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Spontaneous Cortical Network Dynamics is Unaltered in a Mouse Model of Fragile X Syndrome

Review

On the Higg's Field and the Origin of Mass

Interview

At the Crack of Dawn: A Look at the Unmanned Space Mission to the Asteroids Ceres and Vesta

Spontaneous Cortical Network Dynamics is Unaltered in a Mouse Model of Fragile X Syndrome

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Fragile X Syndrome (FXS) is an inherited form of mental retardation caused by a mutation of the Fmr1 gene in humans. Although a number of abnormal neuronal properties have been reported in humans with FXS, the cause of the cognitive deficits remains elusive. Since behavior and cognition ultimately emerge from networks of neurons, synaptic or cellular abnormalities underlying FXS should influence the network-wide spatiotemporal patterns of activity—that is, neural dynamics. Here, spontaneous network activity in a mouse model of FXS was analyzed by recording from pyramidal neurons in neocortical organotypic slice cultures made from Fmr1 knockout mice and littermate controls during the first 4 weeks in vitro. Different 'modes' of spontaneous activity were observed, including sustained periods of network-wide activity referred to as Up states, which are believed to play an important role in normal cortical function. Both control and mutant networks exhibited a significant increase in the amount of spontaneous activity –including an increase in the frequency and amplitude of Up states–during weeks 2-4 in vitro. Although no significant differences in the amount of spontaneous activity were detected between mutant and control groups, there were changes in the variability of spontaneous activity during the 4th week. Our results indicate that cellular alterations in Fmr1 knockout mice do not alter levels of spontaneous activity in neocortical slices.

activity in the network. Experiments involving simultaneous intracellular and extracellular multiunit recordings (which measure the activity of neural populations) have demonstrated that Up states in intracellular recordings coincide with similar events in multiunit recordings, indicating that Up states are a network phenomenon (Sanchez-Vives and McCormick, 2000). Thus, intracellular recordings from single neurons provide information about the level of network activity as well as its spatiotemporal patterns. Up states have been observed in vivo under anesthesia, sleep and quiet wakefulness (Sanchez-Vives and McCormick, 2000; Cossart et al. 2003; Petersen et al., 2003; Shu et al., 2003; Massimini et al., 2004; Volgushev et al., 2006), and in vitro in both acute and organotypic slices (Plenz and Kitai, 1998; Echevarria and Albus, 2000; Shu et al., 2003; Kerr and Plenz, 2004; Johnson and Buonomano, 2007). It has recently been shown in rat neocortical organotypic slices that spontaneous activity, including Up state frequency, increases during development in vitro (Johnson and Buonomano, 2007). The presence of Up states in vivo and in vitro indicates that mechanisms are in place to generate these states; moreover, it supports the idea that these spontaneous events are functionally important.

Due to evidence that FMRP plays an important role in normal circuit function and that neural computations and cognition rely on network dynamics, we examined whether or not cortical networks from mutant slices exhibit altered spontaneous dynamics was examined. Furthermore, given that physiological phenotypes such as the dendritic spine abnormalities appear during specific developmental windows, and that spontaneous activity is known to increase with development, we considered the possibility that differences in network activity due to *Fmr1* mutation may manifest in an age-dependent manner.

MATERIALS AND METHODS

Organotypic slice preparation Organotypic slices were prepared using the interface method (Stoppini et al., 1991). Postnatal day 5 (P5) mice were anesthetized using Isoflurane and decapitated. The brain was removed and placed in chilled cutting media. Coronal slices (400 μm) from primary somatosensory cortex were cut using a vibratome and placed on Millipore (Billerica, MA) filters (MillicellCM) with 1 mL of culture media. Culture media was changed 1 hour, then 24 hours after cutting, and every 2-3 days thereafter. Cutting media consisted of Eagle's minimum essential media (EMEM; cat. Num. 15-010; MediaTech, Herndon, VA) plus 3 mM $MgCl_2$, 10 mM glucose, 25 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), and 10 mM Tris base. Culture media consisted of: EMEM plus 4 mM glutamine, 0.6 mM $CaCl_2$, 1.85 mM $MgSO_4$, 20 mM glucose, 30 mM HEPES, 0.5 mM ascorbic acid, 20% horse serum, 10 μg/L penicillin, and 10 μg/L streptomycin. Slices were incubated in 5% CO_2 at 35°C for 7-28 days.

Electrophysiology Recordings were made from regular-spiking, pyramidal neurons located less than 400 μm from the external surface of the cortex using infrared differential interference contrast visualization. Experiments were performed in artificial cerebrospinal fluid (ACSF) consisting of 125 mM NaCl, 2.5 mM KCl, 2 mM $MgSO_4$, 26.2 mM $NaHCO_3$, 1 mM NaH_2PO_4, 25 mM glucose and 2.5 mM $CaCl_2$. Internal solution for whole-cell recordings contained 100 mM K-gluconate, 20 mM KCl, 4 mM ATP-Mg, 10 mM phospho-creatine, 0.03 mM GTP-Na, and 10 mM HEPES. The pH was adjusted to 7.3 and the osmolarity was adjusted to 300 mOsm. All analyses were performed using custom-written software in Matlab (MathWorks, Natick, MA). Sequential recordings from one mutant and one littermate control slice were made during every experiment.

Because slices tend to exhibit little to no spontaneous activity during the first week in vitro, recordings were made starting at the beginning of the second week. Furthermore, a recent study in rat neocortical slices showed that levels of spontaneous activity reached a plateau after the 4th week in vitro (Johnson and Buonomano, 2007); therefore, recordings after the fourth week were not considered in this study.

Quantification of Intrinsic Excitability Prior to recording spontaneous activity, the intrinsic excitability of neurons was probed using 250 ms depolarizing current injections of 0.05, 0.10, 0.15, 0.20, 0.25, 0.30 nA. The number of action potentials elicited was counted in an offline analysis.

