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EEG Spectral Features in Sleep of Autism Spectrum Disorders in Children with Tuberous Sclerosis Complex

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Conflict of Interest:

Dr. Cook discloses that he has received research support from Covidien (formerly Aspect Medical Systems), National Institutes of Health, and NeoSync, Inc. within the past three years; he has been an advisor/consultant/reviewer for Arctica Health, Cerêve, HeartCloud, NeuroDetect, NeuroSigma, NIH (ITVA), U.S. Departments of Defense and Justice, and the VA (DSMB); he is editor of the Patient Management section of the American Psychiatric Association's FOCUS journal; his biomedical intellectual property is assigned to the Regents of the University of California, and he has stock options in NeuroSigma, where he has served as Chief Medical Officer (on leave); he is employed by the University of California, Los Angeles and also has an appointment as a Staff Psychiatrist, Neuromodulation and Mood Disorders programs, Greater Los Angeles Veterans Administration Health System.

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Dr. Leuchter discloses that within the past 36 months he has received research support from the National Institutes of Health, Neuronetics, Department of Defense, CHDI Foundation, and NeuroSigma, Inc. He has served as a consultant to NeoSync, Inc., Ionis Pharmaceuticals, Inc., and ElMindA. He is Chief Scientific Officer of Brain Biomarker Analytics LLC (BBA). Dr. Leuchter owns stock options in NeoSync, Inc. and has equity interest in BBA.

Dr. Sahin has received grant support from Novartis Pharmaceuticals, Roche, Pfizer, Ipsen and LAM Therapeutics and Quandrant Biosciences. He has served on scientific advisory boards for Sage, Roche, and Takeda.

Mr. Wilson, Dr. Goyal, Dr. Northrup declare no conflicts of interests.

Abstract

Tuberous sclerosis complex (TSC) is a multisystem disorder with increased prevalence of autism spectrum disorders (ASDs). This project aimed to characterize the autism phenotype of TSC and identify biomarkers of risk for ASD. Because abnormalities of EEG during sleep are tied to neurodevelopment in children, we compared electroencephalographic (EEG) measures during stage II sleep in TSC children who either did (ASD+) or did not (ASD-) exhibit symptoms of ASD over 36-month follow up. Relative alpha band power was significantly elevated in the ASD+ group at 24 months of age with smaller differences at younger ages, suggesting this may arise from differences in brain development. These findings suggest that EEG features could enhance the detection of risk for ASD.

Keywords: Autism, TSC, EEG, Biomarkers

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Abstract

Tuberous sclerosis complex (TSC) is a multisystem disorder with increased prevalence of autism spectrum disorders (ASDs). This project aimed to characterize the autism phenotype of TSC and identify biomarkers of risk for ASD. Because abnormalities of EEG during sleep are tied to neurodevelopment in children, we compared electroencephalographic (EEG) measures during stage II sleep in TSC children who either did (ASD+) or did not (ASD-) exhibit symptoms of ASD over 36-month follow up. Relative alpha band power was significantly elevated in the ASD+ group at 24 months of age with smaller differences at younger ages, suggesting this may arise from differences in brain development. These findings suggest that EEG features could enhance the detection of risk for ASD.

EEG Spectral Features in Sleep of Autism Spectrum Disorders in Children with Tuberous Sclerosis Complex

Autism spectrum disorders (ASDs) are common neurodevelopmental conditions, and are estimated to be present in approximately 1 in 68 school-aged children (14.6 per 1000, Christensen et al. 2016). Though some children develop symptoms soon after birth, there is considerable heterogeneity in clinical presentation, both in terms of specific symptoms and the age at which they emerge (American Psychiatric Association 2013), and multiple risk factors have been identified (Modabbernia, Velthorst and Reichenberg 2017; Mitchell, Barton, Harvey and Williams 2017).While interventions can mitigate disability associated with ASD, these appear to be most effective when introduced early in childhood (Landa 2018; Catalano, Holloway and Mpofu 2018). Genetically defined syndromes with increased prevalence of ASD provide unique opportunities to examine the neurophysiologic patterns associated with the symptoms of ASD. Tuberous Sclerosis Complex (TSC) is a multisystem genetic disorder, in which some individuals exhibit ASD (Fernandez and Scherer 2017), making it particularly useful for expanding our neurobiological understanding of ASD, potentially including molecular pathway and neurogenetic features.

