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Research Report

# Degraded speech sound processing in a rat model of fragile X syndrome

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ABSTRACT

Fragile X syndrome is the most common inherited form of intellectual disability and the leading genetic cause of autism. Impaired phonological processing in fragile X syndrome interferes with the development of language skills. Although auditory cortex responses are known to be abnormal in fragile X syndrome, it is not clear how these differences impact speech sound processing. This study provides the first evidence that the cortical representation of speech sounds is impaired in *Fmr1* knockout rats, despite normal speech discrimination behavior. Evoked potentials and spiking activity in response to speech sounds, noise burst trains, and tones were significantly degraded in primary auditory cortex, anterior auditory field and the ventral auditory field. Neurometric analysis of speech evoked activity using a pattern classifier confirmed that activity in these fields contains significantly less information about speech sound identity in *Fmr1* knockout rats compared to control rats. Responses were normal in the posterior auditory field, which is associated with sound localization. The greatest impairment was observed in the ventral auditory field, which is related to emotional regulation. Dysfunction in the ventral auditory field may contribute to poor emotional regulation in fragile X syndrome and may help explain the observation that later auditory evoked responses are more disturbed in fragile X syndrome compared to earlier responses. Rodent models of fragile X syndrome are likely to prove useful for understanding the biological basis of fragile X syndrome and for testing candidate therapies.



## 1. Introduction

Fragile X syndrome is the most prevalent cause of inherited intellectual disability in the world. Fragile X syndrome results from the loss of function of the *FMR1* gene, which encodes the RNA binding protein, fragile X mental retardation protein (FMRP). FMRP regulates synaptic development and plasticity. Many of the cognitive and behavioral features of fragile X syndrome emerge during childhood and are associated with abnormal organization of cortical connections. Reduced ability

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to maintain and manipulate phonological representations in fragile X syndrome limits the ability to acquire vocabulary and effective syntax (Pierpont et al., 2011).

Neurophysiology studies have established that both children and adults with fragile X syndrome have cortical deficits in auditory processing (Castrén et al., 2003; Knoth and Lippé, 2012; Rojas et al., 2001; St Clair et al., 1987; Van der Molen et al., 2012a,b) in spite of normal auditory brainstem responses (Roberts et al., 2005). It is not clear how these observed cortical impairments impact speech sound processing. To address this important issue, we evaluated the potential impact of auditory processing abnormalities on speech sound discrimination in rats with a deletion of the *Fmr1* gene. Earlier studies have shown auditory processing abnormalities in rodent models of fragile X syndrome, but it is not known how these disturbances impair speech sound processing.

It is reasonable to investigate neural deficits in speech sound processing in rats because the basic neural mechanism used to processes speech sounds up to the level of auditory cortex appear to be shared with other mammals. While non-human animals or non-vocal learners cannot produce learned speech, they can still process speech sounds in their auditory pathway in a manner similar to vocal learners (Arriaga and Jarvis, 2013; Doupe and Kuhl, 1999; Petkov and Jarvis, 2012). Rats are able to accurately discriminate between English consonant and vowel sounds (Centanni et al., 2013b; Engineer et al., 2008, 2014a,b; Porter et al., 2011; Ranasinghe et al., 2012). Importantly, rat behavioral and neural response thresholds for discrimination of speech in noise, spectrally degraded speech and temporally degraded speech closely parallel human thresholds (Ranasinghe et al., 2012; Shetake et al., 2011). In this study, we evaluated the precise spatio-temporal firing patterns of auditory cortex neurons in response to speech sounds presented to control rats and rats lacking the *Fmr1* gene. We predicted that *Fmr1* mutation would degrade cortical responses to key acoustic features of human speech (Boddaert et al., 2004; Bomba and Pang, 2004; Engineer et al., 2008; Rinaldi et al., 2008).

## 2. Results

### 2.1. *The auditory cortex response to speech is degraded in Fmr1 KO rats*

Individuals with fragile X syndrome exhibit altered auditory evoked potential responses, suggesting that auditory cortex responses may be altered in a rodent model of fragile X syndrome (Knoth and Lippé, 2012; Rotschafer and Razak, 2013). We found that local field potential responses to speech sounds averaged across all auditory cortex recording sites were significantly impaired in *Fmr1* KO rats compared to naïve control rats (Fig. 1). The N1, P2, N2, and P3 component peak amplitudes were all weaker in *Fmr1* rats compared to controls (Fig. 1b).

There is some evidence in individuals with autism and in rodent models of autism that responses in specific auditory fields may be more impaired than other auditory fields (Engineer et al., 2014a,b; Lai et al., 2011). To examine the possibility that this finding extends to a rodent model of fragile X syndrome, we separately analyzed local field potential responses to speech sounds in four auditory cortex fields: anterior auditory field (AAF), primary auditory cortex (A1), ventral auditory field (VAF), and posterior auditory field (PAF). While there were no significant differences in N1 amplitude or P3 amplitude in AAF, both the P2 and N2 amplitudes in AAF were significantly weaker in *Fmr1* KO rats compared to control rats ($p<0.0001$, Fig. 2a). A1 responses were smaller for all four components in *Fmr1* KO rats compared to control rats ($p<0.0001$, Fig. 2b). *Fmr1* KO rats also had weaker amplitudes for all four components in VAF compared to control rats ($p<0.0001$, Fig. 2c). In PAF, while there was no significant difference in N1 amplitude or P2 amplitude, both the N2 and P3 amplitudes were significantly weaker in *Fmr1* KO rats compared to control rats ($p<0.005$, Fig. 2d). The weaker local field potential amplitudes evoked by speech sounds across auditory fields suggest that speech sound processing may be impaired in *Fmr1* KO rats.

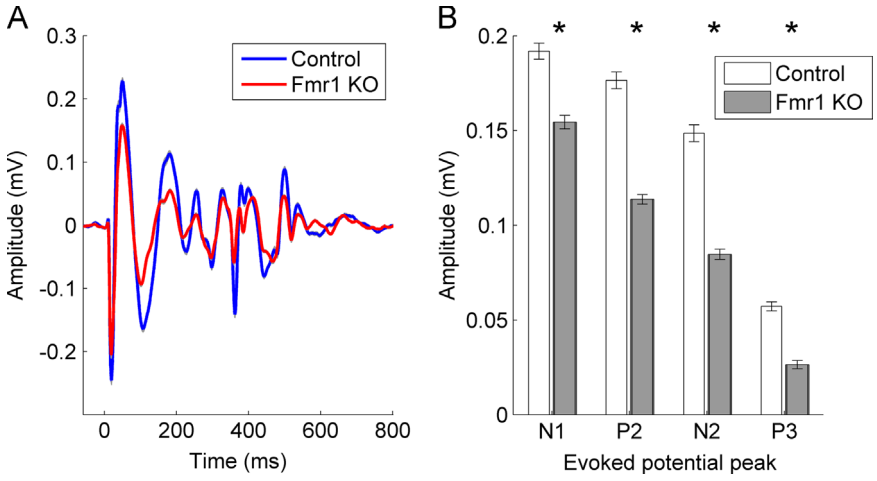


**Fig. 1 – Local field potential responses to speech sounds. (a) The local field potential response to the speech sound 'dad' averaged across the four auditory cortex fields. Gray shading behind each line indicates s.e.m. across recording sites. (b) The N1, P2, N2, and P3 amplitudes are significantly reduced in *Fmr1* KO rats compared to naïve control rats across the four auditory fields ($p<0.0001$). Responses are the average response to the 11 speech sounds presented.**

