

SHORT REPORT**GABA and Glx predict EEG responses of visual sensitivity in autism**Laurie-Anne Sapey-Triomphe^{1,2} | Nicolaas A. J. Puts^{3,4} | Thiago L. Costa^{1,5} | Johan Wagemans¹¹Department of Brain and Cognition, Leuven Brain Institute, KU Leuven, Leuven, Belgium²Université Claude Bernard Lyon 1, CNRS, INSERM, Centre de Recherche en Neurosciences de Lyon CRNL U1028 UMR5292, CAP Team, Bron, France³Department of Forensic and Neurodevelopmental Sciences and the Institute of Psychiatry, Psychology, and Neuroscience, Sackler Institute for Translational Neurodevelopment, King's College London, London, UK⁴MRC Centre for Neurodevelopmental Disorders, King's College London, London, UK⁵Department of Neurosciences, Biomedicine and Movement Sciences, University of Verona, Verona, Italy**Correspondence**Laurie-Anne Sapey-Triomphe, Department of Brain and Cognition, Leuven Brain Institute, KU Leuven, Leuven 3000, Belgium.
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Abstract

The mechanisms underlying atypical sensory processing in autism remain to be elucidated, but research points toward a role of the glutamatergic/GABAergic balance. To investigate the potential relationships between visual sensitivity and its molecular correlates in autism, we combined data from electroencephalography (EEG) and magnetic resonance spectroscopy (MRS) studies. Twenty autistic adults and sixteen neurotypical adults (NT) participated in both an EEG study assessing visual sensitivity (Sapey-Triomphe et al., *Autism Research*, 2023) and in an MRS study measuring Glx and GABA+ concentrations in the occipital cortex (Sapey-Triomphe et al., *Molecular Autism*, 2021). These studies revealed no group differences in neural detection thresholds or in Glx/GABA levels in the occipital cortex. Neural detection thresholds for contrast and spatial frequency (SF) were determined using fast periodic visual stimulations and neural frequency tagging. In the present study, Glx/GABA+ concentrations in the occipital cortex and neural detection thresholds did not differ between groups. Interestingly, lower Glx/GABA+ ratios were associated with lower contrast detection thresholds and higher SF detection thresholds. These correlations were also significant within the neurotypical and autistic groups. This report suggests that the Glx/GABA balance regulates visual detection thresholds across individuals. In both autistic and NTs, lower Glx/GABA ratios in the occipital cortex allow for better detection of visual inputs at the neural level. This study sheds light on the neurochemical underpinnings of visual sensitivity in autism and warrants further investigation.

Lay Summary

Visual sensitivity is atypical in autism, but its underlying neural and molecular mechanisms remain relatively unknown. Here, we demonstrate a relationship between the brain's ability to detect a visual information and the concentrations of two key neurotransmitters. Interestingly, this relationship was observed in both neurotypical and autistic adults, suggesting an overall intact role of these neurotransmitters in processing low-level visual information in autism.

KEYWORDS

autism, electroencephalography, GABA, glutamate, magnetic resonance spectroscopy, sensitivity, vision

Abbreviations: AUT, autism; cpd, cycles per degree; EEG, electroencephalography; GABA, gamma-aminobutyric acid; Glx, glutamate and glutamine; MRS, magnetic resonance spectroscopy; NT, neurotypical; SF, spatial frequency.

INTRODUCTION

In recent years, there has been a growing interest in the sensory specificities encountered in autism, given their high prevalence and significant impact. Elucidating the

underpinnings of sensory processing in autism is challenging, but fundamental, as it holds the potential to improve interventions and diagnostic approaches.