Quantification of spontaneous activity Five minutes of spontaneous activity was recorded from each neuron. Spontaneous events were defined as those in which membrane potential crossed a threshold of 5 mV above resting potential. No quantitative change in the data was detected when using different thresholds. However, a 5-mV threshold enabled us to focus primarily on network activity, as opposed to unitary events, because such events reflect the temporal summation of inputs from the many cells in the network. Standard deviation was calculated using MATLAB. Event area was defined as the average area (in mV*s) of the recorded trace above threshold during a sweep of 30 s as calculated from 10 sweeps. Event time was the percentage of recording time spent above threshold. An Up state was defined as an event that remained above threshold for no less than 500 ms. During a network activation such as an Up state, the membrane potential would often make multiple brief passes above and below this threshold before returning to resting potential. For this reason, a minimum inter-event-interval of 100 ms was used as a criterion for defining separate events. Measurements from neurons within a given slice were averaged to reduce the effects of variability across individual cells. Two and three-way Analysis of Variance (ANOVA) was used to determine the significance of changes in spontaneous activity and intrinsic excitability due to development or genotype.

Structure of spontaneous activity Because younger slices may exhibit little or no spontaneous activity, and some variability exists in the number of spontaneous events in each neuron; this analysis was performed on a subset of neurons that

exhibited at least three well defined events. All spontaneous events were extracted into individual 3-second segments starting at the time of the first threshold crossing. The segments were aligned according to the time of the threshold crossing (aligning according to the peak of the cross-correlation did not alter the results). Correlation coefficients were calculated including a 20 ms window before onset and 20 ms after offset (as defined by the final reverse threshold crossing within the 3-s segment). Note that because segments have different offsets, the correlations were calculated using the offset time for the longest event of each pair. Segments of fixed duration were not used because of the bias that may be produced towards high correlations for short bouts of activity. Only segments with at least 500 ms of activity within the 3-s segments were used. Results were not qualitatively different when only Up state events (i.e., events with at least 500 ms of continuous activation) were used. Based on the correlation coefficient between all segments from within each cell, a correlation matrix was constructed and used to calculate the mean correlation. For visualization purposes, the correlation was used to create a "similarity matrix" by clustering events according to Euclidean distances between the correlation coefficients. ANOVA was used to determine the significance of the changes in mean correlation across genotype and week.

Mouse model *Fmr1* knockout mice used in the current study were originally described in Bakker (1994). All mice were group housed, maintained in a 12:12 light/dark cycle with water and food ad libitum. Since the mutation occurs on the X chromosome, heterozygous females were bred to wild type C57BL/6J males in order to generate mutant and wild-type male offspring. On day P4, tail biopsies were procured from all animals in a litter for genotyping, and mutant males and wild-type male littermates were selected. Wild-type females were selected when male controls were not available.

The following primers were used:
SRY (to genotype male vs. female): 5'-TTG TCT AGA GAG CAT GGA GGG CCA TGT CAA-3' and 5'-CCA CTC CTC TGT GAC ACT TTA GCC CTC CGA-3'

FMR (to genotype Fmr1 knockouts): 5'-ATC TAG TCA TGC TAT GGA TAT CAG C-3' and 5'-GTG GGC TCT ATG GCT TCT GAG G-3'

All experiments were performed in accordance with the institutional guidelines of the University of California at Los Angeles.

RESULTS

Developmental increase in spontaneous activity To characterize the development of spontaneous dynamics in cortical networks, we performed whole-cell recordings in pyramidal neurons of neocortical organotypic slices for 7-28 days *in vitro* (DIV) (2-4 weeks *in vitro*). It was possible to sample network activity by recording the membrane potential of a single postsynaptic neuron because each pyramidal cell receives thousands of synaptic inputs. Thus, the amount and structure of postsynaptic activity of a single neuron can be used to measure network dynamics (Buonomano, 2003; MacLean et al., 2005; Johnson and Buonomano, 2007).

It was hypothesized that knocking out *Fmr1* would result in altered neural dynamics because alterations in cortical function are likely to be expressed at the level of the patterns of activity within neural circuits. We first considered the possibility that overall spontaneous activity may be altered by the mutation. To quantify the degree of overall spontaneous activity (Figure 2A), we measured the standard deviation of the voltage trace as well as the time and area above a predetermined threshold (Figure 1). The standard deviation provided a raw measure of overall activity because it accounts for both positive and negative deflections of the membrane potential. It is also independent of arbitrary assumptions about the data such as the threshold used for other measurements.

We observed a progressive increase in the standard deviation of the voltage in both control and mutant slices through week 4 (Figure 2B; Two-way ANOVA, $F_{(2,46)} = 4.28$, $P<0.05$, week 2, n=22; week 3, n=20; week 4, n = 10; where n is the total number of slices). Similarly, a significant increase in the area and time above threshold in both control and mutant slices (Figure 2C and 2D; Two-way ANOVA, $F_{(2,46)} = 5.61$, $P<0.01$ and $F_{(2,46)} = 11.52$, $P<0.001$, for area and time above threshold respectively) was observed. ANOVA revealed that there were no differences between controls and mutants in standard deviation, total area or total time above threshold ($P>.05$) in the genotype and interaction (Week*Genotype) factors. Therefore, while overall spontaneous activity was not altered by the mutation, it did increase with development in both control and mutant slices, which is consistent with previous results in rats (Johnson and Buonomano, 2007).

The above measurements quantified spontaneous activity but were not sensitive to the spatiotemporal structure and hence qualitative aspects of the activity. That is, five separate events of 10 mV that lasted 100 ms would result in the same value as one 10 mV event that lasted 500 ms. Therefore, we used measurements that were designed to specifically quantify Up states in order to analyze network dynamics (Figure 3A; Johnson and Buonomano, 2007). These included Up state frequency, mean duration and area. Up state frequency increased significantly in both control and mutant slices between weeks 2-4 (Figure 3B; Two-way ANOVA, $F_{(2,46)} = 9.912$, $P<0.0001$; week 2, n=22, week 3, n=20, week 4, n = 10). There was a concomitant increase in mean Up state duration and total Up state area from week 2 to 4 (Figure 3C and 3D; Two-way ANOVA, $F_{(2,42)} = 10.797$, $P<0.0001$; $F_{(2,42)} = 4.425$, $P<0.05$; week 2, n=18; week 3, n=20; week 4, n = 10 for duration and area, respectively). No significant differences were detected with respect to genotype ($P>.05$) for genotype or interaction (Week*Genotype) factors in an ANOVA, consistent with previous findings (Johnson and Buonomano, 2007). Thus, Up state-specific measurements, which measure network activity, increased with development in both control and mutant slices.

