQ ($P > 0.5$). Occipital GABA levels did not correlate with any of the tactile tasks ($P > 0.2$ for all comparisons).

Discussion

In this study, we aimed to investigate whether children with ASD have reduced GABA levels compared to TDC and whether GABA levels are associated with sensory abnormalities in ASD. Our results show reduced GABA levels in children with ASD in sensorimotor cortex, but not in OCC, replicating a previous study [Gaetz et al.,

2014]. Our results also show GABA levels are associated with impaired tactile performance in these children, specifically in tasks we previously showed group

Figure 3.

differences in performance [Puts et al., 2014a] and hypothesized were driven by GABAergic inhibitory function. In this study we link abnormal tactile function to abnormal brain GABA levels.

Several studies have suggested altered GABA function in ASD, although the pathophysiological origin of these alterations remains unclear. Our study confirms that reductions in bulk GABA concentration are present in ASD, and that they are not shown globally throughout the brain.

Detection Threshold

The correlations presented in this article show that GABA levels in ASD are associated with the effect of sub-threshold stimulation on detection threshold, where children with lower GABA levels show an absence (or even reversal) of the effect of sub-threshold stimulation. It has been suggested that sub-threshold stimulation predominantly affects feed-forward inhibitory mechanisms [Blankenburg et al., 2003; Connors et al., 1988; Favorov & Kursun, 2011; Tommerdahl et al., 2010; Zhang & Sun, 2011], and that detection threshold is raised by sub-threshold stimulation due to the activation of inhibitory drive. It is therefore consistent that participants with higher GABA levels show increased dynamic detection threshold and an increased effect of sub-threshold stimulation, and therefore that children with ASD, having lower GABA levels, show a reduced effect. Interestingly, this correlation (between GABA levels and the dynamic-static detection threshold increment) is significant for both groups combined and ASD alone, but not for TDC alone, which may stem from reduced variance (and thus power to reveal correlation) in controls. Interestingly, while GABA plays a role in static detection, as GABA may decrease the amount of neuronal noise in the system [Tavassoli et al., 2016], no correlation is seen in this study. It is also possible that this process occurs via, e.g., thalamic mechanisms rather than cortical.

Adaptation and Amplitude Discrimination

Children with ASD typically do not show an effect of adaptation on amplitude discrimination. While GABA levels did not correlate with the effect of adaptation per se, they did correlate with amplitude discrimination per-
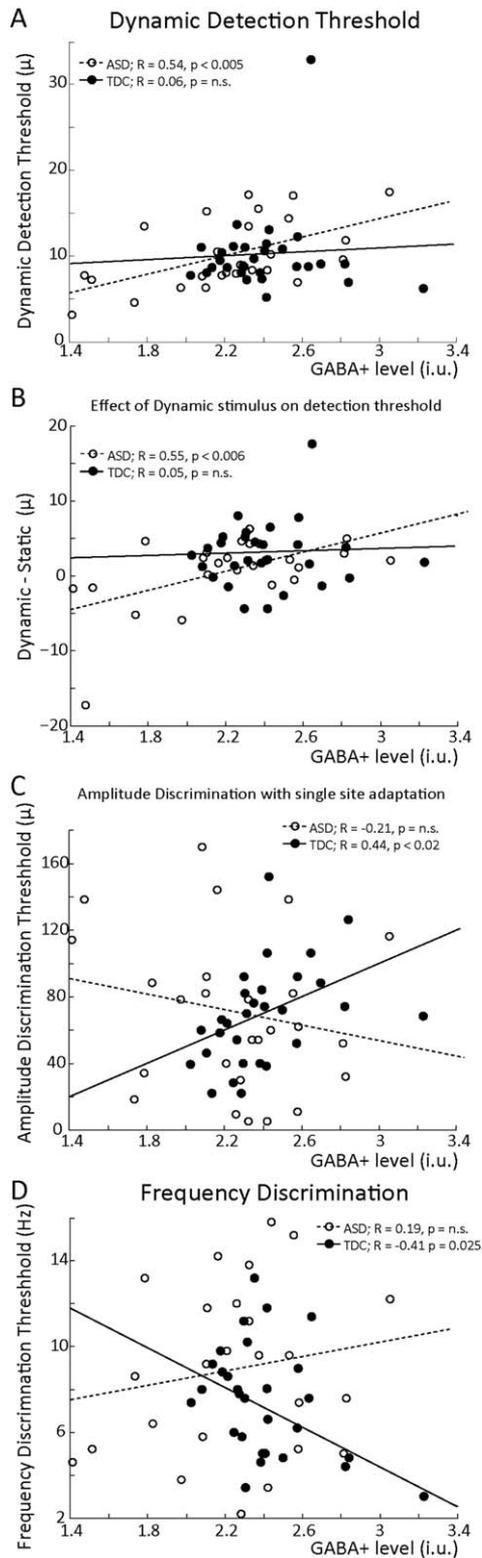
Figure 3. Correlative analysis. (A) GABA concentration is correlated with dynamic detection threshold in cohorts combined as well as in ASD separately. (B) GABA concentration is positively correlated with the difference between a static and dynamic detection threshold in cohorts combined and ASD separately, with higher GABA levels leading to a stronger effect. (C) GABA concentration correlates with single-site adaptation amplitude discrimination performance in TDC but not in ASD. (D) GABA concentration correlates with frequency discrimination performance in TDC but not in ASD.

