BRAIN COMMUNICATIONS

Epileptiform discharges relate to altered functional brain networks in autism spectrum disorders

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Many individuals with autism spectrum disorders have comorbid epilepsy. Even in the absence of observable seizures, interictal epileptiform discharges are common in individuals with autism spectrum disorders. However, how these interictal epileptiform discharges are related to autistic symptomatology remains unclear. This study used magnetoencephalography to investigate the relation between interictal epileptiform discharges and altered functional brain networks in children with autism spectrum disorders. Instead of particularly addressing individual brain regions, we specifically examine network properties. For this case-control study, we analysed 70 children with autism spectrum disorders (52 boys, 18 girls, 38–92 months old) and 19 typically developing children (16 boys, 3 girls, 48–88 months old). After assessing the participants' social reciprocity using the Social Responsiveness Scale, we constructed graphs of functional brain networks from frequency band separated task-free magnetoencephalography recordings. Nodes corresponded to Desikan–Killiany atlas-based 68 brain regions. Edges corresponded to phase lag index values between pairs of brain regions. To elucidate the effects of the existence of interictal epileptiform discharges on graph metrics, we matched each of three pairs from three groups (typically developing children, children with autism spectrum disorders who had interictal epileptiform discharges and those who did not) in terms of age and sex. We used a coarsened exact matching algorithm and applied adjusted regression analysis. We also investigated the relation between social reciprocity and the graph metric. Results show that, in children with autism spectrum disorders, the average clustering coefficient in the theta band was significantly higher in children who had interictal epileptiform discharges. Moreover, children with autism spectrum disorders who had no interictal epileptiform discharges had a significantly lower average clustering coefficient in the theta band than typically developing children had. However, the difference between typically developing children and children with autism spectrum disorder who had interictal epileptiform discharges was not significant. Furthermore, the higher average clustering coefficient in the theta band corresponded to severe autistic symptoms in children with autism spectrum disorder who had interictal epileptiform discharges. However, the association was not significant in children with autism spectrum disorders who had no interictal epileptiform discharge. In conclusion, results demonstrate that alteration of functional brain networks in children with autism spectrum disorders depends on the existence of interictal epileptiform discharges. Interictal epileptiform discharges might 'normalize' the deviation of altered brain networks in autism spectrum disorders, increasing the clustering coefficient. However, when the effect exceeds tolerance, it actually exacerbates autistic symptoms.

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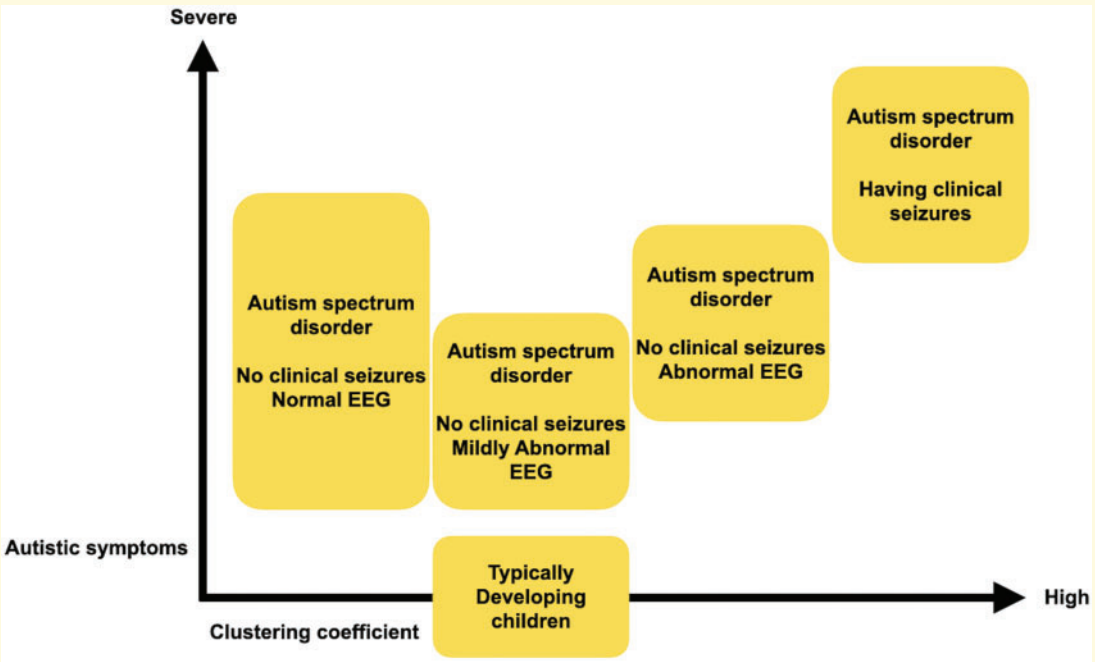
Abbreviations: ASD− =autism spectrum disorder with no interictal epileptiform discharge; ASD+ =autism spectrum disorder with interictal epileptiform discharge; *C* =average clustering coefficient; CEM =coarsened exact matching; IED =interictal epileptiform discharge; K-ABC =Kaufman Assessment Battery for Children; *L* =average shortest path length; MEG =magnetoencephalomagnetoencephalography; PLI =phase lag index; SRS =Social Responsiveness Scale; SW= small world-ness; SWN =Small-World Network; TD children =typically developing children

Graphical Abstract



Introduction

Autism spectrum disorder, a neurodevelopmental syndrome, is characterized by impaired social cognition and communication as well as repetitive or obsessive behaviour and interests.[1] Signs of autism spectrum disorder emerge early in life, typically during infancy. Whereas symptoms might change over the course of development, autism spectrum disorder is generally considered a chronic, lifelong condition with no known spontaneous remission. Furthermore, its prevalence is increasing constantly.[2] People with autism spectrum disorder require intensive support. Investigating its biological underpinnings is becoming increasingly important to establish tools for early diagnosis and for effective intervention.

