Altered A-Type Potassium Channel Function Impairs Dendritic Spike Initiation and Temporoammonic Long-Term Potentiation in Fragile X Syndrome

Gregory J. Ordemann, Christopher J. Apgar, Raymond A. Chitwood, and Darrin H. Brager

Department of Neuroscience, Institute for Neuroscience, Center for Learning and Memory, University of Texas at Austin, Austin, Texas 78712

Fragile X syndrome (FXS) is the leading monogenetic cause of cognitive impairment and autism spectrum disorder. Area CA1 of the hippocampus receives current information about the external world from the entorhinal cortex via the temporoammonic (TA) pathway. Given its role in learning and memory, it is surprising that little is known about TA long-term potentiation (TA-LTP) in FXS. We found that TA-LTP was impaired in male *fmr1* KO mice. Although there were no significant differences in basal synaptic transmission, synaptically evoked dendritic calcium signals were smaller in KO neurons. Using dendritic recording, we found no difference in complex spikes or pharmacologically isolated Ca^{2+} spikes; however, the threshold for fast, Na^+ -dependent dendritic spikes was depolarized in *fmr1* KO mice. Cell-attached patch-clamp recordings found no difference in Na^+ channels between wild-type and *fmr1* KO CA1 dendrites. Dendritic spike threshold and TA-LTP were restored by blocking A-type K^+ channels with either 150 μM Ba^{2+} or the more specific toxin AmmTx3. The impairment of TA-LTP shown here, coupled with previously described enhanced Schaffer collateral LTP, may contribute to spatial memory alterations in FXS. Furthermore, as both of these LTP phenotypes are attributed to changes in A-type K^+ channels in FXS, our findings provide a potential therapeutic target to treat cognitive impairments in FXS.

Key words: CA1; calcium; dendrite; FMRP; LTP

Significance Statement

Alterations in synaptic function and plasticity are likely contributors to learning and memory impairments in many neurologic disorders. Fragile X syndrome is marked by dysfunctional learning and memory and changes in synaptic structure and function. This study shows a lack of LTP at temporoammonic synapses in CA1 neurons associated with biophysical differences in A-type K^+ channels in *fmr1* KO CA1 neurons. Our results, along with previous findings on A-type K^+ channel effects on Schaffer collateral LTP, reveal differential effects of a single ion channelopathy on LTP at the two major excitatory pathways of CA1 pyramidal neurons. These findings expand our understanding of memory deficits in FXS and provide a potential therapeutic target for the treatment of memory dysfunction in FXS.

Introduction

Fragile X syndrome (FXS), caused by transcriptional silencing of the *fmr1* gene and loss of the Fragile X mental retardation

protein (FMRP; Martin and Bell, 1943; Lubs, 1969; Verkerk et al., 1991; Tassone et al., 2000), is the leading monogenetic cause of autism and intellectual disability, affecting ~1 in 4000 males and 1 in 6000 females (Martin and Bell, 1943; Brown et al., 1982; Kemper et al., 1988; Turner et al., 1996; Rogers et al., 2001). FMRP controls many neuronal proteins, including those involved in synaptic structure, function, and plasticity, through translational regulation of target mRNAs (Bassel and Warren, 2008; Darnell et al., 2011) and direct protein–protein interactions (Ramos et al., 2006; Brown et al., 2010; Deng et al., 2013, 2019; Brandalise et al., 2020). Given its high level of FMRP expression, well-defined circuitry, and relevance to learning and memory processes, the hippocampus has been critical to understanding synaptic changes in FXS (Scoville and Milner, 1957; Squire, 1992; Ludwig et al., 2014). The Schaffer collateral CA3 to CA1 synapse has been extensively studied in FXS (Huber et al., 2002; Shang et al., 2009; Brager et al., 2012; Routh et al., 2013; Bostrom et al.,

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R.A. Chitwood's present address: Department of Neuroscience, Baylor College of Medicine, Houston, Texas 77030.

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Correspondence should be addressed to Darrin H. Brager at dbrager@mail.clm.utexas.edu.

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2015; Toft et al., 2016; Wang et al., 2016). By contrast, few studies have investigated the temporoammonic (TA) entorhinal cortex to CA1 synapses. Wahlstrom-Helgren and Klyachko, 2015 found no difference in TA synaptic transmission using somatic whole-cell recording; whereas Booker et al. (2020), found that TA synaptic transmission was reduced in *fmr1* knock-out (KO) mice using extracellular field potential recording. Neither of these studies, however, investigated TA long-term potentiation (LTP). Given the lack of LTP studies and the critical involvement of the TA pathway in the consolidation of long-term memory (Remondes and Schuman, 2004) and its necessity for the generation of hippocampal CA1 place fields (Bittner et al., 2017), we asked whether TA-LTP is altered in FXS.

CA1 pyramidal neuron dendrites express Na^+ , K^+ , Ca^{2+} , and h-channels, which play crucial roles in dendritic integration and the induction of LTP. We previously showed that the functional expression of dendritic h-channels (I_h) is higher in *fmr1* KO CA1 neurons (Brager et al., 2012; Brandalise et al., 2020). We also demonstrated that the current carried by dendritic A-type K^+ channels (I_{KA}) is reduced in *fmr1* KO CA1 neurons (Routh et al., 2013). These changes alter the local integrative properties and increase the backpropagation of action potentials, respectively. Although the effect of these changes in dendritic h-channels and I_{KA} on Schaffer collateral LTP were previously described (Brager et al., 2012; Routh et al., 2013), the impact on TA-LTP remains unknown.

Using somatic and dendritic recording, we found that TA-LTP following theta-burst stimulation was impaired in *fmr1* KO mice. The lack of LTP was not because of the higher expression of h-channels as a block of I_h with ZD7288 did not rescue LTP. Two photon imaging during bursts of TA stimulation revealed that dendritic Ca^{2+} signals were smaller in *fmr1* KO neurons. Although complex and pharmacologically isolated Ca^{2+} spikes recorded in the dendrites were not different, the threshold for fast dendritic spikes (dspikes) was more depolarized in *fmr1* KO CA1 pyramidal neurons. Application of extracellular Ba^{2+} or AmmTx3 to block I_{KA} rescued dspike threshold and TA-LTP in *fmr1* KO CA1 pyramidal neurons, implicating A-type K^+ channels.

Materials and Methods

Animals. The University of Texas at Austin Institutional Animal Care and Use Committee approved all animal procedures. Male wild-type and *fmr1* KO C57/B6 mice from 8 to 16 weeks old were used for all experiments. *fmr1* KO male and homozygous female *fmr1* KO mice were paired to produce litters of *fmr1* KO animals. Mice were weaned at postnatal day 20. Animals were housed in single-sex groups at room temperature with *ad libitum* access to food and water and set on a reverse 12 h light cycle in the University of Texas at Austin vivarium located in the Norman Hackerman Building.

