BRAIN COMMUNICATIONS

A neurophysiological model of speech production deficits in fragile X syndrome

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Fragile X syndrome is the most common inherited intellectual disability and monogenic cause of autism spectrum disorder. Expressive language deficits, especially in speech production, are nearly ubiquitous among individuals with fragile X, but understanding of the neurological bases for these deficits remains limited. Speech production depends on feedforward control and the synchronization of neural oscillations between speech-related areas of frontal cortex and auditory areas of temporal cortex. Interaction in this circuitry allows the corollary discharge of intended speech generated from an efference copy of speech commands to be compared against actual speech sounds, which is critical for making adaptive adjustments to optimize future speech. We aimed to determine whether alterations in coherence between frontal and temporal cortices prior to speech production are present in individuals with fragile X and whether they relate to expressive language dysfunction. Twenty-one participants with full-mutation fragile X syndrome (aged 7–55 years, eight females) and 20 healthy controls (matched on age and sex) completed a talk/listen paradigm during high-density EEG recordings. During the talk task, participants repeated pronounced short vocalizations of 'Ah' every 1–2 s for a total of 180 s. During the listen task, participants passively listened to their recordings from the talk task. We compared pre-speech event-related potential activity, N1 suppression to speech sounds, single trial gamma power and fronto-temporal coherence between groups during these tasks and examined their relation to performance during a naturalistic language task. Prior to speech production, fragile X participants showed reduced pre-speech negativity, reduced fronto-temporal connectivity and greater frontal gamma power compared to controls. N1 suppression during self-generated speech did not differ between groups. Reduced pre-speech activity and increased frontal gamma power prior to speech production were related to less intelligible speech as well as broader social communication deficits in fragile X syndrome. Our findings indicate that coordinated pre-speech activity between frontal and temporal cortices is disrupted in individuals with fragile X in a clinically relevant way and represents a mechanism contributing to prominent speech production problems in the disorder.

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Abbreviations: C-units = communication units; ELS = Expressive Language Sampling; ERSP = event-related spectral perturbation; ERP = event-related potential; FXS = fragile X syndrome; GCA = Granger causality analyses; IFG = inferior frontal gyrus; TDC = typically developing control

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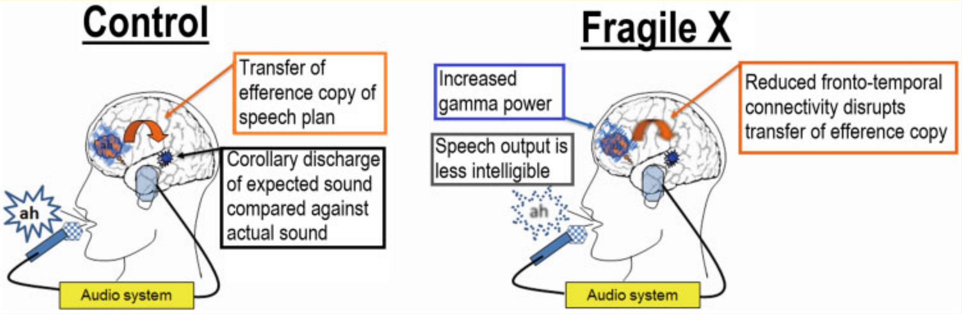
Graphical Abstract



Introduction

Fragile X syndrome (FXS) is the most common inherited intellectual disability and monogenic cause of autism spectrum disorder (Crawford *et al.*, 2001; Fernandez-Carvajal *et al.*, 2009). The disorder results from CGG trinucleotide repeat expansion in the 5′-untranslated region of the Fragile X Mental Retardation 1 (*FMR1*) gene, causing hyper-methylation and silencing of FMR protein production (Pieretti *et al.*, 1991; Park *et al.*, 2008; Kao *et al.*, 2010). Through its regulation of protein synthesis, FMR protein is critical to both neural development and synaptic function (Bassell and Warren, 2008; Zukin *et al.*, 2009; Darnell *et al.*, 2011). The absence of FMR protein has widespread effects on synapse maturation and experience-dependent modification of neural circuitry (Kooy *et al.*, 2000; Ronesi *et al.*, 2012). At the neural systems level, these local circuit alterations disrupt functional integration within brain networks and thus account for a wide range of neurobehavioural dysfunctions in FXS. Among these dysfunctions, deficits in speech production are a prominent clinical observation (Abbeduto *et al.*, 2007), but the disruptions in functional brain circuitry that contribute to speech deficits in FXS are not well understood.

High-density EEG studies offer a non-invasive approach to examine functional brain connectivity with high temporal resolution. This is important for studying speech production, which depends on feedforward control mechanisms and the rapid synchronization of neural oscillations within frontal and temporal regions of eloquent language cortex (Wang *et al.*, 2014). Prior to speech onset, the following two parallel processes occur: (i) a command generated from speech-related areas of inferior frontal gyrus (IFG) is sent to motor cortex to produce the intended speech sound and (ii) an efferent copy of the intended speech sound is transmitted from IFG to the superior temporal gyrus. The corollary discharge of the intended speech sound is compared against the actual speech sound, with the difference being used to minimize disparity between intended and future speech sounds (Houde and Jordan, 2002; Eliades and Wang, 2003, 2005; Ford and Mathalon, 2005; Ford *et al.*, 2010; Price *et al.*, 2011; Wang *et al.*, 2014). These processes for optimizing species-specific vocalizations crucial for social communication have been examined in non-human primates, songbirds, some marine mammals, bats and crickets (Suga and Shimozawa, 1974; Poulet and Hedwig, 2002; Eliades and Wang, 2003; Schneider *et al.*, 2014; Schneider and Mooney, 2015).

Previous EEG studies of typically developing individuals have shown that the forward model/corollary discharge process is reflected in a negative-going signal originating from IFG that oscillates with phase delay in auditory cortex (Ford *et al.*, 2010; Chen *et al.*, 2011; Wang *et al.*, 2014). In humans, the online self-monitoring process linked to fronto-temporal circuitry is critical for correcting errors in articulation, prosody and pitch (Osberger and McGarr, 1982; Oller and Eilers, 1988; Doupe and Kuhl, 1999). Disruptions in the functional connectivity between frontal and temporal cortex may interfere with speech development in clinical populations, such as individuals with FXS, and contribute to their chronic and pervasive expressive language deficits.

In addition to increased low-frequency coherent activity between frontal and temporal cortices before speech onset (Wang *et al.*, 2014), increased gamma band activity over motor/language regions of the frontal lobe occurs just prior to speech onset, consistent with the idea that phasic synchronization in gamma oscillations is related to speech as it is to multiple higher-level functions (Morillon *et al.*, 2010). This high-frequency activity is temporally locked to the pre-speech period and spatially locked to frontal regions and, therefore, may represent a critical component in the forward model/corollary discharge process. Previous research has demonstrated abnormal sensory-evoked and resting gamma oscillations in patients with FXS and *FMR1 Knockout* (KO) mice (Hou *et al.*, 2006; Osterweil *et al.*, 2010; Choi *et al.*, 2011; Ethridge *et al.*, 2016, 2017; Wang *et al.*, 2017; Lovelace *et al.*, 2018). Investigating alterations in both low- and high-frequency oscillations during speech production in patients with FXS may elucidate pathophysiological alterations related to speech production deficits.