At a neurobiological level, atypical sensory processing in autism may be related to an increased excitatory/inhibitory ratio (Dickinson et al., 2016; Rubenstein & Merzenich, 2003). This ratio depends on complex interactions and involves the main excitatory and inhibitory neurotransmitters, namely glutamate and gamma-aminobutyric acid (GABA). The cortical balance of excitation to inhibition plays a central role in detecting and discriminating stimuli (Rubenstein & Merzenich, 2003). In particular, GABAergic inhibition improves perceptual performance by decreasing variability or noise, thereby suppressing irrelevant signals while enhancing the representation of relevant neural information (Hammett et al., 2020). In the visual cortex, lateral inhibition mediated by GABA plays a key role in enhancing visual contrast or sharpening orientation detection (Edden et al., 2009; Sillito, 1975). As a consequence, an imbalance in favor of an increased excitatory-to-inhibitory ratio could impair perception.

Autism is characterized by alterations in neurometabolite concentrations in several brain regions, with a tendency toward decreased GABA and increased Glx (i.e., glutamate and glutamine) levels (Du et al., 2023). In autism, whereas GABA concentration is decreased in the somatosensory cortex and associated with atypical tactile sensitivity (Puts et al., 2016; Sapey-Triomphe et al., 2019), GABA and Glx concentrations are typical in the occipital cortex (Du et al., 2023; Puts et al., 2016; Sapey-Triomphe et al., 2019) despite atypicalities in self-reported visual sensitivity (Sapey-Triomphe et al., 2023; Simmons et al., 2009). Yet, a binocular rivalry study suggested that GABAergic action in the occipital cortex might be disrupted in autism (Robertson et al., 2016). These studies rely on magnetic resonance spectroscopy (MRS), which allows a noninvasive *in vivo* quantification of metabolites in pre-defined brain regions.

Interestingly, 16 neurotypical and 20 autistic adults (AUTs) who participated in our MRS study (Costa et al., *n.d.*; Sapey-Triomphe et al., 2021) measuring occipital Glx and GABA levels also participated in our electroencephalography (EEG) study assessing visual sensitivity (Sapey-Triomphe et al., 2023). We considered this overlap of datasets as a great opportunity to investigate the relationships between neural visual detection thresholds and their neurobiological correlates. In our EEG study, participants were presented with fast periodic visual stimulations that were initially imperceptible due to low contrast or high spatial frequency (SF), but became progressively visible (Sapey-Triomphe et al., 2023). As the stimuli became more visible, a steady-state response emerged, which allowed implicitly determining the contrast and SF thresholds at which the visual cortex detected these stimuli (Hempton et al., 2018; Sapey-Triomphe et al., 2023). Our study did not reveal any group differences in neural thresholds

between neurotypical (NT) and autistic individuals (Sapey-Triomphe et al., 2023). Yet, self-reported sensitivity was increased in autistic participants, as well as behavioral sensitivity for SF but not for contrast (Sapey-Triomphe et al., 2023). These heterogeneous results suggest that visual sensitivity, as addressed with detection thresholds for contrast and SF (a particularly sensitive probe of early and low-level processing), might not simply be increased in autism, but may depend on additional factors (Sapey-Triomphe et al., 2023).

Here, we aimed to investigate whether Glx/GABA ratios in the occipital cortex were correlated with EEG visual detection thresholds in autistic and NT adults, based on data from our previous studies (Sapey-Triomphe et al., 2021; Sapey-Triomphe et al., 2023). As sensory signals are primarily conveyed through glutamatergic signaling, and GABA reduces intrinsic noise via lateral inhibition (Hammett et al., 2020), we hypothesized that a relative increase in inhibitory processes would correlate with improved detection sensitivity. Specifically, lower Glx/GABA ratios within the visual cortex should be associated with lower contrast and higher SF detection thresholds. As there were no group differences in neural detection thresholds (Sapey-Triomphe et al., 2023) and in metabolite levels (in our previous study (Sapey-Triomphe et al., 2021) and in the literature (Du et al., 2023; Puts et al., 2016; Sapey-Triomphe et al., 2019)), we predicted similar trends in both groups. Yet, we acknowledged the possibility of inefficient GABAergic action in autism (Robertson et al., 2016), which would result in weaker or nonexistent correlations in autistic individuals.