The Autism Centers of Excellence (ACE) Program is a trans-institute initiative funded by the U.S. National Institutes of Health. Its objectives include the execution of large-scale, multi-site, multidisciplinary studies in ASD. As part of the ACE Program, we examined electroencephalographic (EEG) data recorded longitudinally in children at five participating centers who were followed for several years, starting as young as one month of age. Our

objective was to identify neurophysiologic differences between TSC youth with symptoms of ASD (ASD+) and those without (ASD-) and the time course of the development of these neurophysiologic features. Our goal was to better characterize the autism phenotype of TSC and identify biomarkers that may predict risk for development of autism in children with TSC.

In furtherance of this goal, this project focused on EEG recordings from sleep, and in particular, during Stage II sleep. Sleep disturbances are reported commonly in a range of neurodevelopmental disorders, with high rates identified in children with Smith-Magenis syndrome and Angelman syndrome as well as TSC and ASD (Tricket et al., 2018). Based on questionnaire data, patterns of disturbance were reported to vary among these groups, with difficulties with sleep onset and sleep maintenance characterizing children with ASD while those with TSC were characterized by daytime sleepiness, parasomnias, and night walking (Tricket et al. 2018). Given these reported clinical differences, we sought to examine neurophysiologic data during sleep in a way that could be reliably and consistently acquired across the age range of our subjects.

Neurophysiologically, stage II sleep is generally characterized by an abundance of diffuse slow wave activity in delta and theta bands, constituting the majority of relative power in that brain state, with a smaller amount of energy in the alpha band, arising from activity including sleep spindles (Armitage, Trivedi and Rush 1995). Objective abnormalities of sleep are widely reported in children with ASD (Köse, Yilmaz, Ocakoğlu, and Özbaran 2017; Cohen, Conduit, Lockley, Rajaratnam, and Cornish 2014) and include derangements of sleep architecture as well as paroxysmal abnormalities (Çetin, Korkmaz, Alev, and Demirbilek 2017), although these can be found in waking recordings as well (Yasuhara 2010). In longitudinal work, it had been found that sleep disturbances in children with ASD tend to co-occur with core symptoms of ASD,

rather than precede them, and that children with ASD generally experience worsening of clinical sleep problems as they age, whereas typically-developing children tend to have a decrease (Verhoeff et al., 2018). Epileptiform discharges have been observed in the EEGs of children with TSC, along with alterations sleep architecture (e.g., Hunt, 1993; Bruni et al., 1995; Kharoshankaya et al., 2016). To examine differences among subjects against this landscape of sleep and EEG abnormalities, in this exploratory work we sought to focus on a feature that would be present in all subjects, would require only minimal cooperation by the subjects, and could be reliably identified in the recordings from all groups.

Methods

Compliance with Ethical Standards

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

Participants

Consonant with the ACE Centers' objective of study children with TSC with regard to the presence or absence of ASD, a group of 158 youths between the ages of one and 36 months were enrolled at five clinical sites: Boston Children's Hospital (BCH), Cincinnati Children's Hospital Medical Center (CCHMC), University of Alabama at Birmingham (UAB), University of California, Los Angeles (UCLA), and University of Texas at Houston (UTH). Subjects in the study were assigned a categorical diagnosis (ASD or typically-developing) and a degree of confidence by the clinician making the diagnosis. Of the 158 children, we restricted our analyses

by first considering only those individuals who had been assigned to a diagnostic category with a high degree of clinician-rated confidence (4 or 5 on a 5-point Likert scale), totaling a sample of 70 individuals. Of these 70, we considered those characterized either as typically-developing (N=59, ASD-) or with a probable diagnosis of ASD (N=11, ASD+) by their 36 month visit. Subjects without this degree of diagnostic clarity by the 36 month visit were not included in analyses to identify candidate EEG features. Finally, we considered all the EEG recordings at the 24, 18, and 12 month time points that fulfilled our inclusion criteria (below), ultimately allowing comparisons between of 9 ASD+ and 17 ASD- children. Other time points were not analyzed due to low numbers of interpretable EEG recordings for these subjects. Subject information is summarized in Table I.