EEG studies of speech production in typically developing individuals have shown that the neural response in auditory cortex to self-generated speech is highly reduced relative to the neural response to identical externally generated speech sounds (i.e. 'N1 suppression'; Ford *et al.*, 2010). Increased N1 suppression reflects effective tagging of speech sounds as self-generated versus externally generated, which is believed to facilitate differential processing of self-generated speech. Furthermore, greater auditory responses to self-generated speech occur when there are mismatches between intended and actual speech sounds, thought to alert the forward model to make necessary adjustments for future speech production (Eliades and Wang, 2005; Chang *et al.*, 2013). Synchronous pre-speech fronto-temporal oscillations are related to effective N1 suppression (Ford and Mathalon, 2005; Heinks-Maldonado *et al.*, 2005; Ford *et al.*, 2010; Wang *et al.*, 2014). Thus, assessing N1 suppression provides a way to assess whether speech is effectively tagged as self-generated sounds, a tagging that is abnormal in some neuropsychiatric disorders including schizophrenia (Ford *et al.*, 2010).

In the present study, we used a talk/listen paradigm (Ford *et al.*, 2010; Wang *et al.*, 2014) to compare neurophysiological responses to self-generated speech versus passive listening to the same speech sounds. We aimed to (i) determine the extent to which individuals with FXS generate coherent low-frequency neural oscillations between speech (frontal) and auditory (temporal) regions and increased frontal gamma oscillations prior to speech onset and (ii) determine whether there is a reduction in N1 suppression for self-generated speech. We hypothesized that individuals with FXS would demonstrate reduced coherent fronto-temporal activity, increased gamma power and reduced negative-going activity in inferior lateral frontal regions prior to speech onset and reduced N1 suppression following speech production compared with healthy controls. Finally, we predicted that abnormal neural responses prior to speech onset would be related to speech disturbances in a naturalistic speech production task previously used in FXS research (Abbeduto *et al.*, 1995; Berry-Kravis *et al.*, 2013) and to other clinically relevant features of FXS.

Materials and methods

Participants

Twenty-one right-handed participants with full-mutation FXS (>200 CGG repeats; eight females, age range 10–55 years; Table 1) and 20 age- and gender-matched right-handed healthy control participants (12 females, age range 14–56 years) completed the study. Healthy typically developing controls (TDC) were recruited through web-based fliers from the local community and were matched on sex and age within 4 years to the participants with FXS. TDC had no known prior diagnosis of or treatment for developmental or neuropsychiatric disorders. No participant had a history of seizure disorder or current use of anticonvulsant medication, benzodiazepine or novel potential treatment for FXS (i.e. minocycline). Some participants with FXS were being treated with psychiatric medications for behavioural issues: atypical antipsychotics (5), antidepressants (10) and stimulants (7) at a stable dose for at least 4 weeks before testing. Prior studies of these drugs do not indicate robust effects of these drug treatments on our electrophysiological parameters (Ethridge *et al.*, 2016, 2017; Wang *et al.*, 2017). For this reason and to maximize representativeness of our patient sample, all participants were included in final analyses. Participants or their legal guardians provided informed written consent and verbal assent, when appropriate, according to the Declaration of Helsinki. The local Institutional Review Board approved the study.

Table 1 Demographic characteristics of patients with FXS and TDC

	FXS ($n = 21$)	TDC ($n = 20$)
Age (range 10–55)	22.5 (10)	24.5 (12)
Gender, *n* (%, male)	13 (67)	15 (75)
Handedness (%, right)	100	100
Abbreviated IQ	60.2 (20)***	106.8 (10)
Deviation full scale IQ	49.4 (28)***	105.0 (8)
SCQ	11.6 (8)***	2.1 (2)
ELS: lexical	91.4 (43)**	153.3 (52)
ELS: syntax	6.8 (2)***	13.2 (3)
ELS: % unintelligibility	12 (1)**	1 (1)
ELS: talkativeness	13.5 (6)*	9.3 (2)
ELS: % dysfluency	23 (15)*	37 (17)
WJ: auditory attention	69.1 (11)	–

Mean (SD), unless otherwise denoted. IQ = intelligence quotient; SCQ = Social Communication Questionnaire; WJ = *Woodcock Johnson*, Third Edition.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Psychological measures

Intellectual functioning was assessed with the *Stanford-Binet Intelligence Scale*, 5th Edition. *Stanford-Binet Intelligence Scale*, 5th Edition, standard scores were converted to deviation scores based upon expected age-related performance to estimate intellectual ability in participants with FXS for whom reducing floor effects in scores is important (Sansone *et al.*, 2014). Expressive language abilities were assessed using the Expressive Language Sampling (ELS) protocol (Abbeduto *et al.*, 1995) in which participants spontaneously generated speech while narrating a wordless picture book as previously done in FXS research (Kover *et al.*, 2012). Language samples were recorded and transcribed using Systematic Analysis of Language Transcripts software (Miller and Iglesias, 2008). All speech was segmented into communication units (C-units; an independent clause and all its modifiers, including dependent clauses, rather than utterances to avoid over-estimating language abilities in highly verbal individuals; Abbeduto *et al.*, 1995). Syntactic complexity (mean length of C-units in morphemes), lexical diversity (i.e. number of different word roots in up to 50 C-units), fluency (percentage of C-units with filled pauses and sound repetitions) and intelligibility (percentage of C-units that were partly or completely unintelligible to the transcribers) were computed for each participant (see Kover *et al.*, 2012 for details about ELS scores). ELS scores were not available for one participant with FXS due to technical issues during recording. Primary caregivers of individuals with FXS completed the Social Communication Questionnaire (Rutter *et al.*, 2003), Aberrant Behavior Checklist (Aman *et al.*, 1985) and Vineland Adaptive Behavior Scales (VABS; Sparrow) to rate their child's social and psychological functioning.

Procedure

The talk/listen paradigm (Ford *et al.*, 2010) was presented using Presentation software (www.neurobs.com/presentation). During the talk task, participants repeatedly pronounced short (<300 ms), sharp vocalizations of the phoneme 'Ah' in a self-paced manner, every 1–2 s, for a total of 180 s. Vocalizations were recorded using a microphone and transmitted back to participants through earphones in real time (zero delay). Participants practiced the task prior to testing. During the listen task, participants passively listened to their own recordings from the talk task. Sound intensity was kept equivalent across talk and listen tasks for each participant by ensuring that a 1000-Hz tone (generated by a Quest QC calibrator) produced equivalent dB intensities. Trigger codes were inserted into the continuous EEG file at vocalization onsets to time-lock speech epochs and EEG data.

EEG recording

EEG data were obtained using a 128-channel HydroCel Geodesic Sensor Net and NetAmps 400 amplifiers (Electrical Geodesics Inc., Eugene, OR, USA). Recordings were referenced to the vertex sensor (Cz). As is standard with high input impedance amplifiers like those from EGI, sensor impedances were <50 kΩ. Data were recorded continuously throughout testing, digitized at 1000 Hz and stored for off-line analysis.

