Impaired Dendritic Expression and Plasticity of h-Channels in the fmr1⁻/y Mouse Model of Fragile X Syndrome

Darrin H. Brager,¹,* Arvin R. Akhavan,¹ and Daniel Johnston¹
¹Center for Learning and Memory, The University of Texas at Austin, Austin, TX 78712, USA
*Correspondence: dbrager@mail.cim.utexas.edu
DOI 10.1016/j.celrep.2012.02.002

SUMMARY

Despite extensive research into both synaptic and morphological changes, surprisingly little is known about dendritic function in fragile X syndrome (FXS). We found that the dendritic input resistance of CA1 neurons was significantly lower in fmr1⁻/y versus wild-type mice. Consistent with elevated dendritic Ih, voltage sag, rebound, and resonance frequency were significantly higher and temporal summation was lower in the dendrites of fmr1⁻/y mice. Dendritic expression of the h-channel subunit HCN1, but not HCN2, was higher in the CA1 region of fmr1⁻/y mice. Interestingly, whereas mGluR-mediated persistent decreases in Ih occurred in both wild-type and fmr1⁻/y mice, persistent increases in Ih that occurred after LTP induction in wild-type mice were absent in fmr1⁻/y mice. Thus, chronic upregulation of dendritic Ih in conjunction with impairment of homeostatic h-channel plasticity represents a dendritic channelopathy in this model of mental retardation and may provide a mechanism for the cognitive impairment associated with FXS.

INTRODUCTION

Fragile X syndrome (FXS) is the most common form of inherited mental retardation with a variety of phenotypes including impaired cognitive ability, problems with working memory, autistic behavior, and increased incidence of epilepsy. FXS is characterized by the absence of the RNA-binding protein fragile X mental retardation protein (FMRP) (Bell et al., 1991). Mice lacking FMRP, such as the fmr1 knockout (fmr1⁻/⁻) mouse, perform poorly in hippocampal-dependent spatial learning tasks (Bakker et al., 1994). Interestingly, the performance of fmr1⁻/⁻ mice is similar to mice lacking proteins involved in long-term potentiation (LTP), a proposed cellular correlate of learning (e.g., Silva et al., 1992).

Several FMRP-target mRNAs are localized in neuronal dendrites and encode for voltage-gated channels or proteins involved in the regulation of channel expression and/or function (Darnell et al., 2011; Bassell and Warren, 2008). Incorrect gene expression, channel function, and/or loss of posttranslational channel regulation can have substantial effects on the control of cellular excitability. The physiological regulation of cellular excitability and dendritic integration through plasticity of voltage-gated ion channels is hypothesized to maintain the input-output characteristics of a neuron within normal limits (Turrigiano and Nelson, 2000). However, changes in dendritic channels in neurological disorders or disease can also alter the input-output relationship and result in a pathophysiological state (Bernard et al., 2004; Jung et al., 2007). One channel that undergoes activity-dependent plasticity and is altered in neurological disease is the h-channel (van Welie et al., 2004; Brager and Johnston, 2007; Fan et al., 2005; Jung et al., 2007; Shin et al., 2008).

h-channels are widely distributed in the central nervous system. In the hippocampus, h-channels are composed primarily of HCN1 and HCN2 subunits (Santoro et al., 2000). In CA1 pyramidal neurons the density of h-channels increases with distance from the soma along the apical dendrite (Magee, 1998). The high density of h-channels in the dendrites allows Ih (the current mediated by h-channels) to significantly contribute to the total membrane conductance and thereby exert strong influence over neuronal function in the subthreshold voltage range near rest. Subtle modifications in the physiology of h-channels can produce significant changes in synaptic integration and neuronal excitability (Santoro et al., 2000; Magee, 1999; Poolos et al., 2002).

Using whole-cell recording from CA1 pyramidal neuron apical dendrites, we show that dendritic physiology in the fmr1⁻/⁻ mouse is altered in a manner consistent with elevated dendritic Ih. Differences in hippocampal HCN1 protein expression were consistent with our physiological results. Interestingly, activity-dependent increases in Ih following LTP induction were absent in fmr1⁻/⁻ mice. Elevated dendritic expression of Ih coupled with impaired h-channel plasticity might thus indicate some of the cellular mechanisms underlying the cognitive impairments associated with FXS.

