Neocortical localization and thalamocortical modulation of neuronal hyperexcitability contribute to Fragile X Syndrome

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Fragile X Syndrome (FXS) is a monogenetic form of intellectual disability and autism in which well-established knockout (KO) animal models point to neuronal hyperexcitability and abnormal gamma-frequency physiology as a basis for key disorder features. Translating these findings into patients may identify tractable treatment targets. Using source modeling of resting-state electroencephalography data, we report findings in FXS, including 1) increases in localized gamma activity, 2) pervasive changes of theta/alpha activity, indicative of disrupted thalamocortical modulation coupled with elevated gamma power, 3) stepwise moderation of low and high-frequency abnormalities based on female sex, and 4) relationship of this physiology to intellectual disability and neuropsychiatric symptoms. Our observations extend findings in Fmr1−/− KO mice to patients with FXS and raise a key role for disrupted thalamocortical modulation in local hyperexcitability. This systems-level mechanism has received limited preclinical attention but has implications for understanding fundamental disease mechanisms.

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loss of the fragile X mental retardation protein (FMRP), mainly caused by the silencing of the fragile X mental retardation 1 (Fmr1) gene, leads to Fragile X Syndrome (FXS). FXS is an X-linked disorder that displays variable penetrance and variable expression with affected individuals characterized by a high prevalence of intellectual disability, anxiety disorders, communication impairments, sensory hypersensitivities, and autism. FMRP is a polyribosome-associated RNA binding protein that regulates the levels of many pre- and postsynaptic proteins. FMRP indirectly regulates proteins that maintain neuronal excitability and directly interact with membrane-bound ion channels. The loss of FMRP has been associated with neuronal hyperexcitability. The Fmr1−/− knockout (KO) mouse, for example, is susceptible to audiogenic seizures, displays increased spontaneous network spiking, and demonstrates elevation in resting-state electroencephalography (EEG) gamma power (>30 Hz). As the Fmr1−/− KO is central to preclinical development, it is essential to understand parallels in humans with the mouse model, but there is also an urgent need to extend our understanding of system-level dynamics in humans. EEG is a highly feasible method to postulate whole-brain hypotheses in populations such as FXS, where invasive recordings are not available, and other neuroimaging may pose selection bias.

In FXS, thalamic abnormalities have been previously reported, including lower fractional anisotropy between thalamus and neocortex, alterations in T-type calcium channels, reduced thalamic gray matter density, reduced thalamic GABA-A receptor density. The implications of many of these structural and physiological changes are not well understood but identifying electrophysiological evidence of TCD in FXS could provide a larger context from which to interpret and investigate these findings.

Herein, we provide evidence of TCD in FXS. Compared to previous reports, we have substantially increased the sample size, conducted source localization, and modeled cortical regions and functional networks. We expected to confirm changes in theta, alpha, and gamma activity signatures consistent with TCD. Finally, we expected to find evidence that TCD-related alterations would demonstrate clinical correlation with core disorder features, including cognition and neuropsychiatric symptoms. These findings provide parallels to the Fmr1−/− KO and implicate subcortical contributions to the pathophysiology of FXS, which has thus far been under-reported in the literature.

**Results**

**Participants and analysis overview.** We compared eighty seconds of artifact-free resting-state EEG data (Fig. 1) between 70 individuals with a genetic diagnosis of FXS (without seizures or on antiepileptics) and 71 age- and sex-matched typically developing control participants (Table 1). Other clinical phenotypic differences between groups were estimated by neuropsychiatric measures (Fig. 2a and Supplementary Table 1). Raw data were handled blindly, and there were no differences in preprocessing characteristics between groups (Supplementary Table 2). We divided spectral power into seven frequency bands: delta (2–3.5 Hz), theta (3.5–7.5 Hz), alpha1 (8–10 Hz), alpha2 (10–12.5 Hz), beta (13–30 Hz), and gammal (30–55 Hz), and gammag2 (65–90 Hz). To optimize the detection of neurogenic activity from the gamma band, we followed best practices to address myogenic contamination. Unless otherwise specified, independent variables that were analyzed by linear mixed effect model included group (FXS or control), sex (male or female), and frequency band (delta, theta, alpha1, alpha2, beta, gammal, and gammag2) as fixed effects and subject as the random effect. We have organized the results to report three primary analyses: 1) changes in spectral power, 2) evaluation of peak alpha frequency (PAF), and 3) alterations of CFC. We conclude with a summary of clinical correlations across analyses.

**Evidence of reduced alpha, increased theta, and gamma power in FXS from scalp EEG**

**Scalp EEG Relative Power.** Before source modeling, we considered the temporal and spatial properties of the scalp EEG recording by examining the topographical distribution and scalp-averaged spectrograms of relative power across groups. Relative power (proportion of band power to total power) is generally reported to reduce inter-subject variation and facilitate group comparisons in human studies. Scalp spectrogram and PAF: As in other examples of TCD, visual inspection revealed a global leftward shift in PAF towards the theta band in males and females with FXS (Fig. 2b). The model confirmed that PAF was significantly reduced in FXS with a significant group x sex interaction effect (F[1,137] = 5.597; p = 0.02), but no effect of electrode region. Estimates of PAF (mean Hz ± std. error) in male participants with FXS (7.8 ± 0.17) were lower than either control males (9.2 ± 0.17) or females with FXS (8.8 ± 0.19). Scalp topography: We first examined power split by frequency bands between FXS and control groups. We found that in FXS, widespread alpha power decreases, theta power increases, and clusters of increased gamma power (Supplementary Fig. 1a).
These analyses 1) con-
cally similar, but there were patches of increased alpha1 and 
spectral power topography: Group comparison of scalp topo-
power irrespective of activity in other frequency bands. Scalp 
alpha2 power is visible in males with FXS. Thus, participants with 
Fig. 1 Overview of Methodology. Principal steps of investigation: Following blinded preprocessing, artifact-free EEG data and a cortical lead field matrix 
were used to construct a weighted minimum norm estimate (MNE) source model to estimate current source density and spectral power by frequency band 
at each vertex of a triangular cortical mesh. Based on hypotheses, subsequent analyses were performed at four different levels: raw vertices, parcellation of 
vertex points into Desikan–Killiany atlas-based nodes, cortical regions (contiguous anatomical grouping of nodes), and/or resting-state networks (RSN) 
(functional non-continuous grouping of nodes).