METHODS

Participants

Twenty autistic and sixteen NT adults participated in both an EEG study assessing visual detection thresholds (Sapey-Triomphe et al., 2023) and an MRS study measuring Glx and GABA+ concentrations in the visual cortex (Costa et al., *n.d.*; Sapey-Triomphe et al., 2021). The two groups did not differ in age (NT: 31.2 ± 6.5 years old, AUT: 31.7 ± 10.5 years old; $p = 0.87$), sex ratio (NT: 11 male, AUT: 11 male; $p = 0.28$), nor total intelligence quotient (NT: 108.9 ± 14.6 , AUT: 110.8 ± 17.3 ; $p = 0.76$) assessed by the Wechsler Adult Intelligence Scale IV (WAIS-IV, Wechsler, 2008). Among these 36 participants, one NT and three autistic participants had poor Glx fits, and one autistic participant had a poor GABA fit. Therefore, the analyses of Glx/GABA+ ratio include 31 participants, comprising 15 neurotypical, and 16 autistic participants.

Autistic participants received their diagnoses from a multidisciplinary Expertise Center for Autism. Inclusion criteria included being between 18 and 55 years old, reporting normal or corrected-to-normal vision, and

scoring above 70 at the WAIS-IV. None of the NT reported being diagnosed with a psychiatric or neurological disorder or being under current neuropsychiatric medication. The time between the MRS and EEG acquisitions ranged between 3 and 12 months. This time gap did not differ between groups and did not correlate with the EEG or MRS measurements.

The studies were approved by the medical Research Ethical Committee of the University Hospital UZ/KU

Leuven (Belgium). All participants provided written informed consent before starting the experiment.

EEG data

Detection thresholds in contrast and SF were implicitly measured using EEG (Figure 1a), as in (Hemtinne et al., 2018). The description of the paradigm, data