EEG analyses

Consistent with our prior studies (Ford *et al.*, 2010; Chen *et al.*, 2011; Wang *et al.*, 2014), raw EEG data were filtered using a 1-Hz high-pass filter, a 50-Hz low-pass filter and a 60-Hz notch filter, using the EEGLAB toolbox to remove non-stationary drift and line noise (Delorme and Makeig, 2004). EEG data were transformed to an average reference and subjected to Fully Automated Statistical Thresholding for EEG artefact Rejection (Nolan *et al.*, 2010). This method has shown >90% sensitivity and specificity for the detection of contaminated epochs (Nolan *et al.*, 2010).However, because limiting the contamination of muscle artefact is important, especially high-frequency jaw/mouth movement artefacts in the gamma range, we followed this process with visual inspection of raw data to ensure that no epochs with muscle artefact were missed.

Data were epoched from −800 to 800 ms with respect to the onset of each 'Ah' and baseline corrected using data from the −800 to −500 ms epoch preceding vocalization (Wang *et al.*, 2014). Analyses were carried out using EEGLAB, SPM12 for MEG/EEG (www.fil.ion.ucl.ac.uk/spm/) and FieldTrip (Donders Institute for Brain, Cognition and Behaviour, Radboud University, Nijmegen, The Netherlands; http://www.ru.nl/neuroimaging/fieldtrip/). All raw EEG data were pre-processed without knowledge of participant clinical and demographic information.

Connectivity analyses

Guided by prior work with healthy individuals indicating pre-speech activity in auditory cortex is synchronized with activity in frontal regions (Wang *et al.*, 2014), pairwise connectivities were separately calculated between temporal seed electrodes (average of T7 and T8) and all the other electrodes (Harper *et al.*, 2017). Connectivity values were quantified by calculating the debiased weighted phase lag index in FieldTrip toolbox, with the first and last 200 ms of data in each epoch trimmed to reduce edge effects. This method minimizes artefacts resulting from spurious inflation of scalp EEG connectivity caused by volume conduction, and it has minimum sample size bias to improve the ability to detect phase synchronization patterns (Vinck *et al.*, 2011). For each electrode pair, debiased weighted phase lag index was calculated every 10 ms for consecutive 400 ms timespans with a frequency resolution of 2.5 Hz (Vinck *et al.*, 2011). Debiased weighted phase lag index results were normalized with a baseline of −600 to −400 ms before speech onset when assessing the time period from −400 to 0 ms during which the pre-speech event-related potential (ERP) occurs (Harper *et al.*, 2017). Independent *t*-tests compared connectivity between temporal seed electrodes and each other electrode between FXS and TDC groups in talk and listen tasks separately. Adjacent electrodes with time–frequency data exceeding alpha level (0.05) were grouped into a cluster (minimum of two adjacent electrodes). To correct for multiple comparisons and identify the significance of each cluster, we used a Monte Carlo method, a cluster-based permutation test in Fieldtrip thresholded to $P < 0.05$, for statistical comparisons (5000 permutations; Maris *et al.*, 2007).

Granger causality analyses

To estimate the directional information flow from significant functional connectivity findings, we performed

Granger causality analyses (GCA) using the Fieldtrip toolbox. Using sliding windows similar to our connectivity analyses (400-ms width with 10-ms step), we obtained the time dimension of GCA results. Then, GCA results were normalized with a baseline of −600 to −400 ms before speech onset and averaged within significant clusters identified in connectivity analyses described above.

Event-related potential analyses

For each group, ERP averages were generated using a robust averaging approach (Wager *et al.*, 2005). Similar to our previous study (Wang *et al.*, 2014), inspection of grand average ERP waveforms indicated three components. A slow negative component occurred in a 400-ms time period before speech onset, N1 peaked ~100 ms after speech onset and P2 peaked ~200 ms after speech onset. Based upon previous findings (Ford and Mathalon, 2005; Ford *et al.*, 2010; Wang *et al.*, 2014), we extracted mean amplitudes of the pre-speech component (−400 to 0 ms) from a 10-electrode cluster surrounding Fpz and mean amplitudes of both N1 (80–120 ms) and P2 (170–210 ms) components from a 10-electrode cluster surrounding Cz for each subject.

Due to greater blink and movement artefacts in patients with FXS, the number of artefact-free epochs used in analyses was fewer for FXS than TDC ($\text{Epochs}_{\text{TDC\_talk}} = 94$; $\text{Epochs}_{\text{TDC\_listen}} = 93$; $\text{Epochs}_{\text{FXS\_talk}} = 68$; $\text{Epochs}_{\text{FXS\_listen}} = 61$). However, as one of the first performance-based EEG tasks completed in this patient population, we retained all participants with FXS to have the most representative patient sample for analyses. Furthermore, we established that unequal numbers of valid trials did not account for our results following a randomization procedure used in previous studies (Liu *et al.*, 2012).

Time–frequency analyses

To examine the non-phase-locked neural oscillatory dynamics of representative ERP components, we conducted time–frequency analyses using the same 10-electrode Fpz and Cz clusters used for ERP analyses. For each channel, event-related spectral perturbation (ERSP) was calculated using the EEGLAB toolbox and all ERSP values were averaged separately across the 10-electrode Fpz and Cz clusters. Power spectrum of the spectral estimate for frequencies from 3 to 50 Hz was calculated with 1 Hz frequency resolution using a modified Morlet wavelet transformation in the single trial data and then averaged across trials. The length of wavelets increased linearly from one cycle at 3 Hz to eight cycles at 50 Hz. To account for multiple comparisons, we used a cluster-based non-parametric permutation approach to test the significance of ERSP effects in each task × group analysis thresholded to $P < 0.05$ (2000 permutations; Cohen and van Gaal, 2014). Based on our previous EEG/ERP findings in FXS (Ethridge *et al.*, 2016, 2017; Wang *et al.*, 2017), our primary interest was in lower gamma frequency activity (30–50 Hz). Due to concerns regarding potential contamination of muscle artefact from speaking in the lower gamma frequency, we conducted broad regional analyses to help verify source of power did not originate from lateral jaw/mouth regions.

Pitch analyses

Using methods from previous speech studies of individuals with neurodevelopmental disabilities (Bonneh *et al.*, 2011), we calculated fundamental frequency, or pitch, using the VoiceBox speech processing toolbox separately for speech during the talk condition and during ELS tasks (frequency resolution: 50 Hz). Sound recordings were epoched based on vocalization onsets and offsets for each participant and then concatenated into a speech-only recording. Due to the time-varying nature of spectral information present in speech, we used short-time Fourier transform to calculate the power of concatenated speech, sliding forward in 10 ms step with 371.5 ms fast Fournier transform (FFT) window length to ensure over-sampling for good interpolation. For each participant, we computed the following five pitch variables: (i) mean pitch, or pitch strength; (ii) pitch range, the difference between maximum and minimum pitch values; (iii) pitch SD, the SD of pitch; (iv) normalized (divided by the total number of pitch samples) histograms of pitch values in 12 bins span from 0 to 400 Hz; and (v) coefficient of variation in pitch. As coefficient of variation is not normally distributed, we used a non-parametric test (Scheirer–Ray–Hare) for statistical analyses.