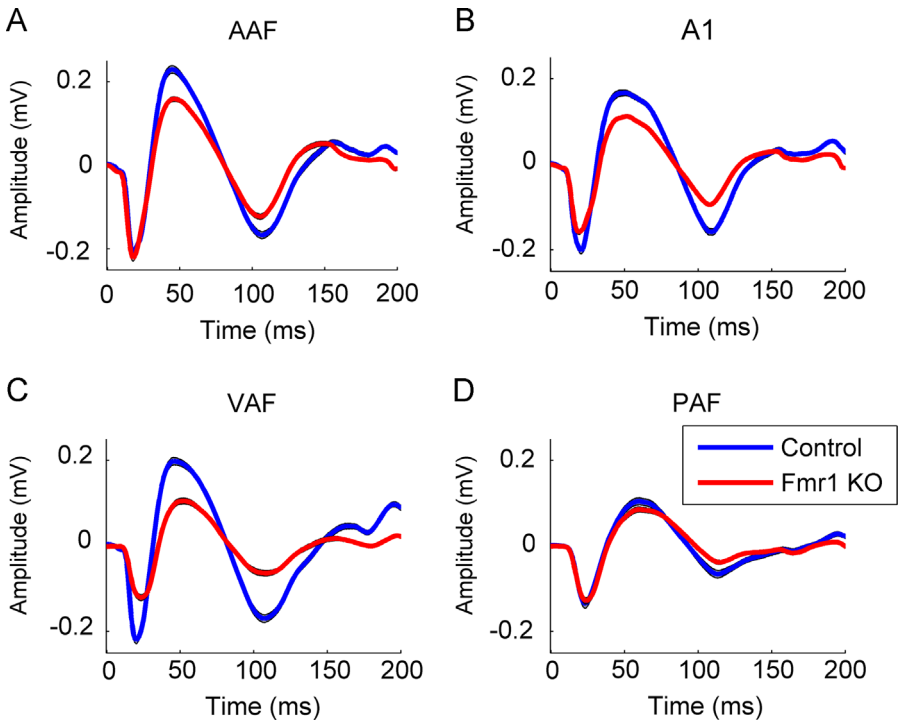


**Fig. 2 – Local field potential recordings in response to speech sounds. (a) The N1–P2 amplitude in *Fmr1* KO rats is significantly reduced compared to naïve control rats in anterior auditory field. Gray shading behind each line indicates s.e.m. across recording sites. Responses are the average response to the 11 speech sounds presented. All figures depicting multiple auditory fields are ordered by ascending onset latency to tones in naïve control rats (AAF, A1, VAF, and PAF). (b) The N1–P2 amplitude in *Fmr1* KO rats is significantly reduced compared to naïve control rats in primary auditory cortex. (c) The N1–P2 amplitude in *Fmr1* KO rats is significantly reduced compared to naïve control rats in ventral auditory field. (d) The N1–P2 amplitude in *Fmr1* KO rats is unimpaired compared to naïve control rats in posterior auditory field.**

Multiunit responses evoked by a set of 11 speech sounds were recorded in each of the four auditory fields. The onset response strength to speech sounds was significantly weaker by 29% in AAF, 36% in A1, and 69% in VAF in *Fmr1* KO rats compared to controls ($p<0.0005$, Figs. 3 and 4a–c). In contrast, in PAF, the onset response to speech sounds in *Fmr1* KO rats was not significantly different compared to controls (Fig. 4d). Onset latency responses to speech sounds were significantly slower by 1 ms in VAF and PAF in *Fmr1* KO rats compared to controls (VAF $15.7\pm0.4$ ms vs. $14.6\pm0.2$ ms, $p=0.01$; PAF $18.5\pm0.3$ ms vs. $17.5\pm0.4$ ms, $p=0.04$). The onset latency to speech sounds in the other fields was not significantly slower in *Fmr1* KO rats ($p>0.05$). Responses to speech sounds are both weaker and slower in *Fmr1* KO rats, particularly in ventral auditory field.

Many previous studies have documented that the neural similarity between pairs of speech sounds predicts behavioral discrimination ability (Centanni et al., 2013b; Engineer et al., 2008; Perez et al., 2013; Ranasinghe et al., 2012; Shetake et al., 2011). We hypothesized that a neural classifier would be less accurate at speech sound discrimination using the impaired auditory cortex responses from *Fmr1* KO rats compared to control rats. We found that classifier performance was impaired in each of the fields that exhibited weaker responses to speech sounds. Classifier performance was worse by 4% in AAF, 5% in A1, and 8% in VAF in *Fmr1* KO rats compared to controls ($p<0.001$, Fig. 5a–c). Although PAF responses to speech sounds were slower, they were not weaker, and the neural classifier performance was unimpaired in this field ($p=0.25$, Fig. 5d). These results confirm that the auditory cortex processing of speech sounds is impaired in *Fmr1* KO rats.

### 2.2. *Temporal and spectral processing are degraded in Fmr1 KO rats*

Previous studies have documented sensory gating abnormalities in individuals with fragile X syndrome (Frankland et al., 2004). We recorded auditory cortex responses to trains of noise bursts presented at varying rates in *Fmr1* KO and control rats. Based on the impaired responses to speech sounds in *Fmr1* KO rats, we predicted that responses to stimuli with rapid temporal changes would also be impaired.

The response to the first noise burst in a train of six noise bursts was 25% weaker in AAF and 46% weaker in VAF in *Fmr1* KO rats compared to controls (Figs. 4a and c and 6). In A1 and PAF, the response to the first noise burst was not significantly different in *Fmr1* KO rats ($p>0.77$, Figs. 4b and d and 6).