EEG Recordings and Power Spectra

EEGs were recorded at the five clinical centers using different EEG recording devices (Nihon Kohden, Stellate, Natus/Xltek) with a heterogeneous set of recording montages (see Supplemental Material). All recordings contained a common core set of 23 electrode locations defined in the 10-20 system (Fp1, F7, T7, P7, O1, F3, C3, P3, Fz, Cz, Fp2, F8, T8, P8, O2, F4, C4, P4, and Pz, along with A1, A2, ground, and reference), and these channels were used for analysis. All EEG files were converted into the common European Data Format (EDF) for reviewing and analysis using the BrainVision Analyzer 2 software package (BVA2, Brain Products GmBH, Gilching, Germany). EEG data files were brought into BVA2, and high pass (0.5Hz), low pass (70Hz), and notch (60Hz) filters were applied. Signals were then visually inspected by raters blinded to subject group, for quality and absence of epileptiform discharges; between ten and thirty 2-second artifact-free epochs containing sleep spindles were extracted for spectral analysis, to ensure comparison in a similar brain state across all subjects. Subjects were

included in our analyses only if their recording had 20 seconds or more of artifact-free data in Stage II sleep. Pragmatic reasons for employing Stage II sleep in our analyses include that it could reliably be ascertained across individuals without the need for subject cooperation, it is abundant in most sleep recordings, and it could be found in both ASD- and ASD+ subjects. Again, no epileptiform discharges were included in the analyzed epochs.

Absolute and relative power values were calculated using BVA2 with custom scripts, using power bands defined as: delta 0.5-4 Hz; theta 4-8 Hz; alpha 8-12 Hz; beta 12-20 Hz; gamma 20-55 Hz.

Identification of features that differed between groups was conducted using the following steps, using SPSS (v24, IBM, Armonk NY) to perform our analyses. First, an omnibus one-way ANOVA was used to determine which frequency bands, if any, contained significant group differences; to reduce the number of comparisons, topography was collapsed across the different electrode sites into regional modules: frontal (Fp1, Fp2, Fz, F3, F4, F7, F8), central (C3, Cz, C4) temporal (T7, T8), and parieto-occipital (P3, P4, P7, P8, Pz, O1, O2), and left and right hemispheric submodules (omitting midline channels). These ANOVA tests were performed separately for absolute power and relative power measures, at 24-, 18-, and 12- month time frames. As a second step, each measure with a detected difference (0.05 alpha level) was then examined for the regions that drove those differences, using False Discovery Rate (FDR) techniques to identify which regions survived multiple comparisons. For modules that did survive, we examined the channels within that regional module to determine which specific electrode locations exhibited differences.

Results

All subjects were included in the 24-month analysis, one ASD- subject did not have analyzable EEG data at the 18-month point, and a different ASD- subject lacked analyzable EEG data for the 12-month analysis.

At the 24-month recording, significant group differences emerged in the alpha band in relative power. No modules met our FDR requirements for multiple comparisons in delta, theta, beta, or gamma bands, or in alpha absolute power, though frontal gamma absolute power was elevated at an uncorrected level (F 4.229, p=0.049). As shown in Table II and Figure I, these findings exhibited a band-dependent topography. In the alpha band, differences were found centrally and parieto-occipitally, on both left and right sides and temporally on the left (F ranging 6.28 – 6.85). Figure II shows the FDR analysis in alpha absolute power at the 24-month recording. Figure III shows the topography of these alpha-band findings.

In both the 18- and 12-month analyses, no statistically significant group differences were detected that met our stringent FDR selection criteria. The brain regions that exhibited significant alpha-band differences at the 24-month recording showed a stronger tendency to differ at 18-month recording (Central: F 1.508, p=0.232; Frontal: F 1.194, p=0.286; Parieto-Occipital: F 0.662, p=0.424; Temporal: F 1.460, p=0.239) than at the 12-month assessment (Central: F 0.715, p=0.406; Frontal: F 1.258, p=0.274; Parieto-Occipital: F 1.536, p=0.228; Temporal: F 1.973, p=0.173), using F score magnitudes as representations of the degree of separation between groups.