INTRODUCTION

Fragile X syndrome (FXS) is an X-linked inherited form of mental retardation that occurs in approximately 1 in 3600 human males and 1 in 6000 in females (Crawford, 2001). FXS patients exhibit a number of behavioral, sensory, and cognitive abnormalities including low IQ, hyperactivity, and attention deficits in addition to hypersensitivity and poor adaptation to sensory stimuli. Patients also show physical symptoms including elongated face, macroorchidism and low muscle tone. The disease is caused by a mutation of the Fmr1 gene that silences the expression of Fragile X Mental Retardation Protein (FMRP), which is a RNA binding protein that is known to regulate translation and RNA trafficking locally in neuronal dendrites (Jin and Warren, 2000; Verheij et al., 1993).

An animal model of FXS has been developed by knocking out the *Fmr1* gene in mice. *Fmr1* knockout (*FMR1-KO*) mice lack FMRP and show molecular, morphological and behavioral alterations similar to human patients including hyperactivity, abnormal anxiety-related responses, and impaired motor coordination, making them a robust model to study the disease (Bakker et al., 1994; Peier et al., 2000; Irwin et al., 2002; McKinney et al., 2005). More recently, *FMR1-KO* mice have been shown to be deficient in an escape/avoidance task, which tests learning and memory ability. The mice have also performed poorly in an object recognition task, suggesting that mutant mice develop cognitive impairments analogous to those observed in human patients (Ventura et al., 2004; Brennan et al, 2006).

A number of molecular and cellular phenotypes in FXS have been documented in areas of the brain, including neocortex, hippocampus, and the cerebellum, which are involved in higher cognitive function, learning and memory, and motor control (Bakker et al., 1994; Irwin et al., 2000; Peier et al., 2000; Mineur et al., 2002; Huber, 2006). For example, abnormalities in the morphology and distribution of dendritic spines, which are small protrusions from a neuron's dendrite and the postsynaptic component of excitatory synapses, have been observed in both humans and mice lacking FMRP (Hinton et al, 1991; Bakker et al., 1994; Comery et al., 1997; Irwin et al, 2001). Normal cortical development has been characterized by an overproduction of immature spines followed by a pruning process (Murphy and Magness, 1984; Schuz, 1986; Galofre and Ferrer, 1987; Horner, 1993). In mice lacking FMRP, spines in the neocortex and hippocampus have been shown to be significantly longer during the first two postnatal weeks in vivo (Nimchinsky et al., 2001). However, this effect disappeared by the fourth week suggesting that the pruning process is delayed in *Fmr1* mutants. Given the severity of cognitive deficits in FXS, these studies suggest that normal spine development plays a critical role in cortical ontogenesis and cognitive function.

Hippocampal and neocortical neurons lacking FMRP have also been reported to exhibit altered synaptic plasticity, which is the ability of neurons to adaptively regulate synaptic strength. Specifically, FMRP deficiency is known to result in an increased threshold for synaptic potentiation and to facilitate synaptic depression (Huber et al., 2002; Meredith et al., 2007; Hinton et al., 1991; Bakker et al., 1994; Comery et al., 1997; Nimchinsky et al., 2001; Galvez and Greenough, 2005; Koekkoek et al., 2005; Grossman et al., 2006). It has been suggested that abnormalities with both dendritic spines and synaptic plasticity may be produced by increased metabotropic glutamate receptor activity (Dolen et al., 2007). Together, the current data suggest that FMRP is important in synaptic physiology; hence, it plays an important role in network function and neural processing.

While the neuropathies associated with FXS have been studied at the level of individual neurons and synapses, the explicit causes of the cognitive impairments at the network level remain elusive. Specifically, neural computations that underlie behavior and cognition are not the product of isolated neurons, but emerge from functional interactions between individual neurons in large-scale neuronal networks. Therefore, the abnormal synaptic and cellular properties in FXS must ultimately be expressed at the network level as alterations in spatiotemporal patterns of activity, which are referred to as neural dynamics. It is unknown whether the behavioral and cognitive deficits are produced by local abnormalities in isolated brain areas or by global alterations involving multiple brain areas. Furthermore, the level of complexity at which alterations in neural dynamics should be observed is unclear. However, in order to fully understand the neural basis of cognitive impairments associated with FXS, it is critical to examine network dynamics in FMRP-lacking circuits (Belmonte and Bourgeron, 2006).

Studies of neural dynamics have focused in part on spontaneous patterns of activity referred to as "Up" states which are observed both in vivo and in vitro (Metherate and Ashe, 1993; Kerr and Plenz, 2004; Johnson and Buonomano, 2007). Up states observed in intracellular recordings are defined as sustained periods of depolarization of the membrane potential and reflect barrages of synaptic inputs produced by recurrent

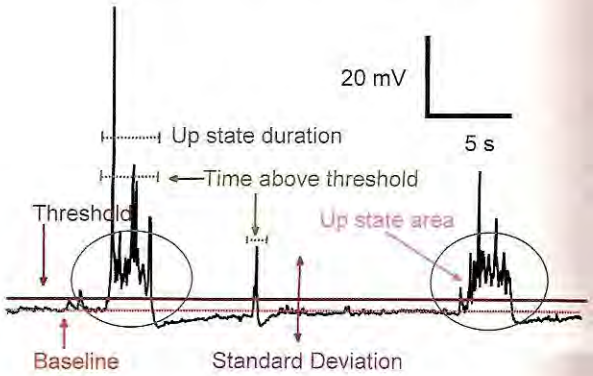

Figure 1. A 20-second voltage trace from a pyramidal neuron. *Voltage traces were analyzed by quantifying standard deviation as well as total time and total area above a predetermined threshold. Baseline was defined as the median of a trace, and threshold was set to be 5 mV above baseline. Up state area, mean duration as well as the number of Up states were also analyzed. Up states are circled in the diagram, and were defined as sustained periods of depolarization for a minimum of 500 ms; events with transient 'dips' below threshold that were less than 100 ms were also included.*