In addition to social impairment, many people with autism spectrum disorder have co-occurring epilepsy. As many as 5–46% of children with autism spectrum disorder develop epilepsy. That prevalence is much higher than in typically developing (TD) children, which is only 2–7%.[3–5] Also, 32% of patients with epilepsy meet the diagnostic criteria of autism spectrum disorder.[6] That association has attracted attention: abnormal neurophysiological activities are often investigated in individuals with autism spectrum disorder. In this context, electroencephalography and magnetoencephalography (MEG) are used to visualize those abnormal brain activities. Such abnormal activities include interictal epileptiform discharges (IEDs), defined as sharp waves or complex peak waves that occur in the absence of observable changes in behaviour. It is particularly

interesting that 6.7–50% of patients with autism spectrum disorder show IEDs in their EEG,[7,8] even in the absence of co-occurring epilepsy. This rate is much higher than that of TD individuals, which is 1–4%.[9,10]

The limited available information related to association between autistic symptomatology and IEDs is complicated.[11–16] Our earlier study[11] investigated the association between IEDs and cognitive function in children with and without autism spectrum disorder who did not have epilepsy. For that study, after we recruited 163 TD children (84 boys, 79 girls, 32–89 months old) and 107 children with autism spectrum disorder (85 boys, 22 girls, 36–98 months old), we evaluated the association between their numbers of IEDs (per unit time) and their level of intelligence. Regarding the TD group, we observed that higher numbers of IEDs corresponded to lower intelligence. However, the opposite relation was found for the autism spectrum disorder group. In the group, higher numbers of IEDs corresponded to higher intelligence. For a subsequent study, we recruited 40 TD children and 26 children with autism spectrum disorder in the similar age range. Results demonstrated that higher numbers of IED corresponded to better sociality.[12] Hartley-McAndrew and Weinstock[15] reported similar results, but Milovanovic et al.[13] reported that the association was not significant. Other reports described an opposite association (i.e. existence of EEG abnormalities correlated with severe autistic symptoms).[14,16] Although association between IED and either intelligence or autistic symptoms remains unclear, it is noteworthy that large-scale studies consistently show the existence of epilepsy (i.e. observable clinical seizures) as associated with severe autistic symptoms. For instance, after Yasuhara[17] investigated 1064 children with autism spectrum disorder, the author reported that epilepsy is more likely to occur among children with autism who also have lower intelligence. More recently, after Ewen et al.[18] examined 6975 children with autism spectrum disorder for their large-scale cohort study, they reported that severe autism spectrum disorder symptoms is an independent risk factor of epilepsy. Combining those results from earlier reports of the literature reveals that severe autistic symptoms are likely to be associated with epileptic discharges causing observable seizures (possibly representing severer forms of epileptic activity). However, epileptic discharges in the absence of clinical seizures (possibly representing milder forms of epileptic activity) might or might not be beneficial for autistic symptoms. The present study investigated the neurological underpinnings of this association using MEG.

In the field of brain imaging, researchers have specifically emphasized the characterization of the functions of individual brain regions. However, accumulating evidence suggests that autism spectrum disorder is a disorder of abnormal brain connectivity, i.e. dysfunction in coordination over widely distributed brain regions.[19] Brain connectivity is a multi-faceted concept,[20] but in the field of MEG or EEG, connectivity usually refers to statistical dependencies that have been identified between time series of electromagnetic signals from different brain regions.[21] This 'functional' connectivity has been inferred as arising from, for example, the synchronization of presynaptic potentials in a set of neurons, which enhances their effect on postsynaptic neurons in targeted areas.[22] In terms of brain connectivity, a popular hypothesis is that autism spectrum disorder is one of long-range underconnectivity combined with local overconnectivity.[23] However, in light of the brain's inherent complexity, any hypothesis based on such a simple measure (i.e. mean of the strength of connectivity) might be an oversimplification. To meet this challenge and to describe the properties of complex networks on a large scale, the field of neuroscience has provided network theory.[24]

Using network theory, also designated as graph theory, one can describe properties of a given complex system using the same parameters, irrespective of their constituent elements.[25] Particularly in the field of EEG/MEG, we consider the complex networks of brain regions, in which brain region activity is expressed as electrophysiological signals. Brain regions are mutually interacting over the course of time. Within the framework of graph theory, such a complex network is reduced to a set of nodes and edges called a 'graph'.[26] Nodes represent brain regions. Edges represent functional connectivity linking any pair of nodes. The information included in the graph can be summarized further using various measures. Well-established measures include the mean clustering coefficient (C) and average shortest path length (L). These derive originally from statistical physics, although they have been used widely in the field of neuroscience during recent decades. C represents the tendencies of nodes to form local clusters. In networks of the brain, higher C indicates functionally segregated information processing.[27] L stands for the average shortest 'distance' between two nodes (i.e. number of edges to cross to get from one node to another), which indicates integration of information from remote brain regions.[27] A graph having high C and short L is called a 'small-world network (SWN)'. Conceptually, SWN is somewhere between an ordered (high C and long L) and a random (low C and short L) network.[28] In this sense, SWN is well-connected both locally and globally. For that reason, it is regarded as a balance of integration and segregation. It is particularly interesting for our purposes that the existing literature suggests that a healthy human brain network possesses SWN properties,[26,29] which have been attributed to evolutionary processes gradually culminating in an optimal balance between cost and efficiency.[26] Furthermore, reports of some reports have described that brain networks deviate from the SWN property when affected by some neurological diseases such as Alzheimer's disease,[30] depression[31] and schizophrenia.[32] In people affected by those disorders, C and L are reportedly smaller than in healthy controls.

Many EEG/MEG researchers have examined interictal brain networks in patients with epilepsy. Their results suggest that the interictal epileptic brain network is also

deviated from the SWN property, but in the opposite direction. *C* in epilepsy is reportedly larger than in healthy controls. Nevertheless, reports describe conflicting results for *L*. For instance, Pegg et al.[33] reported in their systematic review that studies of idiopathic generalized epilepsy tend to report higher *C* in lower frequency bands and decreased *L* in the beta band. Particularly, among four studies of idiopathic generalized epilepsy brain network in which *C* was evaluated,[34–37] two studies demonstrated increased *C* in the theta[34] and extended alpha (5–15 Hz) bands[36] in patients with idiopathic generalized epilepsy compared to healthy controls. The other two studies found no significant difference. In terms of *L*, among three studies of idiopathic generalized epilepsy[34,35,37] that evaluated *L*, results of only one study[35] indicated a decreased *L* in the beta band. The other two found no significant difference. Higher *C* in lower frequency bands was also reported for patients with focal epilepsy, but here also the results for *L* were conflicting. Particularly, Horstmann et al.[38] reported higher *C* and increased *L* in patients with epilepsy than in healthy controls. The difference was most evident in the delta band, but was observed in other frequency bands, except for the alpha band. Quraan et al.[39] reported higher *C* in the theta band and decreased *L* in the alpha band. Also, Wang and Meng[40] found higher *C* in the theta and beta bands and increased *L* in alpha and theta bands for patients with epilepsy. Although it is difficult to compare these results directly because of methodological differences such as sample size, participant characteristics, imaging modalities (EEG/MEG), measures of synchronization, properties of graphs (weighted or unweighted), behavioural condition (eyes-closed or eyes-open), a consensus holds that interictal brain networks in focal or generalized epilepsy show higher *C* in lower frequency bands. The results for *L* are inconsistent depending on the experimental conditions. A possible exception is benign epilepsy with centrotemporal spikes. Three studies particularly addressing patients with benign epilepsy with centrotemporal spikes consistently showed lower *C* in the theta and other frequency bands for the patient group.[41–43]