Responses to the subsequent noise bursts in the train, however, were significantly weaker in VAF and significantly stronger in PAF (Figs. 6 and 7). The noise burst trains were presented at 4 speeds: 7, 10, 12.5, and 15 Hz. For each of the four auditory fields in both *Fmr1* KO and control rats, there

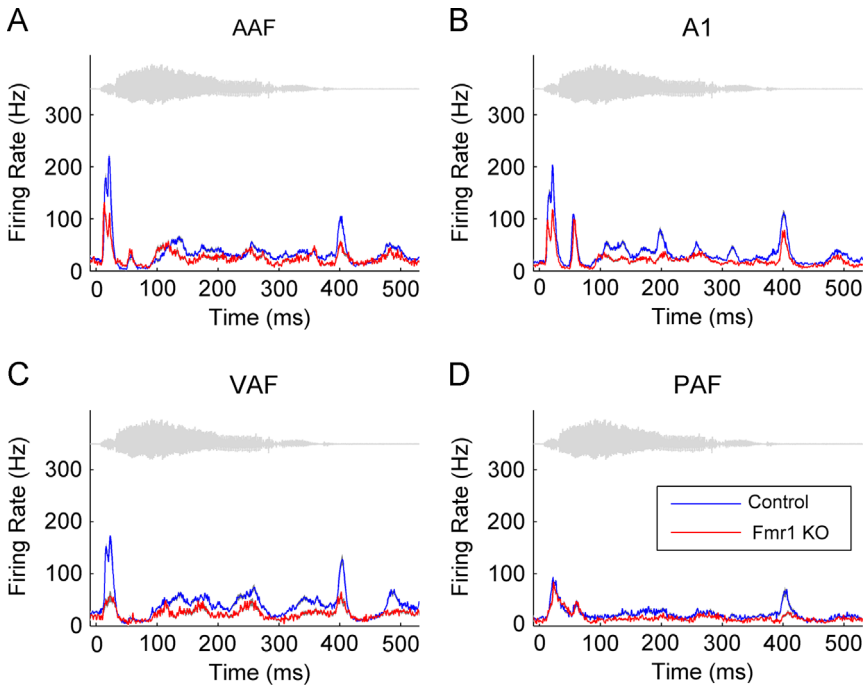


**Fig. 3 – Post stimulus time histograms (PSTHs) in response to the speech sound 'deed'. (a) The response strength to speech sounds in *Fmr1* KO rats is significantly reduced compared to naïve control rats in anterior auditory field. Gray shading behind each line indicates s.e.m. across recording sites. The waveform for the sound 'deed' is plotted for reference at the top of each subplot. (b) The response strength to speech sounds in *Fmr1* KO rats is significantly reduced compared to naïve control rats in primary auditory cortex. (c) The response strength to speech sounds in *Fmr1* KO rats is significantly reduced compared to naïve control rats in ventral auditory field. (d) The response strength to speech sounds in *Fmr1* KO rats is unimpaired compared to naïve control rats in posterior auditory field.**

were more spikes per noise burst at slower speeds compared to faster speeds. In both AAF and A1, responses to the subsequent noise bursts were not significantly different between *Fmr1* KO and control rats ($p>0.05$, Fig. 7a and b). There were significantly fewer spikes per noise burst in VAF in *Fmr1* KO rats compared to controls across all four presentation rates ($p<0.05$, Fig. 7c). In PAF, there were significantly more spikes per noise burst at the slower rates of presentation in *Fmr1* KO rats compared to naïve controls ($p<0.05$, Fig. 7d).

Responses to tones were significantly weaker in *Fmr1* KO rats compared to controls (Fig. 4). Responses to tones played at 60 dB were weaker in AAF, A1, and VAF in *Fmr1* KO rats ($p<0.05$, Fig. 4a–c). Across a range of intensities, responses to tones were the most severely impaired in VAF (Fig. 8c), but were also significantly weaker in A1 (Fig. 8b) and AAF (Fig. 8a). The auditory processing impairments observed in *Fmr1* KO rats are not specific to speech sounds. This matches previous research in children with fragile X syndrome showing an inability to use a preceding tone to predict the onset of a loud noise burst, despite the ability to hear the tone (Frankland et al., 2004). Both temporal processing and spectral processing are degraded in *Fmr1* KO rats compared to controls.

### 2.3. *Speech sound discrimination performance is unimpaired*

While language delays are common in fragile X syndrome (Hinton et al., 2013), phoneme discrimination is intact (Barnes et al., 2009). In this study, we trained 11 rats to discriminate individual phoneme sounds. Rats were trained to press a lever in response to the sound 'dad', and refrain from lever pressing in response to sounds differing in consonant (for example, 'bad') or vowel (for example, 'deed'). *Fmr1* KO rats took significantly fewer days to reach the shaping criteria compared to naïve control rats (1.3 days vs. 3.1 days, $p=0.007$). While counterintuitive, this finding matches earlier observations in Fmr1 KO mice and children with autism showing superior performance on simple auditory tasks (Frankland et al., 2004; Ouimet et al., 2012). The rats next advanced to a 'dad' detection stage that lasted until the rat was able to accurately press the lever in response to 'dad' with a $d'$ performance value $\geq 1.5$ for 10 sessions. *Fmr1* KO rats performed this task equally as well as naïve control rats, and both groups of rats took the same amount of time to reach the detection stage performance criteria (10.1 days vs. 10.9 days, $p=0.69$).

The final stage of training was a discrimination task where rats learned to discriminate consonants and vowels. *Fmr1* KO rats performed these discrimination tasks as well as naïve control rats, and there was no significant difference in the number of days each group took to reach 70% correct performance ($3.0\pm0.5$ days for *Fmr1* KO rats vs. $4.0\pm0.6$ days for control rats, $p=0.21$). Rats performed each discrimination task for 3 weeks before switching to their second discrimination task. *Fmr1* KO rats did not perform significantly different compared to naïve control rats on the last week of training for