RESULTS

Dendritic Properties of fmr1⁻/⁻ Mice Are Significantly Different from Wild-Type

The intrinsic properties of hippocampal pyramidal neurons, established in part by the contribution of dendritic voltage-gated...
ion channels, strongly influence the manner in which synaptic inputs are combined and propagated from the dendrites to the soma. In particular a proportion of the total population of h-channels is active at rest and contributes to the resting membrane potential ($V_m$), input resistance ($R_N$), and membrane time constant ($\tau_m$) of CA1 neurons (Magee, 1998). We used somatic and dendritic whole-cell recording to test whether there were significant differences in the $V_m$, $R_N$, and $\tau_m$ of CA1 neurons between $fmr1^{-/-}$ and wild-type (WT) mice. We found that $V_m$ was significantly more depolarized, $R_N$ was lower, and $\tau_m$ was faster, in the dendrites versus the soma for both WT and $fmr1^{-/-}$ mice; consistent with a higher density of h-channels in the apical dendrite. There were no significant differences between WT and $fmr1^{-/-}$ mice using somatic recordings (see Figure S1 available online). When we compared the dendrites between $fmr1^{-/-}$ and WT mice, however, we found that the dendritic $V_m$ of $fmr1^{-/-}$ mice was significantly depolarized versus WT (knockout [ko], $-61 \pm 0.6$mV; WT, $-64 \pm 0.6$mV; $p < 0.05$). Furthermore, dendritic $R_N$ was significantly lower (Figures 1A–1C) and dendritic $\tau_m$ was significantly faster (WT, $17 \pm 2$ ms; ko, $8 \pm 1$ ms; $p < 0.05$) in $fmr1^{-/-}$ mice versus WT.

To test the hypothesis that the differences in dendritic properties were due to elevated $I_h$, we took advantage of several unique characteristics of h-channels and measured electrophysiological parameters that are sensitive to the amount of $I_h$ (Brager and Johnston, 2007). Voltage sag (sag) during step current injections can be due to the relatively slow activation/deactivation kinetics of $I_h$ (Magee, 1998). We found that dendritic sag from hyperpolarizing current steps was 38% higher in $fmr1^{-/-}$ mice versus WT (Figures 1D–1F). Rebound amplitude following a hyperpolarization is due in part to the slow closing of h-channels. The amount of rebound from a given steady-state hyperpolarization is increased with larger $I_h$ (Brager and Johnston, 2007). Dendritic rebound was 43% higher in $fmr1^{-/-}$ mice versus WT (Figures 1G–1I). The same kinetic properties that underlie sag and rebound allow $I_h$ to act as a resonator conductance, and differences in resonance frequency ($f_R$) reflect differences in $I_h$ (Hutcheon and Yarom, 2000; Narayanan and Johnston, 2007). Similar to sag and rebound, dendritic $f_R$ was 42% higher in $fmr1^{-/-}$ mice versus WT (Figures 1J–1L). $I_h$ reduces temporal summation by repolarizing the membrane during an excitatory postsynaptic potential (EPSP) (Magee, 1998). Consistent with elevated $I_h$, we found that dendritic temporal summation was significantly lower in $fmr1^{-/-}$ mice versus WT (Figures 1M–1O). When measured from the soma, we found no significant difference in sag, rebound, $f_R$, or summation between WT and $fmr1^{-/-}$ mice (Figure S1).

As a further test of the contribution of $I_h$ to the differences in dendritic properties, we compared WT and $fmr1^{-/-}$ dendritic recordings before and after application of the h-channel blocker ZD7288. Extracellular application of 20 $\mu$M ZD7288 significantly increased $R_N$ in both WT and $fmr1^{-/-}$ dendrites (Figure 1C). Furthermore, block of $I_h$ significantly decreased sag, rebound, and $f_R$ and increased temporal summation in both WT and $fmr1^{-/-}$ dendrites (Figures 1F, 1L, 1L, and 1O). After block of $I_h$ by ZD7288, dendritic properties were no longer significantly different between WT and $fmr1^{-/-}$ dendrites. These electrophysiological results suggest that dendritic, but not somatic, $I_h$ is significantly higher in $fmr1^{-/-}$ mice.