Preparation of acute brain slices. Mice were anesthetized with acute isoflurane exposure followed by injection of a ketamine/xylazine cocktail (100/10 mg/kg i.p.). Mice were then perfused through the heart with ice-cold saline consisting of the following (in mM): 2.5 KCl, 1.25 NaH_2PO_4 , 25 NaHCO_3 , 0.5 CaCl_2 , 7 MgCl_2 , 7 dextrose, 205 sucrose, 1.3 ascorbate, and 3 Na^+ pyruvate (bubbled with 95% O_2 /5% CO_2 to maintain pH at ~ 7.4). The brain was removed, trimmed, and sectioned into 300- μm -thick transverse slices of the middle hippocampus using a vibrating tissue slicer (Vibratome 3000, Vibratome; Brager et al., 2012; Brandalise et al., 2020). Slices were held for 30 min at 35°C in a chamber filled with artificial cerebral spinal fluid (aCSF) consisting of the following (in mM): 125 NaCl, 2.5 KCl, 1.25 NaH_2PO_4 , 25 NaHCO_3 , 2 CaCl_2 , 2 MgCl_2 , 10 dextrose, and 3 Na^+ pyruvate (bubbled with 95% O_2 /5% CO_2) and then held at room temperature until the time of recording.

Electrophysiology. Slices were submerged in a heated (32–34°C) recording chamber and continually perfused (1–2 ml/min) with bubbled aCSF containing the following (in mM): 125 NaCl, 3.0 KCl, 1.25 NaH_2PO_4 , 25 NaHCO_3 , 2 CaCl_2 , 1 MgCl_2 , 10 dextrose, 3 Na^+ pyruvate, 0.005 CGP 55845, and 0.002 gabazine. To reduce recurrent excitation, a cut was made between area CA3 and area CA1. CA1 pyramidal neuron dendrites or somata were visually identified using differential interference contrast or DAPI contrast optics.

Whole-cell current-clamp recordings. Patch pipettes (4–8 M Ω somatic, 7–11 M Ω dendritic) were pulled from borosilicate glass and filled with the following (in mM): 120 K-Gluconate, 16 KCl, 10 HEPES, 8 NaCl, 7 K_2 phosphocreatine, 0.3 Na-GTP, 4 Mg -ATP (pH 7.3, with KOH). Neurobiotin (2%; Vector Laboratories) was included in the internal recording solution to determine the recording location during *post hoc* morphologic reconstruction. Neurons that had a significant portion of the oblique or apical dendrites cut were excluded from analysis. In some cases, Alexa Fluor 594 was used to provide real time feedback of dendritic morphology.

Data were acquired using a Dagan BVC-700A amplifier and AxoGraph X or custom data acquisition software written using Igor Pro (WaveMetrics). Data were sampled at 10–50 kHz, filtered at 3–5 kHz, and digitized using an ITC-18 interface (InstruTECH). Pipette capacitance and series resistance were monitored and adjusted throughout each recording. Series resistance was monitored throughout each experiment, and the experiment was discarded if series resistance exceeded 30 M Ω (50 M Ω for dendritic recordings). Experiments in which the resting membrane potential was more depolarized than -50 mV were discarded. The liquid junction potential was estimated to be 14.3 mV (Patcher's Power Tools Igor Pro) and was not corrected.

Extracellular stimulation was performed using bipolar sharp tungsten electrodes (5 M Ω , 8° taper; A-M Systems) connected to an NL800A current stimulus isolator (Digitimer). The temporoammonic inputs were targeted by visually locating the axon fibers in the stratum lacunosum moleculare (SLM) region (≥ 250 μm from CA1 stratum pyramidale) and lowering the tungsten electrode until the tip was ~ 10 μm below the surface of the tissue. Stimulation intensity was increased until reliable EPSP (1–2 mV for somatic recordings and 2–4 mV for dendritic recordings) was elicited. To isolate NMDAR-dependent EPSPs (see Fig. 4) MgCl_2 was removed from the extracellular aCSF and 20 μM DNQX added to block AMPA receptors.

Induction of long-term potentiation. Long-term potentiation was performed using theta burst stimulation (TBS) as previously described (Tsay et al., 2007). Baseline EPSPs were stimulated at 0.067 Hz for 5 min. LTP was induced using TBS with bursts of 10 stimuli at 100 Hz, performed in five trains at 5 Hz, and each set of TBS was repeated four times at 20 s intervals. EPSPs were then recorded for ~ 30 min post TBS. The change in EPSP slope was plotted normalized to the baseline period.

Analysis of current-clamp data. Data were analyzed using AxoGraph X or custom analysis software written in Igor Pro. EPSP summation was quantified as the ratio of the amplitude of the fifth EPSP to the amplitude of the first EPSP. Paired-pulse ratio was calculated as the slope of the second EPSP divided by the slope of the first EPSP. Input-output was measured by increasing the magnitude of stimulus and measuring the slope of the resulting EPSP. For a given input-output experiment (see Figs. 1, 4, 5) the same stimulation electrode was used throughout to reduce variability within the experiment (But note that stimulation amplitudes differed across the experiments.).

Complex spikes were elicited by delivering square current injections (50–450 pA for 1 s). The width of a complex spike was calculated differentially depending on the number of Ca^{2+} -dependent events following the initial fast spike. For one slow spike, the width was calculated as the half-width from the initiation point of the spike to the peak of the event. When two or three Ca^{2+} -dependent spikes occurred, the width was calculated using methods for the measurement of dendritic Ca^{2+} plateau width modified from Takahashi and Magee (2009). For these events, width was taken as the halfway point between the initiation point of the first slow event and the lowest trough between events (see Fig. 6B).

Dendritic Ca^{2+} spikes were isolated with the addition of 0.5 μM TTX and 50 μM 4-AP. Spikes were generated by injecting square current

Figure 1. TA-LTP is impaired in *fmr1* KO CA1 pyramidal neurons. **A**, Diagram of recording configuration for somatic EPSP measurements. **B**, EPSP slope is increased 30 min after TBS in wild-type but not *fmr1* KO CA1 neurons; baseline (mean of 5 min before TBS), post-TBS (mean of 5 min at the end of recording; wild type: Wilcoxon, $W = 28$, $p = 0.02$, $\eta^2 = 0.79$; *fmr1* KO: baseline, 0.23 ± 0.04 , post-TBS, 0.24 ± 0.05 mV/ms, Wilcoxon, $W = -3$, $p = 0.92$). **C**, Normalized change in EPSP slope for wild-type and *fmr1* KO CA1 neurons. Inset, Representative EPSP traces from baseline (a) and post-TBS (b). **D**, Graph of area under the curve during TBS (Mann–Whitney, $U = 13$, $p = 0.06$). **E**, Input output of TA inputs measuring EPSP slope (two-way RM ANOVA, $F_{(1,7)} = 0.65$, $p = 0.45$). Inset, representative experiments from wild-type and *fmr1* KO neurons. **F**, Paired pulse ratio as a function of interstimulus interval (ISI; two-way RM ANOVA, $F_{(1,9)} = 4.23$, $p = 0.07$). Inset, Representative 50 ms ISI traces. **G**, Temporal summation of TA EPSPs as a function of frequency (two-way RM ANOVA, $F_{(1,9)} = 0.55$, $p = 0.48$). Inset, Representative 50 Hz traces.

pulses 1000 ms long and varying between 50 and 700 pA with 50 pA intervals. Because Ca^{2+} -dependent spikes have a much slower time course compared with Na^{+} -dependent spikes, measurements that used the second derivative of the voltage response (Gasparini et al., 2004) are not reliable. The threshold of somatically recorded action potentials occurs at $\sim 8\%$ of the maximum dV/dt . We therefore estimated the Ca^{2+} spike threshold using the time when dV/dt was 8% of maximum. Amplitude was measured as the difference from baseline membrane potential to the peak of the first Ca^{2+} spike.