Discussion

The primary finding of our analyses was that TSC children with ASD exhibited significantly higher levels of alpha relative power during stage II sleep, with a broad topography involving central, temporal, and parieto-occipital regions, in comparison with similarly aged TSC children without ASD at 24 months. Additionally, this pattern of group differences was present at attenuated (non-significant) levels in the same children at younger ages (18 and 12 months), suggesting that this neurophysiologic measure may reflect neurodevelopmental characteristics that emerge over time and may be associated with ASD symptoms. Secondarily, these differences might be useful as a diagnostic biomarker that would be reliable once children reached age 24 months.

In studies of typically-developing (TD) children with neither TSC nor ASD, a pattern of changes in sleep EEG has been described that emerges over development. Specifically, a posterior-to-anterior progression of change in spectral power as children grow has been previously reported (Kurth et al. 2010; Novelli et al. 2016). Buchman and colleagues (Buchmann et al. 2010) have reported that spectral power decreases along with cortical maturation, as assessed via structural neuroimaging, and that these changes were present with both slow wave energy and with alpha power in a group of somewhat older TD children (8-19 years). Our observation of considerably higher posterior alpha power in our ASD+ subjects (Figure III) is consistent with theories that ASD is related to maturational trajectories that differ from children without ASD. Recent work using waking EEG (Tierney et al., 2012; Levin et al., 2017; Gabard-Durnam et al., 2019) supports differing maturational trajectories in children at high risk vs low risk for developing ASD. Future studies could record whole-night polysomnography data to expand this line of work in children who do not have additional neurological diagnoses, as well as children with TSC.

Others have described altered levels of resting gamma activity in ASD compared with subjects without ASD, but both elevations (van Diessen, Senders, Jansen, Boersma, and Bruining 2015) and reductions (Maxwell et al. 2015) have been reported. We observed a tendency towards elevated gamma power in a frontal module in ASD+, but this observation did not survive correction for multiple comparisons. It has been suggested that there is greater variability within an individual for those with ASD (David et al. 2016) which may confound identification of group-level differences in this frequency range.

Pragmatically, and from a biomarker development perspective, stage II sleep was the brain state most reliably determined and standardized in the EEGs of these subjects. While the maximally awake and alert state in TD subjects generally can be determined with both behavioral and EEG means, it is more difficult to make this determination in young children with ASD, and cooperation in performing an attentional task can be variable across the spectrum. For a biomarker to be useful in clinical work, it is critical for the underlying measurements to be performed reliably, using methods that are well tolerated by individuals in the intended clinical population. Scalp EEG can be measured noninvasively with low-cost equipment that is easily used in outpatient, pediatric settings, with well-defined staff training, offering potential advantages over other neurophysiologic and neuroimaging measurement techniques that are well suited to scientific discovery research.

Some authors have discussed ethical questions in relationship to the diagnosis of ASD, including whether it is better viewed as a disorder or an identity (Hens et al., 2018). This project has focused on biological aspects of the condition and has studied individuals who are experiencing life impairments; our findings are best viewed as observations about biological variability between ASD+ and ASD- groups, and particularly differences which may emerge

early in life. While it is premature to resolve questions of how such a biomarker could be used to mitigate impairment and suffering, it is worthwhile to ensure that a broad-based discussion take place about the full ethical, philosophical, biomedical, and clinical ramifications of any biological marker that might identify those at elevated risk for the future development of the findings of ASD.

Limitations of the current work include the sample size, potential variability across sites in both clinical and EEG technique, and issues around generalizability of findings from subjects with TSC who are enrolled at specialized academic centers. To evaluate the use of elevated alpha relative power in stage II sleep as a marker to aid in the evaluation of children for possible ASD, additional research is needed. This would involve confirmatory work with children drawn from a variety of clinical settings (general pediatric offices as well as academic centers of excellence) and followed to ages greater than 36 months. Both ASD+ and ASD- groups in this sample were predominantly Caucasian, limiting generalization to other populations. A further concern is that nearly all the ASD+ individuals persisted in the study, while a substantial fraction of the ASD- subjects were lost to follow-up; while the range of reasons for this loss are not known, it is possible that the ASD+ subjects' behavioral disturbances led families to be more connected with the study, while the children developing without ASD symptoms and impairments were less so, with potential impact on the generalizability of our findings because of the different drop out rates.