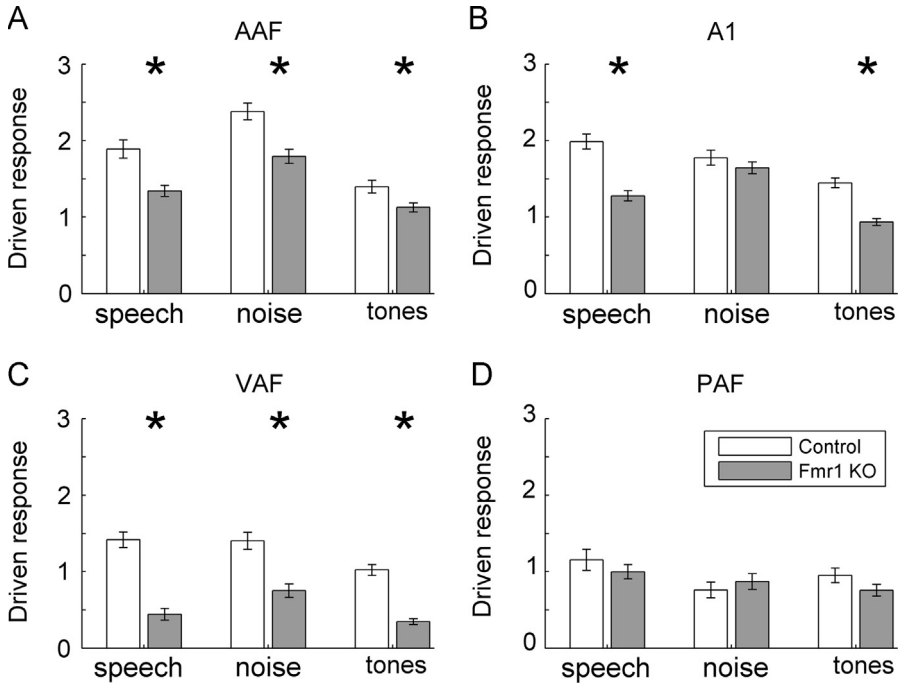


**Fig. 4 – Weaker responses to auditory stimuli in *Fmr1* KO rats compared to naïve control rats. (a) The driven response to speech sounds, noise bursts, and tones in AAF is weaker in *Fmr1* KO rats compared to naïve controls. Error bars indicate s.e.m. across recording sites. Stars indicate sounds that evoke significantly weaker activity in *Fmr1* KO rats ($p<0.05$). (b) The driven response to speech sounds and tones in A1 is weaker in *Fmr1* KO rats compared to naïve controls. There is no difference in response strength to noise bursts between the two groups. (c) The driven response to speech sounds, noise bursts, and tones in VAF is weaker in *Fmr1* KO rats compared to naïve controls. (d) There is no difference in driven response to speech sounds, noise bursts, or tones in PAF in *Fmr1* KO rats compared to naïve controls ($p>0.05$).**

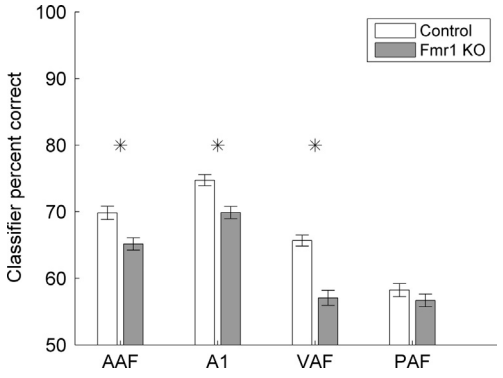


**Fig. 5 – Neural discrimination of speech sounds is impaired in *Fmr1* KO rats compared to naïve control rats. Average neural percent correct across four consonant discrimination pairs for each of the four auditory fields in both *Fmr1* KO and naïve control rats. Consonant discrimination accuracy is significantly impaired in AAF, A1 and VAF in the *Fmr1* KO rats compared to naïve controls ($p<0.05$). Error bars indicate s.e.m. across recording sites.**

the consonant discrimination task (91.7±1.8% correct for *Fmr1* KO rats vs. 86.5±3.1% correct for control rats, $p=0.17$, Fig. 9) or the vowel discrimination task (85.8±1% correct for *Fmr1* KO rats vs. 82.1±2.4% correct for control rats, $p=0.27$, Fig. 9). These results are consistent with the literature showing normal discrimination of phonemes in fragile X syndrome (Barnes et al., 2009).

### 2.4. *No auditory cortex plasticity after speech training*

Responses were also recorded from 181 auditory cortex sites in the four speech trained *Fmr1* KO rats following the completion of speech training. Responses to speech sounds, noise bursts, and tones in speech trained *Fmr1* KO rats were largely indistinguishable from responses in untrained *Fmr1* KO rats ($p>0.01$, Bonferroni correction). Compared to naïve control rats, speech trained *Fmr1* KO rats still exhibit a weaker response strength to speech sounds by 21% in AAF, 35% in A1, and 86% in VAF ($p<0.05$). Neural classifier accuracy did not improve following speech training and still exhibited a 6% deficit in AAF, 7% deficit in A1, and 9% deficit in VAF compared to control rats ($p<0.005$). While neural classifier accuracy did not significantly change between untrained *Fmr1* KO rats and speech trained *Fmr1* KO rats in AAF or VAF, neural classifier accuracy was significantly impaired after training by 2% in A1 and 5% in PAF ($p<0.05$). The observation that several weeks of speech training is insufficient to significantly alter A1 responses to speech sounds in *Fmr1* KO rats is consistent with observations in wild-type rats (Engineer et al., 2014a,b).

## 3. Discussion

Fragile X syndrome is the most common inherited form of intellectual disability and the leading genetic cause of autism. Impaired phonological processing in fragile X syndrome

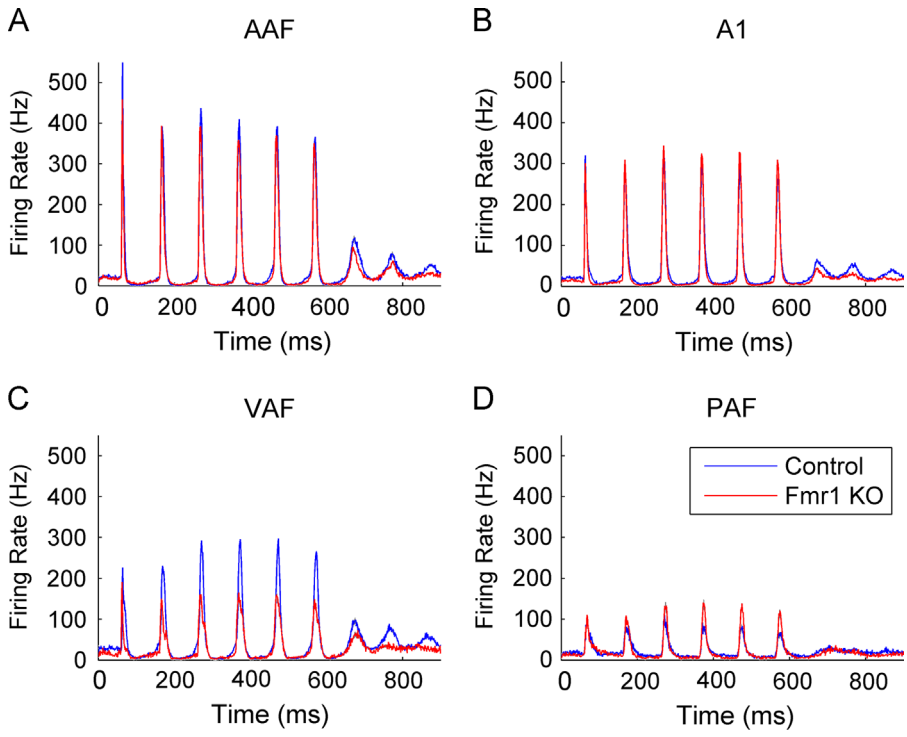


**Fig. 6 – Responses to noise burst trains presented at 10 Hz in *Fmr1* KO rats compared to naïve control rats. The response to noise bursts in AAF (a), A1 (b), VAF (c), and PAF (d) in *Fmr1* KO rats (red line) compared to naïve control rats (blue line). Gray shading behind each line indicates s.e.m. across recording sites.**