Table 1 Demographic and clinical features of the EEG 
dataset.

| Measure | FXS     | Controls | t   | p     | adj. p.
|---------|---------|----------|-----|-------|-------|
| Age (Years) | 20.5 ± 10.0 | 22.2 ± 10.7 | <0.001 | 0.35 | 0.70 |
| Sex      | F: 32 M: 38 | F: 30 M: 41 | 0.17 | 0.72 | 0.72 |
| FSIQ     | 49.5 ± 30.2 | 103.2 ± 9.2 | <0.001 | <0.001 | <0.001 |
| NVIQ     | 40 ± 35.7 | 103.4 ± 10.7 | −12.56 | <0.001 | <0.001 |
| VIQ      | 58.9 ± 28.7 | 103.1 ± 12.8 | −10.45 | <0.001 | <0.001 |
| SCQ      | 13.6 ± 7.7 | 21.2 ± 2.2 | 9.94 | <0.001 | <0.001 |
| ADAMS-Anxiety | 7.2 ± 5.0 | 21.2 ± 2.6 | 6.37 | <0.001 | <0.001 |
| ADAMS-OCD | 2.3 ± 2.5 | 0.4 ± 1.1 | 5.12 | <0.001 | <0.001 |

Showing mean (±standard deviation) group t-tests by group. 
FXS Fragile X Syndrome, F female, M male, FSIQ Full Scale IQ, NVIQ Non-verbal intelligence 
scale, VIQ verbal intelligence scale, SCQ Social Communication Questionnaire, WI-3 Woodcock 
III Tests of Cognitive Abilities, ADAMS Anxiety, Depression, and Mood Scale, t t-statistic 
following independent Student t-tests, p unadjusted significance, adj. p p-value following 
Bonferroni correction.

Scalp EEG absolute power. To ascertain if these changes in relative 
alpha and theta power were dependent on the proportion of 
gamma power, we next examined absolute power in which each 
frequency band is considered independently. Scalp spectrogram 
and peak alpha frequency (PAF): The absolute power spectro-
gram displayed an increase in FXS participants across most fre-
cuencies (Fig. 2c). However, a significant decrease in absolute 
alpha2 power is visible in males with FXS. Thus, participants with 
FXS (exemplified in males) show similar or lower levels of alpha2 
power irrespective of activity in other frequency bands. Scalp 
spectral power topography: Group comparison of scalp topo-
graphy demonstrated elevation of absolute power, except within 
the alpha range where FXS and controls were generally statisti-
cally similar, but there were patches of increased alpha1 and 
decreased alpha2 in FXS (Supplementary Fig. 1b). The results of 
these analyses 1) confirm that relative power differences (espe-
cially within the alpha band) are not dependent on the proportion 
of gamma power and 2) the need for spectral normalization to 
account for large baseline differences in absolute power at the 
group level. Absolute power is more influenced by head 
group level. Absolute power is more influenced by head 
geometry and skull thickness, which vary considerably across 
participants, and such factors are present in FXS. Thus, we 
primarily performed relative power normalization in subsequent 
sections to mitigate subject- and group-specific biases, evident in 
absolute power analyses.

Use of source localization to resolve scalp-level findings. 
Though evidence from scalp EEG findings suggests cortical 
hyperexcitability, concluding the spatial distribution of these 
changes is limited as electrode activity represents a volume-
conducted instantaneous linear mixture of underlying brain 
sources. We employed source modeling to overcome the 
limitations of scalp EEG analysis and localize significant group 
differences by frequency band. As predicted by the TCD 
model, we hypothesized that changes in low-frequency bands 
would be global, but increased gamma activity would be more 
localized. A depth-weighted minimum norm estimate model 
based on the cortical envelope of the Montreal Neurological 
Institute (MNI) ICBM152 common brain template was used to 
perform source localization. The result was a triangular mesh 
of 15,002 vertices, with each vertex representing a time series of 
current source density. Vertices were parcellated into 68 cortical 
regions according to the Desikan–Killiany (DK) atlas. Thus, analyses were performed at either the 
vertex, node, region, or network level based on hypotheses.

Source localization reveals a global decrease in alpha activity 
and localized changes in gamma power. Vertex level. We first performed a high-resolution comparison of 
the relative power of the cortical envelope at the vertex level to 
examine overall group effects. Figure 2d depicts 5% false dis-
covexy rate (FDR)-corrected significance between-group contrasts 
(FXS-Controls). In participants with FXS, we observed a global reduction in alpha2 power and a global increase in theta power. In contrast, increases in gamma activity in FXS were primarily
restricted to the bilateral temporal lobes and regions of the parietal and occipital lobes. We again confirmed that the decrease in alpha2 was not due to relative power normalization by comparing source-localized absolute power between groups. Even considering that mean absolute power was increased across all frequency bands in FXS, at the group level, alpha1 and alpha2 activity were higher only in lateral regions in FXS (Supplementary Fig. 2). In a subgroup of males with FXS, absolute alpha2 power had significant clusters of increased and decreased alpha2 absolute power compared to control males (Fig. 2f). We examined additional sex effects at the region and network-level based on these significant group effects.