formance after adaptation in TDC, but not ASD. Strictly, this did not follow our predictions that it is the "effect of adaptation" that is predicted by GABA levels. However, studies have suggested that single site adaptation makes separation of subsequent signals more difficult through inhibition of firing rates (representing stimulus intensity) [Simons et al., 2005; Tannan et al., 2008], but also that amplitude discrimination in itself is driven by lateral inhibition [Tommerdahl et al., 2010; Whitsel et al., 2003]. Therefore, performance in the amplitude discrimination with adaptation tasks likely involves both lateral inhibitory connectivity (to separate signals) and GABA-driven reductions in firing rate due to adaptation, and may therefore be reflected by bulk GABA more clearly than those separate mechanisms. Given this possibility, it is therefore expected that participants with higher GABA levels show worse performance, as is shown in TDC. It must be noted that while the jackknife correlation is significant on a one-sided alpha level of 0.1, it is not at 0.05 and specific individuals may drive the correlations, although behaviorally there was no reason for exclusion. Behavioral performance of children with ASD in the adaptation condition itself is not different from TDC, but they do not show an effect of adaptation compared to the unadapted condition.

Frequency Discrimination

Finally, we show that GABA levels correlate with frequency discrimination performance in TDC but not ASD. GABAergic inhibition plays an important role in encoding tactile frequencies [McLaughlin & Juliano, 2005], and participants with higher GABA levels may be able to better encode tactile frequencies, therefore improving their ability to distinguish them. This TDC result is consistent with our previous work showing this correlation in healthy adults [Puts et al., 2011], and while the jackknife correlation is not significant at all comparisons at 0.05, the directionality does follow our previous work, and behaviorally there was no reason for exclusion of specific individuals. TDC and ASD do not show significant differences in frequency discrimination performance, so the different correlative outcomes may reflect differences in mechanisms underlying frequency discrimination that warrant further investigation.

One common feature of these results is the effects of sub-threshold stimulation, adaptation, and frequency discrimination; rely on either temporal changes in neuronal signals or changes in synchrony. Modulatory effects of prior stimulation (whether sub- or supra-threshold) are behaviorally absent in ASD. The absence of sub-threshold activity is reflected in reduced GABA levels. Correlations between GABA levels and differences in (supra-threshold) adaptation, and differences in frequency discrimination, are absent in ASD while they do exist in TDC. This may suggest that children with ASD respond abnormally to changes in sensation which may be driven by impaired GABA-driven adjustments in cortical processing. These autism-associated impairments in GABA-mediated tactile functions might reflect impaired synchronization or modulation of incoming neuronal signals, or the inhibitory capacity of cortex to filter, or habituate to, to sensory information. (However, it should be emphasized that these mechanisms do not solely depend on GABAergic inhibition). Performance in TDC may be linked more closely to specific inhibitory mechanisms, than it is in ASD, as discussed in more detail below.

The behavioral data presented here follow a similar pattern as shown in our previous study that used larger cohorts (and includes several of the current cohort that underwent MRI scans). In this smaller cohort, static detection threshold is also higher in ASD, and children with ASD do not show a significant increase in detection threshold whereas TDC do. Amplitude discrimination without adaptation is only significantly different between groups at $P = .05$ and does not pass correction for multiple comparisons. However, the trend is similar to the statistically significant data presented in previous work [Puts et al., 2014a]. Differences in study populations might help to explain the discrepancies. Inclusion in the current study required understanding instructions and being able to lie still in the scanner. It is therefore possible that the current study sample of children with ASD were less affected as compared with those in the previous study.

Interestingly, while occipital GABA levels appear normal, studies have shown differences in visual processing in ASD [Dickinson, Jones, & Milne, 2014; Freyberg, Robertson, & Baron-Cohen, 2015; Robertson, Ratai, & Kanwisher, 2016], so it remains unclear how normal GABA levels associate with abnormal visual processing. Robertson et al. [Robertson et al., 2016] did, however, show that occipital GABA levels correlate with visual binocular rivalry in healthy participants, but not in ASD, showing an absence of association between GABA and visual processing in ASD. This study did not find reduced GABA levels in OCC (consistent with our results), yet, still showed abnormal inhibitory visual signaling in ASD. GABA reductions (as detected by MRS) are not global throughout the brain, and are not universally linearly linked to altered sensory processing. It is important to emphasize that the behavioral tasks used in this study were specifically chosen to tap into inhibitory mechanisms, based on prior human and animal work [McLaughlin & Juliano, 2005; Tommerdahl et al., 2010; Zhang & Sun, 2011]. In our own findings, we do see that reduced sensorimotor GABA levels predicting altered tactile function in one task (the effect of sub-threshold stimulation), but that, similar to

Robertson et al., [Robertson et al., 2016] correlative brain-behavior correlations that are seen in TDC may be absent in ASD. It is important to note that behavioral performance in ASD is not always reduced [Dickinson & Milne, 2014; Puts, Wodka, Tommerdahl, Mostofsky, & Edden, 2014b] and that all behaviors arise from the complex interaction of multiple neurotransmitter systems across broad networks.