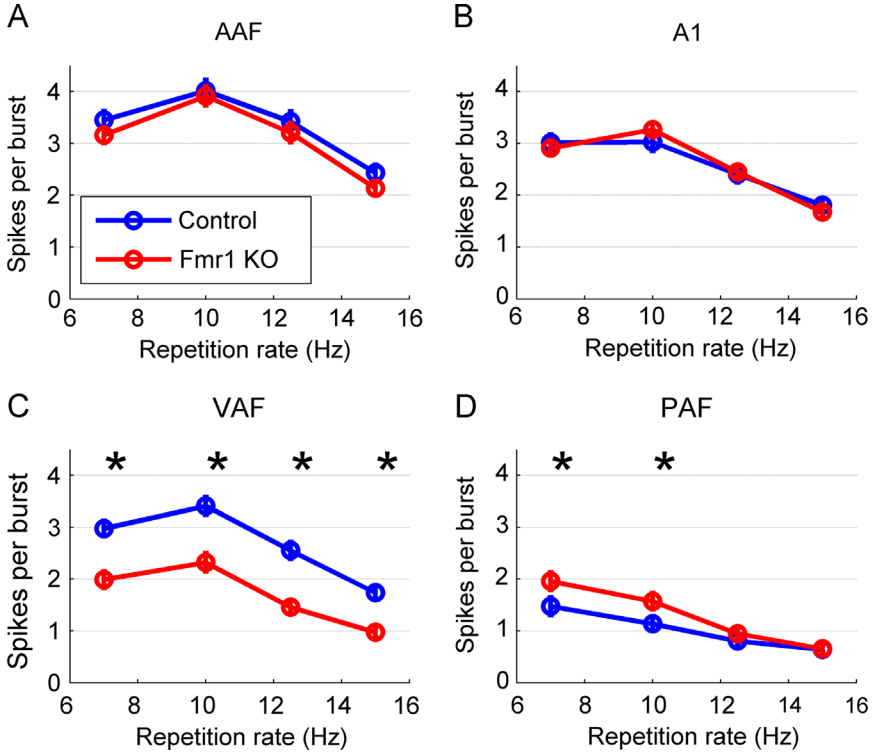


**Fig. 7 – Repetition rate transfer functions for noise burst trains. (a) The number of spikes per noise burst plotted as a function of the noise burst repetition rate in AAF in *Fmr1* KO and naïve control rats. Noise burst trains were presented at 7, 10, 12.5, and 15 Hz. Error bars represent s.e.m. across recording sites. (b) The number of spikes per noise burst plotted as a function of the noise burst repetition rate in A1 in *Fmr1* KO and naïve control rats. (c) The number of spikes per noise burst across repetition rates was reduced in VAF in *Fmr1* KO rats compared to naïve control rats. (d) The number of spikes per noise burst at the slower repetition rates was increased in PAF in *Fmr1* KO rats compared to naïve control rats.**

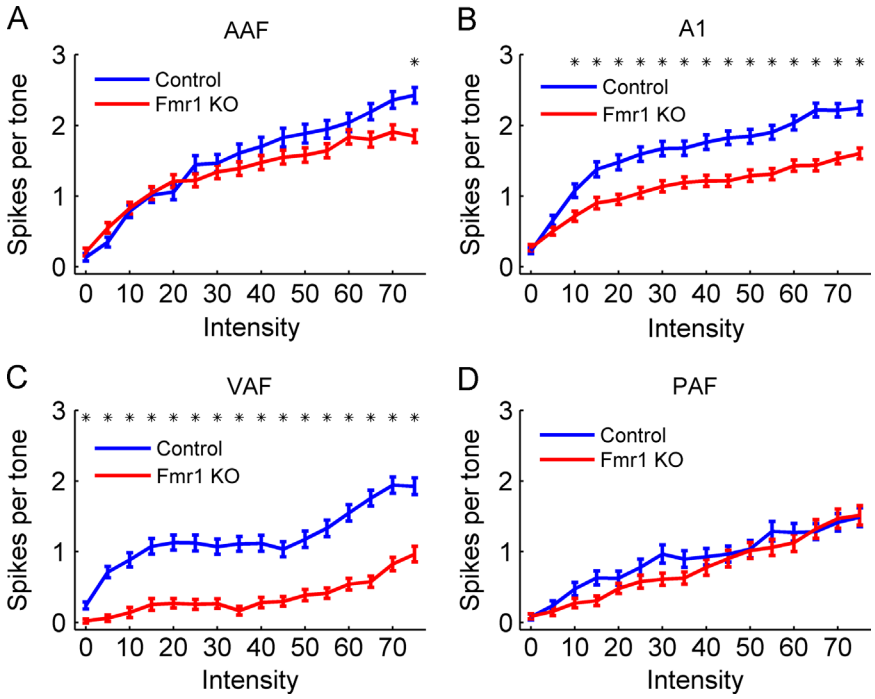


**Fig. 8 – Tone rate intensity functions for *Fmr1* KO and naïve control rats. (a) *Fmr1* KO rats evoke fewer spikes per tone at 75 dB in AAF compared to control rats. The number of spikes evoked per tone is plotted at each intensity (0–75 dB). Responses to tones within ½ octave of each site's characteristic frequency were averaged together. Error bars indicate s.e.m. across recording sites. Stars indicate statistically significant differences between *Fmr1* KO and control responses ($p < 0.0031$, Bonferroni correction). (b) The number of spikes evoked per tone in A1 is significantly decreased in *Fmr1* KO rats compared to naïve control rats. (c) The number of spikes evoked per tone in VAF is significantly decreased in *Fmr1* KO rats compared to naïve control rats. (d) The number of spikes evoked per tone in PAF is unimpaired in *Fmr1* KO rats compared to naïve control rats.**

interferes with the development of language skills (Pierpont et al., 2011). Our recordings in *Fmr1* knockout rats provide the first evidence that the cortical representation of speech sounds is impaired in fragile X syndrome. Neural responses to speech sounds, noise burst trains, and tones were reduced in several cortical regions, including primary auditory cortex, anterior auditory field, and ventral auditory field. Behavioral discrimination of simple speech sounds was normal in *Fmr1* knockout rats. These physiological and behavioral observations are consistent with earlier reports in fragile X syndrome. This new model may prove useful in elucidating the neural mechanisms that contribute to fragile X syndrome and could be used to evaluate potential treatments for fragile X syndrome.