In summary, our finding of an age-related elevation in alpha power in TSC children with ASD supports the possibility of using sleep EEG as a window into the neurophysiology of ASD. This has potential both for understanding the developmental and maturational aspects of the syndrome, and for identifying potential therapeutic molecular targets and measuring engagement

with them. Additional work could replicate and extend these observations in populations with TSC and as well as with children without co-occurring neurological disorders.

Figure Captions

Figure I. Group differences at 24 months between ASD+ and ASD- subjects. ASD+ subjects exhibited higher values of alpha power which were significant centrally and parieto-occipitally, on both left and right sides, and temporally on the left.

Figure II. Example False Discovery Rate plot at 24 months. From this analysis, it was determined that up to seven modules could merit exploration among the alpha relative power measures.

Figure III. Topography of Differences in Alpha Absolute Power at 24 months

Figure I top

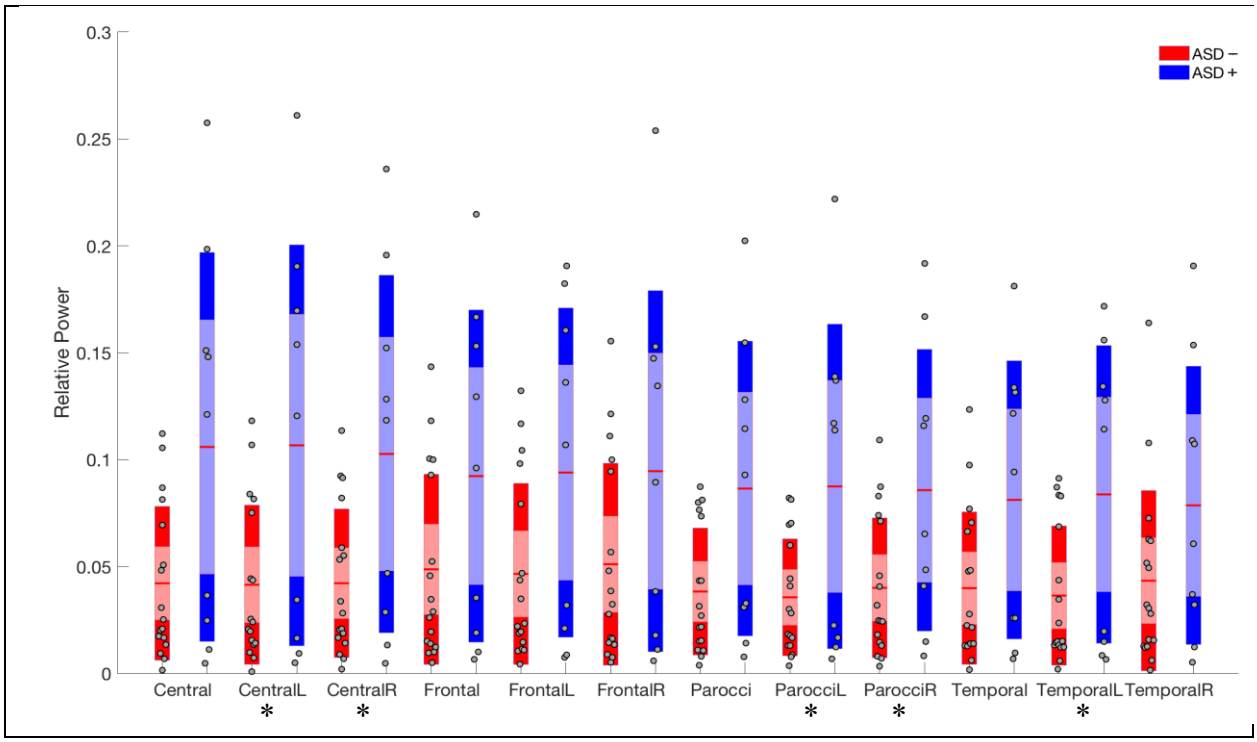

Fig I Group differences at 24 months. ASD+ subjects exhibited higher values of alpha power which was significant centrally and parieto-occipitally, on both left and right sides, and temporally on the left