Although few MEG/EEG studies of brain networks of autism spectrum disorder have been reported, evidence suggests that brain networks in autism spectrum disorder are also deviated from the SWN property, but in a different direction. The first report was that of a study by Pollonini et al.[44] They investigated young adults with high function autism spectrum disorder and reported lower *C* and increased *L* (in broadband signal) in an autism spectrum disorder group than in healthy controls. Tsiaras et al.[45] reported a non-significant trend in the same direction for adolescents with autism spectrum disorder. For a subsequent study in which adults with autism spectrum disorder were recruited, Barttfeld et al.[46] reported lower *C* (in the theta combined with alpha band, and beta band) and increased *L* (in beta band). Furthermore, Boersma et al.[47] analysed children with autism spectrum disorder and reported lower *C* (in the theta, alpha and beta bands) and increased *L* (in the beta band and broadband) compared to healthy controls. It is noteworthy that one study including children with autism spectrum disorder, but not explicitly excluding children with autism spectrum disorder having epilepsy, found no significant difference in either *C* or *L* in an autism spectrum disorder group[48] compared to healthy controls. Similarly, after Takahashi et al.[49] analysed children with autism spectrum disorder, but without excluding children who had epilepsy, they reported that neither the difference in *C* nor in *L* was significant. Here again, although direct comparison of these results is difficult, the existing literature suggests that brain networks of autism spectrum disorder show lower *C* and increased *L* over widely various frequencies in a broad age range, but they might depend on the presence of epilepsy. A notable exception is a report of a study conducted by Ye et al.[50] Based on their examination of autism spectrum disorder in adolescents with IQ higher than 65 and without epilepsy, they reported higher *C* and shorter *L* in the theta band.

This study was designed to assess the role of IEDs on functional brain networks in children with autism spectrum disorder in relation to autistic symptoms. Based on the evidence presented above, we speculated that IEDs can 'normalize' altered functional brain networks in children with milder autism spectrum disorder by increasing *C*. Furthermore, the relation between *C* and autistic symptoms can be an inverted U shape (i.e. excessively higher *C*, represented as having clinical seizures, corresponding to severe autistic symptoms) (Fig. 1). We constructed functional brain networks using MEG for three groups of young children: children with autism spectrum disorder who have IEDs but who have no observable epilepsy, children with autism spectrum disorder who have no IED, and TD children. We hypothesized the following: (i) In children with autism spectrum disorder who have no IED, *C* is lower than in TD children and children with autism spectrum disorders who have IEDs. (ii) In children with autism spectrum disorder who have IEDs, higher *C* corresponds to severe autistic symptoms. In children with autism spectrum disorder who have no IED, higher *C* corresponds to milder autistic symptoms. We also analysed *L* and small world-ness (*SW*, adjusted ratio of *L* and *C*) for completeness, but we formulated no particular hypothesis for those measures because the results for *L* in earlier studies are inconsistent. In addition, strictly speaking, paths in graphs of functional networks merely represent sequences of statistical associations. Therefore, paths might not correspond to information flow, and consequently, might be less straightforward to interpret.[27]

Among imaging modalities, MEG has great merit for this study. First, MEG reportedly has higher sensitivity for IEDs than EEG does.[51] Second, MEG has higher spatial resolution than EEG, which is a great merit when measuring functional connectivity because higher spatial

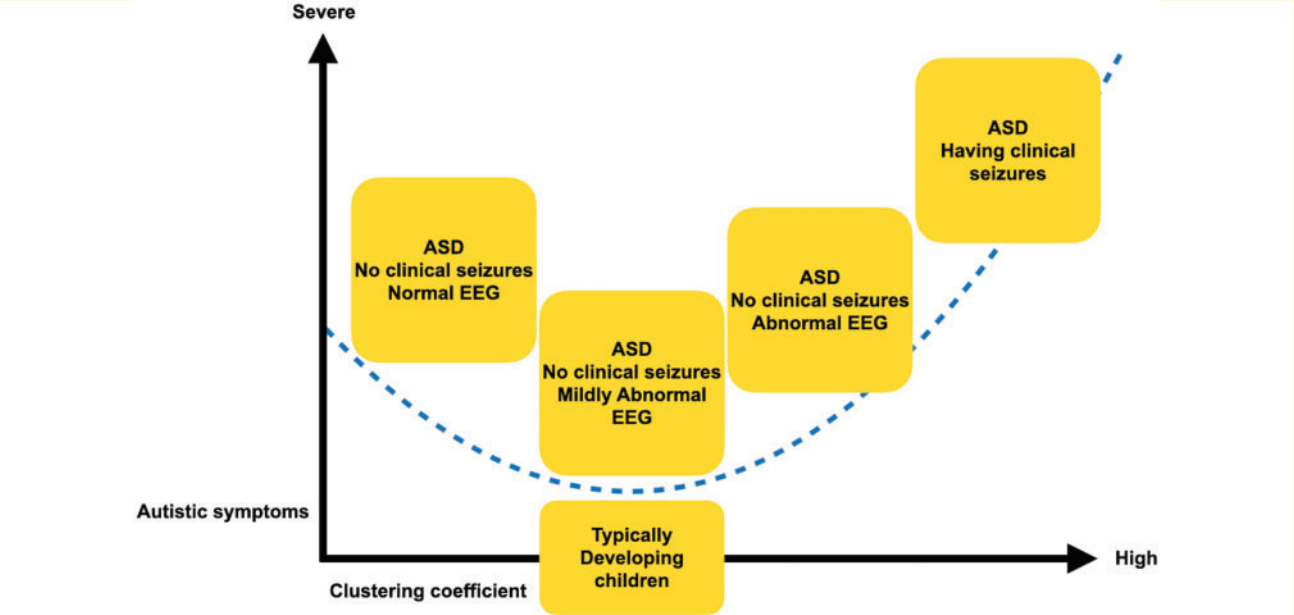


Figure 1 Estimated inverted U-shaped relation between average clustering coefficient in lower frequency and autistic symptoms. Interictal epileptiform discharges might 'normalize' the deviation of autism spectrum disorders brain by increasing *C*. However, when the effect goes beyond tolerance, it can exacerbate autistic symptoms. ASD = autism spectrum disorders; IED = interictal epileptiform discharge

resolution yields more precise signal source mapping. Third, unlike fMRI, MEG is particularly suitable for young children because it is completely silent. Moreover, it uses no radioactive agent, as positron emission tomography and single-photon emission computed tomography do.