**Region level.** A recent study examining TCD signatures in Parkinson’s disease, neuropathic pain, tinnitus, and depression found spectrally equivalent but spatially distinct forms of TCD depending on the disorder\(^1\). After parcellation of nodes into cortical regions, we examined the group by sex differences of spectral activity by frequency band. Based on predicted lower or absent FMRP levels and higher burden of neuropsychiatric symptoms, we expected higher deviations of spectral power in males with FXS than in controls. We found a robust interaction effect supporting our hypothesis between group, sex, frequency band, and region (\(F_{78,66587} = 1.64, p < 0.001\)). Results from pairwise comparisons of same-sex groups showed that participants
with FXS had increased theta and decreased alpha power across cortical regions, but this was more pronounced among males with FXS (Table 2). In addition, males with FXS, but not females with FXS, exhibited increased gamma power (Fig. 3a). Gamma power peaked across temporal regions but did not reach statistical significance in prefrontal (gamma1 and gamma2) or left occipital regions (gamma2).

Network level. Neuroimaging studies have identified modular brain networks related to higher-order cognitive, affective, and motor functions. Thus, we extended our analysis to EEG-based RSNs, representing standing functional networks rather than contiguous anatomical regions (Supplementary Table 15). The interaction of group, sex, frequency band, and RSN was a significant linear predictor of relative log power ($F_{0.068811} = 2.21$, $p < 0.001$; Fig. 3b). Females with FXS exhibited fewer and more modest changes from control females than their male counterparts. Females with FXS, for example, had similar gamma1 and gamma2 power across auditory and visual networks and decreased gamma power across cognitive networks to control females. In contrast, males with FXS demonstrated significant elevations in gamma power across all RSNs.

Modulation of alpha and gamma power abnormalities by sex. We hypothesized that sex, a key determinant of phenotype in FXS, is associated with stepwise changes in EEG. We examined the above models for within-group spectral power contrasts.
Fig. 3 Relative power differences by cortical region or resting-state network. Bar plots represent 5% FDR corrected pairwise contrasts of model estimates of log relative power between FXS (n = 70) and control (n = 71) participants. For region plots, left and right of individual bars correspond to the right and left hemispheres, respectively. Positive $t$-values indicate that log relative power estimates in FXS are greater than those in control. **A** Sex-matched group differences in log relative power by cortical region. Males with FXS demonstrate region-specific increases in gamma power compared to controls with sparing of prefrontal regions. **B** Sex-matched group differences in log relative power across RSNs. A significant increase in theta and gamma power, as well as a decrease in alpha power, were observed across cognitive and sensory RSNs of males with FXS. Compared to control females, females with FXS had only modest changes in RSNs including similar gamma levels in visual and auditory networks. Obligate mosaicism in Fmr1 in females with full mutation FXS may attenuate EEG alterations. **C** We explored sex differences in relative power within the FXS group by region and RSN. Positive $t$-values indicate that log relative power estimates in males with FXS are greater than in females with FXS. There were fewer differences between males and females with FXS than in control comparisons, except for decreased alpha and that gamma activity remained elevated in most regions and all RSNs in males. Resting-state network abbreviations: DMN default mode network, DAN dorsal attention network, SAN salient affective network, VIS visual attention network, AUD auditory network.
between males and females with FXS by region and network (Fig. 3c).

**Peak alpha frequency at the source level reveals reduced frequency and loss of posterior-anterior configuration**

**Region level.** Reports of TCD have observed alterations in alpha activity, including 1) leftward shift of PAF towards the theta frequency and 2) shifted spatial distribution (known as anteriorization) of slow alpha frequencies. Our goal was to determine how group, sex, and posterior-to-anterior cortical regions (occipital, parietal, central, and frontal) affect source localized PAF. A three-way interaction was present \(F_{3,4354} = 4.303; p = 0.005\), suggesting a linear relationship between PAF and the interaction of group, sex, and region. Frequency reduction of source PAF in FXS: To confirm the presence of reduced or slowed PAF in FXS, we examined univariate pairwise contrasts between PAF within each posterior-anterior region between sex-matched groups. Both males and females with FXS had reduced PAF compared to controls, with the largest decrease in the parietal and central regions of males with FXS (Fig. 4a). The spatial configuration of source PAF is lost in FXS; We hypothesized that PAF would be reduced and lose its characteristic asymmetrical posterior-anterior topography, as with other TCD syndromes. In male and female controls and, to a lesser extent, females with FXS, PAF was peaked over parietal and central regions (Fig. 4b). In contrast, males with FXS displayed a relatively flat profile of PAF across posterior to anterior regions. PAF in males with FXS was mildly depressed from occipital to parietal regions and not significantly different between posterior and central regions.

**Node level.** Given differences along the posterior-anterior axis, we next examined contrasts in PAF in all 68 atlas nodes to clarify the spatial distribution of PAF reduction (Supplementary Table 15). Interestingly, we found a significant interaction effect between group and node (group x node; \(F_{7,9378} = 1.44, p = 0.01\), but no effect of sex. In approximately 51% of nodes (35/68), participants with FXS had a significantly lower PAF than controls (Supplementary Table 3). An atlas-based visualization is presented in Fig. 4c, which depicts t-scores of the FXS-control contrast. The greatest difference in PAF between FXS and controls were found in the left parietal region including the supramarginal gyrus \((F_{140} = -4.7, 5\% \text{ FDR} < 0.001)\), inferior parietal gyrus \((F_{140} = -4.4, 5\% \text{ FDR} < 0.001)\) and precuneus \((F_{140} = -4, 5\% \text{ FDR} < 0.001)\).