* Indicates statistically significant difference

Figure II top

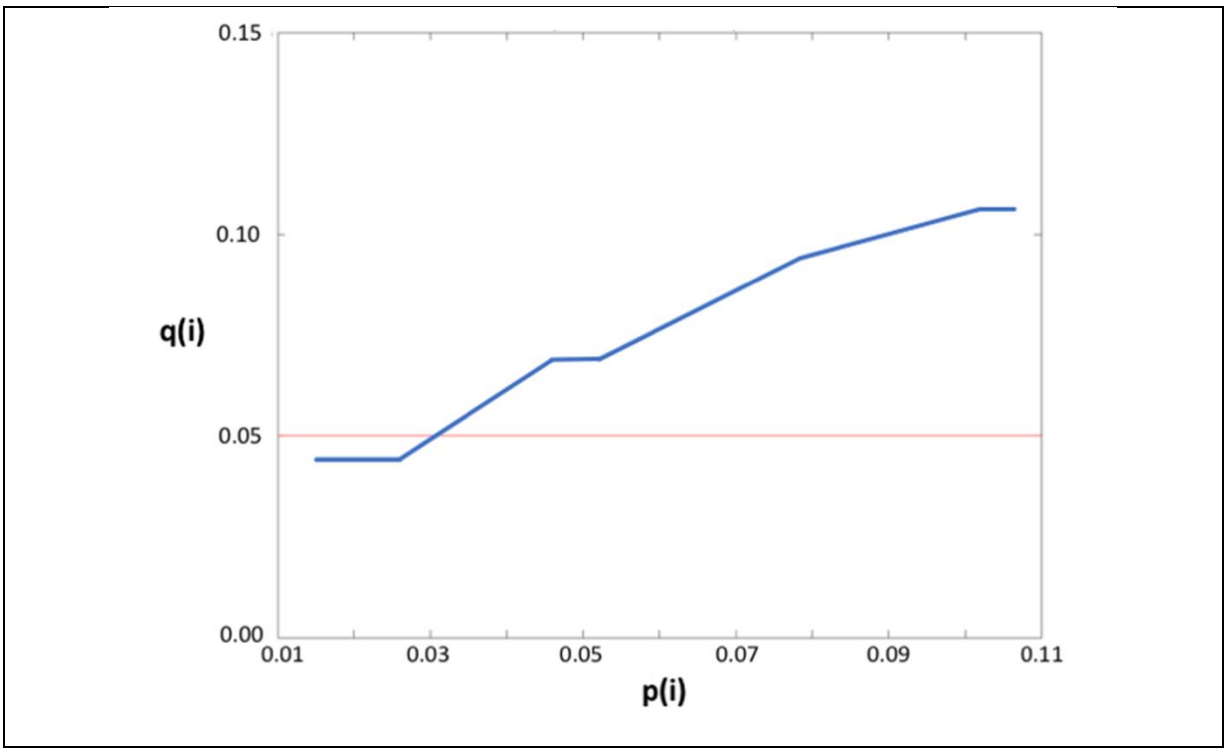

Fig II Example False Discovery Rate plot at 24 months. From this analysis, it was determined that up to seven modules could merit exploration among the alpha relative power measures