Materials and methods

Participants

From Kanazawa University and affiliated hospitals, we recruited 78 children with autism spectrum disorders (58 boys, 20 girls, 38–92 months old) and 19 TD children (16 boys, 3 girls, 48–88 months old). Four boys with autism spectrum disorders were unable to complete MEG recording because of their severe psychomotor agitation. We were unable to identify the electrical dipole current for P100M for one boy and two girls with autism spectrum disorders, as described below in co-registration of MEG on MRI imaging. One boy with autism spectrum disorders was excluded from the analysis in the course of MEG data pre-processing, as described below in the description of pre-processing. These children were excluded from statistical analyses. Therefore, we analysed 70 children with autism spectrum disorders (52 boys, 18 girls, 38–92 months old) and 19 TD children (Table 1). We described sample size calculation in Supplementary materials.

The autism spectrum disorder diagnosis was made according to the Diagnostic and Statistical Manual of Mental Disorders (4th edition)[52] using the Diagnostic Interview for Social and Communication Disorders[53] or the Autism Diagnostic Observation Schedule-Generic.[54] The exclusion criteria were (i) blindness, (ii) deafness, (iii) any other neuropsychiatric disorder including epilepsy and (iv) ongoing medication. Written informed consent was obtained from parents before participation by children. The Ethics Committee of Kanazawa University Hospital approved the methods and procedures, all of which were conducted in accordance with the Declaration of Helsinki.

We are continually recruiting participants as part of a single large project (Bambi plan, http://bambiplan.w3.kanazawa-u.ac.jp/pdf/jusen_english.pdf). As a result, some participants overlap with those in our earlier studies,[11,55] but no results overlap. In addition, the emphases of those earlier studies were completely different from those of this study.

Assessment of social reciprocity and intelligence

We assessed participants' social reciprocity using the Social Responsiveness Scale (SRS),[56] a 65-item rating scale that measures sociality and autistic mannerisms, as a quantitative trait for TD children and children with autism spectrum disorders. A parent of each participant filled out the SRS. We used gender-normed T scores

Table 1 Characteristics of the participants

	ASD		TD	χ^2 or t			P		
	ASD with no IEDs	ASD with IEDs		ASD+ versus ASD−	ASD+ versus TD	ASD− versus TD	ASD+ versus ASD−	ASD+ versus TD	ASD− versus TD
N	52	18	19						
Sex (% male)[a]	79%	61%	84%	2.20	2.50	0.25	0.138	0.114	0.615
Age in months[b]	65.5 (11.8)	62.1 (10.2)	68.1 (10.7)	1.11	1.75	0.83	0.270	0.089	0.410
SRS total score[b]	73.0 (11.6)	70.8 (9.3)	52.1 (8.2)	0.74	−6.40	−7.04	0.464	<0.001*	<0.001*
K-ABC scores									
Mental Processing scale[b]	92.3 (20.4)	102.4 (19.1)	104.4 (10.5)	−1.78	0.40	2.40	0.080	0.689	0.019*
Achievement scale[b]	94.8 (19.7)	99.5 (16.5)	101.9 (14.7)	−0.87	0.47	1.40	0.386	0.642	0.167

Numbers are mean (standard deviation) or counts. * Indicate significant difference ($p < 0.05$).
[a]Chi-square test.
[b]Student's *t*-test.
ASD, autism spectrum disorder; ASD−, autism spectrum disorder with no IEDs; ASD+, autism spectrum disorder with IEDs; IEDs, interictal epileptiform discharges; K-ABC, Kaufman Assessment Battery for Children; SRS, Social Responsiveness Scale; TD, Typically developing children.

(SRS-T). Higher scores represent greater difficulty with social reciprocity.

Using the Japanese version of the Kaufman Assessment Battery for Children (K-ABC), we assessed the intelligence of the participants.[57] With the K-ABC, the set of skills for problem-solving abilities is interpreted as intelligence, which is measured on the Mental Processing Scale (MPS). However, knowledge of facts, defined as achievement, is measured on the Achievement Scale (ACH). Those scores are provided as age-adjusted standardized scores, normalized to have mean of 100 and standard deviation of 15.

Magnetoencephalography recordings

MEG data were recorded using a 151-channel Superconducting Quantum Interference Device (SQUID), whole-head coaxial gradiometer MEG system for children (PQ 1151R; Yokogawa/KIT, Kanazawa, Japan) in a magnetically shielded room (Daido Steel Co., Ltd, Nagoya, Japan) installed at the MEG Center of Ricoh Co., Ltd (Kanazawa, Japan). During the recording, three coils were attached at each of the bilateral mastoid processes and nasion of the child. In reference to the specific magnetic field generated by the coils, we were able to ascertain the child's head position within the helmet. The band-pass-filtered MEG data (0.16–200 Hz) were collected at a 2000 Hz sampling rate.

Each child lay supine on a bed and viewed the selected video programme projected onto a screen. Each video programme itself was muted. Instead, an auditory syllable sound stimulation was given in the same way as in our earlier study[58] because the observed auditory evoked field was used to co-register MEG data on anatomical MRI images. Four boys with autism spectrum disorder were unable to complete the MEG recording. On average, MEG was recorded for 300 s for each child.

To keep a young child motionless during recording, great effort was necessary. Details of the recording procedure and auditory evoked stimuli are presented in Supplementary materials.