**Elevated HCN1 Levels in the CA1 Region of the $fmr1^{-/-}$ Mouse Hippocampus**

Our electrophysiological data support the hypothesis that $I_h$ is higher in the dendrites of CA1 neurons from $fmr1^{-/-}$ mice versus WT mice. We sought to further test this hypothesis by examining h-channel proteins using immunohistochemistry and western blotting. We used antibodies to label the h-channel subunits HCN1 and HCN2. As expected, the intensity of HCN1 and HCN2 labeling increased with distance from the cell body layer indicative of the h-channel gradient. Measuring the intensity along a line perpendicular to, but not including, the pyramidal cell body layer in CA1 allows for a comparison of the distribution of HCN1 and HCN2 staining between slices (Shin et al., 2008). We found that the intensity of the HCN1 fluorescence, but not HCN2, was higher in the distal dendrites of $fmr1^{-/-}$ mice versus WT mice (Figures 2A–2F). The difference in HCN1 staining between $fmr1^{-/-}$ and WT mice persisted when the HCN1 signal was normalized to dendritic marker MAP2 (Figure S2). We used western blot analysis to determine if there was higher HCN protein levels in the dendritic field of the CA1 region of the $fmr1^{-/-}$ mouse versus WT. We found that HCN1, but not HCN2, protein levels were significantly higher in homogenates from the dendritic region of CA1 in $fmr1^{-/-}$ versus WT (Figures 2G and 2H). These results suggest that the higher dendritic $I_h$ in $fmr1^{-/-}$ mice is due in part to an increase in the distal dendritic expression of the h-channel subunit HCN1.

**Intrinsic Plasticity Is Impaired in $fmr1^{-/-}$ Mice**

Homeostatic mechanisms that maintain neuronal output are essential for the stabilization of neural networks during classical Hebbian synaptic plasticity (Turrigiano, 2011). We previously demonstrated that activation of group I metabotropic glutamate receptors (mGluRs) results in long-term depression (LTD) and an accompanying decrease of $I_h$ in CA1 neurons (Brager and Johnston, 2007). In the hippocampus of $fmr1^{-/-}$ mice, group I mGluR-dependent LTD is enhanced, whereas NMDA receptor-dependent LTD is unaffected (Huber et al., 2002). To test whether mGluR-dependent h-channel plasticity is altered in $fmr1^{-/-}$ mice, we measured intrinsic properties before and after activation of group I mGluRs (100 $\mu$M DHPG for 10 min). Application of DHPG to either WT or $fmr1^{-/-}$ CA1 neurons produced a transient depolarization that recovered by 30 min after wash (Figure 3A). This transient activation of mGluRs decreased EPSP slope by 23% in WT and 40% in $fmr1^{-/-}$ mice 30 min after DHPG application (Figures 3B and 3C). In agreement with our previous observations in rat hippocampus (Brager and Johnston, 2007), we found that mGluR activation significantly increased $R_N$ by 85% in WT mice and by 83% in $fmr1^{-/-}$ mice (Figure 3D). Consistent with a decrease in $I_h$, $f_R$, rebound, and sag were significantly decreased after DHPG application in WT, and $f_R$ and rebound were significantly decreased in $fmr1^{-/-}$ mice (Figures 3E–3G). Based on these results, we conclude that mGluR-dependent plasticity of $I_h$ occurs in both WT and $fmr1^{-/-}$ mice.
Figure 1. h-Channel Dependent Dendritic Properties Are Significantly Different between WT and fmr1−/− Mice

(A) Diagram of recording locations and representative dendritic recordings from WT and fmr1−/− mice.

(B) Group data showing that dendritic RN was significantly lower in fmr1−/− mice (n = 6) versus WT (n = 6).

(C) Representative dendritic whole-cell recordings showing that there was more sag (arrow) in the dendrites of fmr1−/− mice versus WT.

(D) Group data showing that application of ZD significantly increased RN in both WT (n = 4) and fmr1−/− mice (n = 4).

(E) Group data showing that dendritic sag was significantly higher in fmr1−/− mice.