### 3.1. *Speech discrimination in rodents*

Both this study and studies in individuals with fragile X syndrome report normal discrimination of simple speech sounds, but impaired cortical auditory processing. This observation is puzzling because many previous studies have shown that the performance of the neural classifier is correlated with speech discrimination performance (Centanni et al., 2013a,b, 2014; Engineer et al., 2008, 2014a,b; Perez et al., 2013; Ranasinghe et al., 2012; Shetake et al., 2011). This correlation between neural classifier and performance is even observed in degraded listening conditions, such as speech in noise (Shetake et al., 2011) or using vocoded speech (Ranasinghe et al., 2012), and is present in multiple auditory fields (Centanni et al., 2013b). Neural classifier performance also accurately predicts the behavioral impairment in an environmental rodent model of autism, prenatal valproate exposure (Engineer et al., 2014a,b). The observation that neural classifier performance does not predict behavioral performance in *Fmr1* KO rats suggests that further studies are necessary to explore the link between auditory processing and behavioral performance, and may be due in part to the well-known dysfunctional plasticity mechanisms in *Fmr1* KO rodents (Harlow et al., 2010; Kim et al., 2013; Meredith et al., 2007; Padmashri et al., 2013).

It has previously been shown that rats have intact speech discrimination ability following the removal of auditory cortex using the same task used in present study (Porter et al., 2011). It is important to note that the sounds used in both studies are clearly spoken natural speech sounds containing multiple redundant acoustic cues. While Porter et al. found that speech discrimination was intact following auditory cortex lesion, when they shortened the sounds to include only the 40 ms onset of the consonant, speech discrimination was impaired. So, while speech discrimination using clear, full-length exemplars is intact, it is possible that *Fmr1* KO rats would be impaired at speech discrimination using the same sounds presented with background noise or using truncated speech sounds that force the rats to use only the consonant. Many children with learning disabilities have abnormal cortical responses and normal clear speech

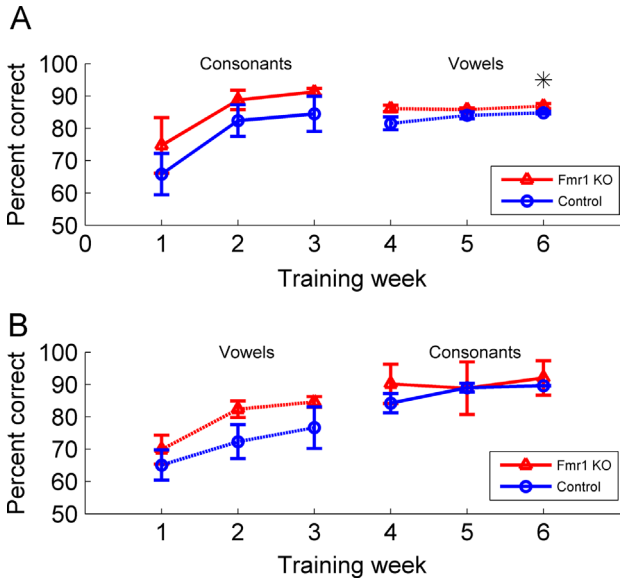


**Fig. 9 – Speech discrimination performance is unimpaired in *Fmr1* KO rats. (a) *Fmr1* KO rats are not impaired at consonant and vowel discrimination. Percent correct performance is shown for rats trained on a consonant discrimination task for three weeks, followed by a vowel discrimination task for three weeks. Solid lines indicate performance on the consonant discrimination task, while dashed lines indicate performance on the vowel discrimination task. Error bars indicate s.e.m. across rats. (b) Percent correct performance is shown for rats first trained on a vowel discrimination task for three weeks, followed by a consonant discrimination task for three weeks.**

discrimination ability and only exhibit phoneme processing impairments in noisy backgrounds or when required to manipulate phonemic information (Russo et al., 2009).

### 3.2. *Speech problems in fragile X syndrome*

Substantial language delays are common in fragile X syndrome, but the underlying neurological cause is not known (Hinton et al., 2013; Murphy and Abbeduto, 2003; Sudhalter et al., 1991). Impairments in nonword repetition, sequencing, and inference suggest that a specific deficit in working memory contributes to poor language processing in fragile X syndrome (Johnson-Glenberg, 2008; Keenan and Simon, 2004; Pierpont et al., 2011). The recent demonstration that phonological working memory is worse than visual-spatial working memory in fragile X syndrome confirms that impaired phonological processing likely contributes to language delays (Baker et al., 2011). Our observation that discrimination of consonant and vowel sounds is normal in *Fmr1* knockout rats is consistent with clinical observations. Future studies are needed to determine if *Fmr1* knockout rats have impaired auditory short term memory (Sakurai, 1994).

### 3.3. *Imaging in individuals with fragile X syndrome*

Anatomical and physiological studies have confirmed significant abnormalities in the central auditory system of individuals with fragile X syndrome. Cortical evoked responses to tones differ from controls (Castrén et al., 2003; Rojas et al., 2001; St Clair et al., 1987; Van der Molen et al., 2012a,b). However, the specifics of the experimental design appear to influence which peaks in the evoked potential are significantly larger and which are significantly smaller than controls (Knoth and Lippé, 2012). A review of the auditory electrophysiology studies in individuals with fragile X syndrome suggest a cascade of impaired auditory processing that affects later auditory potentials to a greater degree than earlier auditory potentials (Knoth and Lippé, 2012). It is not clear how these abnormalities would impact the neural representation of speech. Evoked potentials to speech sounds have not been reported in fragile X syndrome.

The superior temporal gyrus is reduced in size in individuals with fragile X syndrome (Gothelf et al., 2008; Reiss et al., 1994). Since superior temporal gyrus neurons are normally highly sensitive to phonemes (Chang et al., 2010; DeWitt and Rauschecker, 2012), an impairment to this region could contribute to language processing problems in fragile X syndrome. A recent study found that individuals with fragile X syndrome exhibit reduced connectivity among the regions of the language network, which includes the right middle temporal gyrus, left posterior supramarginal gyrus, and right anterior superior temporal gyrus (Hall et al., 2013). It is possible that abnormal activity in these regions contribute to poor phonological working memory in fragile X syndrome (Rämä et al., 2004).