Statistical analyses

Separate repeated-measures ANOVAs were used to examine our primary EEG variables (coherence measures, component amplitude, etc.) with group (FXS versus TDC) as the between-subject factor and task (talk versus listen) as the within-subject factor when appropriate. All repeated-measures tests included Greenhouse–Geisser correction, and significant interaction effects were probed with *post hoc* t-tests corrected for multiple comparisons. Two-tail alpha-level was set to $P < 0.05$. Sex and age were entered as a factor or covariate in statistical models; however, no significant findings emerged for either measure so both were removed from the final models. To determine the inter-relationships between EEG, pitch and clinical variables, Pearson correlations were conducted. These were considered exploratory heuristic analyses so a nominal statistical threshold was employed.

Data availability

Data are available on the NDAR database at NIH and from the corresponding author upon reasonable request.

Results

Connectivity analyses

During the talk task, patients with FXS demonstrated reduced debiased weighted phase lag index phase coherence relative to controls between temporal seed electrodes (T7 and T8) and frontal electrodes (cluster-level test statistic from permutation test = 2339.3, $P = 0.03$; Fig. 1A). This occurred during the interval from −400 to 0 ms before speech onset and at frequencies ranging from 5 to 15 Hz (peak time = −200 ms; peak frequency = 10Hz; Fig. 1B). Reduced fronto-temporal coherence was supported by finding from our GCA analyses documenting a direction × group interaction ($F(1, 37) = 6.71$, $P = 0.014$; Fig. 1C). That is, greater frontal → temporal information flow was found in TDC compared with FXS ($t(39) = 3.45$, $P = 0.001$; Fig. 1C). Temporal → frontal synchrony did not differ between groups ($t(39) = 1.86$, $P = 0.07$). No significant group differences ($Ps > 0.41$) or interactions ($Ps > 0.07$) were found during the listen task for connectivity (Fig. 1B) or GCA analyses.

Event-related potential analyses

ERP responses to speech sound onset during talk for TDC and FXS are shown in Fig. 2A. We found a significant three-way interaction of group × task × ERP component ($F(2, 78) = 3.33$, $P = 0.043$). *Post hoc t*-tests revealed that pre-speech negativity was significantly greater during the talk task than the listen task for TDC ($t(19) = 4.77$, $P < 0.001$) and that pre-speech negativity did not differ between talk and listen tasks for FXS ($t(20) = 0.62$, $P = 0.54$). Both groups showed reduced N1 amplitudes (TDC: $t(19) = 4.01$, $P = 0.001$; FXS: $t(20) = 4.81$, $P < 0.001$) and P2 amplitudes (TDC: $t(19) = 3.10$, $P = 0.006$; FXS: $t(20) = 5.06$, $P < 0.001$) during the talk task than the listen task. Contrary to our hypothesis, N1 suppression ($N1_{Talk} - N1_{Listen}$) did not differ between groups ($F(40) = 1.35$, $P = 0.255$). Thus, despite reduction in the efferent copy signal from frontal to temporal cortex in FXS, patients did not show a reduction in the ability to identify and process self-generated speech differently. In addition, a group × task interaction was observed for the P2 component ($F(40) = 4.50$, $P = 0.040$). TDC participants had marginally higher P2 amplitudes during the talk task, whereas participants with FXS had marginally higher amplitudes during the listen task.

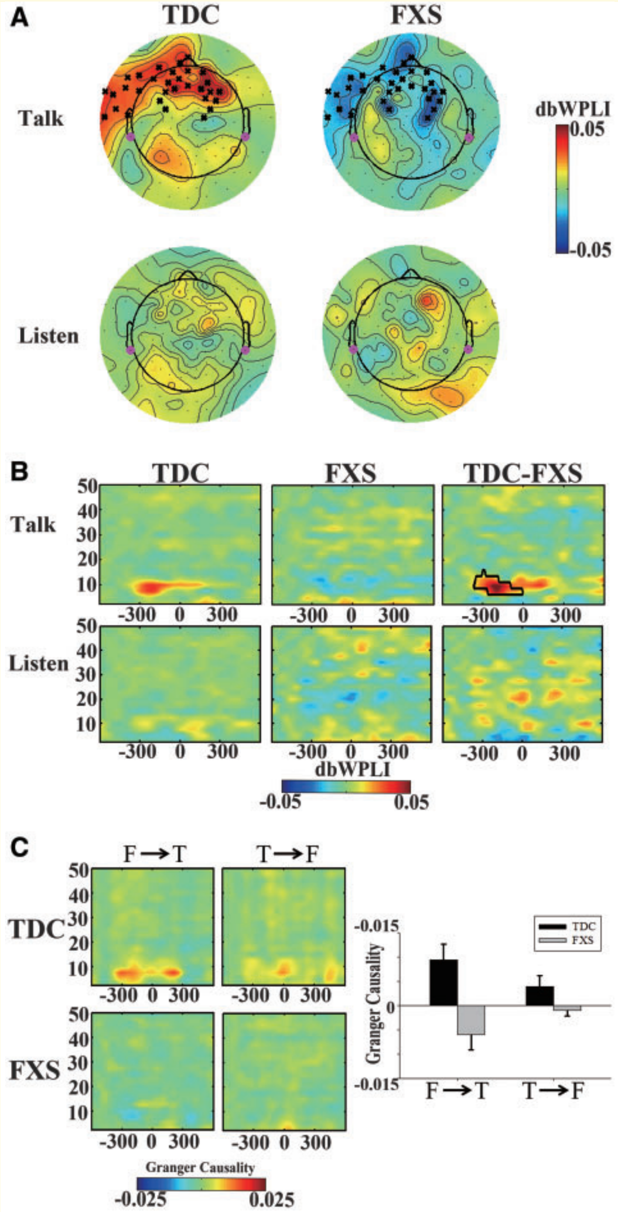


Figure 1 Connectivity findings for TDC and FXS. **(A)** Significant coherence between temporal seed electrodes (pink circle) and frontal electrodes (black 'X') for TDC and FXS participants. **(B)** Time–frequency plot of significant fronto-temporal electrode pairs for TDC, FXS and the comparison of TDC and FXS. Black-outlined area depicts significant clusters in group comparisons. **(C)** Time–frequency plot of Granger causality analyses in frontal to temporal (F → T) and temporal to frontal (T → F) directions presented on the left. Bar graph showing mean and standard error GCA values for TDC (black) and FXS (grey) on the right. Across all plots, warmer colours depict stronger coherence values.

Time–frequency EEG power

In the Fpz cluster, a significant group × task interaction was observed during the pre-speech period (peak frequency = 44 Hz, peak time = −244 ms, time range = −282 to −214 ms, $Fs(1, 78) > 11.71$, $Ps < 0.0009$; Fig. 2B). During the talk task, ERSP in the gamma range was elevated in FXS compared with TDC in this region prior to speech production ($t(39) = 4.02$, $P < 0.001$). No group differences or interactions were significant for the Cz cluster or for broader regional analyses in lateral-temporal regions.