(F) Group data showing that application of ZD significantly decreased sag in both WT and fmr1−/− mice.

(G) Representative dendritic whole-cell recordings (left) and plot used to calculate rebound slope (right) from WT and fmr1−/− mice.

(H) Group data showing that dendritic rebound slope was significantly higher in fmr1−/− mice.

(I) Group data showing that application of ZD significantly decreased rebound in both WT and fmr1−/− mice.

(J) Representative voltage traces and ZAP used to determine fR (dashed line). Note the rightward shift in the fmr1−/− (red) versus the WT (black).

(K) Group data showing that dendritic fR was significantly higher in fmr1−/− mice.

(L) Group data showing that application of ZD significantly decreases fR in both WT and fmr1−/− mice.

(M) Representative traces used to measure temporal summation of fmr1−/− mice versus WT.

(N) Group data showing that dendritic temporal summation was significantly less in fmr1−/− mice.

(O) Group data showing that application of ZD significantly increased dendritic summation in both WT and fmr1−/− mice.

Group data are presented as mean ± SEM. *p < 0.05; **p < 0.01; ***p < 0.005 significantly different from WT. †p < 0.05 significantly different from baseline.
In CA1 neurons, theta-burst firing of action potentials paired with EPSPs (theta-burst pairing, TBP) results in an NMDA-dependent increase in Ih (Fan et al., 2005). To test whether the higher dendritic Ih in fmr1/C0/y mice occludes the TBP-dependent increases in Ih, we induced TBP-LTP in CA1 neurons from both WT and fmr1/C0/y mice. CA1 neurons from both WT and fmr1/C0/y mice displayed a significant increase in EPSP slope following TBP (Figures 4A and 4B). In agreement with previous studies (Fan et al., 2005; Narayanan and Johnston, 2007), TBP was accompanied by a significant decrease in R_N in WT mice (baseline, 82 ± 7 MΩ; TBP, 68 ± 6 MΩ; n = 5, p < 0.05) (Figures 4C and 4D). Interestingly, there was no significant change in R_N in fmr1/C0/y mice. Consistent with an increase in Ih, TBP significantly increased f_R, rebound, and sag in WT mice (Figures 4E–4G). In contrast, there was no significant change in f_R, rebound, or sag in fmr1/C0/y neurons after TBP. We thus conclude that the activity-dependent expression of Ih plasticity that normally occurs following TBP-LTP is absent in fmr1/C0/y mice.

DISCUSSION

Neurons from patients and animal models of FXS show significant morphological and synaptic alterations. Here, we show that a significant change to the intrinsic properties of the dendrites of CA1 neurons occurs in the fmr1/C0/y mouse model of FXS. Electrophysiological data support the hypothesis that these changes are due in part to a dendritic enhancement of the hyperpolarization-activated nonselective cationic current, Ih, in the distal dendrites. Furthermore, this elevation in Ih appears to be due to increased distal dendritic expression of the HCN1 subunit. Interestingly, activity-dependent increases in Ih are absent, whereas activity-dependent decreases in Ih remain. These data represent the first physiological description of a channelopathy involving dendritic h-channels associated with FXS.

**Impact on Dendritic Function**

Ih plays a complex role in controlling membrane excitability. What is the impact of elevated Ih in FXS on dendritic function? Elevated Ih will reduce dendritic excitability by decreasing R_N as well as reducing temporal and spatial summation of subthreshold EPSPs. Ih also constrains distal dendritic spikes

---

**Figure 2. Distal Dendritic HCN1 Expression in Area CA1 Is Higher in fmr1/C0/y Mice**

(A) Representative fluorescent images showing HCN1 staining from a WT (A1) and an fmr1/C0/y mouse section (Sub, subiculum; hf, hippocampal fissure; DG, dentate gyrus). Fluorescent intensity was measured along the dashed line.

(B) Representative fluorescent images showing HCN2 staining from a WT (B1) and an fmr1/C0/y (B2) mouse section.

(C and D) Region indicated by the box in (A1) and (A2). sp, stratum pyramidale; sr, stratum radiatum; slm, stratum lacunosum-moleculare.