Existence of interictal epileptiform discharges in magnetoencephalography recordings

One investigator (author T.H.) reviewed the raw MEG signals: time versus amplitude waveforms. He had been trained in EEG/MEG and epilepsy for 13 years and had extensive experience in distinguishing epileptiform discharges from other non-epileptic waveforms. He assessed the MEG recordings in the same manner as that used in our earlier study.[11] During review, T.H. was blinded to patient information. To review the MEG record, a band pass filter (0.5–70 Hz) was applied. We divided all sensor pairs into eight groups: frontal, temporal, vertex, occipital regions of the left and right sides. T.H. reviewed every group of channels by switching the montage display. The investigator was allowed to move the records backward and forward at any time to confirm his findings. Typically, this procedure required 30 min per child. Epileptiform discharges were detected manually by application of the same general principles recommended by the International Federation of Clinical Neurophysiology,[59] which were used for standard EEG interpretation. Epileptiform discharges are described as sharp, transient and clearly different from background activity with an 'epileptiform' morphology and a logical spatial distribution. The IED location was defined as an intermediate point of sink and source.

Co-registration of magnetoencephalography on substituted MRI image

We were unable to obtain the participants' brain structural MRI image because it is difficult for young children to perform MRI recordings without sedation. Instead, we

used a suitable MRI brain template for each child based on the individual head surface shapes. We co-register the MEG and template MRI image according to the electrical dipole current location, which is generated at the supratemporal auditory cortex in response to auditory syllable sound stimulation. We were unable to identify the electrical dipole current for P100M for one boy and two girls with autism spectrum disorders. These children were excluded from subsequent analysis. Details of this procedure are described in Supplementary materials. This procedure was the same as that used for one of our earlier studies.[60]

Magnetoencephalography data analysis

The following MEG analyses were performed using Brainstorm,[61] which is documented and freely available for download online under the GNU general public license (http://neuroimage.usc.edu/brainstorm).

Pre-processing

After we downsampled MEG recordings to 500 Hz, we pre-processed the MEG data in compliance with that described in the Brainstorm tutorial (https://neuroimage.usc.edu/brainstorm/Tutorials/). During the acquisitions, signal quality was poor in some sensors. First, we excluded noisy sensors from the analysis. Second, we applied a notch filter to remove the 60 Hz harmonics (i.e. 60, 120 and 180 Hz); then, we applied a band-pass filter (0.5–100 Hz). Third, we used the Independent Component Analysis method to remove the cardiac and blink artefacts. Fourth, segments containing apparent motion noise were excluded based on visual inspection of the waveforms. At this step, the motion noise in one boy with autism spectrum disorders was excessive. We excluded this child from subsequent analyses. Fifth, segments containing IEDs were excluded based on visual inspection of the waveforms.

Graph construction and graph metrics

Details of atlas-guided source reconstruction and segmenting are described in Supplementary materials. A graph is a basic topographical representation of a network consisting of 'nodes' and 'edges' connecting pairs of nodes. In this study, nodes corresponded to 68 brain regions. Edges were weighed by corresponding phase lag index (PLI) values between pairs of brain regions. We provided a detailed description of PLI in Supplementary materials. Particularly for each epoch, undirected weighted functional connectivity matrix (68×68) was constructed based on PLI for each frequency band (i.e. delta, theta, alpha, beta and gamma). The first step in applying graph theoretical analysis to these functional connectivity matrices consists of converting the matrix into a binary graph, in which the edges either exist or do not exist: they have no graded values. We binarize the matrix because binary networks are, in most cases, simpler to characterize. More importantly, weak connectivity might represent spurious connections, particularly in functional networks. These connections tend to obscure the topology of strong and significant connections.[62] Therefore, we discarded such weak connections by application of a proportional weight threshold. We set the proportion of total connections retained, κ, as 20% according to our earlier study.[49]

Among all the available graph metrics, we adopted the most commonly used: The clustering coefficient (C), the characteristic path length (L) and small world-ness (SW).[27] Our primary interest was on C, but in pursuit of completeness, and for comparison with earlier studies, we also investigated L and SW. We describe what those metrics quantify in Supplementary materials, but we omit their complex mathematical definitions. A formal mathematical definition is presented elsewhere.[27,28]

Statistical analysis

We used Student's t-tests to examine differences in ages and scores in K-ABC and SRS between children with autism spectrum disorders who had IEDs and those who did not. Sex differences were tested using chi-square tests. Then, we compared each of those two groups similarly with TD children.

To investigate the effects of existence of IEDs on graph metrics in children with autism spectrum disorders, we matched the two groups (children with autism spectrum disorders who had IEDs and those who did not) in terms of age and sex. To improve the balance, we used coarsened exact matching (CEM).[63] Then, we performed adjusted regression analysis. Particularly, we predicted each graph metric based on the condition (with and without IEDs) with the CEM weight for weighting. In the CEM algorithm, we temporarily coarsen (or categorize) each variable using Sturge's rule as a binning algorithm. Each participant is then assigned to one of a specified set of strata in which the participant characteristics are matched exactly on a set of coarsened variables. A weighting variable (CEM weight) is generated to equalize the number of treated and control cases in one stratum. It is used for subsequent regression analysis.[63] This report describes the degree of imbalance before and after matching by measuring the multivariate L1 distance. The L1 distance represents how two groups are balanced in terms of matched variables (in our case, sex and age). The L1 distance is a value between zero and one, with a smaller value representing better balance. We tested for three graph metrics (i.e. C, L and SW) for each frequency band (i.e. delta, theta, alpha, beta and gamma). Therefore, significance was inferred for $P < 0.0033$ after Bonferroni correction was applied for 15 comparisons. We similarly compared graph metrics in TD children with those in each of two autism spectrum disorders groups.

Table 2 Difference between ASD+ and ASD− in graph metrics for matched participants

Frequency band	Graph metrics	Coeff.	SE	95% CI	t	P
Delta	*C*	0.006	0.011	−0.017 to 0.028	0.51	0.611
	L	0.006	0.007	−0.008 to 0.019	0.83	0.410
	SW	0.003	0.031	−0.059 to 0.065	0.09	0.931
Theta	*C*	0.042	0.013	0.015 to 0.069	3.14	0.0028*
	L	0.011	0.008	−0.005 to 0.029	1.41	0.164
	SW	−0.013	0.021	−0.055 to 0.029	−0.63	0.532
Alpha	*C*	0.019	0.024	−0.028 to 0.067	0.83	0.413
	L	0.026	0.011	0.003 to 0.049	2.31	0.025
	SW	0.018	0.021	−0.026 to 0.061	0.82	0.418
Beta	*C*	0.163	0.011	−0.005 to 0.038	1.52	0.013
	L	0.006	0.015	−0.024 to 0.036	0.38	0.703
	SW	−0.010	0.030	−0.069 to 0.050	−0.32	0.749
Gamma	*C*	0.008	0.019	−0.030 to 0.045	0.40	0.689
	L	−0.006	0.022	−0.050 to 0.039	−0.26	0.800
	SW	−0.016	0.027	−0.069 to 0.038	−0.59	0.560

ASD− = Autism Spectrum Disorder with no interictal epileptiform discharges; ASD+ = Autism Spectrum Disorder with interictal epileptiform discharges; *C* = average clustering coefficient; *L* = average shortest path lengths; *SW* = small world-ness.
*Indicates significant effect ($p < 0.0033$).