**Theta, not alpha, power is predominantly associated with gamma power in FXS.** Power-power CFC is the association (in normalized Fisher’s Z-transformed correlation coefficients) between time series of EEG power between two frequencies\(^47\). In TCD syndromes, theta power is more strongly correlated with
gamma power than is alpha power. These alterations have been associated with task-related disruptions in the functional activity and cognitive processing. We evaluated the effects of group and sex on theta-gamma1, alpha1-gamma1, and alpha2-gamma1 CFC. As alterations of low-frequency power in FXS occur globally, we specifically examined whole-brain theta, alpha1, and alpha2 CFC with node-level and network-level gamma1 activity.

Node CFC. We first examined the effect of the lower frequency band (theta, alpha1, or alpha2), diagnostic group, sex, and cortical node on gamma1 CFC. Unexpectedly, sex did not have a significant main or interaction effect and was not retained in the final model. We found that the lower frequency band, group, and cortical node had a significant interaction effect on gamma1 CFC. Participants with FXS (n = 70) showed a significantly higher magnitude of inverse theta-gamma1 CFC compared to controls (n = 71), especially over the parietal and central regions. An accompanying mean ± standard error model plot of group by CFC type across RSN demonstrates a prominent increase in inverse theta-gamma1 CFC and an increase in direct alpha2-gamma CFC in the FXS group.

Network CFC. To better understand the functional impact of altered node-level CFC, we also compared CFC measured across RSNs. We found a similar trend to our individual node analysis, with a significant interaction effect of the lower frequency band, group, and RSN (F_{10,28589} = 3.02, p < 0.001). Across all functional networks, contrasts of model estimates revealed that participants with FXS had a greater magnitude of inverse theta CFC and increased alpha2 CFC with gamma1 (Fig. 5C and Supplementary Table 5). Clinical correlations. Due to the broad age range of the sample, partial Spearman’s correlations were conducted to control for the effects of age on findings between EEG features and clinical measures. We first examined 5% FDR corrected correlations between (1) spectral power (Supplementary Tables 6–9), (2) PAF (Supplementary Tables 10–12), and (3) CFC (Supplementary Tables 13 and 14) values across region and RSN for all participants with FXS. FDR correction was performed on the entire correlation table for each of the three main EEG features: source estimated band power, PAF, and CFC. We next conducted an uncorrected exploratory correlation analysis on a subgroup of full-mutation, non-mosaic males (FM) to eliminate confounding with potential mosaic effects and examine findings in a more homogenous group that parallels Fmr1^{−/−} KO mouse. We have hosted an interactive statistical R web application.
Intelectual Quotient (IQ). All FXS: After FDR adjustment, there was a trend-level association such that increased verbal IQ (VIQ) was associated with elevated PAF (VIQ; SAN: r(62) = 0.39; p = 0.002, adj. p = 0.081). FM subgroup: Reduced NVIQ was inversely associated with elevations in gamma power across multiple regions and RSNs (NVIQ; DMN, DAN, AUD, VIS, Temporal, Parietal, Occipital: r(20) = −0.44 to −0.60, p ≤ 0.05; for exemplar Fig. 5d). CFC demonstrated significant correlations with NVIQ and VIQ. A larger relationship between increased theta and gamma1 power (theta-gamma1 CFC) was associated with increased NVIQ (SAN, DAN: theta and gamma1 power (theta-gamma1 CFC) was associated with lower PAF across multiple regions and RSNs (NVIQ; DMN, DAN, and VIS; r(60) = −0.37 to −0.52; for exemplar Fig. 5d). FM subgroup: Greater impairments in social-communication functioning were associated with alpha1 (RPF; r(22) = −0.43; p ≤ 0.05) and alpha2 power (RPF, LPF, RC, LF, RL, LL, DMN, DAN, and SAN; r(60) = −0.39 to −0.41; adj. p ≤ 0.05) and alpha2 power (RPF, LPF, RC, LF, RL, LL, DMN, DAN, and SAN; r(60) = −0.37 to −0.52; for exemplar Fig. 5d). Social communication. The Social Communication Questionnaire is a brief screening instrument to evaluate social-communication skills across the lifetime where higher values indicate greater impairment51. All FXS: More impaired social-communication functioning was associated with decreased alpha1 (LPF, RPF, LT, RT, DMN, DAN, SAN, and VIS; r(60) = −0.39 to −0.41; adj. p ≤ 0.05) and alpha2 power (RPF, LPF, RC, LF, RL, LL, DMN, DAN, and SAN; r(60) = −0.37 to −0.52; for exemplar Fig. 5d).

Auditory attention. Selective auditory attention (including speech-sound discrimination and resistance to auditory-stimulus) was measured using the Woodcock-Johnson III auditory attention task, with higher scores indicating better performance52. All FXS: We found that improved auditory attention performance was associated with alpha1 power (SAN; r(56) = 0.39, adj. p ≤ 0.05; for example Fig. 5d). FM subgroup: Reduced auditory attention performance was associated with decreased alpha1 (LO: r(21) = −0.52, p ≤ 0.05), but increased theta (LO, RO, LP, and RP; r(21) = 0.45 to 0.52, p ≤ 0.05), gamma1 (LO, RP, and VIS; r(21) = −0.42, p ≤ 0.05), and gamma2 power (LO, RO, RP, and VIS; r(21) = −0.44, p ≤ 0.05).