Dendritic Na^{+} spike experiments were performed on dendritic recordings between 200 and 300 μm from the soma. Spikes were generated by injecting a series of double exponential currents ($\tau_1 = 0.1$ ms $\tau_2 = 2$ ms) ranging in amplitude between 500 pA and 5000 pA at 100 or 500 pA intervals. The threshold for dspikes was calculated as 20% of the second peak of the second derivative of the voltage response (Gasparini et al., 2004).

Input resistance (R_N) was calculated from the linear portion of the current–voltage relationship generated in response to a family of current injections (-50 to $+50$ pA, 10 pA steps).

2-Photon Ca^{2+} imaging. Ca^{2+} imaging experiments were performed on a Prairie Ultima Two-Photon Imaging system (Bruker) arranged for *in vitro* patch-clamp recording. An ultra-fast, pulsed laser beam (Mai Tai, Spectra-Physics) was used at 920 nm for imaging. Recording pipettes were filled with Oregon Green BAPTA-1 (OGB-1; 100 μM ; Invitrogen) and Alexa Fluor 594 (40 μM ; Invitrogen). Line scans across the distal dendrites were performed at 500 Hz with a dwell time of 4 μs for between 400 and 1200 ms. Imaging location was chosen ~ 50 μm more proximal to the soma in reference to the extracellular stimulating electrode.

Analysis of line scans were performed using ImageJ (National Institutes of Health) by placing a line through the fluorescent signal and plotting the profile before converting to a number array (Schneider et al., 2012). Changes in Ca^{2+} were quantified using $\Delta F/F$, where F is the

baseline fluorescence before stimulation and ΔF is the change in fluorescence during neuronal stimulation. Ca^{2+} signal traces were smoothed using a Savitsky-Golay function (Igor Pro, WaveMetrics).

Voltage-clamp recordings of Na^{+} current. Cell attached voltage-clamp recordings were performed using an AxoPatch 200B Amplifier (Molecular Devices). Data were acquired at 50 kHz and filtered at 2 kHz and then digitized using an ITC-18 interface (InstruTECH) and recorded using custom Igor Pro software (Igor Pro version 7, WaveMetrics). Pipette solution for cell attached Na^{+} channel recordings consisted of the following (in mM): 140 NaCl, 2.5 KCl, 2 CaCl_2 , 1 MgCl_2 , 10 HEPES, 10 TEA, 1 3,4-Diaminopyridine, 1 4-AP. Na^{+} currents were elicited using depolarizing voltage commands from -70 to 20 mV in 10 mV steps from a holding potential of -90 mV. Steady-state inactivation was measured using depolarizing test pulses to a fixed potential (0 mV) preceded by a series of prepulse conditioning potentials ranging from -100 to -20 mV in 10 mV increments. Linear leakage and capacitive currents were digitally subtracted by scaling traces at smaller voltages in which no voltage dependent current was activated. Activation data were plotted as normalized conductance and steady-state inactivation as normalized current. Activation and inactivation data were fit to a single Boltzmann function using a least squares program.

NEURON modeling. We used a previously validated model (Gasparini et al., 2004) on dspike initiation to test the effects of A-type K^{+} channel alteration on dspike threshold. Modeling was performed using the NEURON environment (<https://neuron.yale.edu/neuron/>; Carnevale and Hines, 2009). The model used in this study was obtained from ModelDB (accession #44050; McDougal et al., 2017). Minor alterations were made to the model parameters to better reflect experimental procedures in the present study. A single, double exponential stimulus injection ($\tau_1 = 0.1$ ms $\tau_2 = 2$ ms) was delivered to the apical dendrite of a model neuron, and the stimulus amplitude increased until a dspike was generated. Dspike data were exported to Igor Pro (WaveMetrics) and analyzed as described above for current-clamp recordings. Under

normal wild-type conditions, A-type K^+ channel $V_{1/2}$ is -0.1 mV, and the conductance density (G_{density}) is 0.008 pS/cm². To mimic the known phenotype of A-type K^+ channels in *fmr1* KO CA1 pyramidal neurons (Routh et al., 2013), $V_{1/2}$ was hyperpolarized to -10.9 mV and G_{density} decreased to 0.0035 pS/cm². No other neuron properties were altered to isolate the effects of altered A-type K^+ channel properties on dspsikes.

DAB reaction and cellular reconstruction. Slices with cells filled with Neurobiotin (Vector Laboratories) during current-clamp experiments were fixed in 3% glutaraldehyde for a minimum of 24 h. Slices were washed in 0.1 M phosphate buffer (PB) and incubated in 0.5% H₂O₂ for 30 min. Slices were then washed in PB and incubated in ABC reagent (Vector Laboratories) containing Avidin DH and biotinylated horseradish peroxidase H for 24–48 h at 4°C. Slices were then incubated in DAB solution (Vector Laboratories) in the presence of H₂O₂ and monitored for a visible color change to the slices and Neurobiotin-filled cell. Slices were dehydrated in glycerol and mounted on glass slides for imaging. Identifiable neurons were reconstructed at 40× magnification using a Leitz Diaplan microscope with Neurolucida version 6.0 software (MBF Bioscience). The Sholl radius was set so there were 10 and 25 concentric circles measuring the basal dendrites and apical dendrites, respectively.

Experimental design and statistical analysis. The use of male wild-type and *fmr1* KO mice was interleaved during each set of experiments. Where possible, experimenters were blind to the condition of the animal during experimentation and analysis. Experiments were designed as a comparison between wild-type and *fmr1* KO mice.

Either the Mann–Whitney rank sum test or Wilcoxon matched pairs (for paired, non-normal data) were used to avoid errors arising from non-normal data distributions. Two-way repeated measures (RM) ANOVA was applied to experiments with multiple test variables for each genotype. Sidak's multiple comparison test was used to compare row means between groups. Data are presented as mean $\pm$ SE. Alpha was set to 0.05 for all experiments. Effect size, a measure of the amount of variance accounted for by differences between groups, is reported as η^2 only when $p < 0.05$; η^2 effect sizes are defined as small, 0.01; medium, 0.06; and large, 0.14. (Cohen, 1988). Statistics were calculated using Prism software (GraphPad).

Results

Long-term potentiation of TA synapses is impaired in *fmr1* KO mice

We made somatic whole-cell current-clamp recordings from CA1 pyramidal neurons in wild-type and *fmr1* KO male mice and recorded TA EPSPs before and after TBS to induce TA-LTP (Tsay et al., 2007; Fig. 1A). In wild-type neurons, TA EPSP slope increased 30 min post-TBS (Fig. 1B,C; wild type: $n = 7$ from 6 mice; pre-TBS: 0.24 ± 0.054 mV/ms; post-TBS: 0.84 ± 0.19 mV/ms. Wilcoxon, $W = 28$, $p = 0.02$, $\eta^2 = 0.79$). By contrast, in *fmr1* KO CA1 neurons TA EPSP slope was not increased after TBS (Fig. 1B,C; *fmr1* KO: $n = 9$ from 7 mice; pre-TBS: 0.23 ± 0.04 mV/ms, post-TBS: 0.24 ± 0.05 mV/ms. Wilcoxon, $W = -3$, $p = 0.92$). The somatic depolarization during the TBS (area under the curve) was not different between wild-type and *fmr1* KO CA1 pyramidal neurons (Fig. 1D; wild type: 12.97 ± 2.38 mV*ms; *fmr1* KO: 5.93 ± 1.48 mV*ms; Mann–Whitney, $U = 13$, $p = 0.06$). In agreement with previous work (Wahlstrom-Helgren and Klyachko, 2015), we found no difference in postsynaptic responsiveness to single TA stimuli (Fig. 1E; wild type: $n = 5$ from 4 mice; *fmr1* KO: $n = 6$ from 4 mice; two-way RM ANOVA, $F_{(1,7)} = 0.65$, $p = 0.45$), baseline paired-pulse ratio (Fig. 1F; wild type: $n = 5$ from 4 mice; *fmr1* KO: $n = 6$ from 4 mice; two-way RM ANOVA: $F_{(1,9)} = 4.23$, $p = 0.07$) or temporal summation (Fig. 1G; wild type: $n = 5$ from 4 mice; *fmr1* KO: $n = 6$ from 4 mice; two-way RM ANOVA, $F_{(1,9)} = 0.55$, $p = 0.48$) between wild-type and *fmr1* KO neurons. Paired-pulse ratio was also not changed post-