For children with autism spectrum disorders, if a significant effect of IEDs was found for any graph metric, we investigated the relation between social reciprocity and the graph metric. Particularly, we applied linear regression models to predict the graph metric based on the participant condition (i.e. with or without IEDs), SRS-T score, interaction between condition and SRS-T score, age and sex. If a significant interaction effect was found, then we applied *post hoc* analysis for further elucidation of the relation between SRS and graph metric. In *post hoc* analysis, we predicted the metric based on SRS-T score controlling for age and sex in each condition (i.e. with or without IEDs). Significance was inferred for $P < 0.025$ after Bonferroni correction was applied. For exploratory analysis, we also investigated the relation between social reciprocity and the graph metric in TD children. The relations between K-ABC scores (MPS and ACH) and the graph metric are analysed similarly.

Before we applied linear regression, we verified that our data meet the assumptions for regression analysis. Specifically, we used standard methods to verify linearity, normality, homogeneity of variance, model specifications, influence and collinearity. Results show that the assumption of homogeneity was violated for some regression models. Therefore, for those models, we used heteroscedasticity-robust standard errors.[64] All statistical analyses were conducted using software (Stata ver. 15.0; Stata Corp., College Station, TX, USA).

Data availability

Raw data were generated at Kanazawa University. Derived data supporting the findings of this study are available as Supplementary information. Stata (Stata ver. 15.0; Stata Corp., College Station, TX, USA) will be needed to use to open the file.

Results

For three children with autism spectrum disorders having no IED, we were unable to obtain SRS scores because their parents became unreachable after MEG recording. Three children (two children with no IED and one child with IEDs) were unable to complete K-ABC because of severe psychomotor agitation. The SRS total score was significantly lower in TD children than children with autism spectrum disorders having no IED [$t(65) = -7.04$, $P < 0.001$], and children with autism spectrum disorders having IEDs [$t(34) = -6.40$, $P < 0.001$]. The MPS score was significantly higher in TD children than in children with autism spectrum disorders having no IED [$t(66) = 2.40$, $P = 0.019$]. We found no significant difference in any other factor. Table 1 presents the results. Supplementary Table 1 presents the distribution of IEDs.

Group differences in graph metrics in matched participants

For comparison between children with autism spectrum disorder having no IED (ASD−) and children with autism spectrum disorder having IEDs (ASD+), after improving balance using the CEM algorithm, 38 children with ASD− and 17 children with ASD+ were the matched participants. The L1 distance improved from 0.474 to 0.272. After matching, we used linear regression with CEM weights to predict the graph metrics based on the condition (i.e. with or without IEDs). The main effect of the condition [$t(53) = 3.14$, $P = 0.0028$] was found to be significant only for the model predicting *C* in the theta band. Table 2 presents the results. Children with ASD+ had significantly higher *C* in the theta band than ASD− had.

For comparison between children with ASD− and TD children, after improving balance using the CEM algorithm, 41 children with ASD− and 18 TD children were

Table 3 Difference between ASD− and TD in graph metrics for matched participants

Frequency band	Graph metrics	Coeff.	SE	95% CI	t	P
Delta	*C*	0.012	0.010	−0.009 to 0.033	1.17	0.247
	L	0.014	0.007	−0.000 to 0.028	1.96	0.056
	SW	−0.022	−0.237	−0.694 to 0.026	−0.92	0.361
Theta	*C*	−0.036	0.011	−0.058 to −0.014	−3.22	0.0021*
	L	−0.005	0.010	−0.026 to 0.016	−0.48	0.633
	SW	−0.027	0.020	−0.068 to 0.014	−1.33	0.188
Alpha	*C*	−0.017	0.019	−0.055 to 0.021	−0.88	0.384
	L	−0.013	0.010	−0.033 to 0.006	−1.41	0.166
	SW	0.012	0.021	−0.030 to 0.053	0.57	0.571
Beta	*C*	0.012	0.011	−0.011 to 0.034	1.01	0.315
	L	−0.007	0.016	−0.038 to 0.025	−0.42	0.680
	SW	0.019	0.034	−0.050 to 0.880	0.55	0.583
Gamma	*C*	0.037	0.020	−0.003 to 0.077	1.87	0.067
	L	0.006	0.018	−0.029 to 0.042	0.35	0.728
	SW	−0.028	0.027	−0.083 to 0.027	−1.03	0.308

ASD− = autism spectrum disorders having no interictal epileptiform discharges; TD = Typically developing children; *C* = average clustering coefficient; *L* = average shortest path length; *SW* = small world-ness.
*Indicates significant effect ($p < 0.0033$).

Table 4 Difference between ASD+ and TD in graph metrics for matched participants

Frequency band	Graph metrics	Coeff.	SE	95% CI	t	P
Delta	*C*	−0.004	0.010	−0.025 to 0.018	−0.35	0.732
	L	0.019	0.011	−0.004 to 0.042	1.70	0.105
	SW	−0.041	0.037	−0.118 to 0.037	−1.09	0.288
Theta	*C*	0.034	0.028	−0.024 to 0.091	1.22	0.238
	L	0.009	0.015	−0.023 to 0.040	0.58	0.568
	SW	0.000	0.025	−0.053 – 0.052	−0.01	0.991
Alpha	*C*	−0.013	0.031	−0.077 to 0.051	−0.42	0.676
	L	0.022	0.014	−0.007 to 0.052	1.57	0.131
	SW	−0.069	0.042	−0.156 to 0.018	−1.65	0.114
Beta	*C*	0.000	0.016	−0.033 to 0.033	0.01	0.993
	L	−0.010	0.023	−0.057 to 0.038	−0.42	0.679
	SW	−0.005	0.043	−0.095 to 0.084	−0.13	0.900
Gamma	*C*	−0.005	0.028	−0.062 to 0.052	−0.17	0.868
	L	−0.028	0.035	−0.101 to 0.045	−0.80	0.431
	SW	−0.136	0.041	−0.221 to 0.050	−3.31	0.035

ASD+ = autism spectrum disorder with interictal epileptiform discharges; TD = typically developing children; *C* = average clustering coefficient; *L* = average shortest path lengths; *SW* = small world-ness.

the matched participants. The L1 distance improved from 0.242 to 0.067. After matching, we used linear regression with CEM weights to predict graph metrics based on the condition (i.e. ASD− or TD). The model predicting *C* in the theta band was the only one for which the main effect of the condition [$t(57) = -3.22$, $P = 0.0021$] was found to be significant. TD children had significantly higher *C* in the theta band than ASD− children had. Table 3 presents the results.