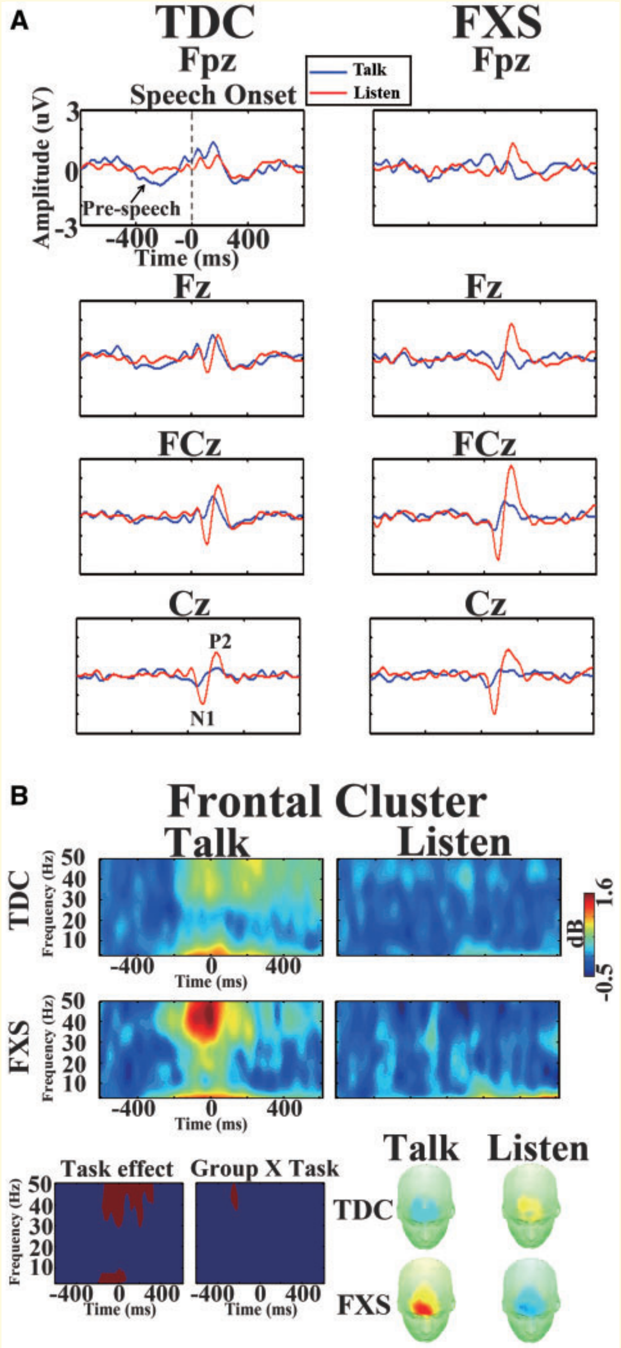


Figure 2 ERP and Gamma Findings for TDC and FXS. (**A**) ERP plots for TDC and FXS participants for both the talk task (blue) and listen task (red). Components of interest are labelled in regions in which amplitudes were largest. (**B**) ERSP time–frequency plots during both the talk and listen tasks (top). Significant task and group × task interaction effects depicted for the Fpz cluster (left), which occurred primarily in frontal regions in FXS participants (right).

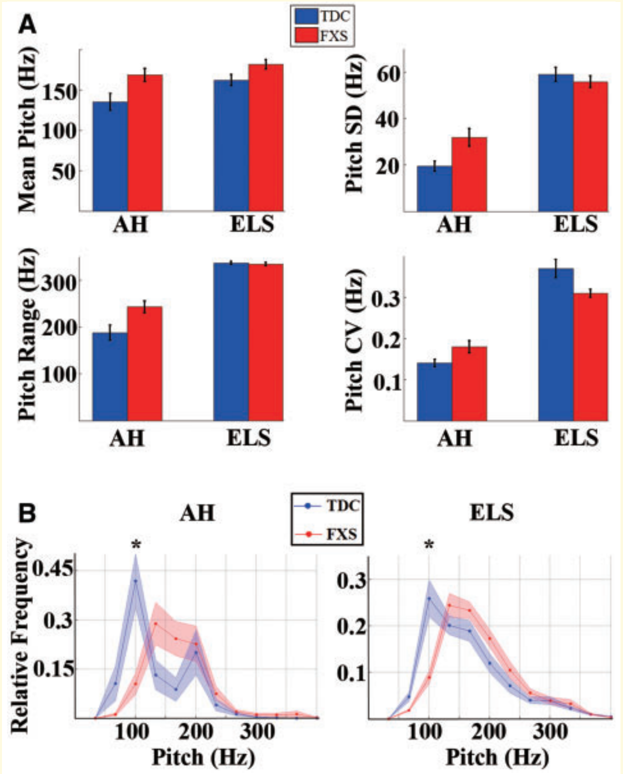


Figure 3 Pitch analysis of speech production. (**A**) Bar graphs for each pitch variable (mean and standard error) analysed for both TDC (blue) and FXS (red) participants in the 'Ah'/talk task and ELS task. (**B**) Normalized pitch histograms for TDC (blue) and FXS (red) participants for each speech production task. Asterisk identifies frequency at which groups significantly differed.

Pitch analysis of speech production

Figure 3A presents the group averages of the normalized vocal pitch histograms in both FXS and TDC during the 'Ah'/talk task and the naturalistic ELS task. During 'Ah'/talk task, frequency histograms of speech production in TDC showed a sharp peak of ∼100 Hz, whereas the pitch histograms of patients with FXS were shallower and more variable (Fig. 3B). FXS had less power relative to TDC ∼100 Hz during both the 'Ah'/talk task ($t(39) = 3.65$, $P = 0.0007$) and during the ELS task ($t(38) = 4.12$, $P = 0.0002$). Participants with FXS also had higher mean pitch than TDC during both 'Ah'/talk and ELS tasks ($F(1, 38) = 7.47$, $P = 0.009$; Fig. 3A). Pitch SD ($t(39) = 2.78$, $P = 0.008$), pitch range ($t(39) = 2.69$, $P = 0.010$) and pitch coefficient of variation ($z(39) = 2.14$, $P = 0.032$) were larger for participants with FXS than TDC during the 'Ah'/talk task but not the ELS task ($ts < 1.56$, $Ps > 0.12$; Fig. 3A).

Correlations among EEG measurements

Relationship between pre-speech connectivity and other EEG measurements

For the TDC group, N1 reduction in Talk versus Listen was significantly correlated with greater pre-speech fronto-temporal connectivity ($r(20) = 0.50$, $P = 0.026$;