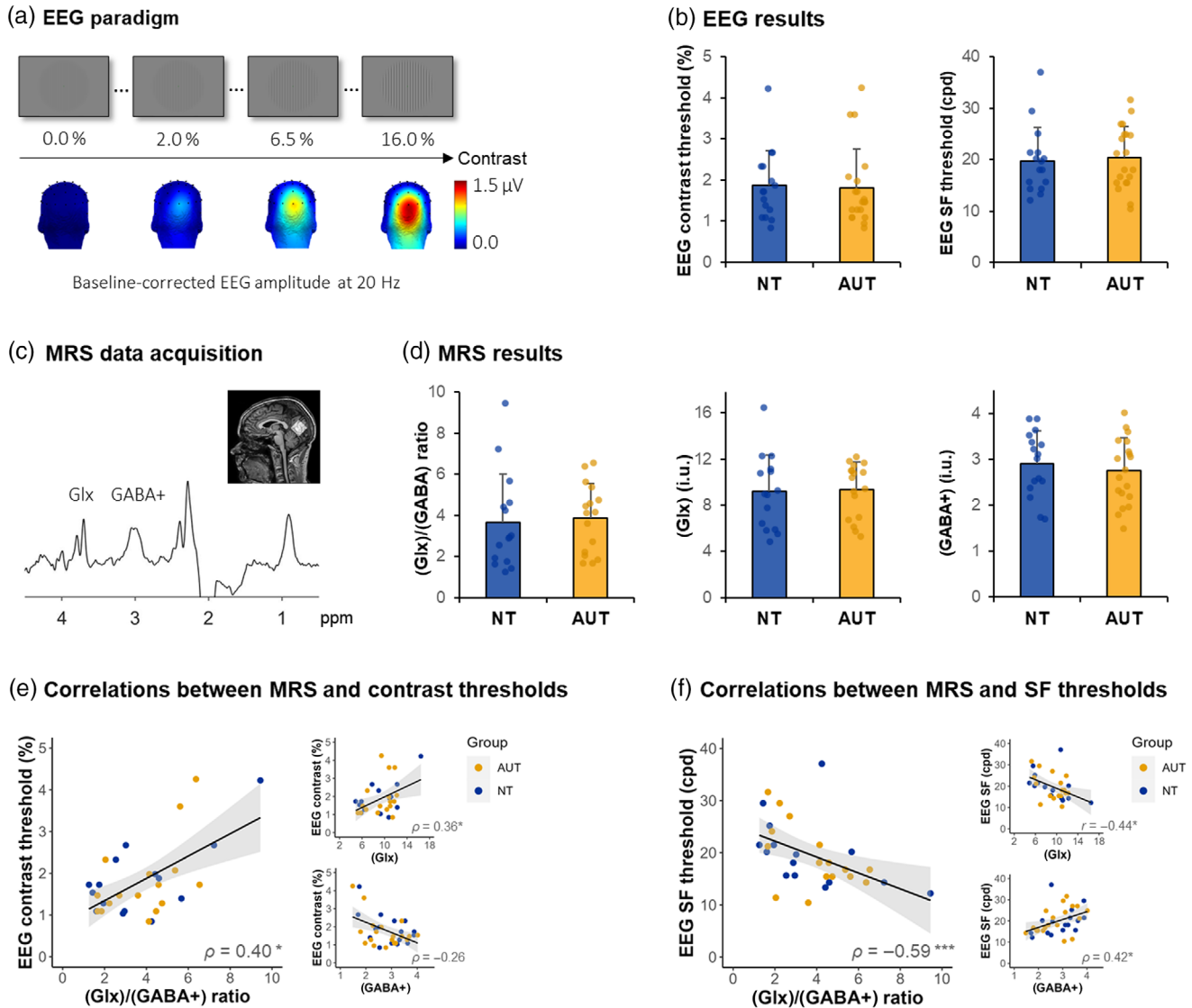


FIGURE 1 Electroencephalography (EEG) and magnetic resonance spectroscopy (MRS) results in the neurotypical (NT) and autistic (AUT) groups. (a) Example of trial used in the EEG paradigm (here, where contrast-reversing gratings increased in contrast). The gratings could gradually increase or decrease in contrast or spatial frequency (SF) during a trial (20 s long). The fast-periodic visual stimulation triggered a steady-state response at 20 Hz in the visual cortex, as shown with the topography of the NT group (see Sapey-Triomphe et al., (2023) for more details). (b) Mean detection threshold in contrast (left, values in percentage of Michelson contrast) and in SF (right, in cycles per degree—cpd), determined from the baseline-corrected EEG amplitude at 20 Hz in the occipital cortex. (c) Example of MRS voxel ($3 \times 3 \times 3 \text{ cm}^3$) localization centered over the medial occipital cortex and of MR spectrum (see Sapey-Triomphe et al., (2021) for more details). (d) MRS results: Glx/GABA+ ratio (left), Glx concentration (middle), and GABA+ concentration (right), given in institutional units (i.u.). (e) Correlations between EEG detection thresholds in contrast and Glx/GABA+ ratios, Glx, and GABA concentrations. Within-group correlations with Glx/GABA+ ratios: NT: $\rho = 0.40$ and AUT: $\rho = 0.50^*$. (f) Correlations between EEG detection thresholds in SF and Glx/GABA+ ratios, Glx, and GABA concentrations. Within-group correlations with Glx/GABA+ ratios: NT: $\rho = -0.66^{**}$ and AUT: $r = -0.56^*$. Note that gratings are more difficult to perceive at a low contrast or at a high SF, which explains the opposite direction of the correlations. Pearson correlation tests were used for normally distributed data (r values), whereas Spearman correlation tests were used otherwise (ρ values). Error bars indicate standard deviations. $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$.

acquisition and analyses are given in (Sapey-Triomphe et al., 2023) and are summarized below.

Participants were presented with vertical sinewave pattern-reversing gratings (10 Hz). In 24 trials, the SF was set at 1.5 cycles per degree (cpd), whereas the contrast increased (or decreased) from 0.10% to 16%. In 24 trials, the contrast was set at 30%, whereas the SF increased (or decreased) from 2.7 to 40 cpd.