For comparison between ASD+ children and TD children, after improving balance using the CEM algorithm, 11 ASD+ children and 11 TD children comprised the matched participants. The L1 distance improved from 0.503 to <0.001. After matching, we used linear regression with CEM weights to predict the graph metrics based on condition (i.e. ASD− or TD). We found no significant effect for any model. Table 4 presents the results. Results show that TD children had higher *SW* in the gamma band than ASD+ children, but the difference was not significant after Bonferroni correction.

Effects of Social Responsiveness Scale scores on *C* in the theta band

For children with autism spectrum disorders, we applied linear regression models to predict *C* in the theta band based on the participant condition (i.e. with or without IEDs), SRS-T score, interaction between condition and SRS-T score, age and sex. For this model, the interaction effect between condition (i.e. with or without IEDs) and the SRS total T-score was found to be significant [$t(61) = 2.28$, $p = 0.026$]. The main effect of the condition [$t(61) = -2.14$, $P = 0.037$] was also found to be significant. No other factor was found to be significant. However, the *F* statistic was not significant [$F(5,61) = 1.19$, $P = 0.323$]. Therefore, to elucidate the relation between social cognition

Table 5 Effect of SRS-T score on *C* in theta band for children with ASD

Children with ASD	**versus *C* in theta band**	**Coeff.**	**Robust SE**	**95% CI**	**t**	***P***	***F***	***P***	**R^2**
	SRS-T	0.000	0.001	−0.001 to 0.001	−0.52	0.606	1.19	0.323	0.168
	Condition	−0.174	0.081	−0.336 to −0.011	−2.14	0.037*			
	Condition × SRS-T	0.003	0.001	0.000 to 0.005	2.28	0.026*			
	Sex	0.034	0.019	−0.003 to 0.071	1.82	0.074			
	Age (months)	−0.001	0.001	−0.002 to 0.000	−1.31	0.195			
	Post hoc analysis								
	ASD having no IEDs								
	SRS-T	0.000	0.000	−0.001 to 0.001	−0.26	0.795	0.47	0.703	0.019
	Sex	−0.012	0.014	−0.040 to 0.017	−0.83	0.412			
	Age (months)	0.000	0.000	−0.001 to 0.001	0.78	0.439			
	ASD having IEDs								
	SRS-T	0.005	0.001	0.002 to 0.007	3.84	0.002*	6.18	0.007*	0.488
	Sex	0.088	0.022	0.040 to 0.013	3.95	0.002*			
	Age (months)	−0.002	0.002	−0.006 to 0.002	−0.96	0.351			
TD children	versus *C* in theta band								
	SRS-T	−0.001	0.002	−0.005 to 0.004	−0.29	0.774	0.05	0.984	0.010
	Sex	0.012	0.032	−0.057 to 0.081	0.37	0.713			
	Age (months)	−0.000	0.001	−0.003 to 0.002	−0.24	0.811			

ASD = autism spectrum disorder; TD children = typically developing children; *C* = average clustering coefficient; IED = Interictal Epileptiform Discharge; SRS-T = Social Responsiveness Scale Total T score.
*Indicates significant effect ($p < 0.05$ for the first regression model, and $p < 0.025$ for the post hoc analysis)

and *C* in the theta band, we applied linear regression to predict *C* in the theta band based on the SRS total T-score, age and sex in each group (i.e. ASD+ and ASD−). For children with no IED, no factor was found to be significant. For children with IEDs, significant main effects were found for the SRS total T-score [$t(14) = 3.84$, $P = 0.002$] and sex [$t(14) = 3.95$, $P = 0.002$]. No other factor was found to be significant. Those results indicate that higher SRS T-score and being male are associated with higher *C* in the theta band in the presence of IEDs.

We applied the same model for TD children. In this group, no factor was found to be significant. The results are presented in Table 5.

Effects of Kaufman Assessment Battery for Children scores on *C* in the theta band

In the three groups, no factor was found to be significant. The results are presented in Supplementary Table 2.

Discussion

For TD children and children with autism spectrum disorders, we constructed brain networks based on PLI and analysed the properties of those networks to explore the role of IEDs. In children with autism spectrum disorders, *C* in the theta band was significantly higher in the presence of IEDs. In addition, children with autism spectrum disorders having no IED showed significantly lower *C* in the theta band than TD children did. However, the difference was non-significant between TD children and children with autism spectrum disorders having IEDs. Furthermore, in children with autism spectrum disorders having IEDs, higher *C* in the theta band corresponded to severe autistic symptoms. However, the association was non-significant in children with autism spectrum disorders having no IED. The effects of K-ABC scores on *C* in the theta band were non-significant in all three groups: autism spectrum disorders with IEDs, autism spectrum disorders without IEDs and TD children.