Figure 2. CA1 pyramidal neuron morphology is not different between wild-type and *fmr1* KO mice. **A**, Representative neuronal reconstructions of wild-type (black) and *fmr1* KO (red) CA1 pyramidal neurons. **B**, **C**, Total dendritic length (**B**; Mann–Whitney, $U = 16$, $p = 0.82$) and surface area (**C**; Mann–Whitney, $U = 15$, $p = 0.7$) are not different between wild-type and *fmr1* KO CA1 neurons. **D**, Dendritic branching is not different between wild-type and *fmr1* KO CA1 pyramidal neurons (two-way RM ANOVA, $F_{(1,10)} = 0.19$, $p = 0.67$).

TBS consistent with a postsynaptic locus of LTP (wild type: two-way RM ANOVA, $F_{(1,4)} = 3.53$, $p = 0.13$; *fmr1* KO: two-way RM ANOVA, $F_{(1,5)} = 3.98$, $p = 0.1$).

Dendritic morphology is not different in wild-type and *fmr1* KO CA1 pyramidal neurons

We filled CA1 pyramidal neurons with neurobiotin during whole-cell recording for *post hoc* morphologic reconstruction and analysis (Fig. 2A). There was no significant difference in the neuronal surface area (Fig. 2B; wild type: $n = 6$ from 4 mice; 13.3 ± 1.1 mm²; *fmr1* KO: $n = 6$ from 4 mice; 12.9 ± 1.2 mm²; Mann–Whitney, $U = 16$, $p = 0.82$) or somato-dendritic length, measured as straight line distance from the soma to the tip of the most distal dendrite, (Fig. 2C; wild type: 426.6 ± 25.01 μm; *fmr1* KO: 441.3 ± 24.56 μm; Mann–Whitney, $U = 15$, $p = 0.7$) between wild-type and *fmr1* KO CA1 pyramidal neurons. We used Sholl analysis to compare dendritic branching between wild-type and *fmr1* KO neurons. There was no difference in dendritic morphology between wild-type and *fmr1* KO CA1 pyramidal neurons (Fig. 2D; two-way RM ANOVA, $F_{(1,10)} = 0.19$, $p = 0.67$).

Figure 3. Block of h-channels by 20 μM ZD7288 does not rescue TA-LTP in *fmr1* KO CA1 pyramidal neurons. **A**, Recording paradigm during TA-LTP experiments. **B**, EPSP slope is significantly increased 30 min after TBS in wild-type but not *fmr1* KO CA1 neurons (wild type: Wilcoxon, $W = 21$, $p = 0.03$, $\eta^2 = 0.81$; *fmr1* KO: Wilcoxon, $W = -3$, $p = 0.81$). **C**, Normalized EPSP slope 5 min before and 30 min after TBS of TA inputs. Inset, Representative baseline and post-TBS EPSPs from wild-type and *fmr1* KO CA1 neurons. **D**, Graph of the area under the curve during TBS in the presence of ZD7288 (Mann–Whitney, $U = 11$, $p = 0.84$).

Together, these data show that although TA-LTP is impaired in *fmr1* KO CA1 neurons, basal TA synaptic transmission and CA1 neuron morphology are normal.

Block of I_h does not rescue TA-LTP in *fmr1* KO neurons

The expression of h-channels in the distal dendrites of CA1 pyramidal neurons constrains TA inputs and LTP (Tsay et al., 2007). We previously showed that dendritic I_h is elevated in *fmr1* KO CA1 pyramidal neurons compared with wild type (Brager et al., 2012; Brandalise et al., 2020). Higher I_h in *fmr1* KO CA1 neurons may reduce the effectiveness of TA synapses in the distal dendrites and impair LTP (Magee, 1998, 1999). To test this hypothesis, we repeated the TBS TA-LTP experiments with I_h blocked by 20 μM ZD7288 (Fig. 3A). With I_h blocked, TBS significantly potentiated TA EPSPs in wild-type ($n = 6$ from 4 mice; pre-TBS: 0.076 ± 0.018 mV/ms, post-TBS: 0.23 ± 0.073 mV/ms. Wilcoxon, $W = 21$, $p = 0.03$, $\eta^2 = 0.81$) but not *fmr1* KO CA1

pyramidal neurons (Fig. 3B,C; $n = 5$ from 4 mice; pre-TBS: 0.13 ± 0.029 mV/ms, post-TBS: 0.12 ± 0.041 mV/ms. Wilcoxon, $W = -3$, $p = 0.81$). Our results showed no difference in the area under the curve during TBS of TA inputs in the presence of ZD7288 (Fig. 3D; wild type: 12.3 ± 3.8 mV*ms, *fmr1* KO: 10.5 ± 2.4 mV*ms. Mann–Whitney, $U = 11$, $p = 0.84$). These results suggest that higher dendritic I_h alone does not account for the lack of TA-LTP in *fmr1* KO CA1 pyramidal neurons.

Ca^{2+} entry into *fmr1* KO neurons is reduced at TA synapses

A rise in intracellular Ca^{2+} during TBS is necessary for the induction of TA-LTP (Golding et al., 2002; Remondes and Schuman, 2003; Takahashi and Magee, 2009). We used two-photon imaging to directly measure changes in dendritic Ca^{2+} during TA TBS in wild-type and *fmr1* KO CA1 neurons (Fig. 4A). We varied the initial EPSP amplitude and triggered bursts TA EPSPs (10 at 100 Hz; Tsay et al., 2007). Small but detectable Ca^{2+} signals were observed at EPSP amplitudes >1 mV for both wild-type and *fmr1* KO neurons. In both wild-type and *fmr1* KO dendrites, the Ca^{2+} signal increased with increasing EPSP amplitude; however, the Ca^{2+} signal was smaller in *fmr1* KO compared with wild-type dendrites (Fig. 4B,C; wild type: $n = 11$ from 8 mice; *fmr1* KO: $n = 10$ from 7 mice; two-way RM ANOVA, $F_{(1,19)} = 13.14$, $p = 0.002$, $\eta^2 = 0.13$. Interaction, $F_{(2,38)} = 4.86$, $p = 0.013$, $\eta^2 = 0.1$. Sidak's test, 4 mV: $p = 0.04$, $\eta^2 = 0.29$; wild type: $52.73 \pm 13.38 \Delta\text{F}/\text{F}$; *fmr1* KO: $12.26 \pm 2.77 \Delta\text{F}/\text{F}$).