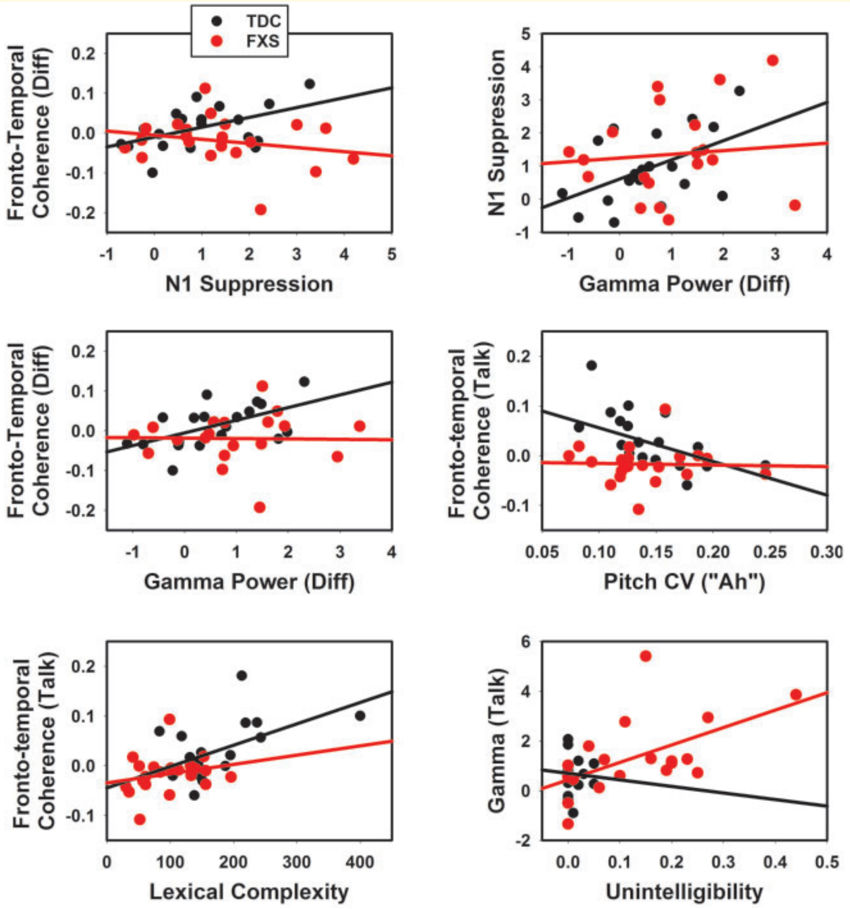


Figure 4 Proposed model of speech production deficits in FXS. Schematic representation for forward model/corollary discharge processes prior to speech production in TDC (left) and FXS (right) participants.

Fig. 4A) and marginally with greater pre-speech negativity ($r(20) = -0.43$, $P = 0.056$) in talk than listen tasks. These results are consistent with prior studies of healthy individuals in indicating that both greater pre-speech low-frequency negative-going activity and greater pre-speech fronto-temporal connectivity are related to greater N1 suppression following speech onset (Wang *et al.*, 2014). Neither of these relationships with N1 suppression were significant in FXS ($|r|\text{s} < 0.17$, $P\text{s} > 0.32$), suggesting that although N1 suppression was present in patients, it had a reduced relationship with the strength of pre-speech activity and fronto-temporal connectivity.

Relationship between pre-speech frontal gamma and other EEG measurements

We averaged pre-speech gamma activity from the Fpz cluster that demonstrated a significant task effect for gamma power across groups (talk > listen; time range = −162 to 0 ms). For TDC, greater pre-speech gamma power in talk than listen conditions was significantly correlated with greater N1 suppression ($r(20) = 0.50$, $P = 0.024$; Fig. 4B) and increased fronto-temporal connectivity ($r(20) = 0.56$, $P = 0.011$; Fig. 4C). However, neither relationship was significant in FXS ($|r|\text{s} < 0.13$, $P\text{s} > 0.58$).

Correlations with speech production

During the ‘Ah’/talk task, greater pre-speech fronto-temporal connectivity was related to lower speech pitch coefficient of variation in TDC ($r(20) = -0.49$, $P = 0.028$; Fig. 4D), indicating that greater functional coherence between frontal and temporal cortices prior to speech production was associated with more consistent speech output. This relationship was not significant among participants with FXS ($r(21) = 0.16$, $P = 0.488$), suggesting that their reduced level of frontal-to-temporal connectivity was not facilitating consistent pronunciation of speech sounds. Reduced pre-speech negativity during the ‘Ah’/talk task was related to lower pitch SD on the ELS task in patients with FXS ($r(21) = -0.49$, $P = 0.024$). This indicates that patients demonstrating the least amount of negative-going pre-speech activity during the experimental ‘Ah’/talk task had more monotonic speech during a naturalistic discussion in which greater pitch variability would be expected. Reduced pre-speech gamma power during 'Ah'/talk task was associated with a smaller pitch range during the ELS task in participants with FXS ($r(21) = 0.72$, $P < 0.001$).

Furthermore, our EEG and pitch measures of forward model/corollary discharge processes during the Talk task were related to ELS scores (see Supplemental material). Among TDC participants, increased pre-speech fronto-temporal connectivity was associated with greater lexical diversity and reduced dysfluency during natural speech. In contrast, among participants with FXS, reduced connectivity was linked to higher percentages of unintelligible speech (Fig. 4E) and lower lexical complexity (number of C-units produced) during naturalistic speech (ELS). Together, these findings suggest the functional significance of our EEG measures with regard to speech production in both experimental and natural conditions.

Correlations with demographic and clinical variables

For individuals with FXS, greater pre-speech gamma ERSP during 'Ah'/talk task was related to higher social and communication deficits (Social Communication Questionnaire scores; $r(21) = 0.53$, $P = 0.037$; Fig. 5A) and lower verbal ($r(21) = -0.60$, $P = 0.007$; Fig. 5B), but not nonverbal ($r(21) = -0.20$, $P = 0.412$), intelligence quotient. This suggests exaggerated frontal gamma power prior to speech production relates to multiple aspects of dysfunction in FXS, ranging from unintelligible speech to broader indications of impaired social communication and verbal skills. In addition, exuberant frontal gamma power during the talk task was associated with more severe parent reports of behavioural issues, including repetitive speech ($r(17) = 0.60$, $P = 0.014$), irritability ($r(17) = 0.55$, $P = 0.027$) and hyperactivity ($r(17) = 0.67$, $P = 0.005$; Fig. 5C). Lower N1 suppression in FXS was related to higher clinically rated externalizing maladaptive behaviours on the VABS ($r(17) = -0.64$, $P = 0.006$; Fig. 5D). Together, these results indicate that disruptions in forward model/corollary discharge processes are related to a broad range of behavioural issues in FXS. Within each group, no significant correlations for EEG and pitch measurements were observed with age or sex.

Discussion

Speech output relies on efficient coupling between frontal and temporal cortex. Efferent pre-speech activity in frontal cortex provides a copy of intended speech to produce a corollary discharge in auditory cortex against which actual speech sound is compared. This forward model/corollary discharge process is reflected in the following three neural signatures occurring prior to speech output: (i) a fronto-central low-frequency negative component; (ii) increased theta/alpha coherence between frontal and temporal cortices; and (iii) increased frontal high-frequency (gamma) power (Giraud *et al.*, 2007; Ford *et al.*, 2010; Llorens *et al.*, 2011; Wang *et al.*, 2014; Flinker *et al.*, 2015; Lu *et al.*, 2016). In the first study of its kind to investigate the neural dynamics of speech production in FXS, we document alterations in all three pre-speech neural signatures in FXS, each of which was associated with speech production abnormalities and broader clinical features associated with this neurodevelopmental disorder. Based on our findings, we propose a model of speech production deficits in individuals with FXS in which disrupted functional coordination of pre-speech activity between frontal and temporal cortices interferes with the brain's ability to implement sensorimotor adaptations to refine optimal and consistent speech production (Fig. 6).