(E) A plot of intensity as a function of location showing that distal dendritic HCN1 staining was higher in fmr1/C0/y mice versus WT mice.

(F) Intensity profile showing that there was no difference in HCN2 staining between fmr1/C0/y and WT mice.

(G) The total amount of HCN1 protein in the CA1 region of the fmr1/C0/y hippocampus was significantly higher than WT.

(H) Representative western blot from WT and fmr1/C0/y mice for HCN2 and tubulin. There was no significant difference in the total amount of HCN2 protein between fmr1/C0/y and WT hippocampus.

Group data are presented as mean ± SEM. **p < 0.01.
by increasing inactivation of both T- and N-type Ca$^{2+}$ channels (Tsay et al., 2007). The normal gradient of h-channels regulates the propagation of signals between the dendrites and the soma, and changes in the amount of dendritic $I_h$ will strongly influence somato-dendritic coupling (Magee, 1998, 1999; Pooilos et al., 2002). The increase in dendritic $I_h$ in FXS would decrease somato-dendritic coupling, reducing the likelihood of synaptic inputs eliciting an action potential at the soma.

Elevated $I_h$ will not only have inhibitory influences on dendritic excitability but also increase membrane excitability by depolarizing the membrane potential closer to the threshold for dendritic spike initiation and by augmenting the depolarizing rebound potential that occurs following a hyperpolarization. We found that rebound from hyperpolarization was higher in the dendrites of $fmr1^{-/-}$ mice. This increase in rebound either alone (Chen et al., 1999) or in concert with the activation of low-threshold voltage-dependent calcium channels could elicit a dendritic spike or dendritic plateau potential (Berger et al., 2003; Tsay et al., 2007). There is a high density of GABAergic synapses and GABAB receptors in the distal dendrites of CA1 neurons (Otmakhova and Lisman, 2004). It is, therefore, possible that in $fmr1^{-/-}$ mice the normal inhibitory input into the distal dendrites of CA1 neurons will in fact result in significant dendritic excitation.

These two opposing actions of elevated $I_h$ on cellular excitability will have the complicated impact of decreasing $R_N$. 

---

**Figure 3. mGluR-Dependent Plasticity Is Present in Both WT and $fmr1^{-/-}$ Mice**

(A) Time course of membrane potential change following a 10 min DHPG application.

(B) Representative traces from WT and $fmr1^{-/-}$ mice before and after DHPG application showing LTD of EPSPs and increased $R_N$.

(C) Group data showing the normalized decrease in EPSP slope 30 min after DHPG washout for both WT (n = 4) and $fmr1^{-/-}$ mice (n = 4). Inset shows expanded time scale of traces in (B).

(D) Group data showing that $R_N$ significantly increased after DHPG washout in both WT and $fmr1^{-/-}$ mice.

(E) Group data showing that $I_h$ significantly decreased after DHPG washout in both WT and $fmr1^{-/-}$ mice.

(F) Group data showing that rebound slope significantly decreased after DHPG washout in both WT and $fmr1^{-/-}$ mice.

(G) Group data showing that sag significantly decreased after DHPG washout in WT but not $fmr1^{-/-}$ mice.

Group data are presented as mean ± SEM. *p < 0.05; **p < 0.01.
temporal and spatial summation, and somato-dendritic coupling, making it harder for synaptic inputs to summate and elicit a somatic action potential, whereas also increasing the likelihood of local dendritic regenerative events. The exact impact of elevated dendritic Ih on information processing in FXS requires further investigation.

**Ih Plasticity in FXS**

Transient and persistent changes in Ih, which alters neuronal intrinsic excitability, occur during and after induction of synaptic plasticity (Brager and Johnston, 2007; Fan et al., 2005). This coordinated change in synaptic and intrinsic excitability is not completely understood, but one current hypothesis is that intrinsic plasticity can alleviate the inherent instability of synaptic plasticity. Following induction of LTP there is a persistent increase in Ih throughout the dendrites of CA1 neurons (Fan et al., 2005; Narayanan and Johnston, 2007). We found that although TBP-dependent LTP was not different between WT and fmr1−/− mice, Ih plasticity was absent in the latter. One possibility is that changes in the intracellular signaling molecules occlude any increase in Ih after TBP-LTP. For example, CAMKII levels are elevated in fmr1−/− mice (Zalfa et al., 2003), and the activation of CAMKII is required for TBP-dependent Ih plasticity (Fan et al., 2005). It is possible that the already higher levels of CAMKII lead to elevated dendritic Ih and that TBP-LTP is unable to further increase in Ih in fmr1−/− mice. The concomitant increase in Ih that normally occurs with LTP is believed to be one mechanism by which homeostatic regulation of neural network stability can be achieved (Narayanan and Johnston, 2007). Our results suggest that despite the appearance of normal