For children with autism spectrum disorders, with and without IEDs, we found significantly higher *C* in the theta band for the former group. Assuming that IED represents a milder form of epileptic activity (because it appears in the absence of clinical seizures), this result is consistent with most earlier graph-theoretical studies for generalized[34,36] and focal epilepsy irrespective of the experimental conditions.[38–40] Evidence from those results of earlier studies suggests that the presence of epilepsy (i.e. stronger form of epileptic activities) corresponds to higher *C*, especially for lower frequency bands. First, it is important that the present and the earlier results be mostly consistent: the epileptic activity must be significantly associated with higher *C*, irrespective of their methodological differences such as EEG or MEG, participant characteristics, synchronization measures, weighted or unweighted graphs, and eyes-open and closed. Considering these results, correspondence of epileptic activity and higher *C* is unlikely to be restricted in patients with epilepsy. In fact, it also occurs in children with autism spectrum disorders. In this sense, the present results extend evidence obtained from the existing literature: the present report is the first describing this association in children with non-epileptic autism spectrum disorders. Second, aetiology of the IEDs appearing in children with autism spectrum disorders apparently differs from that appearing with benign

epilepsy with a centrotemporal spike: earlier studies consistently showed lower *C* in the theta and other frequency bands for this population.[41–43] Alternatively, the effects of IEDs on *C* vary depending on the location. In this case, mostly centrotemporal foci of IEDs in benign epilepsy with a centrotemporal spike might explain that discrepancy. Because of the small sample size (only 18 children had IEDs), we did not separate participants based on IED location. Therefore, further studies must be conducted to ascertain whether IEDs on centrotemporal region specifically lower *C*.

Comparison between children with autism spectrum disorders having no IED and TD children revealed significantly lower *C* in the theta band for children with autism spectrum disorders. Although the methodologies differed, this result is compatible with most reported graph-theoretical studies for autism spectrum disorders.[44–47] It is noteworthy that participants with autism spectrum disorders having epilepsy[44–46] or showing IEDs in their EEG[47] were excluded from analyses in those studies. Among the remaining three studies,[48–50] however, two reports described non-significant difference in *C* (in terms of coherence[48] or PLI-based[49] graphs) between children with and without autism spectrum disorders.[48,49] One report described higher *C* (in terms of weighted-PLI-based graphs) in adolescents with autism spectrum disorders compared to age-matched healthy controls.[50] In contrast to the studies described above,[44–47] participants with autism spectrum disorders having co-occurring epilepsy[48,49] or recordings containing IEDs[50] were not explicitly excluded from those three studies. Considering the results of the present study, which suggest correspondence of higher *C* and IEDs in children with autism spectrum disorders, the possible existence of IEDs in the later three studies[48–50] might explain this inconsistency. In this sense, our results confirm most findings from earlier studies,[44–47] and extend earlier results in that the effects of IEDs on functional brain networks might explain discrepancies that have been reported from earlier studies.[48–50] Correspondence between lower *C* and autism spectrum disorders having no IEDs confirm that the network topology of autism spectrum disorders deviates from the small-world property. Alteration occurs in early childhood at the latest, but considering the present results, this deviation might be 'normalized' in the existence of IEDs.

We applied linear regression models to elucidate the relation between *C* in the theta band and autistic symptoms. In children with autism spectrum disorders having IEDs, higher *C* corresponded to severer autistic symptoms, controlling for age and sex. However, in children with autism spectrum disorders having no IED, the association was non-significant. Considering lower *C* in those who had no IED, the effect of *C* on autistic symptoms seems to have some elasticity. The association between *C* and autistic symptoms might be generally unclear, unless *C* exceeds a certain threshold. Only one report, that of a study by Takahashi et al.,[49,64] included discussion of the association between graph metrics on MEG/EEG-based brain network and autistic symptoms. In contrast to the present results, after they analysed children with autism spectrum disorders, they reported non-significant association for all graph metrics. However, because they did not separate the groups in terms of having IEDs, the different patterns of the association between *C* and autistic symptoms in children with and without IEDs might explain this discrepancy. More explicitly, a possibly larger portion of children having no IED might obscure the significant relation in those who had IEDs. Here, one must be careful of small sample sizes and methodological differences of the studies. As one example of contrast, Takahashi et al. constructed graphs at the sensor space, whereas the graphs in the present study were at the source space. Future large-scale studies including participants with non-epileptic autism spectrum disorders with or without IEDs as well as individuals with autism spectrum disorders having co-occurring epilepsy must be conducted to elucidate this association further.

We found significant difference neither for *L* nor *SW* between each pair of the three experimental groups (i.e. TD children, autism spectrum disorder with and without IEDs). It is unlikely that IEDs have a strong effect on *L* in children with autism spectrum disorder. Similarly, children with autism spectrum disorder might not have much longer or shorter *L* than TD children. It is also possible that, in light of the inconsistent results on *L* from the earlier studies, the effect of IEDs or autism spectrum disorder on *L* are task dependent. From this perspective, a possible effect of IEDs or autism spectrum disorder might be blurred under our experimental condition (i.e. eyes-open under visual stimulation). Either way, one must be cautious when considering results on *L* in functional brain networks. Paths in such graphs might not correspond to information flow,[27] and as such, they might be less straightforward to interpret.

This study has some methodological considerations. First, because of the small sample size (18 children with autism spectrum disorders having IEDs), we did not classify children with autism spectrum disorders having IEDs in terms of the respective location of epileptic activities. To clarify region-specific effects of IEDs, studies examining a larger sample are needed. Second, all of our participants were children. No group consisted of adolescents or adults. The present results should not be generalized unless the results are confirmed for older individuals. Third, none of the TD children in this study showed IEDs. A future study comparing IED effects on non-epileptic autism spectrum disorders and on TD children might produce interesting results elucidate whether effects differ between the two populations. Fourth, we kept the children still in MEG with the aid of visual attention (i.e. showing a video programme) because, otherwise, it was difficult to maintain their cooperation. In this sense, their MEG data were recorded with eyes-open under visual stimulation. As a consequence, the observed brain activity

should be distinguished clearly from 'resting state' brain activity. Studies that employ attention-controlled conditions will provide more reliable evidence, although these conditions will probably be difficult to achieve with young children.

In conclusion, we demonstrated that alteration of functional brain networks in children with autism spectrum disorders depends on the existence of IEDs. Results suggest that IEDs might 'normalize' the deviation of autism spectrum disorders brain by increasing *C*. However, when the effect extends beyond tolerance, it actually exacerbates autistic symptoms. One issue of practical interest is the possible usefulness of *C* in the theta band to facilitate the diagnosis of MEG recorded patients using machine-learning algorithms. This particular application is attractive in clinical practice, but it potentially circumvents many methodological limitations (e.g. reduced electromagnetic signal from deep brain sources). Additional studies must be done in this respect. Such future studies, to confirm and elucidate the relation between *C* and autistic symptoms, must include individuals with non-epileptic autism spectrum disorders having IEDs or not, and include individuals who have co-occurring epilepsy.

Supplementary material

Supplementary material is available at *Brain Communications* online.

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Competing interests

The authors report no competing interests.

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