Fronto-temporal disconnectivity model of speech production deficits in fragile X syndrome

Our demonstrations of impaired fronto-temporal connectivity and forward model/corollary discharge process in FXS indicate that disturbances in these processes represent a critical component of speech production deficits in this disorder. Coordinated pre-speech neural system interaction between IFG and superior temporal gyrus reflects the corollary discharge of intended speech generated from an efference copy of the speech command against which the actual speech sound can be compared (Houde and Jordan, 2002; Eliades and Wang, 2003, 2005; Ford and Mathalon, 2005; Ford *et al.*, 2010; Price *et al.*, 2011; Wang *et al.*, 2014). Updating future speech production is highly dependent on this process, as is development of expressive language skills more broadly (Houde and Jordan, 2002; Hickok *et al.*, 2011). Weakened signal originating in the frontal cortex with information flow to the temporal cortex, as we found in FXS, may be insufficient to compare against actual speech sound. Speech sound discrepancies thus may then go undetected and uncorrected in this patient population. Our finding of increased frontal high-frequency oscillations may degrade signal-to-noise ratio (SNR) of the low-frequency signal, further impairing the ability to compare intended and actual speech sounds (Shergill *et al.*, 2002). Together, these alterations may compromise the ability of individuals with FXS to optimize future speech, consistent with their relations to altered speech in experimental and naturalistic settings observed in the present study.

Abnormal vocal pitch quality

Vocal pitch is adjusted based on the intent and importance of communication. Speech is further optimized for communication based on ongoing evaluation of discrepancies between intended and actual speech sounds used to adjust future speech production. When the ability to

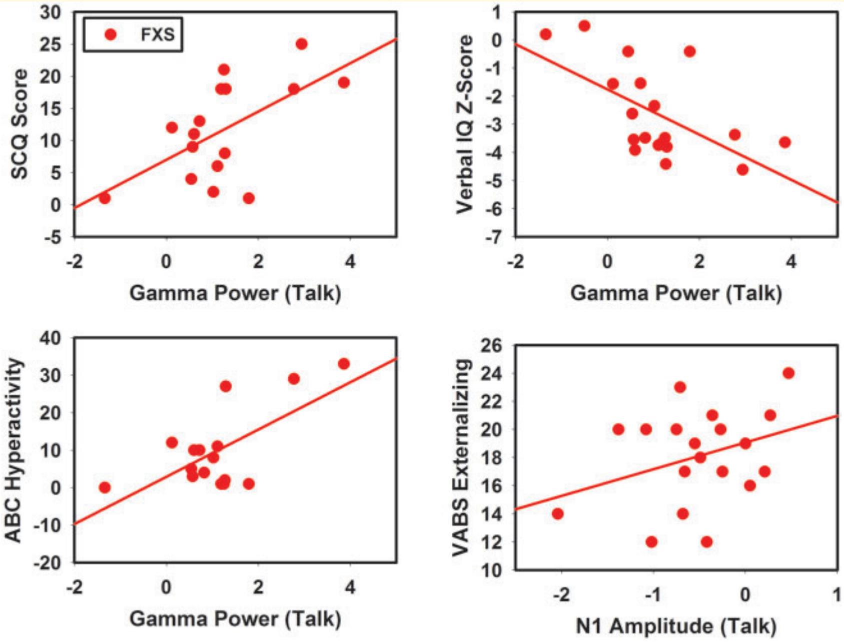


Figure 5 Speech production correlations for FXS and TDC. Scatterplots and linear regression findings depicting correlations of between EEG/ERP measures and between EEG/ERP measures and speech production variables separately for TDC (black) and FXS (red) participants.

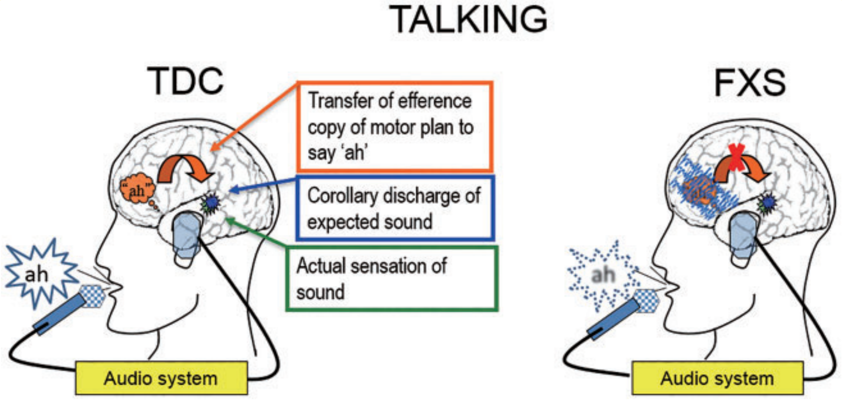


Figure 6 Clinical correlations for FXS. Scatterplots and linear regression lines depicting correlations of between EEG/ERP measures and clinical features associated with FXS.

evaluate speech productions relative to intended speech is compromised, so is the maintenance of consistent vocalizations. Increased pitch variability while repeating a simple phoneme in FXS when pitch is expected to be monotonic may arise from reductions in the fidelity of the forward model. These findings may have a parallel with impaired feedforward sensorimotor mechanisms in oculomotor and manual motor control that we have related to increased variability of motor responses in autism, a related neurodevelopmental disorder (Mosconi *et al.*, 2015; Schmitt *et al.*, 2016).

During a narrative task like ELS when greater variability in vocal pitch is expected for social communication, individuals with FXS demonstrated reduced pitch variability relative to TDC. This reduction was linked to reduced pre-speech negativity, suggesting that a degraded efference copy also may interfere with pitch modulation during social communication, as reflected in clinical observations of abnormal speech productions in patients with FXS (Roberts *et al.*, 2001; Abbeduto *et al.*, 2007; Finestack *et al.*, 2009; McDuffie *et al.*, 2012).

Time-locked high-frequency activity

Gamma oscillations originating in frontal cortex occurring prior to speech onset are thought to help drive fronto-temporal coherence in TDC (Brown *et al.*, 2005;

Chen *et al.*, 2011; Budde *et al.*, 2014; Sengupta and Nasir, 2015). Frontal pre-speech gamma power was observed in both FXS and TDC groups but was increased in the patient group. Our demonstration that increased pre-speech gamma power was related to unintelligible speech, lower verbal but not nonverbal intelligence quotient, and more severe behavioural problems suggest a role for increased frontal high-frequency power in broader language and social development in FXS. While the neural mechanisms for these associations remain to be fully clarified, increased frontal gamma band activity prior to speech onset may be, in part, a compensatory effort since in healthy individuals this activity is positively associated with quality of speech production. In the context of an already weakened pre-speech fronto-temporal signal, this high-frequency activity might further degrade the efferent copy rather than provide positive benefits.

Our gamma band findings in frontal cortex extend the established literature on increased neuronal excitability and 'background' gamma by providing evidence for elevated high-frequency activity beyond sensory cortex in FXS (Hou *et al.*, 2006; Osterweil *et al.*, 2010; Choi *et al.*, 2011; Ethridge *et al.*, 2016, 2017; Wang *et al.*, 2017; Lovelace *et al.*, 2018). To our knowledge, this is the first study to show abnormal task-related high-frequency activity time-locked to behaviour in patients with FXS. An imbalance of excitation, inhibition or exaggerated excitability of pyramidal neurons, is believed to underlie atypical gamma oscillations in FXS based on preclinical observations (Gibson *et al.*, 2008; Goswami *et al.*, 2019), suggesting that current observations of elevated pre-speech gamma also may arise from a similar fundamental mechanism. This could help account for the lack of association between high-frequency activity and alpha/theta band fronto-temporal coherence in FXS, a relationship noted in our TDC sample. In this case, the elevated frontal gamma power and its clinical significance may not be compensatory but reflect a more fundamental characteristic of neocortical excitability in FXS that may contribute to problems of speech production.