![Figure 4. TBP-Dependent Ih Plasticity Is Absent in fmr1−/− Mice](image)
synaptic potentiation, the deficit in intrinsic plasticity may lead to inherent network destabilization. The combined plasticity of \(I_h\) and synaptic strength results in preferential tuning of the dendrites to selected synaptic inputs. This form of homeostatic plasticity is believed to be critical for information storage and processing by neuronal networks (Turrigiano and Nelson, 2000). A loss of normal h-channel plasticity, with or without accompanying deficits in synaptic plasticity, could have profound effects on neuronal signal processing.

**Potential Mechanisms**

How does the absence of FMRP lead to an increase in dendritic \(I_h\) and HCN1 protein? FMRP may bind to and regulate the translation of HCN1 mRNA. Classically, FMRP acts as a translational repressor (but see Gross et al., 2011). If HCN1 mRNA is a target of FMRP, then the loss of FMRP will directly result in elevated HCN1 expression. Indeed, our data show that expression of HCN1 protein is higher in the CA1 region of the fmr1\(^{-/-}\) mouse hippocampus. FMRP may also interact directly with ion channel proteins. In the olfactory bulb and brain stem, FMRP can bind to and regulate the gating of the sodium-activated potassium channel, Slack (Brown et al., 2010). FMRP may bind directly to h-channel proteins or regulate the binding between HCN subunits and auxiliary proteins such as tetratri-cotepide repeat-containing Rab8b-interacting protein (TRIP8b) (Santoro et al., 2004). The interaction between TRIP8b and HCN subunits regulates both the gating and surface expression of h-channels.

The elevated \(I_h\) may also be a compensatory change due to the loss of FMRP-dependent regulation of another protein. FMRP is necessary for normal synapse elimination during development (Pfeiffer and Huber, 2007). One possibility is that excessive synaptic excitation may lead to a homeostatic increase in \(I_h\). Uppregulation of hippocampal h-channels can occur following high levels of AMPA receptor activation (van Welle et al., 2004). Increased excitatory input from CA3 Schaeffer collaterals due to elevated neurotransmitter release (Deng et al., 2011) could lead to excess CA1 AMPA receptor activation in fmr1\(^{-/-}\) mice. Additionally, synaptic inputs from layer III of the entorhinal cortex (EC) onto the distal dendrites of CA1 neurons regulate the normal h-channel gradient (Shin and Chetkovich, 2007). Increased excitatory input from the EC, due to improper synaptic pruning, could lead to a compensatory increase in \(I_h\). Alternatively, elevated \(I_h\) may be in response to loss of another ion channel. Invertebrate studies suggest that there may be homeostatic coregulation of \(I_h\) and \(I_{h,2}\) including functional reciprocity (Harris-Warrick et al., 1995). There are conflicting reports about Kv4.2 expression, the putative subunit of \(I_h\), in the fmr1\(^{-/-}\) hippocampus. One study reported that FMRP promotes translation of Kv4.2 mRNA and that Kv4.2 protein levels are reduced in fmr1\(^{-/-}\) mice (Gross et al., 2011). More recently, a second study reported that FMRP acts as a translational repressor and that Kv4.2 expression is higher in the fmr1\(^{-/-}\) hippocampus compared to WT (Lee et al., 2011). It is possible that changes in Kv4.2 expression results in a compensatory increase in \(I_h\), in part by increased HCN1 expression. We are currently investigating these hypotheses as potential mechanisms underlying the change in dendritic function in FXS.