Discussion

In the present study, we raise several findings derived from resting-state EEG to provide a unifying thalamocortical model to explain alterations of neural activity in FXS. The findings offer spatial and functional context to previously reported scalp-level EEG abnormalities in alpha/theta oscillations and elevated non-myogenic gamma activity. First, we observed clinically associated alpha and gamma activity increases, which varies by sex. Second, we report global changes in alpha and theta power, including a reduction and altered spatial configuration of PAF. Third, we demonstrate a predominance of regional and RSN-based theta-gamma CFC in FXS. These results were obtained from a well-powered sample of individuals with FXS and age- and sex-matched controls, using source modeling to identify effects with anatomical and functional ROIs. Our results highlight system-level features to enhance the development of patient-oriented biomarkers and provide key physiological insight into the neural activity of a prototypical monogenetic neurodevelopmental condition53.

The global increase in theta power, alpha power decrease, and marked PAF reduction into the theta band implicate changes in thalamocortical circuits in FXS. In addition, the association of these changes with increases in gamma activity suggests that thalamocortical modulation may be a key mechanism underlying neocortical hyperexcitability observed in FXS12,13,34. Thalamocortical structures are interconnected by widespread excitatory connections, in which abnormalities have been associated with other examples of cortical hyperexcitability, including epilepsy55. The role of the thalamus has also continued to evolve from a relay station into a dynamic center for contextual modulation of cortical circuits56. Though large-scale direct measurements of thalamic contributions to neocortical excitability in FXS are unlikely, previous invasive experiments in humans, such as with stereotaxic EEG, have bolstered confidence that surface EEG oscillations are a proxy for thalamocortical activity57. In this sense, the observed EEG alterations, including alterations associated with TCD, may reflect the functional implications of the previously reported thalamic findings in FXS28,30,31.

Previously it was assumed that alpha generators resided in the thalamus. However, recent research from invasive recordings in
humans has placed new emphasis on cortical generators while still confirming the central role of the thalamocortical system in driving alpha activity and PAF\textsuperscript{57,58}. Specifically, a PAF between 10–13 Hz appears to have special relevance to functional brain physiology\textsuperscript{29–34}. Like a radio receiver that can only tune specific electromagnetic frequencies, neurons and oscillatory networks also demonstrate a frequency preference\textsuperscript{65,66}. The switch to a dominant peak rhythm at 4–8 Hz in FXS rather than operating peaks closer to 10–13 Hz may be insufficient to drive neural ensembles with an alpha preference\textsuperscript{60,63,67,68}. Conversely, as theta dominance increases, alpha activity may lose its canonical role as an inhibitor of the circuits that provide optimal time for processing sensory and cognitive information\textsuperscript{69,70}.

EEG is an ideal method for studying real-time brain activity at high frequencies, but the addition of source modeling can dramatically improve spatial resolution at the cortical surface\textsuperscript{60}. Increases in gamma power in patients with FXS were localized primarily to the temporal cortices and portions of the parietal and occipital lobes. Gamma oscillations hold a special interest in neurodevelopmental conditions because of their relation to cortical excitability\textsuperscript{5,71}, association with cognitive processes\textsuperscript{24,72}, and analogous measurability in animal models\textsuperscript{6}. The role of gamma oscillations is increasingly nuanced, such that precise synchrony in gamma activity is contributory to higher-order cognition\textsuperscript{61,73} and that a modest degree of asynchrony or noise represents physiological processes\textsuperscript{74–76}. Nevertheless, asynchronous (usually broadband) gamma power, above what is typically expected, has been associated with disease states\textsuperscript{72} as well as with reduced spike precision and spectral leakage of spiking activities in microcircuit preparations\textsuperscript{77}.

Gamma activity varied significantly based on sex and was strongly related to core cognitive and neuropsychiatric symptoms in FXS. As sex is highly predictive of phenotypic differences in FXS\textsuperscript{13} and scalp-level EEG findings\textsuperscript{14,78} we also expected sex to have a large effect on source-localized EEG activity. Unexpectedly, we found that females (obligate mosaics) generally demonstrated similar low-frequency alterations as males with FXS; however, males consistently demonstrated higher gamma power. Indeed, when compared to female controls or males with FXS, females with FXS showed either no difference or a modest reduction in gamma power. We raise at least two possibilities for these findings. First, though theta power (after correction) was similar between males and females with FXS, regional and network alpha power was higher in females. This is further strengthening the association of the presence of alpha power in regulating cortical hyperexcitability. Second, these findings suggest that FMRP expression is associated with variability in gamma power, despite not fully underlying local and system-level mechanisms leading to these changes. Within-group sex differences suggest that full-mutation females, who likely express more FMRP than their male counterparts, may moderate neocortical hyperexcitability as assessed by background gamma activity. Though in females with FXS, some liability to hyperexcitability, are compensatory or causative, so much variation in phenotype may be associated with global changes in alpha activity. In contrast, other disorder-relevant features such as cognition may be more strongly related to gamma disturbances. Since the normalization of gamma activity is associated with increased FMRP expression, we speculate that the pattern of EEG correlations may reflect distinct mechanisms that underlie the variety of cognitive and behavioral phenotypes found in FXS. It may be possible to further explore these hypotheses in future studies which correlate specific EEG findings with domain-specific behavioral tasks.