EEG data were acquired using a BioSemi ActiveTwo amplifier system with 64 electrodes. EEG data were analyzed using Letswave 6 (<https://letswave.cn>) and Matlab 2020b. Seven channels of interest (Iz, Oz, POz, O1, O2, PO7, and PO8) were averaged. Given the pattern reversal nature of the grating stimulus (20 reversals per second), we assessed the amplitude of the steady-state response at 20 Hz in the visual cortex for each contrast or SF level. The significance threshold was set at $Z > 2.33$ (i.e., $p < 0.01$ under a one-tailed hypothesis) as in a study in NT using the same design (Hempton et al., 2018). It allowed assessing whether the EEG amplitude at each epoch was above noise level, in order to determine detection thresholds in contrast and SF (Sapey-Triomphe et al., 2023).

MRS data

The MRS acquisition and analysis details are given in (Sapey-Triomphe et al., 2021) and summarized here. Single voxel MR spectra were acquired in the occipital cortex, placed in the medial of the coronal slice, dorsal to the cerebellum (Figure 1c). MR spectra were acquired using the Hadamard Encoding and Reconstruction of MEGA-Edited Spectroscopy (HERMES) sequence (Chan et al., 2016; Chan et al., 2019; Saleh et al., 2016). MRS spectra were analyzed using Gannet 3.1 (Edden et al., 2014), implemented in Matlab 2020b. GABA+ (i.e., GABA and co-edited macromolecules) and Glx concentrations were quantified relative to the unsuppressed water signal, and corrected for tissue fractions with $\alpha = 0.5$. Metabolite concentrations are given in institutional units (i.u.). The MRS quality metrics and tissue fractions are given as Table S1.

Statistical analyses

All the results are presented as Mean (\pm standard deviation). EEG thresholds and MRS concentrations were compared between groups using Student *t*-tests, and effect sizes are reported as Cohen's *d* (i.e., $d = 0.01$: very small, $d = 0.20$: small, $d = 0.50$: medium, and $d = 0.80$: large (Cohen, 1988)). The normality of the data distribution was assessed using Shapiro–Wilk tests. Correlations were assessed using Pearson correlation tests when the data were normally distributed, and *r*-values were reported ($r = 0.10$: small, $r = 0.30$: medium, and

$r = 0.50$: large effect size). When one of the variables was not normally distributed, Spearman correlation tests were used and ρ were reported. Statistical analyses were performed using R (version 4.3.1).

RESULTS

EEG results

Consistently with Sapey-Triomphe et al., (2023), there were no group differences in EEG contrast detection thresholds (NT: $1.9\% \pm 0.9$; AUT: $1.8\% \pm 1.0$; $t(34) = 0.18$, $p = 0.85$, $d = 0.06$), nor in EEG SF detection thresholds (NT: $19.8 \text{ cpd} \pm 6.5$; AUT: $20.4 \text{ cpd} \pm 6.1$; $t(34) = 0.32$, $p = 0.75$, $d = 0.11$) (Figure 1b).

MRS results

In line with Sapey-Triomphe et al., (2021), the Glx/GABA+ ratios did not differ between groups (NT: 3.7 ± 2.3 ; AUT: 3.9 ± 1.7 ; $t(29) = 0.26$, $p = 0.80$, $d = 0.09$, Figure 1d). There were no group differences in Glx concentrations (NT: $9.2 \text{ i.u.} \pm 3.2$; AUT: $9.4 \text{ i.u.} \pm 2.3$; $t(30) = 0.23$, $p = 0.82$, $d = 0.08$), nor GABA+ concentrations (NT: $2.9 \text{ i.u.} \pm 0.7$; AUT: $2.7 \text{ i.u.} \pm 0.7$, $t(33) = 0.67$, $p = 0.50$, $d = 0.23$).

Correlations between MRS and EEG results

Glx/GABA+ ratios and EEG detection thresholds were positively correlated for contrast ($\rho = 0.40$, $p = 0.025$, Figure 1e) and negatively correlated for SF ($\rho = -0.59$, $p < 0.001$, Figure 1f). In other words, being visually more precise, that is, perceiving gratings at a low contrast or high SF, was associated with having a lower Glx/GABA+ ratio in the visual cortex.