**EXPERIMENTAL PROCEDURES**

**Acute Hippocampal Slices and Electrophysiology**

All experiments were conducted in accordance with the University’s IACUC. Hippocampal slices (300 \(\mu\)m) were prepared from 2- to 3-month-old male WT and fmr1\(^{-/-}\) mice (C57BL/6) as described previously (Fan et al., 2005) (see Extended Experimental Procedures). Slices were placed in a holding chamber filled with ACSF containing (125 NaCl, 2.5 KCl, 1.25 NaHPO\(_4\), 25 NaHCO\(_3\), 2 CaCl\(_2\), 2 MgCl\(_2\), and 12.5 dextrose, bubbled continuously with 95% O\(_2\)/5% CO\(_2\)) warmed to 35°C for 30 min and then kept at RT. Hippocampal slices were placed into a submerged recording chamber and perfused with ACSF (as above except 3.0 KCl and 1.0 MgCl\(_2\)) at 31°C–33°C and viewed with a Zeiss Axioskop. For physiological measurements (Figure 1), 10 \(\mu\)M DNOX, 50 \(\mu\)M AP5, 2 \(\mu\)M SR95531, and 5 \(\mu\)M CGP52432 were present throughout. For mGlurS experiments (Figure 3), AP5 and 10 \(\mu\)M MK-801 were included in the ACSF (Bragger and Johnston, 2007). For LTP experiments (Figure 4), GABAA- and GABA\(_B\)-mediated IPSPs were blocked by SR95531 and CGP55845, and area CA3 was removed. All drugs were made from stock solutions in water or DMSO (final concentration of DMSO \(\leq 0.1\%\)). Drugs were obtained from Ascent Scientific (Bristol, UK).

Pipettes (4–6 M\(\Omega\)) were pulled from borosilicate glass and filled with solution containing 120 mM K-glucanate, 20 mM KCl, 10 mM HEPES, 4 mM NaCl, 4 mM MgATP, 0.3 mM Na-GTP, and 7 mM KCl-phosphocreatine (pH 7.3). Series resistance (\(R_s\)) was monitored throughout the recording, and experiments with \(R_s\) >30 M\(\Omega\) were discarded. EPSPs of 4–6 mV were elicited using tungsten-stimulating electrodes placed <20 \(\mu\)m from the apical dendrite ~100–150 \(\mu\)m from the soma. Action potentials were elicited by current injection into the soma (1–2 nA for 2 ms).

Data were sampled at 20 kHz, filtered at 3 kHz, and digitized by an ITC-18 interface. EPSPs were quantified by linear fit to the initial slope. H-sensitive parameters were measured and analyzed as described in the Extended Experimental Procedures.

**Immunohistochemistry**

Two to 3-month-old male fmr1\(^{-/-}\) and WT mice were anesthetized and perfused with 4% paraformaldehyde (PFA) at 4°C. The brain was removed, post-fixed in PFA for 2 hr at RT, and placed in 30% sucrose in PBS overnight at 4°C. A total of 50 \(\mu\)m near-horizontal thin sections containing the hippocampus were cut on a freezing microtome. Slices were washed and then incubated in a blocking buffer solution containing 5% Normal Goat Serum, 0.1% Triton X-100, and PBS (PBS-T), and then incubated in primary antibody (1:2,000 concentration in PBS-T) against either HCN1 or HCN2 overnight at 4°C. Sections were then washed with PBS then placed in PBS-T containing a secondary antibody overnight at 4°C. The slices were then washed in PBS and mounted on microscope slides. The sections were visualized on a fluorescence microscope; images were captured by CCD camera, and analyzed using ImageJ (National Institutes of Health). The intensity of staining was quantified by measuring the pixel intensity along a line perpendicular to the cell body axis in CA1 (Shin et al., 2008).