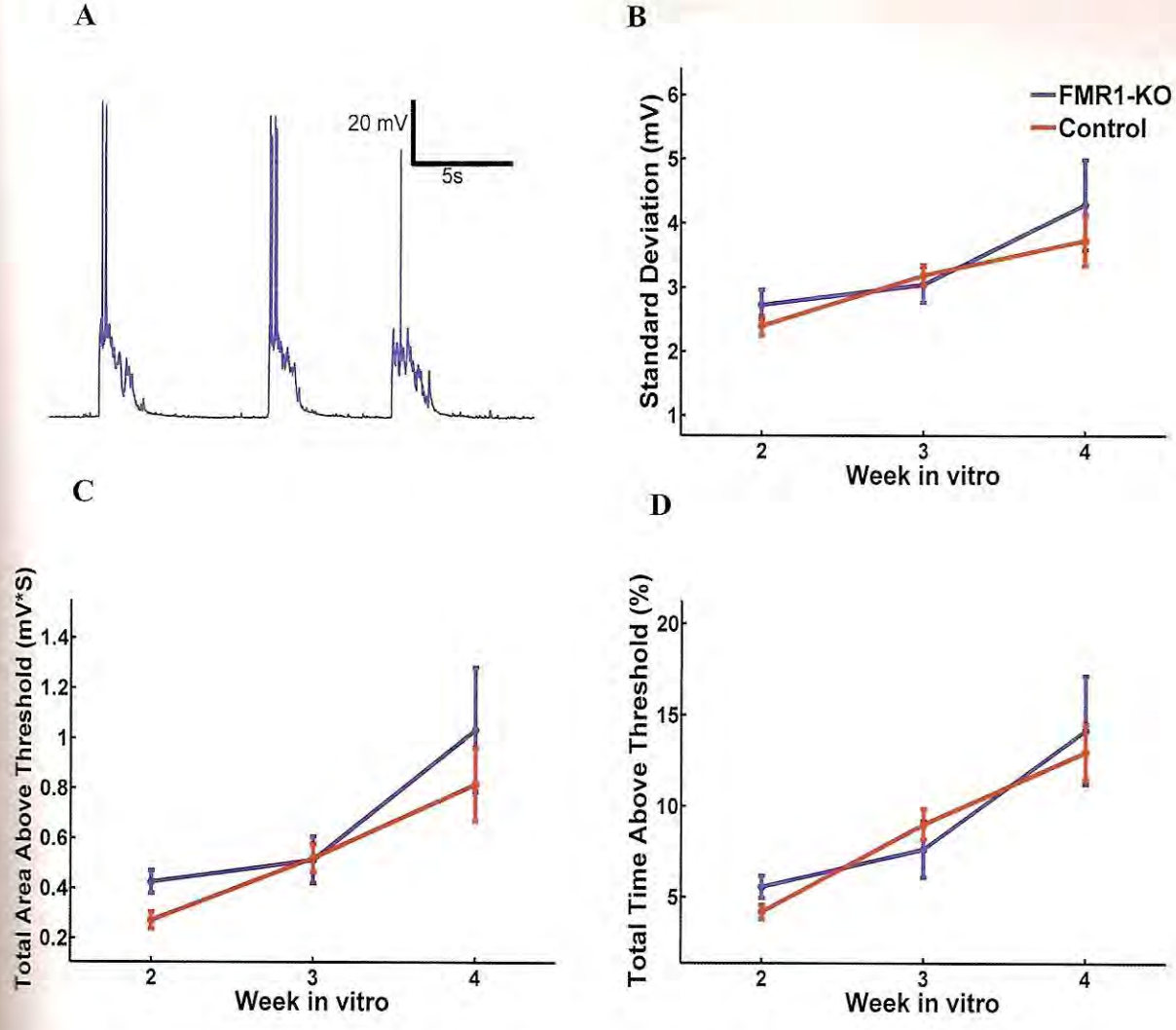

Figure 2. Spontaneous activity increased significantly during development in both controls and FMR1-KO slices.
(A) 30-second segment of a recording from a wild-type neuron displaying overall spontaneous activity. (B) The standard deviation of the membrane potential increased significantly during development in both control and mutant slices (Two-way ANOVA, $F_{(2,46)} = 4.28$, $P<0.05$; week 2, n=22, week 3, n=20, week 4, n = 10, where n is total the number of slices; error bars in S.E.M. (C) The total time spent above threshold, expressed as a percentage of the total recording time (300 seconds), also showed a significant increase throughout development in both groups. (Two-way ANOVA, $F_{(2,46)} = 5.61$, $P<0.001$; week 2, n=22, week 3, n=20, week 4, n = 10) (D) Total area above threshold also increased significantly with development. (Two-way ANOVA, $F_{(2,46)} = 11.52$, $P<0.01$; week 2, n=22, week 3, n=20, week 4, n = 10).

Developmental decrease in intrinsic excitability

Changes in levels of spontaneous activity and the emergence of Up states are independent of external input which implies that there are forms of cellular and synaptic plasticity in place to allow for these developmental changes. The increase in spontaneous activity may be produced by a number of different mechanisms. An increase in the excitability of individual neurons is one possibility; in other words, the same amount of input could elicit more spikes, resulting in an increase in network activity. Thus, we measured the intrinsic excitability of each neuron in order to check whether this could account for changes in the development of spontaneous activity, as well as to examine whether or not intrinsic excitability is regulated differently in *FMR1-KO* neurons.