As in other examples of TCD, we found that theta-gamma CFC, rather than alpha-gamma CFC, is predominant in FXS compared to controls\textsuperscript{81}. We did not find any effect of sex in our final model, which likely reflects the similar low-frequency alterations in males and females with FXS, despite relative differences in gamma activity. It has been proposed that in TCD, theta modulation does not produce the same lateral inhibition of gamma activity as alpha oscillations. This leads to a net increase in asynchroneus, or background, gamma activity and spurious neural activity\textsuperscript{46}. Exploratory correlations in full mutation, non-mosaic males revealed significant associations with CFC between cognitive and neuropsychiatric features. Still, the interpretation of these findings remains complex and will require future experiments to parse. For example, a stronger inverse theta-gamma CFC relationship was associated with reduced cognitive scores, but we cannot infer either directionality or directly compare it with other frequency-specific CFC in the same subject. However, the results provide a rich starting point for developing hypotheses for future connectivity and causality analyses.

As the TCD model has not previously been explored in FXS, considering a theoretical framework from other disorders associated with TCD can provide future direction. In tinnitus, where abnormal sensory perception has been consistently linked with slow-wave oscillations, it has been hypothesized that decreased organized input to the thalamus (i.e., deafferentation or tonic inhibition) leads to excessive theta band activity. When this shift occurs, inappropriate activation of the sensory cortices (reflected by increased gamma activity) results in the perception of tinnitus. Though not directly measured in humans, neocortical changes in the Fmr1\textsuperscript{−/−} KO mouse include intrinsic hyperexcitability of pyramidal neurons\textsuperscript{3} and fast-spiking parvalbumin cell dysfunction\textsuperscript{82} which is critical to sharpening synchronous neuronal activity\textsuperscript{27,83}. Could these changes lead to a noisy cortex and disrupt organized feedback to the thalamus, and thus, thalamocortical signaling, result in alterations observed with EEG? In addition, T-type calcium channels play a central role in either tonic or burst firing of thalamic neurons\textsuperscript{84} and T-type calcium blockade can modulate theta/alpha oscillations in the human brain\textsuperscript{23}. The contribution of calcium channelopathies is increasingly recognized in intellectual disability syndromes\textsuperscript{85}, including known alterations in T-type calcium channels in FXS\textsuperscript{29}. This raises speculation for new avenues of therapeutics, including targeting ion channels and non-invasive perturbation channels, to understand mechanisms that promote and maintain suboptimal brain states.

We cannot determine if the measured changes, which suggest neocortical hyperexcitability, are compensatory or causative, so back-translational approaches are necessary to uncover the underlying mechanisms of these biomarkers. For example, in some Fmr1\textsuperscript{−/−} KO circuitry, compensatory mechanisms may partially restore global homeostasis\textsuperscript{71}. Placed in a larger context, the circuit changes observed in FXS appear to disrupt more specialized circuits for higher-order cognition, emotional regulation, and sensory processing\textsuperscript{86}, rather than to a level representing a common cause of epilepsy (which is rare and mild in FXS). Thus, subpopulations in FXS with residual Fmr1 expression may vary in phenotype based on the extent of protein deficiency,
protein distribution, developmental period, circuit function, and neuronal type.

In general, absolute and relative low and high-frequency power changes were congruent, especially when examining group effects. Some changes, such as sex differences in FXS of theta power, were more prominent in absolute power. However, as seen in Fig. 4c, similar directional sex differences are present in theta power but did not reach statistical significance after multiple comparisons. Furthermore, close examination of scalp topographies (Fig. 2b, c) reveals within-band variability in theta and may support future analyses subdividing theta band into low and high components. Our use of relative power was to account for potential systematic bias due to head circumference and developmental stage while still acknowledging the complex between-group and within-group relationships regarding oscillatory power distribution both across frequencies and across spatial locations. Rather than assess detailed point-to-point connectivity, we used CFC to assess whole-brain averaged alpha or theta power to gamma power across individual cortical nodes to compare to reports of TCD in other disorders. Within the scope of TCD, phase-amplitude (or phase-phase) coupling is not well-understood. Despite ascertaining mosaic status in males, males with mosaicism are a relatively small subset (n = 12) and underpowered for subgroup analysis. Additionally, males with mosaicism can vary phenotypically based on mosaic type (repeat number or methylation), further emphasizing the importance of a well-powered sample. Although the effect of non-epileptic medications on the results cannot be ruled out, a medication naïve sample would preclude the inclusion of more severely affected individuals, given the high rate of medication use in FXS. Previous EEG studies of medication effects in psychiatric populations, including our observations in FXS, do not suggest effects as we have observed.

In summary, the present findings demonstrate evidence of clinically relevant TCD physiology in individuals with FXS syndrome. The changes we have observed may contribute to and maintain abnormal cortical states that reduce functional brain connectivity and regional function necessary for optimal brain functions. The results suggest a central role of thalamocortical regulation of neocortical hyperexcitability in FXS and, thus, have implications for future therapeutic and physiological investigations. These systems-level findings may also guide back-translational approaches for developing and testing new treatments with electrophysiological biomarkers that can transfer directly from the mouse model to patient studies.