Our observation that the ventral auditory field is more impaired than A1 or AAF in *Fmr1* knockout rats is consistent with the observation that higher auditory processing is impaired in fragile X syndrome and lower level auditory processing is largely intact. Higher cortical fields tend to exhibit longer latencies and more complex receptive field structure (Centanni et al., 2013b; Polley et al., 2007). These characteristics could be useful in the performance of higher order auditory processing. For example, lesions restricted to rat auditory association cortex can attenuate vowel discrimination learning, while lesions restricted to the primary auditory cortex have no significant effect on speech discrimination (Kudoh et al., 2006; Porter et al., 2011). It is not clear which regions of auditory cortex contribute to auditory working memory, but a growing body of evidence suggests that spatial and nonspatial auditory tasks preferentially recruit dorsal and ventral brain areas, respectively (Arnott et al., 2005). While deactivation of AAF impairs pattern discrimination, deactivation of PAF impairs sound localization ability (Ahveninen et al., 2013; Lomber and Malhotra, 2008). We found that responses to both speech and non-speech sounds were unimpaired in PAF, suggesting that sound localization abilities may be intact in *Fmr1* knockout rats. The ventral auditory stream in humans shows increasing specificity and invariance for temporally complex speech sounds. Our observation of reduced speech evoked activity in the ventral auditory field of *Fmr1* knockout rats suggests that the rat model of fragile X syndrome could be useful for elucidating the neurological mechanisms responsible for abnormal auditory processing in fragile X syndrome.

### 3.4. *Ventral auditory field*

It is not clear why VAF should be more impaired than A1 or AAF (Table 1). One possibility is that synaptic abnormalities

**Table 1- Summary of neural responses to speech, noise, and tones in *Fmr1* KO rats compared to naïve control rats.**

| Neural response | AAF | A1 | VAF | PAF |
|---|---|---|---|---|
| **Speech LFP amplitude** | ↓ | ↓↓ | ↓↓↓ | – |
| **Speech spikes** | ↓↓ | ↓↓ | ↓↓↓ | – |
| **Speech discrimination** | ↓ | ↓ | ↓ | – |
| **Noise spikes** | ↓↓ | – | ↓↓↓ | – |
| **Noise train spikes** | – | – | ↓↓↓ | ↑↑ |
| **Tone spikes** | ↓↓ | ↓↓ | ↓↓↓ | – |

↓, significantly less.
↓↓, >20% reduction.
↓↓↓, >40% reduction.

accumulate, causing greater disturbance in higher sensory stations compared to lower stations. The observation that PAF responses are normal in *Fmr1* knockout rats is not consistent with this explanation. A second possibility is that the ventral auditory field may develop at a later time than A1, AAF, and PAF. The observation that the switch from depolarizing to hyperpolarizing GABA is delayed in the cortex of fragile X mice provides a potential mechanism for a field specific deficit caused by a delay in this important developmental event (He et al., 2014). A third possibility is that the differential pattern of connectivity in VAF compared to the other fields is responsible for the greater deficit. VAF and A1 receive inputs from non-overlapping regions of the auditory thalamus (Polley et al., 2007; Smith et al., 2012; Storace et al., 2010). VAF is connected to the insula which is involved in emotion, multisensory integration, and representation of vocal communication sounds (Kimura et al., 2010, 2007; Remedios et al., 2009; Rodgers et al., 2008). Activity in the insula is abnormal in fragile X syndrome and autism (Anderson et al., 2010; Hall et al., 2013). It is possible that reduced activity in the ventral auditory field contributes to the decreased gray matter density, decreased connectivity, and decreased low-frequency fluctuations in the insula of individuals with fragile X syndrome (Hall et al., 2013). Inappropriate emotional processing probably contributes to the hyperreactivity to sound observed in fragile X syndrome (Miller et al., 1999; Roberts et al., 2013). Higher cortisol levels in fragile X syndrome are correlated with behavioral problems (Hessl et al., 2002). Treatments that improve normal auditory processing may strengthen language function and reduce behavioral problems in fragile X syndrome.

### 3.5. *Physiology in rodent models of fragile X syndrome*

The molecular and cellular deficits caused by loss of FMRP likely contribute to the reduced cortical responses to speech and non-speech sounds. Loss of FMRP exaggerates mGluR-stimulated protein synthesis and disrupts normal neural activity and plasticity (Bear et al., 2004; Dölen et al., 2007; Harlow et al., 2010; Hays et al., 2011; Osterweil et al., 2010). Abnormal cortical plasticity has been hypothesized to be responsible for the cognitive and behavioral impairments that characterize fragile X syndrome (LeBlanc and Fagiolini, 2011). For example, the mouse model of fragile X syndrome exhibits impaired motor skill learning that is accompanied by increased dendritic spine turnover in motor cortex and reduced training-induced formation of dendritic spines (Padmashri et al., 2013). It is not clear how loss of FMRP leads to reduced speech responses in auditory cortex, but impaired neural plasticity is likely to play a key role.

Fragile X mice exhibit reduced A1 frequency map plasticity when exposed early in life to a pure tone (Kim et al., 2013). Experience-dependent regulation of potassium channels is also impaired in the auditory brainstem (Strumbos et al., 2010), though thresholds and amplitude of the auditory brainstem response are not different in fragile X mice compared to wild-type mice (Kim et al., 2013). Impaired synaptic plasticity in fragile X syndrome is likely exacerbated by abnormally high neural firing and synchrony during sleep (Gonçalves et al., 2013), which is known to be critical for normal plasticity (Frank et al., 2001). Reduced effectiveness of neuromodulators, including dopamine and acetylcholine, may also contribute to impaired regulation of neural plasticity in fragile X syndrome (D'Antuono et al., 2003; Wang et al., 2008). Finally, unreliable calcium signaling in mice lacking the *Fmr1* gene appears to cause an increased threshold for spike-timing-dependent plasticity (Meredith et al., 2007). The cascading effects of many different abnormalities likely results in the greatly reduced VAF responses to speech sounds observed in this study.

### 3.6. *Potential treatments for fragile X syndrome*

As the function of FMRP and the consequences of its loss become better understood, it may be possible to develop behavioral or pharmacological therapies to improve the quality of life for individuals with fragile X syndrome. The rat model of speech sound processing in fragile X syndrome may prove useful for testing these theories because of the rich array of behavioral tasks that rats can perform using speech sounds and their high correlation with neural activity patterns (Centanni et al., 2013b, 2014; Engineer et al., 2008, 2013; Ranasinghe et al., 2012; Shetake et al., 2011). The increasing complexity of speech responses at higher stations of the central auditory pathway provides a valuable model for testing the cascading consequences of FMRP and for testing potential methods to ameliorate them. Treatment with NMDAR co-agonists, $GABA_A$ receptor agonists, mGlu5 antagonists, and other compounds can improve or eliminate many of the neural abnormalities associated with fragile X syndrome (Bostrom et al., 2013; Dansie et al., 2013; Michalon et al., 2012, 2014; Olmos-Serrano et al., 2011). It remains to be seen whether these agents can restore normal operation to complex networks such as the central auditory system (Bagni and Oostra, 2013). The rat model of fragile X syndrome will likely be useful to identify additional physiological and behavioral consequences of FMRP loss and to test potential new therapies, including intensive behavioral training or environmental enrichment (Engineer et al., 2014a,b; Hagerman et al., 2009; Percaccio et al., 2005, 2007).