NMDAR-mediated EPSPs are not different between wild-type and *fmr1* KO TA synapses

NMDAR activation is a key source of Ca^{2+} influx during the induction of TA-LTP (Golding et al., 2002; Remondes and Schuman, 2003; Takahashi and Magee, 2009). Although we previously showed that there was no significant difference in TA EPSPs between wild-type and *fmr1* KO CA1 neurons (Fig. 1), those experiments did not separate the AMPA and NMDAR contributions to the EPSP. To test whether NMDAR-mediated EPSPs are different between wild-type and *fmr1* KO neurons, we stimulated TA inputs in the presence of AMPA receptor blocker DNQX (20 μM) and with 0 mM Mg^{2+} in the extracellular saline (Fig. 4D, left). Application of the NMDAR antagonist D-AP5 (50 μM) confirmed isolation of NMDAR-mediated EPSPs in a subset of experiments (Fig. 4D, right). In agreement with our data in Figure 1E, we found no difference in isolated NMDAR-mediated TA EPSPs between wild-type and *fmr1* KO CA1 pyramidal neurons (Fig. 4E; wild type: $n = 7$ from 3 mice; *fmr1* KO: $n = 7$ from 3 mice; two-way RM ANOVA, $F_{(1,12)} = 0.19$, $p = 0.87$).

Dendritic recordings of TA synaptic transmission and LTP

Using somatic recordings, we showed a clear lack of TA-LTP in *fmr1* KO CA1 pyramidal neurons, and dendritic imaging revealed reduced dendritic Ca^{2+} influx during bursts of TA stimulation. This suggests that the dendrites are the locus of the changes that impair TA-LTP in *fmr1* KO CA1 pyramidal neurons. Many of the dendritic events required for TA-LTP are distorted or undetectable using somatic recording because of the filtering properties of CA1 dendrites. Thus, we performed current-clamp recordings from the apical dendrites of wild-type and *fmr1* KO CA1 pyramidal neurons (Fig. 5A; 40 μM Alexa Fluor 594; distance from soma, wild type: $211.8 \pm 3.52 \mu\text{m}$; *fmr1* KO $212.1 \pm 4.69 \mu\text{m}$; Mann–Whitney, $U = 99.5$, $p = 0.84$). Consistent with our somatic recordings, TA EPSP slope was significantly increased post-TBS in wild-type but not *fmr1* KO

Dendritic Calcium Imaging

Figure 4. Synaptic Ca^{2+} signaling reduced in *fmr1* KO dendrites. **A**, Representative CA1 neuron filled with OGB-1 (100 μM) and Alexa 594 (40 μM). Yellow bar represents location of line scan imaging in the s.l.m. region. **B**, Representative Ca^{2+} and voltage signals during 100 Hz bursts of TA EPSPs using 1 and 4 mV initial EPSP amplitudes. **C**, Group data of peak intracellular Ca^{2+} signal during bursts of synaptic activity as a function of initial EPSP amplitude (two-way RM ANOVA, $F_{(1,19)} = 13.14$, $p = 0.002$, $\eta^2 = 0.13$. Interaction, $F_{(2,38)} = 4.86$, $p = 0.013$, $\eta^2 = 0.1$. Sidak's test, 4 mV, $p = 0.04$). **D**, Representative input output traces of NMDAR-dependent EPSPs in 0 mM Mg^{2+} and the AMPA blocker DNQX (20 μM ; left). Addition of the NMDAR antagonist AP5 (25 μM) confirmed the synaptic response was NMDAR dependent (right). **E**, Slope of NMDAR EPSPs as a function of stimulation intensity was not different between wild-type and *fmr1* KO neurons (two-way RM ANOVA, $F_{(1,12)} = 0.19$, $p = 0.87$).

neurons (Fig. 5B,C; wild type: $n = 8$ from 4 mice; pre-TBS: 0.65 ± 0.15 mV/ms, post-TBS: 2.74 ± 0.59 mV/ms. Wilcoxon, $W = 36$, $p = 0.008$, $\eta^2 = 0.4$; *fmr1* KO: $n = 7$ from 4 mice; pre-TBS: 0.65 ± 0.11 mV/ms, post-TBS: 1.01 ± 0.18 mV/ms. Wilcoxon,

$W = 18$, $p = 0.16$). There was no difference in the area under the curve during TBS of TA synapses between wild-type and *fmr1* KO CA1 pyramidal neurons (Fig. 5D; wild type: 13.33 ± 3.14 mV $\cdot$ ms, *fmr1* KO: 8.78 ± 3.76 mV $\cdot$ ms. Mann–Whitney, $U = 16$, $p = 0.19$). There was no significant difference in the response to single stimuli (Fig. 5E; wild type: $n = 6$ from 2 mice; *fmr1* KO: $n = 7$ from 4 mice; two-way RM ANOVA, $F_{(1,17)} = 0.63$, $p = 0.44$), paired-pulse ratio (Fig. 5F; wild type: $n = 7$ from 4 mice; *fmr1* KO: $n = 7$ from 4 mice; two-way RM ANOVA, $F_{(1,12)} = 0.022$, $p = 0.88$), or summation (Fig. 5G; wild type: $n = 6$ from 2 mice; *fmr1* KO: $n = 7$ from 4 mice; two-way RM ANOVA, $F_{(1,12)} = 0.00001$, $p = 0.99$) between wild-type and *fmr1* KO CA1 neurons. Paired-pulse ratios were not different after TBS (wild type: two-way RM ANOVA, $F_{(1,6)} = 1.89$, $p = 0.22$; *fmr1* KO: two-way RM ANOVA, $F_{(1,6)} = 0.02$, $p = 0.89$). Dendritic recordings, much like in somatic recordings, show a lack of *fmr1* KO TA-LTP. We next used dendritic recording to investigate suprathreshold dendritic events implicated in TA-LTP.

Dendritic complex and Ca^{2+} spikes are not different between wild-type and *fmr1* KO neurons

NMDARs, activated by presynaptic glutamate release, are one source of Ca^{2+} signaling. We found that subthreshold NMDAR-mediated EPSPs activated by stimulation of TA synapses were not different between wild-type and *fmr1* KO neurons. Dendritic voltage-gated channels can also contribute to rises in dendritic Ca^{2+} (Magee and Johnston, 1995; Golding et al., 2002; Remondes and Schuman, 2003; Takahashi and Magee, 2009). The activation of distal synapses in CA1 pyramidal neurons gives rise to complex spikes in CA1 dendrites mediated by voltage-gated Na^+ and Ca^{2+} channels (Andreassen and Lambert, 1995; Golding and Spruston, 1998; Golding et al., 1999; Takahashi and Magee, 2009; Kim et al., 2015). We therefore tested the hypothesis that complex spikes and Ca^{2+} -dependent action potentials are impaired in *fmr1* KO neurons.