These correlations were also significant within-group for SF thresholds in NT ($\rho = -0.66$, $p = 0.008$) and AUT ($r = -0.56$, $p = 0.025$), and for contrast thresholds in AUT ($\rho = 0.50$, $p = 0.048$) but not NT ($\rho = 0.40$, $p = 0.14$).

To further explore whether these correlations were driven by Glx or GABA+, we assessed correlations per metabolite. Glx levels were correlated with EEG thresholds for contrast ($\rho = 0.36$, $p = 0.041$) and SF ($r = -0.44$, $p = 0.011$), that is, lower Glx levels were associated with more precise perception. GABA+ levels were correlated with EEG thresholds for SF ($r = 0.42$, $p = 0.012$), that is, higher GABA levels were associated with more precise perception. GABA levels were not significantly correlated with EEG thresholds for contrast ($\rho = -0.26$, $p = 0.14$).

After applying false discovery rate (FDR) corrections for multiple comparisons (i.e., correlations with contrast

and SF thresholds), all correlations that were initially significant remained significant.

DISCUSSION

To investigate the neurobiological underpinnings of visual sensitivity in autism, we combined data from recent MRS (Sapey-Triomphe et al., 2021) and EEG (Sapey-Triomphe et al., 2023) studies. We showed that lower Glx/GABA+ ratios in the visual cortex were associated with lower contrast and higher SF detection thresholds assessed with EEG. These correlations were statistically significant for both variables (contrast and SF) in the expected directions, even though they relied on two different acquisition techniques (MRS and EEG) and data collected months apart. These findings highlight the central role of basal Glx and GABA concentrations in the occipital cortex in explaining inter-individual differences in visual sensitivity. This explanation applies to both autistic and NT adults.

Interestingly, lower Glx and higher GABA levels were associated with better detection of visual stimuli at the neural level. A decrease in the excitatory/inhibitory ratio may have contributed to finely tuning functions in the visual cortex, and allowing a neural response above noise level. The discrimination of the stimuli may have been facilitated through a process of divisive normalization, which is a computation that divides a neuron's activity by the activity of the neuronal population in which it is embedded (Rosenberg et al., 2015).

Furthermore, both within-group correlations were significant, suggesting similar neurochemical underpinnings in neurotypical and autistic individuals for detecting this kind of visual stimuli. Unlike binocular rivalry in autism (Robertson et al., 2016), this suggests that GABAergic action is not disrupted in autism for detecting low-level visual stimuli. It also points toward typical processing of early visual inputs in autism, whereas differences might occur at later stages of processing, which could explain certain specificities in visual sensitivity in autism.

We acknowledge the relatively small sample size, but we highlight that it allowed us to find the expected correlations across and within-groups. Concerning the EEG data, there were no group differences in visual areas, but we cannot exclude that differences might exist in other networks or would not have been detected with this method. Finally, a limitation of MRS studies is that GABA might be co-edited with macromolecules, whereas glutamate is co-edited with glutamine. MRS provides information on metabolite concentrations, but differences may exist at other levels (e.g., receptor density or functioning, organization of local neuronal networks, etc.).

In conclusion, we demonstrated relationships between Glx/GABA+ ratios and detection thresholds in the visual cortex, which did not differ between neurotypical and autistic adults. These findings suggest that early visual

processing relying on this balance is typical in autism. Our approach combined neural and molecular investigations of visual sensitivity and relied on robust and implicit methods that could be applied to different populations (i.e., nonverbal individuals). These results call for further investigations in other sensory modalities, particularly in those where the sensory cortex shows reduced GABA levels in autism, as it should lead to reduced neural selectivity. Using larger samples will be needed to replicate these findings and extend them to the endophenotypes of autism. Further investigations into these neuromolecular correlates of sensory processing in autism could contribute to alleviating the impact of sensory symptoms.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Zenodo at <https://doi.org/10.5281/zenodo.10683141>.

ETHICS STATEMENT

This study was approved by the medical Research Ethical Committee UZ/KU Leuven (Research projects S61857 and S63024). Participants provided written informed consent before the start of the experiment, in accordance with the Declaration of Helsinki.

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SUPPORTING INFORMATION

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