Unexpected intact N1 suppression

Despite alterations in pre-speech neurophysiology, patients with FXS did not demonstrate reduced N1 suppression to self-generated speech compared with TDC. This finding was surprising, given that the temporal dynamics and tight linkage between N1 suppression and pre-speech activity are well-documented in TDC (Houde and Jordan, 2002; Eliades and Wang, 2003, 2005; Ford and Mathalon, 2005; Ford *et al.*, 2010; Chen *et al.*, 2011; Price *et al.*, 2011). One possibility is that the efferent copy may provide a sufficiently robust signal to distinguish self-generated speech from externally generated speech (as indicated by intact N1 suppression) but impaired higher-level auditory processing in FXS (as reflected in differences in P2 between FXS and TDC). N1 and P2 represent functionally distinct aspects of auditory processing, with N1 reflecting early sensory processing and P2 reflecting more higher-level auditory processing (Crowley and Colrain, 2004; Tremblay *et al.*, 2014; Wang *et al.*, 2014). We observed lower-relative P2 during the talk task compared with the listen task in patients only, consistent with our previous findings of altered tone processing in FXS (Ethridge *et al.*, 2016, 2017). Because P2 is more sensitive than N1 in detecting perturbations during vocalization (Behroozmand *et al.*, 2009, 2011), this may contribute to the less refined speech output observed in our pitch and ELS findings in patients with FXS.

Mechanistic implications

Our findings of clinically relevant fronto-temporal connectivity alterations and elevated gamma power before speech onset might be best understood in the context of aberrant neural synchronization of auditory cortical responses to tones and increased intrinsic high-frequency activity in patients with FXS and in *FMR1* KO mice in vivo and in vitro (Hou *et al.*, 2006; Osterweil *et al.*, 2010; Choi *et al.*, 2011; Ethridge *et al.*, 2016, 2017; Wang *et al.*, 2017; Lovelace *et al.*, 2018; Goswami *et al.*, 2019). Auditory processing alterations may contribute to the atypical development of speech and language skills in FXS. Our finding of clinically relevant increased pre-speech gamma power in frontal cortex time-locked to behavioural events provides new evidence indicating that elevated high-frequency activity extends beyond previous reports in sensory cortex and be relevant to the prominent behavioural features in FXS.

Evidence from the extensive literature in analogous corollary discharge processes in songbirds offers potential insights for the interpretation of our clinical electrophysiological observations (Doupe and Kuhl, 1999). In juvenile songbirds, a molecular signalling cascade via mechanistic target of rapamycin that involves extracellular signal-regulated kinase occurs just prior to species-specific song learning (London and Clayton, 2008; London, 2019). Mechanistic target of rapamycin is thought to modulate synaptic function, and in turn, cellular plasticity, by regulating protein translation (Shimobayashi and Hall, 2014). In the context of FXS, known increases in mechanistic target of rapamycin phosphorylation, aberrant extracellular signal-regulated kinase activation kinetics and exaggerated metabotropic glutamate receptor long-term depression in FXS (Bear *et al.*, 2004; Weng *et al.*, 2008) may contribute to the observed neuroophysiological and speech production deficits in patients with FXS.

Our electrophysiological findings in FXS have similarities with those in other disorders with prominent speech production deficits. For example, individuals who stutter demonstrate a profile of aberrant fronto-temporal connectivity prior to speech production and under-activation of auditory language regions following speech production (Brown *et al.*, 2005; Budde *et al.*, 2014; Sengupta and Nasir, 2015). In autism spectrum disorder, reduced correlations between frontal and temporal cortex at rest have been documented and related to more severe expressive language impairments and autism spectrum disorder symptomatology (Dinstein *et al.*, 2012). Thus, while having disorder-specific features, alterations in fronto-temporal circuitry may contribute to abnormal speech development in multiple neurodevelopmental disorders (Belmonte *et al.*, 2004). Of note, several recent studies have documented that transcranial direct current stimulation over left IFG including Broca's area reduced articulation errors and increased speech output in both individuals with autism spectrum disorder (Schneider and Hopp, 2011) and individuals with chronic aphasia (Fiori *et al.*, 2011; Marangolo *et al.*, 2011, 2013; Mandelli *et al.*, 2018). *FMR1* KO mice demonstrate altered spectral and temporal properties of and reduced rate of ultrasonic vocalizations compared with wild type (Roy *et al.*, 2012; Hodges *et al.*, 2017; Toledo *et al.*, 2019), the latter which was rescued following drug treatment with minocycline in a recent study (Toledo *et al.*, 2019). The link between abnormal ultrasonic vocalizations and disrupted feedforward control/fronto-temporal connectivity remains to be determined; however, this suggests that pharmacological interventions also may useful in enhancing speech production in FXS.

Limitations

There are certain limitations that need to be considered with regard to this study. Due to speech production limitations in FXS, ∼40% of our patient population with FXS could complete the talk/listen task successfully. This may limit the generalization of study findings. For reasons of anxiety and sound sensitivity, it was not possible to acquire MRI data to investigate relations of electrophysiological alterations to potential neuroanatomic changes. Third, it is possible that enhanced pre-speech gamma activity may in part reflect muscle/jaw artefact during speech production. Though our findings indicate group differences in frontal region of interest (see Fig. 2A) as opposed to anterior–lateral regions near temporal muscles (Muthukumaraswamy, 2013), some caution should be exerted when interpreting these data until replication of the method and further independent analyses of potential artefact contribution are ruled out. Another related factor is that our characterization of gamma did not include the full range of high-frequency gamma power. Fourth, vocalizations of the phoneme 'Ah'in the present paradigm do not depend on semantic language circuitry (Ford and Mathalon, 2005), warranting future studies of more complex speech production in FXS. Relations of our EEG measures to speech characteristics derived from a naturalistic narrative task, however, do suggest that fronto-temporal connectivity disturbances reported here have broader implications regarding real-world expressive language skills in FXS. Lastly, studies are indicated to examine fronto-temporal circuitry related to speech production in younger-aged individuals, in other developmental disordered populations and in regard to sex-specific differences, in part to clarify the specificity of our finding to FXS.

Conclusions

In this study, we provide the evidence of disrupted frontal–temporal connectivity in the theta/alpha frequency range and exaggerated frontal gamma power prior to speech onset and we provide that these alterations were related to alterations in speech production in individuals with FXS. These results provide novel insights into the neural basis of speech production deficits in FXS and support for a model in which disrupted fronto-temporal connectivity and corollary discharge processes interfere with the brain's ability to implement sensorimotor adaptations to refine optimal and consistent speech production. Our findings of reduced fronto-temporal connectivity during a speech production task may provide potential new and important intervention targets using neuromodulation and other strategies aimed at improving expressive language in patients with FXS.

Supplementary material

Supplementary material is available at *Brain Communications* online.

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Competing interests

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