## 4. Experimental procedure

Nine male hemizygous SD-*Fmr1*$^{tm1sage}$ knockout (KO) rats were obtained from SAGE Labs (Boyertown, PA). We trained

11 rats (4 *Fmr1* KO rats and 7 experimentally naïve controls rats) to discriminate speech sounds. Local field potential and multiunit responses to tones, noise burst trains, and speech sounds were recorded from 1114 auditory cortex sites in experimentally naïve rats ($n=10$ rats, 610 auditory cortex sites), untrained *Fmr1* KO rats ($n=5$ rats, 323 auditory cortex sites), and speech trained *Fmr1* KO rats ($n=4$ rats, 181 auditory cortex sites). Speech sounds, training, and anesthetized recording procedures are identical to those used in our previous studies (Centanni et al., 2013b; Engineer et al., 2008; Perez et al., 2013). The University of Texas at Dallas Institutional Animal Care and Use Committee approved all protocols and recording procedures.

### 4.1. *Speech stimuli*

Speech sounds were identical to the sounds used in our previous studies and were spoken by a female native English speaker (Centanni et al., 2013b; Engineer et al., 2008, 2013, 2014a,b; Perez et al., 2013; Ranasinghe et al., 2013). Five consonants were recorded in a '_æd' context ('dad', 'bad', 'gad', 'tad', and 'sad'), and five vowels were recorded in a 'd_d' context (/ae/ 'dad', /ɛ/ 'dead', /ʌ/ 'dud', /i/ 'deed', and /u/ 'dood'). Each sound was presented at 60 dB, measured using the loudest 100 ms of the vowel portion of the stimulus, and the frequency was shifted up by an octave using the STRAIGHT vocoder to better match the rat hearing range (Engineer et al., 2008; Kawahara, 1997; Perez et al., 2013).

### 4.2. *Speech training*

The speech training procedure used in this study is identical to many of our previous studies (Engineer et al., 2008, 2014a,b; Perez et al., 2013; Porter et al., 2011). Four *Fmr1* KO rats and seven experimentally naïve control rats were each trained on both a consonant discrimination task and a vowel discrimination task. Half of the rats were first trained on the consonant task followed by the vowel task while the other rats were first trained on the vowel task followed by the consonant task.

Rats were trained to perform an operant go/no-go lever press task. Each rat trained for two 1 h sessions per day for 5 days a week. Rats first learned to press a lever to receive a food pellet reward (45 mg sugar pellet, Bio-Serv). This shaping stage lasted until the rat was able to independently press the lever 100 times per session for two sessions. The rats next performed a detection task, where they needed to press the lever in response to hearing the sound 'dad'. This stage lasted until the rat was able to accurately perform the task with a $d' \geq 1.5$ for 10 sessions. The final stage of training was a discrimination task, where rats needed to press the lever for the target sound 'dad' and refrain from pressing the lever to all non-target speech sounds ('bad', 'gad', 'tad', and 'sad' for the consonant task; 'dead', 'dud', 'deed', and 'dood' for the vowel task). Rats were rewarded for pressing the lever within 3 s after the presentation of the target sound, and received a time out (program paused and booth lights were extinguished for 6 s) if they pressed the lever after the presentation of a non-target sound. The discrimination stage lasted for 3 weeks per task.

### 4.3. *Auditory cortex recordings*

Auditory cortex responses were recorded from 10 experimentally naïve control rats, 5 untrained *Fmr1* KO rats, and 4 speech trained *Fmr1* KO rats. The recording procedure used in this study is identical to many of our previous studies (Centanni et al., 2013a; Engineer et al., 2012, 2013; Perez et al., 2013; Reed et al., 2011). Responses were recorded from four auditory cortex fields: anterior auditory field (AAF), primary auditory cortex (A1), ventral auditory field (VAF), and posterior auditory field (PAF). Rats were anesthetized with pentobarbital (50 mg/kg), and received dilute supplemental doses throughout the experiment (8 mg/mL). A tracheotomy was performed to ensure breathing throughout the experiment and a cisternal drain was performed to reduce swelling. A craniotomy and a durotomy were performed to expose right auditory cortex. *Fmr1* KO rats had a significant amount of bleeding during the craniotomy surgery. Multiunit and local field potential (0–300 Hz) responses were recorded simultaneously from right auditory cortex using 4 Parylene-coated tungsten microelectrodes (1–2 MΩ, FHC). Tucker-Davis hardware (RA16 and RX5), software (SigGen and Brainware), and speakers (FF1) were used for sound generation and data acquisition. Tone frequency intensity tuning curves (25 ms tones from 1 to 48 kHz in 0.125 octave steps, 0–75 dB SPL in 5 dB steps) were obtained to determine the characteristic frequency of each auditory cortex site. Trains of 6 noise bursts (1–32 kHz range) were presented at 7, 10, 12.5, and 15 Hz. Each noise burst train and speech sound was presented 20 times at each site.

### 4.4. *Data analysis*

The four auditory fields were defined based on tonotopy, relative location, and response latency. AAF recording sites exhibited a characteristic tonotopy of low to high characteristic frequencies from anterior to posterior, as well as fast response latencies (Centanni et al., 2013b; Polley et al., 2007). A1 recording sites exhibited a characteristic tonotopy of high to low characteristic frequencies from anterior to posterior, as well as fast response latencies (Kilgard and Merzenich, 1999). VAF recording sites did not exhibit a characteristic tonotopy, were located between AAF and A1, and had longer response latencies (Centanni et al., 2013b; Polley et al., 2007). PAF recording sites did not exhibit a characteristic tonotopy, were located posterior to AAF and VAF, and had long response latencies (Jakkamsetti et al., 2012; Puckett et al., 2007).

The onset response to speech sounds was quantified as the number of spikes evoked in the first 40 ms of the response. The onset latency was the latency where the firing rate exceeded 3 standard deviations above the spontaneous firing rate. The peak latency was the latency of maximum firing rate. The driven response was calculated as the number of stimulus driven spikes minus the spontaneous firing rate. Neural classifier performance was quantified using a PSTH-based nearest neighbor classifier, as in our previous studies (Centanni et al., 2013b; Engineer et al., 2008; Perez et al., 2013). Each single trial response was compared with the average activity pattern evoked by each of the presented speech stimuli. The neural classifier guessed that the single trial

response (40 ms onset response with 1 ms temporal precision) was evoked by the sound whose average pattern had the minimum Euclidean distance. Speech sound discrimination performance was quantified in units of percent correct, which is defined as the average of the correct lever presses to target sounds and the correct rejections of non-target sounds.

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