Methods

Participants. The dataset included 145 participants drawn from a large federally funded human neurophysiology study in FXS (National Institutes of Mental Health U54 HD082008). Exclusion criteria for FXS (confirmed by Southern Blot and polymers chain reaction) participants included a present history of unstable seizures (any treated seizure within one year) and scheduled use of benzodiazepines. Controls did not have treatment for neuropsychiatric illness as reported via clinical interview. All participants provided written informed consent (or assent as appropriate) prior to participation as approved by the institutional review board of Cincinnati Children’s Hospital Medical Center. Following blinded preprocessing, three recordings were discarded from further analysis due to excessive line-noise artifact (1 FKS, 2 controls) and one due to insufficient data due to intolerance of the EEG procedure (1 FKS). The final dataset consisted of 70 participants with a genetic diagnosis of full mutation FXS (Mean age = 20.5, SD = 10; age range: 5.9–45.7; 32 females; 12 males with mosaicism) and 71 controls (Mean age = 22.2, SD = 10.7; age range: 5.9–48.2; 30 females). Females with full mutation FXS were included in the primary analyses, and effects were confirmed in supplementary analyses of male participants. Age effects were examined in each model for statistically significant fixed effects. Thirty-five FKS patients were on antidepressants, and 18 were receiving antipsycotics. These and other concurrent medications were only permitted if the participant was on stable dosing for at least six weeks.

Data acquisition and preprocessing. Five minutes of continuous EEG data were collected while participants were seated comfortably while watching a silent video (color/contrast modulated for accommodations, if needed). Data were collected at a 1000 Hz sampling rate with an EGI NetAmp 400 with a 128-channel HydroCel electrode net (Magstim/EGI, Eugene, OR). Preprocessing: All data was blinded and coded regarding group, participant, or collection date. Data was exported in EGI raw format and imported into EEGLAB (Figs. 3 and 4; MathWorks Inc., Natick, MA USA). Raw EEG data were filtered using EEGLAB 14.1.290 with a 2 Hz high pass digital zero-phase filter and a 55 to 65 Hz notch filter (with harmonics removed up to Nyquist frequency of the original sampling rate) to remove line noise. Raw data was visually inspected by an assistant who excluded segments with a large amount of movement artifact and interpolated bad channels (no more than 5% per subject) using spherical spline interpolation implemented in EEGLAB 14. Data was average referenced. An artifact subspace reconstruction approach was carried out with the clean_rawdata function (with default parameters) to repair data segments of an artifact by applying a reconstruction mixing matrix from non-interpolated neighboring channels. The mixing matrix is computed from clean segments within the EEG data.90 Blind source separation was performed with temporal Independent Component Analysis on each preprocessed dataset using the extended INFOMAX algorithm96–98 with principal component analysis rank reduction (further reduced for interlateral channels). This approach was recently validated to effectively reduce myogenic contamination from approximately 25–98 Hz.99 The resulting components were manually reviewed and categorized for eye movement/blinks, muscle movement, channel noise, or cardiac artifact based on temporospatial and spectral features and back-projected to remove artifact. The resulting non-artifactual independent components are near-independent in time course activity and resemble dipolar scalp projections, and have been proposed to represent spatially coherent local field activity within a single cortical area. Data was divided into 2-second epochs and manually reviewed for noise artifacts. Summary of artifact cleaning is presented in Supplementary Table 2 and demonstrates no significant differences between preprocessing measures between groups.

Source modeling. Minimum norm estimation is a widely adopted solution to the inverse problem. Current estimates are calculated at every spatial location in source space to minimize the total power across the cortex. Thus, minimum norm models, in contrast to dipole fitting, produce uniform maps across subjects which is well-suited for group comparisons and can provide resolution comparable to magnetoencephalography.100 For each subject, the first 80-s of artifact-free data from each of the EGI 128-channel electrodes were co-registered with a Montreal Neurological Institute (MNI) averaged ICBM152 common brain template.101 The degree of accuracy and precision of EEG source localization is debated, but intracranial recordings during epileptic surgery,102 surface and deep brain stimulation103, and comparisons with functional magnetic resonance imaging (fMRI)104 estimate focal localization at 1.5 cm for superficial cortices. Thus, even with standard head models and spatial smoothing EEG is suitable for studying high-frequency brain activity in vivo clinical studies.105,106 OpenM/EEG107 was used to calculate a 15,000 vertices lead-field mesh incorporating electrode distances. Noise covariance was set as an identity matrix as recommended for scalp resting EEG108,109. Construction of a spatially defined, depth-weighted norm source model to generate a current source density map (units: picoampere-meter) was performed in Brainstorm110 and used to reconstruct time series activations at each vertex.

Anatomical and functional parcellation. Individual vertices from the lead-field mesh were grouped into 68 cortical nodes according to the Desikan–Killiany (DK) atlas.111 We opted to study both the anatomical and functional configuration of the atlas nodes. The anatomical configuration was derived from the DK atlas. It consisted of categorizing the 68 nodes into 14 regions: occipital (O), Limbic (L), parietal (P), temporal (T), central (C), frontal (F), and prefrontal (PF) each with a right (R) or left (L) designation. Functional source EEG resting-state networks have been derived from examining their dynamic properties and similarities to networks identified by other neuroimaging techniques (diffusion tensor imaging, fMRI, and magnetoencephalography).110 Following this template, we assigned 44 cortical nodes into resting-state networks including default mode network (DMN), dorsal attention network (DAN), salient affective network (SAN), auditory network (AUD), and visual network (VIS). The remaining nodes not associated with a functional network are classified as “Other” by convention.