**Western Blotting**

Area CA1 was isolated from three to five individual hippocampal slices frozen on dry ice. Tissue was homogenized in lysis buffer containing protease inhibitors. Homogenates were centrifuged at 80 \(\times\) 10\(^3\) for 10 min, resolved using SDS-PAGE, and transferred to nitrocellulose membranes. Membranes were
blocked in blocking buffer (LI-COR) overnight at 4°C or for 1 hr at RT. Membranes were incubated for 2 hr at RT or overnight at 4°C in primary antibodies diluted in blocking buffer (gp z-HCN1, 1:2,000; gp z-HCN2, 1:2,000; mouse z-tubulin 1:20,000). Membranes were washed in PBS plus 0.05% Tween 20 (PBS+) and then incubated in species-appropriate secondary in PBS+ for 45 min at RT. Membranes were then washed in PBS+ followed by PBS and visualized on an Odyssey imaging system (LI-COR). Blots were analyzed using ImageJ. HCN protein levels were normalized to tubulin levels as a control for protein loading. Homogenates from four fmr1−/− and four WT mice were used for four separate western blots.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Extended Experimental Procedures and two figures and can be found with this article online at doi:10.1016/j.celrep.2012.02.002.

ACKNOWLEDGMENTS

The authors thank Dr. Kimberly Huber for providing the founder fmr1−/− mice; Dr. Dane Chetkovich for the HCN1 and HCN2 antibodies; and Drs. Kimberly Raab-Graham, Kelly Dougherty, Nikolai Dembrow, Raymond Chitwood, and Dr. Kimberly Chetkovich for the HCN1 and HCN2 antibodies; and Drs. Kimberly Raab-Graham, Kelly Dougherty, Nikolai Dembrow, Raymond Chitwood, and Jennifer Siegel for helpful discussions and comments on the manuscript. This work was supported by grants from FRAXA (to D.H.B.), The University of Texas Austin Undergraduate Research Fellowship (to A.R.A.), and the National Institutes of Health Grant MH048432 (to D.J.).

Received: June 25, 2011
Revised: December 27, 2011
Accepted: February 1, 2012
Published online: March 15, 2012

REFERENCES

Bakker, C.E., Verheij, C., Willemsen, R., van der Helm, R., Oerlemans, F., Ver- mey, M., Bygrave, A., Hoogeveen, A.T., and Oostra, B.A. (1994). Fmr1 knockout mice: A model to study fragile X mental retardation. Cell 78, 23–33.

Bassell, G.J., and Warren, S.T. (2008). Fragile X syndrome: loss of local mRNA translation of h-channels reduces the excitability of pyramidal neuron dendrites. Neuron 59, 630–642.

Magee, J.C. (1998). Dendritic hyperpolarization-activated currents modify the integrative properties of hippocampal CA1 pyramidal neurons. J. Neurosci. 18, 7613–7624.

Magee, J.C. (1999). Dendritic Ih normalizes temporal summation in hippocampal CA1 neurons. Nat. Neurosci. 2, 508–514.

Narayanan, R., and Johnston, D. (2007). Long-term potentiation in rat hippocampal neurons is accompanied by spatially widespread changes in intrinsic oscillatory dynamics and excitability. Neuro 56, 1061–1075.

Silva, A.J., Stevens, C.F., Tonegawa, S., and Wang, Y. (1992). Deficient hippocampal long-term potentiation in alpha-calcium-calmodulin kinase II mutant mice. Science 257, 201–206.
Tsay, D., Dudman, J.T., and Siegelbaum, S.A. (2007). HCN1 channels constrain synaptically evoked Ca2+ spikes in distal dendrites of CA1 pyramidal neurons. Neuron 56, 1076–1089.

Turrigiano, G. (2011). Too many cooks? Intrinsic and synaptic homeostatic mechanisms in cortical circuit refinement. Annu. Rev. Neurosci. 34, 89–103.

Turrigiano, G.G., and Nelson, S.B. (2000). Hebb and homeostasis in neuronal plasticity. Curr. Opin. Neurobiol. 10, 358–364.

van Welie, I., van Hooft, J.A., and Wadman, W.J. (2004). Homeostatic scaling of neuronal excitability by synaptic modulation of somatic hyperpolarization-activated Ih channels. Proc. Natl. Acad. Sci. USA 101, 5123–5128.

Zalfa, F., Giorgi, M., Primerano, B., Moro, A., Di Penta, A., Reis, S., Oostra, B., and Bagni, C. (2003). The fragile X syndrome protein FMRP associates with BC1 RNA and regulates the translation of specific mRNAs at synapses. Cell 112, 317–327.