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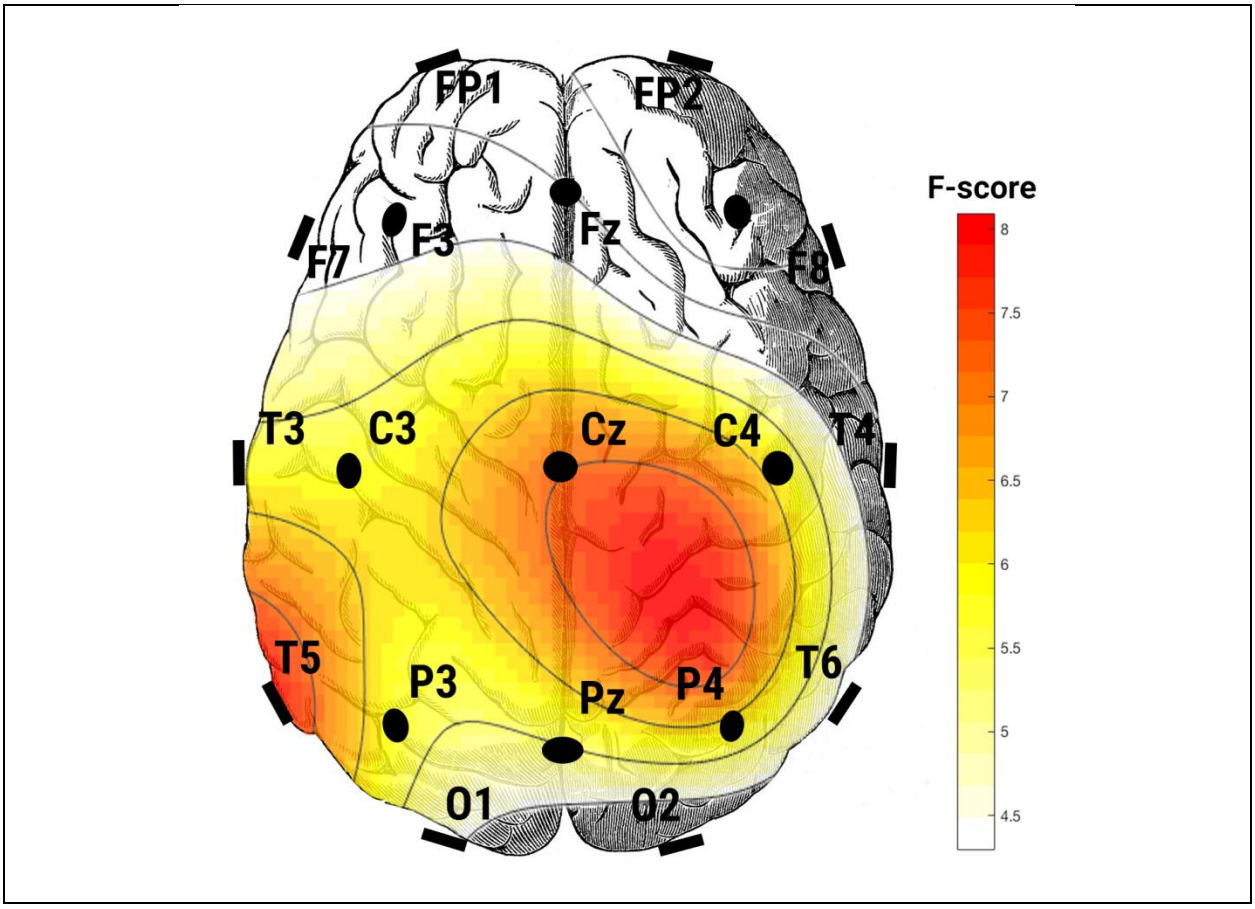

Fig III Topography of Differences in Alpha Absolute Power at 24 months

Table I. Subject characteristics

Group	24 Month Visit	18 Month Visit	12 Month Visit
ASD+	N=9 4F:5M age 23.78 (sd=0.67) mo. Race: 7 White, 1 African American, 1 Asian. Ethnicity: Hispanic 1	N=9 4F:5M age 17.56 (sd=0.73) Race: 7 White, 1 African American, 1 Asian.	N=9 4F:5M age 11.78 (sd=0.83) Race: 7 White, 1 African American, 1 Asian.
ASD-	N=17 8F:9M age 24.29 (sd=1.61) mo. Race: 16 White, 1 Asian.	N=16 8F:8M age 17.63 (sd=0.72) Race: 15 White, 1 Asian.	N=16 8F:8M age 12.13 (sd=0.81) Race: 15 White, 1 Asian.