We examined the intrinsic excitability of neurons using depolarizing current steps (Figure 4A). Specifically, the number of action potentials elicited from 250 ms current injections of increasing intensity was recorded. Due to our selection criteria, there was insufficient data for the week 4 group; therefore, this analysis used cells from weeks 2 and 3. A three-way ANOVA with repeated measures on one factor (current intensity) did not reveal any significant main factor or interaction effects due to developmental stage or genotype. However, there was a trend for controls to exhibit a decrease in excitability that was absent in the *FMR1-KO* neurons (Figure 4B and 4C, respectively). In agreement with previous findings (Johnson & Buonomano, 2007), these results indicate that the developmental increase in spontaneous activity is not produced by increased intrinsic

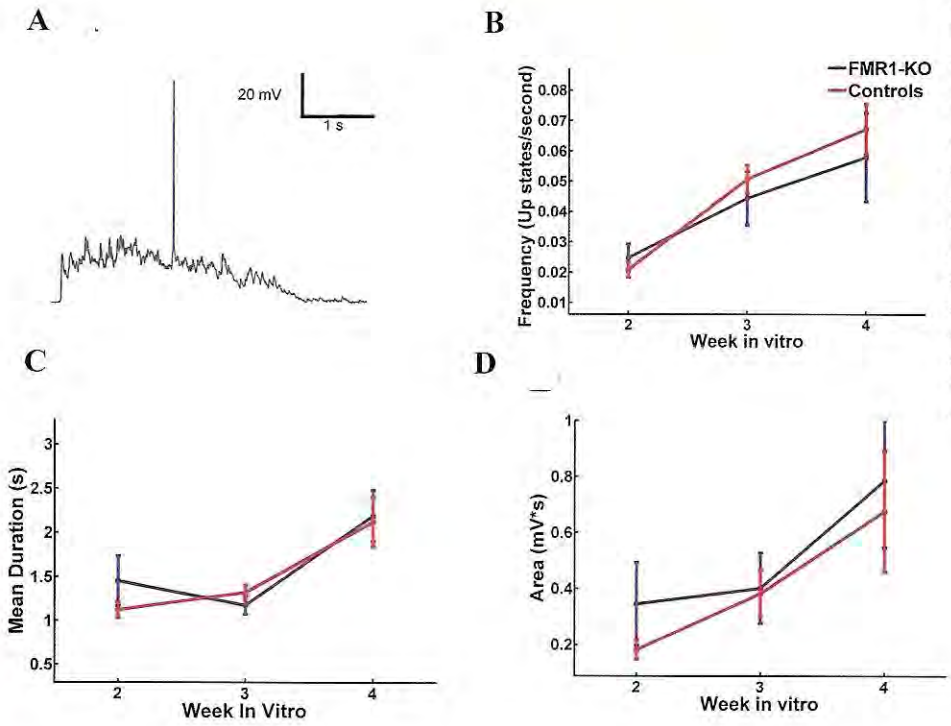

Figure 3. Up state frequency and intensity increased during development in both controls and FMR1-KO slices.
(A) *3-second segment of a recording from a wildtype neuron displaying a typical Up state.* **(B)** *The frequency of Up states increased significantly over development. (Two-way ANOVA, $F_{(2,46)}$ = 9.912, P<0.001; week 2, n=22, week 3, n=20, week 4, n = 10).* **(C)** *The mean duration of Up states also increased significantly from week 2 to week 4 (Two-way ANOVA, $F_{(2,42)}$ = 10.797, P<0.001; week 2, n=18; week 3, n=20; week 4, n = 10)* **(D)** *Up state area increased significantly as well (Two-way ANOVA, $F_{(2,42)}$ = 4.425, P<0.001; week 2, n=18; week 3, n=20; week 4, n = 10).*

excitability, suggesting that intrinsic excitability may be differentially regulated in mutant networks.

Altered Variability of Patterns of Activity A critical issue regarding both the mechanisms and function of spontaneous dynamics is whether there is structure to spontaneous activity. On one hand, patterns could reflect completely random activity, meaning that the spatiotemporal patterns of activity would result from the activation of a random and variable subset of neurons. On the other hand, spontaneous activity could reflect precisely repeating patterns, meaning that the spatiotemporal patterns of activity could be produced by stereotyped neural trajectories. Recent evidence in rat neocortical slices indicates that while there is some structure to spontaneous events, most of the structure is contained in the statistical properties of the events such as the mean amplitude and average duration. This suggests that there is significant variability in the underlying patterns (Johnson and Buonomano, 2007). To quantitatively examine event structure, the correlation coefficient between pairs of events from within a given cell (Figure 5A and 5B) was computed. This coefficient can be thought of as a measure of the "reproducibility" of spontaneous events, because it indicates the degree of similarity between a pair of events, and it is also inversely related to the variability of the spontaneous events. Correlation matrices were constructed in order to visualize the similarity between all pairs of events in a given cell (Figure 5C).

The mean correlation for both genotypes and across developmental stage was relatively low (R=0.29). There was no significant main factor effect on the mean correlation due to development or genotype. However, there was a significant interaction between them. Specifically, the mean correlation between pairs of events in control slices sharply increased from week 3 to week 4, while the same was not observed in mutants (Figure 5D; Two-way ANOVA, $F_{(2,38)}$ = 4.775, P<0.05, week 2, n=16, week 3, n=18, week 4, n = 10, where n is total the number of slices). Together these results indicate that while the average level of activity was the same in both wild-type and *FMR1-KO* slices and that there was a significant difference in the variability of the patterns later in development. In other words, the spontaneous events from the wild-type slices exhibited less variability than mutants during week 4.

DISCUSSION

Development of Spontaneous Dynamics Consistent with previous observations in rat slices (Johnson and Buonomano, 2007), the current results establish that mouse neocortical organotypic slices also exhibit significant developmental changes in spontaneous dynamics. Specifically, cultured neocortical slices from both wild-type and *FMR1-KO* animals showed a significant increase in the level of spontaneous activity during the first month *in vitro*. This increase was independent of the measurement used to quantify network dynamics; both general measurements of overall activity and measurements specific to Up states progressively increased from weeks 2 to 4. Thus, in the absence of external input, mouse neocortical networks will spontaneously generate recurrent activity that increases in intensity and frequency over development. Furthermore, because mice are important for transgenic experiments such as this one, these findings provide an important foundation for future combined electrophysiological and transgenic studies.