In CA1 pyramidal neurons, dendritic complex spikes consist of a fast initial Na^+ spike that triggers 1–3 slower Ca^{2+} mediated spikes (Golding et al., 1999). We used dendritic current injection (1 s) to compare complex spikes between wild-type and *fmr1* KO CA1 neurons (Fig. 6A). Previous studies using rat hippocampus showed that only a subpopulation of CA1 dendrites fire complex spikes (Andreassen and Lambert, 1995; Golding et al., 1999). We found that approximately half of mouse CA1 neurons fired complex spikes and that the proportion was not different between wild-type and *fmr1* KO CA1 pyramidal neurons (wild type: 51.6%, *fmr1* KO: 47.6%). There was no difference in the width of the complex spikes between wild-type and *fmr1* KO CA1 dendrites (Fig. 6B–D; wild type: $n = 16$ from 14 mice; *fmr1* KO: $n = 11$ from 10 mice; two-way RM ANOVA, $F_{(1,24)} = 0.00002$, $p = 0.67$; Takahashi and Magee, 2009). To isolate the dendritic Ca^{2+} spike component of the complex spike, we applied 0.5 μM TTX to block voltage-gated Na^+ channels and 50 μM 4-AP to block voltage-gated K^+ channels and bias the dendrites toward firing dendritic Ca^{2+} spikes (Benardo et al., 1982; Andreassen and Lambert, 1995; Golding et al., 1999). Dendritic Ca^{2+} spikes were evoked by depolarizing current injections and confirmed to be mediated by voltage-gated Ca^{2+} channels by the addition of 200 μM Cd^{2+} (Fig. 6E). The number of elicited Ca^{2+} spikes was not different between wild-type and *fmr1* KO CA1 pyramidal neuron dendrites (Fig. 6F). The first Ca^{2+} spike elicited was selected for further analysis (Fig. 6G–K). Dendritic Ca^{2+} spike amplitude

Figure 5. Dendritic recordings show a lack of TA-LTP in *fmr1* KO CA1 pyramidal neurons. **A**, Representative dendritic recording from a CA1 neuron filled with Alexa Fluor 594 (40 μ m). **B**, EPSP slope is increased 30 min post-TBS in wild-type but not *fmr1* KO CA1 neurons (wild type: Wilcoxon, $W = 36$, $p = 0.008$, $\eta^2 = 0.4$; *fmr1* KO: Wilcoxon, $W = 18$, $p = 0.16$). **C**, Normalized EPSP change in EPSP slope for wild-type and *fmr1* KO CA1 neurons. Inset, Representative EPSP traces from baseline (a) and post-TBS (b). **D**, Graph of area under the curve during TBS (Mann–Whitney, $U = 16$, $p = 0.19$). **E**, Input output of EPSP slope at TA inputs in dendritic recordings (two-way RM ANOVA, $F_{(1,17)} = 0.63$, $p = 0.44$). Inset, Representative dendritic experiments. **F**, Paired-pulse ratio is not different between wild-type and *fmr1* KO CA1 pyramidal neurons (two-way RM ANOVA, $F_{(1,12)} = 0.022$, $p = 0.88$). Inset, 50 ms ISI paired pulse. **G**, Temporal summation is not different between wild-type and *fmr1* KO CA1 pyramidal neurons (two-way RM ANOVA, $F_{(1,12)} = 0.0001$, $p = 0.99$). Inset, 50 Hz temporal summation experiment.

(Fig. 6H; wild type: $n = 6$ from 5 mice, 35.32 ± 1.08 mV; *fmr1* KO: $n = 7$ from 6 mice, 38.08 ± 1.84 mV; Mann–Whitney, $U = 9$, $p = 0.18$), maximum rate of rise (Fig. 6I; wild type: 7.66 ± 1.06 mV/ms, *fmr1* KO: 7.79 ± 0.51 mV/ms. Mann–Whitney, $U = 20.5$, $p = 0.98$), maximum rate of decay (Fig. 6J; wild type: -4.52 ± 0.70 mV/ms, *fmr1* KO: -4.22 ± 2.80 mV/ms. Mann–Whitney, $U = 19$, $p = 0.84$) and estimated threshold (Fig. 6K; wild type: -16.47 ± 2.25 mV, *fmr1* KO: -14.98 ± 3.76 mV. Mann–Whitney, $U = 19$, $p = 0.84$) were not different between wild-type and *fmr1* KO neurons. These results suggest that impairment of dendritic complex spikes or Ca^{2+} spikes does not contribute to the reduced dendritic Ca^{2+} signal in *fmr1* KO CA1 pyramidal neuron bursts of TA stimulation.

Block of inwardly rectifying K^+ channels rescues TA-LTP in *fmr1* KO neurons

Our results thus far suggest that there are no differences in NMDARs at TA synapses, dendritic complex spikes, or dendritic Ca^{2+} spikes between wild-type and *fmr1* KO CA1 pyramidal neurons. Furthermore, despite higher dendritic expression, block of I_h in *fmr1* KO neurons does not rescue TA-LTP. Inwardly rectifying K^+ (K_{IR}) channels are open at or near the resting membrane potential, expressed in CA1 pyramidal neuron dendrites, and contribute to the induction of hippocampal LTP (Hoffman et al., 1997; Chen and Johnston, 2004; Malik and Johnston, 2017). In rats, K_{IR} channels in the dendrites constrain dendritic nonlinear events and control LTP (Malik and Johnston, 2017). To test whether K_{IR} channels contribute to the lack of TA-LTP in *fmr1* KO neurons, we performed TBS TA-LTP experiments in the presence of $25 \mu\text{M}$ Ba^{2+} to block K_{IR} s (Malik and Johnston, 2017; Fig. 7A). Block of K_{IR} rescued TA-LTP in *fmr1* KO CA1 pyramidal neurons (Figure 7B,C; wild type: $n = 5$ from 4 mice;

pre-TBS: 0.16 ± 0.029 mV/ms, post-TBS: 0.37 ± 0.98 mV/ms. Wilcoxon, $W = 15$, $p = 0.03$, $\eta^2 = 0.81$; *fmr1* KO: $n = 6$ from 3 mice; pre-TBS: 0.11 ± 0.015 mV/ms, post-TBS: 0.31 ± 0.05 mV/ms. Wilcoxon, $W = 21$, $p = 0.03$, $\eta^2 = 0.81$). The area under the curve during the induction protocol was not different between wild-type and *fmr1* KO CA1 pyramidal neurons in the presence of $25 \mu\text{M}$ Ba^{2+} (Fig. 7D; wild type: 21.89 ± 8.17 mV*ms, *fmr1* KO: 9.78 ± 1.49 mV*ms. Mann–Whitney, $U = 11$, $p = 0.54$).

One potential explanation for the rescue of TA-LTP by Ba^{2+} is that there is a higher dendritic expression of K_{IR} s in *fmr1* KO CA1 pyramidal neurons. To test whether dendritic K_{IR} s were different between wild-type and *fmr1* KO CA1 neurons, we measured the dendritic resting membrane potential (V_m) and input resistance (R_N) before and after application of $25 \mu\text{M}$ Ba^{2+} (Fig. 7E). Extracellular Ba^{2+} depolarized V_m in both wild-type and *fmr1* KO dendrites (Fig. 7F; wild type: $n = 7$ from 3 mice; pre- Ba^{2+} : -59.29 ± 2.53 mV, post- Ba^{2+} : -55.29 ± 2.47 mV. Wilcoxon, $W = 28$, $p = 0.016$, $\eta^2 = 0.79$; *fmr1* KO: $n = 7$ from 2 mice; pre- Ba^{2+} : -59.86 ± 1.68 mV, post- Ba^{2+} : -54.00 ± 1.6 mV. Wilcoxon, $W = 28$, $p = 0.016$, $\eta^2 = 0.79$). The effect of Ba^{2+} on V_m was however, not significantly different between wild-type and *fmr1* KO neurons (Fig. 7G; wild type: 4.00 ± 1.02 mV, *fmr1* KO: 5.86 ± 0.94 mV. Mann–Whitney, $U = 13.5$, $p = 0.16$). Extracellular Ba^{2+} also increased R_N in both wild-type and *fmr1* KO dendrites (Fig. 7H,I; wild type: $n = 7$ from 3 mice; two-way RM ANOVA, $F_{(1,5)} = 11.81$, $p = 0.019$, $\eta^2 = 0.12$; *fmr1* KO: $n = 7$ from 2 mice; two-way RM ANOVA, $F_{(1,6)} = 10.13$, $p = 0.019$, $\eta^2 = 0.1$). As for V_m , the change in R_N was not significantly different between wild-type and *fmr1* KO dendrites (Fig. 7J; two-way RM ANOVA, $F_{(1,11)} = 0.005$, $p = 0.95$). These results demonstrate that, although block of K_{IR} s by extracellular Ba^{2+} rescued TA-LTP in *fmr1* KO neurons, the functional