Table II. Group Differences at 24 months

Band (measure)	**Module**	**Values (mean (sd)) F score, P value**	**Channel**	**F score, P value**
Alpha (relative power)	Central	ASD+ 0.106 (0.091) ASD- 0.042 (0.036) F 6.609, p 0.017		
	Central left	ASD+ 0.107 (0.094) ASD- 0.041 (0.037) F 6.489, p 0.018		
	Central right	ASD+ 0.103 (0.084) ASD- 0.042 (0.035) F 6.854, p 0.015		
			C3	ASD+ 0.112 (0.109) ASD- 0.042 (0.040) F 5.773, p 0.024
			C4	ASD+ 0.104 (0.088) ASD- 0.043 (0.036) F 6.381, p 0.019
			Cz	ASD+ 0.101 (0.081) ASD- 0.041 (0.035) F 7.061, p 0.014
	Par-Occ	ASD+ 0.086 (0.069) ASD- 0.038 (0.030) F 6.284, p 0.019		
	Par-Occ left	ASD+ 0.087 (0.076) ASD- 0.036 (0.027) F 6.543, p 0.017		
	Par-Occ right	ASD+ 0.086 (0.066) ASD- 0.040 (0.033) F 5.684, p 0.025		
			P3	ASD+ 0.085 (0.082) ASD- 0.033 (0.026) F 5.792, p 0.024
			P4	ASD+ 0.093 (0.082) ASD- 0.033 (0.032)

				F 7.596, p 0.011
			P7	ASD+ 0.098 (0.075) ASD- 0.033 (0.023) F 8.094, p 0.009
			Pz	ASD+ 0.087 (0.076) ASD- 0.034 (0.029) F 6.573, p 0.017
			O1	ASD+ 0.089 (0.078) ASD- 0.041 (0.037) F 4.583, p 0.043
	Temporal	ASD+ 0.081 (0.065) ASD- 0.040 (0.036) F 4.439, p 0.046		
	Temporal left	ASD+ 0.084 (0.070) ASD- 0.036 (0.033) F 5.670, p 0.026		
			T7	ASD+ 0.084 (0.070) ASD- 0.036 (0.033) F 5.670, p 0.026
Gamma (absolute power)	Frontal	ASD+ 46.51 (32.36) ASD- 26.10 (18.25) F 4.292, 0.049		
			F3	ASD+ 50.45 (30.42) ASD- 29.31 (21.61) F 4.244, p 0.050
(not significant after FDR adjustment)	F4	ASD+ 55.59 (34.87) ASD- 28.10 (20.84) F 5.990, p 0.022		

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Supplemental Materials

EEG Recording Equipment and Data Format Conversion

Investigators at BCH, CCHMC, and UAB used the Natus EEG recording system (Natus Medical, Inc., Pleasanton, CA). EEG event markers were exported using the 'Natus Wave' software, and formatted for import into BVA. All Natus EEG files (natively in ERD format) were converted to EDF in BVA and comments are then imported.

The UTH program used the Nihon-Koden recording system (Nihon Koden America, Irvine, CA). These files were converted to EDF format using the BVA software.

The program at UCLA used the Stellate Harmonie 7.0 recording system (Stellate Systems, Inc., Montreal, Quebec). The tool 'Stellate Signal File Browser' was used to convert this system's files to EDF format.

Recording Montages (Channels Included):

Sites: Birmingham (UAB), Boston (BCH), Cincinnati (CCHMC), Houston (UTH), and UCLA

All Sites Recorded the Following Channels:
A1, A2, C3, C4, Cz, F3, F4, F7, F8, Fp1, Fp2, Fz, O1, O2, P3, P4, Pz, T7 (T3), T8 (T4), P7 (T5), P8 (T6)

Non-Overlapping Channels

Channels: Fpz
Recorded at: Birmingham (UAB), Boston (BCH), Cincinnati (CCHMC), Houston (UTH)

Channels: T1, T2
Recorded at: Houston (UTH) and UCLA

Authors Note

IC, JP, MG, MB, HN, DK, AL, and MS conceived of the study and participated in its design and coordination. IC and AL drafted the manuscript. All authors performed the measurements. IC, AW, and AL participated in data analysis and the interpretation of the results. All authors read, edited and approved of the final manuscript.

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