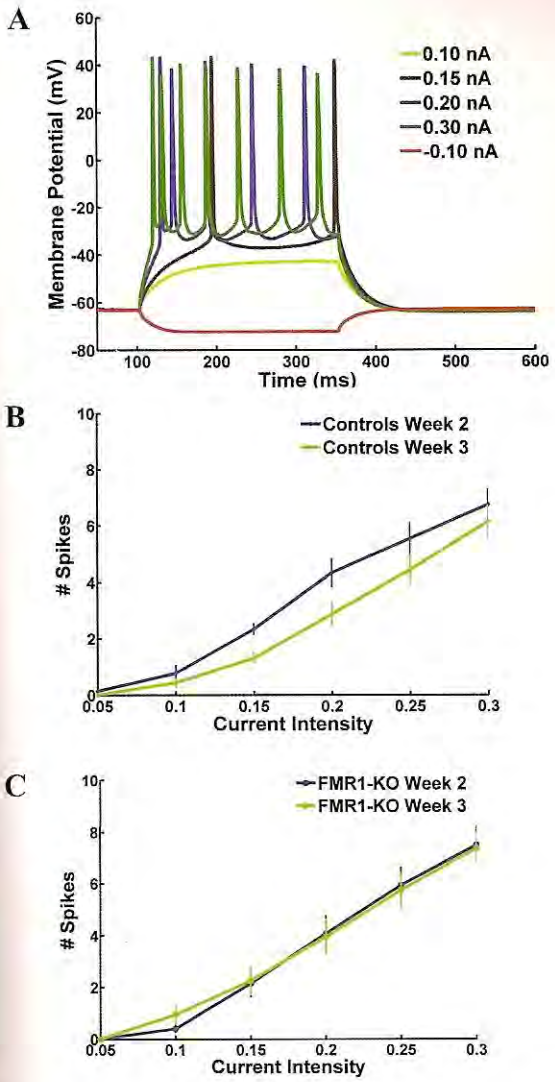

Figure 4. Wild-type neurons exhibited a developmental decrease in intrinsic excitability. **(A)** *Increasing depolarizing current steps elicit an increasing number of spikes. The mean number of spikes elicited is plotted as a function of intensity for week 2 and week 3 groups:* **(B)** *wild-type and* **(C)** *FMR1-KO cells. A three-way ANOVA with repeated measures on current intensity did not reveal any significant effect of genotype, developmental stage, or interaction between the two.*

Effects of *Fmr1* mutation on spontaneous dynamics Studies on the synaptic and cellular properties of FXS cannot fully explain the disease because behavior and cognition must ultimately rely on network dynamics (Belmonte and Bourgeron, 2006). The current study is among the first attempts to examine FXS from a network perspective by studying neural dynamics. However, our results did not reveal any robust genotype-specific differences in neural dynamics as measured by the amount of spontaneous activity. This negative result establishes that baseline spontaneous activity in somatosensory organotypic slices is unaltered by the mutation. This does not suggest that neural network dynamics are unaffected in FXS. Moreover, there are a number of potential reasons as to why there were no observable changes in the amount spontaneous activity. One explanation is that neocortical organotypic cultures are unsuitable as a model system of emergent network-level phenotypes associated with *Fmr1* mutation. Indeed, a study that showed how dendritic spine morphology and distribution are altered by the mutation in vivo failed to observe similar changes in neocortical organotypic slices (Nimchinsky et al., 2001). Thus, it is possible that some aspect of organotypic cultures may create an environment where the effects of the mutation are not fully expressed. If this is the case, then it is possible that studying neural dynamics at a higher level of complexity such as in an intact animal could reveal differences in network performance.

We did find that spontaneous events were on average more correlated in control slices during a later developmental stage. Specifically, during week 4, wild-type exhibited a sharp increase in the mean correlation between bouts of activity, which is an indicator of the similarity between the spontaneous events observed in a given cell. The late onset of this effect is consistent with the notion that alterations due to FMRP deficiency may appear in an age-dependent manner. One interpretation of this result is that the spatiotemporal patterns of activity within the network exhibit less variability (or are more consistent) in controls. Therefore, it is possible that information may not be as well preserved in the spatiotemporal patterns of activity of mutant networks. This observation motivates further examination of the behavior of cortical organotypic circuits lacking FMRP; moreover, it suggests that there may be yet unnoticed alterations of network dynamics in these circuits.

Homeostatic Plasticity The absence of robust differences between wild-type and *FMR1-KO* slices may be related to homeostatic plasticity. The term "homeostatic plasticity" refers to the mechanisms that are in place for a neuron to maintain normal function over a long period of time in the face of ongoing change in its internal and external environment. For example, homeostatic plasticity allows neurons and networks to compensate for decreases or increases in levels of activity. A large number of cellular and synaptic properties have been reported to undergo homeostatic plasticity, including intrinsic excitability (Turrigiano et al., 1998; Marder and Goaillard, 2006; Karmarkar and Buonomano, 2006; Pratt and Aizenmann, 2007). Furthermore, it has been proposed that homeostatic mechanisms present at the cellular level ultimately exist to keep average levels of neural activity within a range that is optimally suited for neural processing (Turrigiano and Nelson, 2004). It is thus possible that mutant networks have homeostatically regulated synaptic and cellular parameters in order maintain normal levels of network activity. In other words, mutant circuits may have achieved network behavior similar to controls through an alternative parameter-space solution. The observation that the intrinsic excitability of mutant neurons did not tend to decrease from week 2 to week 3 is consistent with this notion because it suggests that cellular properties were differentially tuned in the *FMR1-KO* slices.

If mutant networks are indeed arriving at an alternative "solution" in order to achieve normal levels of activity, then it may be possible to detect genotype-specific differences by