Figure 6. Dendritic complex and Ca^{2+} spikes are not different between wild-type and *fmr1* KO CA1 pyramidal neurons. **A**, Dendritic recording schematic. **B**, Method for measuring complex spike width (see above, Materials and Methods). **C**, Complex spikes elicited by dendritic current injection in wild-type and *fmr1* KO neurons (left) and example complex spikes (boxes) shown on expanded time scale (right). **D**, Complex spike width as a function of number of events (1–3; two-way RM ANOVA, $F_{(1,24)} = 0.00,002$, $p = 0.67$). **E**, Representative traces of isolated dendritic Ca^{2+} spikes in the presence of 500 nM TTX and 50 μM 4-AP. Application of 200 μM Cd^{2+} confirmed that spikes were generated by voltage-gated Ca^{2+} channels. **F**, The number of Ca^{2+} spikes as a function of current amplitude (two-way RM ANOVA, $F_{(1,11)} = 0.07$, $p = 0.8$). **G**, **H–K**, Representative dendritic Ca^{2+} spike traces (left). Single Ca^{2+} spike in the box at left on expanded time scale used for analyses in **H–K** (right). Black arrows show the estimated threshold. **H–K**, Dendritic Ca^{2+} spike amplitude (**H**; Mann–Whitney, $U = 9$, $p = 0.18$), maximum rate of rise (**I**; Mann–Whitney, $U = 20.5$, $p = 0.98$), minimum rate of decay (**J**; Mann–Whitney, $U = 19$, $p = 0.84$), and threshold (**K**; Mann–Whitney, $U = 19$, $p = 0.84$) were not significantly different between wild-type and *fmr1* KO neurons.

expression of dendritic K_{IRs} is not different between wild-type and *fmr1* KO CA1 pyramidal neurons.

Dendritic depolarization rescues TA-LTP in *fmr1* KO CA1 pyramidal neurons

The block of K_{IRs} depolarized dendritic V_m , increased dendritic R_N and rescued TA-LTP. In rat CA1 neurons, direct manipulation of dendritic V_m was able to reproduce the effects of K_{IRs} on dendritic function (Malik and Johnston, 2017). To test whether

the depolarization would mimic the effects of Ba^{2+} on TA-LTP, we depolarized the dendritic V_m by 10 mV during the delivery of TBS using steady-state current injection to mimic the depolarization of oblique dendrites (Golding et al., 2005) during Ba^{2+} wash-on (Fig. 8A). Similar to extracellular Ba^{2+} , dendritic depolarization rescued TA-LTP in *fmr1* KO neurons (Fig. 8B,C; wild type: $n = 6$ from 2 mice; pre-TBS: 0.36 ± 0.048 mV/ms, post-TBS: 1.21 ± 0.16 mV/ms. Wilcoxon, $W = 21$, $p = 0.03$, $\eta^2 = 0.4$; *fmr1* KO: $n = 7$ from 4 mice; pre-TBS: 0.55 ± 0.21 mV/ms, post-TBS: 2.22 ± 0.61 mV/ms. Wilcoxon, $W = 24$, $p = 0.047$, $\eta^2 =$

Figure 7. Low Ba^{2+} rescues TA-LTP in *fmr1* KO CA1 pyramidal neurons. **A**, Recording configuration during low Ba^{2+} TA-LTP experiments. **B**, EPSP slope is significantly increased 30 min after TBS in both wild-type and *fmr1* KO CA1 neurons (wild type: Wilcoxon, $W = 15$, $p = 0.03$, $\eta^2 = 0.81$; *fmr1* KO: Wilcoxon, $W = 21$, $p = 0.03$, $\eta^2 = 0.81$). **C**, Normalized change in EPSP slope for wild-type and *fmr1* KO CA1 neurons in the presence of $25 \mu\text{M}$ extracellular Ba^{2+} . Inset, Baseline and post-TBS EPSP traces from wild-type and *fmr1* KO neurons. **D**, Graph of area under the curve during TBS with Ba^{2+} included in the bath saline (Mann–Whitney, $U = 11$, $p = 0.54$). **E**, Recording configuration (left) and representative voltage traces before and after application of $25 \mu\text{M}$ Ba^{2+} . **F**, Ba^{2+} significantly depolarizes V_m in both wild-type and *fmr1* KO neurons (wild type: Wilcoxon, $W = 28$, $p = 0.016$, $\eta^2 = 0.79$; *fmr1* KO: Wilcoxon, $W = 28$, $p = 0.016$, $\eta^2 = 0.79$). **G**, The effect of Ba^{2+} on V_m is not different between wild-type and *fmr1* KO neurons (Mann–Whitney, $U = 13.5$, $p = 0.16$). **H**, **I**, Ba^{2+} significantly increases R_N in wild-type (**H**; two-way RM ANOVA, $F_{(1,5)} = 11.81$, $p = 0.019$, $\eta^2 = 0.12$) and *fmr1* KO (**I**; two-way RM ANOVA, $F_{(1,6)} = 10.13$, $p = 0.019$, $\eta^2 = 0.1$) dendrites. **J**, The effect of Ba^{2+} on R_N is not different between wild-type and *fmr1* KO neurons (two-way RM ANOVA, $F_{(1,11)} = 0.005$, $p = 0.95$).

0.52). The area under the curve during the induction protocol was not different between wild-type and *fmr1* KO CA1 pyramidal neurons (Fig. 8D; wild type: $8.65 \pm 1.08 \text{ mV} \cdot \text{ms}$, *fmr1* KO: $15.22 \pm 3.6 \text{ mV} \cdot \text{ms}$, Mann–Whitney, $U = 15$, $p = 0.45$). Unlike the Ba^{2+} experiments above, however, dendritic V_m was only depolarized during delivery of the TBS. Together with Ba^{2+} wash-on experiments, these results suggest that *fmr1* KO CA1 dendrites are unable to reach the threshold for TA-LTP induction under normal conditions.

Dspike generation is impaired in *fmr1* KO CA1 pyramidal neurons

The large-scale, rapid influx of Ca^{2+} necessary for the generation of LTP at distal synapses in CA1 neurons is dependent on dendritic Na^+ spikes (Kim et al., 2015). Consequently, one explanation for our results thus far is that dspike generation is impaired in *fmr1* KO neurons. We used a double exponential current injection ($\tau_{\text{rise}} = 0.1 \text{ ms}$; $\tau_{\text{decay}} = 2 \text{ ms}$) to mimic the time course of dendritic EPSPs and trigger dendritic Na^+ spikes in wild-type

and *fmr1* KO dendrites (Fig. 9A; Gasparini et al., 2004). Figure 9B shows dspike threshold as a function of the dendritic recording distance from the soma in wild-type and *fmr1* KO CA1 neurons. Dspikes in *fmr1* KO CA1 neurons had a more depolarized threshold (Fig. 9C; wild type: $n = 7$ from 5 mice; *fmr1* KO: $n = 7$ from 5 mice; wild type: $-48.08 \pm 1.35 \text{ mV}$, *fmr1* KO: $-41.48 \pm 1.87 \text{ mV}$, Mann–Whitney, $U = 6$, $p = 0.02$, $\eta^2 = 0.34$) and slower maximum dV/dt (Fig. 9D; wild type: $60.07 \pm 5.14 \text{ mV/ms}$, *fmr1* KO: $44.89 \pm 2.40 \text{ mV/ms}$, Mann–Whitney, $U = 3$, $p = 0.004$, $\eta^2 = 0.54$) compared with wild-type dendrites.