Spectral power. For all analyses, we divided spectral power into 7 frequency bands: delta (2–3.5 Hz), theta (3.5–7.5 Hz), alpha1 (8–10 Hz), alpha2 (10–12.5 Hz), beta (13–30 Hz), and gamma1 (30–55 Hz), and gamma2 (65–90 Hz). Upper alpha bands are associated with more complex cognitive processing, and lower alpha bands have been primarily related to attentional processes, including alertness, expectancy, and vigilance.112

Scalp EEG. Segmented data (2-s) from 108 scalp EEG channels were detrended, tapered with a Hanning window, and transformed into Fourier coefficients representing 0.5 Hz frequency steps. Fourier coefficients were squared to compute...
absolute power and divided into frequency bins. Relative power was defined as the band-specific cumulative absolute power (V^2/Hz) divided by the total power across all defined bands and then averaged over available trials. For source data, Welch's method (50% overlap with Hanning window) was used to estimate spectral power from each vertex amplitude time series. To facilitate group comparisons, we used a circularly symmetric Gaussian smoothing kernel with a full width half maximum (FWHM) size of 3 mm^3 across all vertices. Relative power calculations were performed identically to scalp electrode power.

**Peak alpha frequency.** The average dominant frequency (i.e., alpha peak) was determined by the findpeak() function in MATLAB to identify the frequency of the maximum absolute logarithmic power between 6–14 Hz from each channel or DK node spectrogram.\(^{106}\)

**Clinical measures.** Stanford-Binet Intelligence Scale 5th Ed. (SBIS)\(^{107}\) was conducted by trained clinicians in both FXS and control participants. Due to floor effects, deviation IQ scores\(^{108}\) were computed to capture variability in cognitive functioning. The primary caregivers completed assessments for FXS patients, including the Social Communication Questionnaire\(^{109}\). Anxiety, Depression, and Mood Scale (ADAMS)\(^{109}\), Woodcock-Johnson III Tests of Cognitive Abilities, Auditory Attention subscale\(^{2}\).

**Statistics and reproducibility.** The dataset consisted of 70 individuals with a genetic diagnosis of FXS (without seizures or on antiepileptics) and 71 age- and sex-matched typically developing control participants. See Data Availability and Code Availability sections for access to publicly hosted datasets and analysis scripts. Eighty seconds of artifact-free resting-state EEG data for each participant was used for analysis.

**Power and sample size.** Differences in gamma1 power in FXS compared to controls in previous studies have effect sizes from 0.63 to 1.75, similar to effects in prior studies of N1 amplitudes in FXS.\(^{12,13,78,110}\) Based on these effect sizes, comparing 70 FXS patients (50% males) and 70 TD controls provides power to detect the primary EEG outcome with approximately power > 0.90 (using an omnibus F-test with an alpha of 0.05). In line with reproducible research guidelines, scripts for the generation of figures and tables are available upon request.

**Topographical electrode power comparison.** Cluster-based permutation analysis\(^{111}\) was used to identify differences in relative or absolute power between FXS and controls. Overall alpha was set at 0.057 (adj. p < 0.007) to account for multiple frequency band comparisons (effective alpha for each tail 0.025).

**Source model power comparison.** Group-level statistical (t-statistic) cortical maps were generated by Monte-Carlo permutation (2000 after independent two-tailed t-tests (alpha set at 0.025 per tail) using the ‘rt’sourceestatistics’ function in FieldTrip\(^{12}\) and threshold at p < 0.05. The resulting p values were globally corrected for a false discovery rate (FDR) of 5% applied over the signals and frequency band dimensions.\(^{113}\)

**Node, region, and RSN comparisons.** Log-transformed power differences were evaluated with generalized linear mixed effect models via NLME library in R.1.1 and confirmed with GLIMMIX procedure in SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Random effect was subject and independent variables varied based on model. Fixed effects included GROUP (FXS vs. control), SEX (male vs. female), or FREQUENCY BAND (delta, theta, alpha1, alpha2, beta, gamma1, and gamma2). When specified, NODE indicates the 68 cortical parcels of the DK atlas (Supplementary Fig. 3). REGION refers to the 14 node groups that represent cortical lobes, and RSN refers to the six functional grouping of nodes (DMN, VIS, DAn, SAN, AUD, Other). In REGION and RSN models, nodes were treated as replicates. To ensure optimal model fit, we examined various structures of intra-subject covariance and rank-function for each model, plots based on the standardized residuals were examined. See Supplementary Fig. 3 for a visual DK atlas key and Supplementary Table 15 for a comprehensive classification table of cortical nodes.

**Cross frequency amplitude coupling.** To examine the potential dependence between low-frequency power and high-frequency activity, we calculated power-power CFC.\(^{17}\) CFC was calculated based on each low-frequency band’s mean whole scalp power (theta, alpha1, and alpha2) compared to gamma1 power within each individual cortical node. The Spearman’s correlation coefficient for each low frequency and gamma1 was calculated using the time series of mean relative power across 2-s epochs. Fisher’s Z-transform was used to normalize group-wise comparisons.

**Correlation analysis.** As a successive step, frequency bands of significance were linearly correlated with clinical and behavioral measures. Primary Analysis: Shapiro-Wilk’s normality test was performed on variables to assess suitability for either Spearman’s rank-order or Pearson’s correlation test. A priori hypotheses for high-frequency bands (beta, gamma1, and gamma2) and low-frequency bands (theta, alpha1, and alpha2) with clinical variables were assessed with correlation tests with values adjusted by FDR for multiple test iterations, and partial correlations were used to adjust all correlations for age.

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability.** EEG datasets and figure data that support the findings of this study are available in Zenodo and Figshare with the identifier(s): https://doi.org/10.5281/zenodo.6385768\(^{114}\) and https://doi.org/10.6084/m9.figshare.19424015.v1\(^{115}\).

**Code availability.** Scripts used for EEG analysis are available at http://github.com/cincibrainlab/ehpS. Time-stamped analysis code is available on Figshare\(^{116}\).

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