There is currently an increased interest in investigating the link between GABA and sensory function in ASD, although the literature is still developing. Auditory GABA has been shown to be reduced in ASD [Gaetz et al., 2014], corresponding to reduction in the oscillatory response to auditory stimulation [Rojas et al., 2008; Rojas, Singel, Steinmetz, Hepburn, & Brown 2013]. MRS of GABA is an emerging methodology and thus far, few studies link GABA levels to (within-domain) sensory impairments in ASD; cross-domain associations remain unexplored. GABA levels do not generally correlate between functionally distinct regions, as shown here and previously [Boy et al., 2011; Harris et al., 2015a]. In our data at least, none of the tactile tasks associated with occipital GABA levels, which may not be expected; occipital GABA levels were only acquired in a subset of participants.

While the behavioral tasks used in this study are all thought to reflect aspects of inhibitory function, there does not exist a unilateral or linear association between brain GABA levels and behavioral performance in TDC and ASD. As described in a previous study [Puts et al., 2015], it is unlikely that our measurements of GABA concentration using MRS reflect simple up- and down-regulation of all GABA function. Detection threshold correlates with GABA along the same axis in both cohorts, but dissociations exist in the adaptation and frequency discrimination outcomes. MRS of GABA suffers from limited spatial resolution (all GABA within a large region is measured), so while our tactile tasks, and their basis in the animal literature, might allow for a discussion of specific GABAergic mechanisms (e.g., feed-forward inhibition), a direct link between MRS-GABA and synaptic GABA levels cannot be drawn. The GABA levels measured by MRS likely reflect inhibitory tone or "the ability of the system to inhibit." It is more likely that TDC and ASD differ in specific genetic or receptor functions, leading to changes in GABAergic processes.

The current measurements of GABA are limited by the fact that edited MRS of GABA has a low signal-to-noise [Mullins et al., 2014) so that the regional specificity of our measurements is limited to a voxel that includes both S1 and M1. In addition, the GABA signal is contaminated by co-edited macromolecular signal (MM) [Mullins et al., 2014] to the order of 50%

[Harris, Puts, Barker, & Edden, 2015c]. While there are techniques that remove MM signal from GABA spectra, these techniques are more susceptible to frequency drift and motion [Harris et al., 2015c] than the GABA+ method, limiting their applicability to pediatric cohorts. In the current study, a typical MEGA-PRESS acquisition was used to retain sufficient SNR [Mullins et al., 2014]. Two slightly different MRS acquisitions were used during the study, albeit with the same pulses and editing parameters. In the current study, no differences between acquisitions were found and while we believe these are interchangeable under circumstances without excessive motion, such as in the case of children with normal IQ and relatively mild ADOS scores as in the current study, the second acquisition with better interleaving and an internal water reference allows for better post-hoc corrections and will perform better in more difficult, e.g., pediatric, studies, for instance in children with more severe autism and/or lower IQ.

A common limitation in studying pediatric neurodevelopmental cohorts are effects of comorbid diagnosis and medication. The most common comorbidity in this cohort is ADHD and it has previously been show that children with ADHD also show reduced GABA levels [Bollmann et al., 2015; Edden, Crocetti, Zhu, Gilbert, & Mostofsky, 2012]. In the current study, there was no clear difference in GABA levels between children with ASD with and without comorbid ADHD. Other measures may provide better discriminatory power to assess differences between these groups. All children were refrained from stimulant medication prior to the experiments, but were allowed to take other medications. While this study is underpowered to determine potential medication effects, it is possible that medications affect GABA levels, and possibly behavioral performance, to some degree and larger studies are therefore needed to examine the impact of medication status.

In summary, we showed that children with ASD have significantly reduced SM1 GABA levels compared to TDC. Further, we show that GABA levels correlated with dynamic detection threshold as well as with the effect of sub-threshold stimulation. GABA levels also correlated with amplitude discrimination after adaptation (an effect absent in autism) and frequency discrimination in TDC, but not in children with autism. These data suggest that cortical inhibition at least in the somatosensory cortex, and especially inhibition related to the synchronization and processing of incoming sensory information, is altered in ASD, and is reflected by a bulk reduction in brain GABA levels. This may reflect behavioral abnormalities in filtering of tactile information and impaired habituation of repetitive stimulation. The altered in vivo GABA levels might therefore explain abnormal tactile information processing in autism. The

GABA system may be a future target for therapies that can alleviate these symptoms.

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M. Tommerdahl discloses that he is cofounder of Cortical Metrics that built and provided the stimulator used in these experiments and that he receives royalties through this.

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