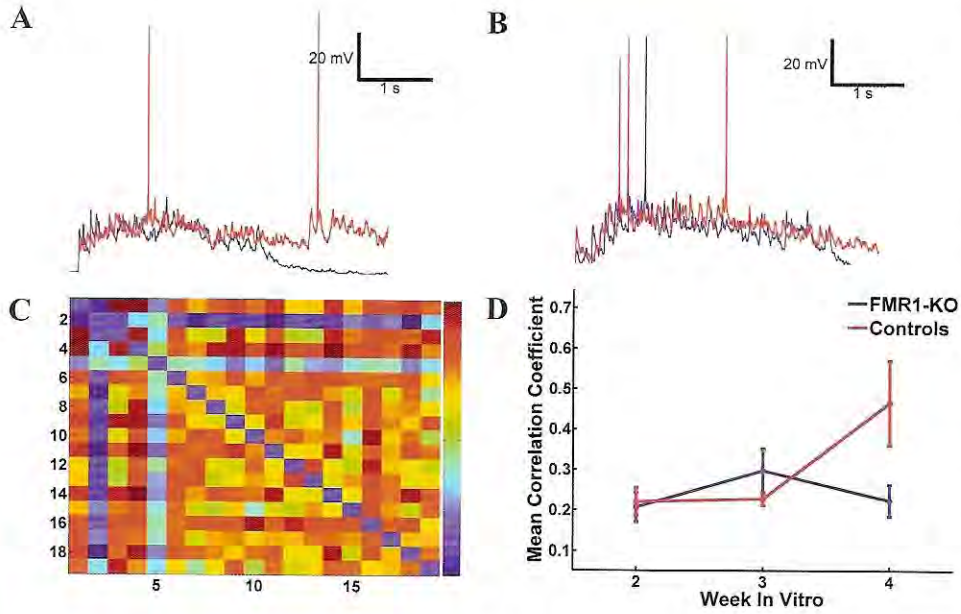

Figure 5. Wild-type neurons showed a significant increase in the mean correlation of spontaneous events during week 4. *Two 3-second segments containing two different superimposed spontaneous events from a wild-type cell that were not highly correlated (R= 0.10).* **(B)** *Two highly correlated events (R = 0.85) from a wild-type cell.* **(C)** *A clustered correlation matrix, which facilitates visualization of the correlation coefficients. The color of each box reflects the relative magnitude of correlation coefficients between different pairs of events within a recording and ranges from blue (low correlation) to red (high correlation).* **(D)** *The mean correlation coefficient of wild-type slices was larger than that of mutants during the fourth week in vitro (Two-way ANOVA, Week*Genotype interaction, $F_{(2,38)} = 4.775$, $P<0.05$; week 2, n=16, week 3, n=18, week 4, n = 10).*

manipulating network activity. To this end, we are currently investigating whether levels of spontaneous activity are altered when networks are "challenged" by chronically inhibiting network activity using pharmacological methods (Turrigiano et al., 1998; Karmarkar and Buonomano, 2006). Specifically, by blocking excitatory neurotransmission for long periods of time, it is believed that a number of cellular and synaptic forms of homeostatic plasticity will be engaged and that a compensatory elevation in network activity will result. The additional "challenge" from the pharmacological treatment may push mutant networks into a parameter-space domain that would result in levels of spontaneous activity different from controls. In other words, it is believed that the *FMR1-KO* networks will cope with the treatment differently than wild-types. If mutant networks are indeed arriving at an alternative "solution" in order to achieve normal levels of activity, then it may be possible to detect genotype-specific differences by manipulating network activity. To this end, we are currently investigating whether levels of spontaneous activity are altered when networks are "challenged" by chronically inhibiting network activity using pharmacological methods (Turrigiano et al., 1998; Karmarkar and Buonomano, 2006). Specifically, by blocking excitatory neurotransmission for long periods of time, it is believed that a number of cellular and synaptic forms of homeostatic plasticity will be engaged and that a compensatory elevation in network activity will result. The additional "challenge" from the pharmacological treatment may push mutant networks into a parameter-space domain that would result in levels of spontaneous activity different from controls. In other words, it is believed that the *FMR1-KO* networks will cope with the treatment differently than wild-type.

As suggested by Belmonte and Bourgeron (2006), it is crucial to study the FXS at the network level because of the inherent complexity of FXS as a developmental disorder. Here, we have attempted to bridge the gap between our understanding of the cellular and physiological phenotypes in the mouse model FXS and the emergence of network-level effects that are likely to exist. We have shown that there were no significant differences in the overall levels of spontaneous activity due to FMRP deficiency, but we did find that there may be differences in the structure or variability of the spontaneous dynamics later in development. These results have established a starting point for further investigations into the emergent effects of cellular and synaptic abnormalities on neural dynamics; and ultimately, how changes in neural dynamics translate to cognitive and behavioral deficits.

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REFERENCES

Bakker, C.E., et al. (1994) *Fmr1* knockout mice: a model to study fragile X mental retardation. Cell 78:23-33.

Belmonte, M.K., and Bourgeron, T. (2006) Fragile X syndrome and autism at the intersection of genetic and neural networks. Nat. Neurosci. 10:1221-1225.

Brennan, F.X., Albeck, D.S. and Paylor, R. (2006) *Fmr1* knockout mice are impaired in a leverpress escape/avoidance task. Genes, Brain and Behavior 5:467-471.

Comery, T.A., et al. (1997) Abnormal dendritic spines in fragile X knockout mice: maturation and pruning deficits. Proc. Natl. Acad. Sci. USA 94:5401-5404.

Cossart, R., Aronov, D., and Yuste, R. (2003) Attractor dynamics of network UP states in the neocortex. Nature 423:283–288.

Crawford, D.C., Acuna, J.M., and Sherman, S.L. (2001) *FMR1* and the Fragile

X syndrome: Human genome epidemiology review. Genet. Med. 3:359-371

Dolen, G., et al. (2007) Correction of Fragile X Syndrome in mice. Neuron 56:955-962.

Echevarria, D., and Albus, K. (2000) Activity-dependent development of spontaneous bioelectric activity in organotypic cultures of rat occipital cortex. Brain Res. Dev. Brain. Res. 123:151–164.

Galofre, E., and Ferrer, I. (1987) Development of dendritic spines in the Vth's layer pyramidal neurons of the rat's somatosensory cortex. A qualitative and quantitative study with the Golgi Method. J. Hirnforsch. 28:653-659.

Galvez, R., and Greenough, W.T. (2005) Sequence of abnormal dendritic spine development in primary somatosensory cortex of a mouse model of the fragile X mental retardation syndrome. Am. J. Med. Genet. 135:155-160.

Grossman, et al. (2006) Hippocampal pyramidal cells in adult *Fmr1* knockout mice exhibit an immature-appearing profile of dendritic spines. Brain Res. 1084:158-164.

Hinton, V.J., et al. (1991) Analysis of neocortex in three males with the fragile X syndrome. Am. J. Med. Genet. 41:289-294.

Horner, C.H. (1993) Plasticity of the dendritic spine. Prog. Neurobiol. 41:281-321.

Huber et al., (2002) Altered synaptic plasticity in a mouse model of fragile X mental retardation. Proc. Natl. Acad. Sci. USA 99:7749-7750.

Huber, K.M. (2006) The Fragile X-Cerebellum connection. Trends in Neurosci. 29:183-185.

Irwin, S.A., et al. (2001) Evidence for altered Fragile-X mental retardation protein expression in response to behavioral stimulation. Neurobiol. Learn. Mem. 74:87-93.

Irwin, S.A., et al. (2002) Dendritic spine and dendritic field characteristics of layer V pyramidal neurons in the visual cortex of fragile-X knockout mice. Amer. J. Med. Gen. 111:140-146.

Irwin, S.A., Galvez, R., and Greenough, W.T. (2000). Dendritic spine structural anomalies in Fragile-X mental retardation syndrome. Cer. Cortex. 10:1038-1044.

Jin, P., and Warren, S.T. (2000) Understanding the molecular basis of Fragile X Syndrome. Hum. Mol. Gen. 9:901-908.

Johnson, H.A., and Buonomano, D.V. (2007) Development and Plasticity of Spontaneous Activity and Up States in Cortical Organotypic Slices. J. Neurosci. 27:5915-5925.

Karmarkar, U.R., and Buonomano D.V. (2006) Different forms of homeostatic plasticity are engaged with distinct temporal profiles. Eur. J. Neurosci. 23:1575-1584.

Kerr, J.N., and Plenz, D. (2004) Action potential timing determines dendritic calcium during striatal up-states. J. Neurosci. 24:877– 885.

Koekkoek, S.K., et al. (2005) Deletion of *FMR1* in purkinje cells enhances parallel fiber LTD, enlarges spines, and attenuates cerebellar eyelid conditioning in Fragile X Syndrome. Neuron 47:339-352.

MacLean, J.N., et al. (2005) Internal dynamics determine the cortical response to thalamic stimulation. Neuron 48:811-823.

Marder, E., and Goaillard, J. (2006) Variability, compensation and homeostasis in neuron and network function. Nat. Rev. Neurosci. 7:563-575.

Massimini, M., et al. (2004) The sleep slow oscillation as a traveling wave. J. Neurosci. 24:6862– 6870.

Meredith, R.M., et al. (2007) Increased Threshold for Spike-Timing-Dependent Plasticity is Caused by Unreliable Calcium Signaling in Mice Lacking Fragile X Gene Fmr. Neuron 54:627-638.

Metherate, R., and Ashe J.H. (1993) Ionic flux contributions to neocortical slow waves and nucleus basalis-mediated activation: whole-cell recordings in vivo. J. Neurosci. 13:5312–5323.

Mineur, Y.S., et al. (2002) Behavioral and neuroanatomical characterization of the Fmr1 knockout mouse. Hippocampus 117:127-136.

Murphy, E.H., and Magness, R. (1984) Development of the rabbit visual cortex: a quantitative Golgi analysis. Exp. Brain. Res. 53:304-314.

Nimchinsky, E.A., Oberlander, A.M., and Svoboda, K. (2001) Abnormal development of dendritic spines in FMR1 knock-out mice. J. Neurosci. 21:5139–5146.

Peier, A.M., et al. (2000). (Over)correction of *FMR1* deficiency with YAC transgenics: behavioral and physical features. Hum. Mol. Genet. 9:1145-1159.

Petersen, C.C., et al. (2003). Interaction of sensory responses with spontaneous depolarization in layer 2/3 barrel cortex. Proc. Natl. Acad. Sci. USA. 100:13638 –13643.

Plenz, D., and Kitai, S.T. (1998) Up and down states in striatal medium spiny neurons simultaneously recorded with spontaneous activity in fast-spiking interneuron in cortex-striatum-substantia nigra organotypic cultures. J. Neurosci. 18:266–283.

Pratt, K.G., and Aizenman, C.D. (2007) Homeostatic Regulation of Intrinsic Excitability and Synaptic Transmission in a Developing Visual Circuit. J. Neurosci. 27:8268-8277.

Sanchez-Vives, M.V., and McCormick, D.A. (2000) Cellular and network mechanisms of rhythmic recurrent activity in neocortex. Nat. Neurosci. 3:1027–1034.

Schuz, A. (1986) Comparison between the dimensions of dendritic spines in the cerebral cortex of newborn and adult guinea pigs. J. Comp. Neurol. 244:277-285.

Stoppini, L., Buchs, P.A., and Muller, D. (1991) A simple method for organotypic cultures of nervous tissue. J. Neurosci. Methods 37:173–182.

Shu, Y., et al. (2003) Barrages of synaptic activity control the gain and sensitivity of cortical neurons. J. Neurosci. 23:10388 –10401.

Turrigiano, G.G., et al. (1998) Activity dependent scaling of quantal amplitude in neocortical neurons. Nature 391:892-896.

Turrigiano, G.G., and Nelson, S.B. (2004) Homeostatic plasticity in the developing nervous system. Nat. Neurosci. Rev. 5:97-107.

Ventura, R., et al. (2004). Object recognition impairment in *Fmr1* knockout mice is reversed by amphetamine: involvement of dopamine in the medial prefrontal cortex. Behav. Pharmacol. 15:433-442.

Verheij C., et al. (1993) Characterization and localization of the *FMR-1* gene product associated with fragile X syndrome. Nature 363:722–724.

Volgushev, M., et al. (2006) Precise long range synchronization of activity and silence in neocortical neurons during slow-wave oscillations [corrected]. J. Neurosci. 26:5665–5672.

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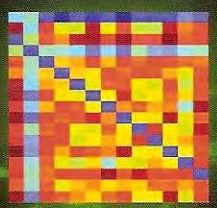

Research

Spontaneous Cortical Network Dynamics is Unaltered in a Mouse Model of Fragile X Syndrome

Review

On the Higg's Field and the Origin of Mass

Interview

At the Crack of Dawn: A Look at the Unmanned Space Mission to the Asteroids Ceres and Vesta