Dendritic voltage-gated Na^+ channels do not differ between wild-type and *fmr1* KO CA1 neurons

CA1 pyramidal neuron dendrites express voltage-gated Na^+ channels, which contribute to dspikes (Magee and Johnston, 1995; Golding and Spruston, 1998). The difference in threshold and maximum dV/dt could be accounted for by differences in dendritic voltage-gated Na^+ channels between wild-type and

Figure 8. Dendritic depolarization rescues TA-LTP in *fmr1* KO CA1 neurons. **A**, Recording configuration. Current injection produced a 10 mV depolarization during TBS. **B**, EPSP slope is significantly increased 30 min after TBS in both wild-type and *fmr1* KO CA1 neurons (wild type: Wilcoxon, $W = 21$, $p = 0.03$, $\eta^2 = 0.4$; *fmr1* KO: Wilcoxon, $W = 24$, $p = 0.047$, $\eta^2 = 0.52$). **C**, Normalized EPSP slope 5 min before and 30 min after TBS of TA inputs. Inset, Representative baseline and post-TBS EPSPs from wild-type and *fmr1* KO CA1 neurons. **D**, Graph of area under the curve during TBS while a steady depolarizing current of 10 mV was applied to the dendritic patch (Mann–Whitney, $U = 15$, $p = 0.45$).

Figure 9. Dspike threshold is more depolarized in *fmr1* KO CA1 pyramidal neurons. **A**, Dendritic voltage response to double exponential current injections (inset) of increasing amplitude (500 pA intervals). Thick lines show voltage traces with dspike and the corresponding current injection. **B**, Graph of distance of dendritic recordings as a function of dspike threshold. **C**, Voltage threshold for dspikes is more depolarized in *fmr1* KO CA1 neurons (Mann–Whitney, $U = 6$, $p = 0.02$, $\eta^2 = 0.34$). **D**, The maximum rate of rise is significantly slower in *fmr1* KO neurons (Mann–Whitney, $U = 3$, $p = 0.004$, $\eta^2 = 0.54$).

fmr1 KO CA1 neurons. To test whether there are differences in dendritic Na^+ channel function, we measured Na^+ current using cell-attached voltage-clamp recordings from wild-type and *fmr1* KO dendrites ($200\text{--}250 \mu\text{m}$ from the soma). The maximum current and voltage-dependence of Na^+ channels we measured in wild-type CA1 pyramidal neurons were in good agreement with those obtained from rat CA1 dendrites (Magee and Johnston, 1995; Gasparini and Magee, 2002). We found no difference in the Na^+ I - V curve between wild-type and *fmr1* KO neurons (Fig. 10A). Additionally, there was no significant difference in the voltage dependence of activation or steady-state inactivation between wild-type and *fmr1* KO Na^+ channels (Fig. 10B). There was no difference in either the $V_{1/2}$ of activation (Fig. 10C; wild type: $n = 10$ from 3 mice, $-19.11 \pm 2.17 \text{ mV}$; *fmr1* KO: $n = 8$ from 3 mice, $-16.2 \pm 4.23 \text{ mV}$. Mann–Whitney, $U = 33$, $p = 0.57$) or the activation slope factor (Fig. 10D; wild type: $7.15 \pm$

Figure 10. Dendritic Na⁺ channels are not different between wild-type and *fmr1* KO CA1 pyramidal neurons. **A**, Current-voltage plot showing no significant difference in Na⁺ current between wild-type and *fmr1* KO dendrites. Inset, Representative Na⁺ current traces in response to voltage steps to -60, -40, -20, and 0 mV. **B**, Na⁺ channel activation and inactivation are not different between wild-type and *fmr1* KO CA1 dendrites. **C**, **D**, Na⁺ channel activation V_{1/2} (**C**, Mann-Whitney, $U = 33$, $p = 0.57$) and slope factor (**D**; Mann-Whitney, $U = 29$, $p = 0.36$) are not different between wild-type and *fmr1* KO dendrites. **E**, **F**, Na⁺ channel steady-state inactivation V_{1/2} (**E**; Mann-Whitney, $U = 11$, $p = 0.31$) and slope factor (**F**; Mann-Whitney, $U = 7$, $p = 0.09$) are not different between wild-type and *fmr1* KO dendrites.

1.08. *fmr1* KO: 8.57 ± 1.13 , Mann-Whitney, $U = 29$, $p = 0.36$) between wild-type and *fmr1* KO CA1 pyramidal dendrites. Similarly, steady-state inactivation properties of V_{1/2} of inactivation (Fig. 10E; wild type: $n = 6$ from 2 animals, -63.47 ± 5.59 . *fmr1* KO: $n = 6$ from 2 animals, -55.51 ± 1.45 . Mann-Whitney, $U = 11$, $p = 0.31$) and inactivation slope (Fig. 10F; wild type: $n = 6$ from 2 mice, -8.93 ± 1.98 ; *fmr1* KO: $n = 6$ from 2 mice, -4.41 ± 1.03 . Mann-Whitney, $U = 7$, $p = 0.09$) were not different between wild-type and *fmr1* KO neurons. Our results suggest that the differences in Na⁺ channel function do not contribute to the threshold and dV/dt differences in dspsikes between wild-type and *fmr1* KO CA1 pyramidal neurons.

A-type K⁺ channel block restores dspsike threshold in *fmr1* KO neurons

A-type K⁺ currents (I_{KA}) are smaller in *fmr1* KO CA1 neurons compared with wild type (Routh et al., 2013). We did not initially

consider differences in I_{KA} as a potential cause for the lack of TA-LTP in *fmr1* KO neurons as we would expect the reduction in I_{KA} to either promote or have no effect on dspsikes. There is, however, a hyperpolarized shift in the activation of A-type K⁺ channels in *fmr1* KO dendrites compared with wild type (Routh et al., 2013). A-type K⁺ channels that activate at more negative potentials could overlap with Na⁺ currents and influence dspsikes in *fmr1* KO neurons. Furthermore, low concentrations of Ba²⁺ have been shown to affect transient K⁺ currents expressed in the heart (Shi et al., 2000). We thus hypothesized that A-type K⁺ channels influence dspsikes in *fmr1* KO but not wild-type CA1 neurons. As an initial test of this hypothesis, we recorded dspsikes under control conditions, 25 μ M Ba²⁺ (used in Fig. 7) and 150 μ M Ba²⁺, a concentration known to block A-type K⁺ channels (Gasparini et al., 2007; Routh et al., 2013; Fig. 11A–C). Neither concentration of Ba²⁺ had an effect on dspsike threshold in wild-type neurons; however, dspsike threshold was significantly hyperpolarized by 25 or 150 μ M Ba²⁺ in *fmr1* KO neurons (Fig. 11A–C; wild type: $n = 7$ from 3 mice; *fmr1* KO: $n = 8$ from 2 mice; two-way RM ANOVA, $F_{(1,13)} = 4.9$, $p = 0.045$, $\eta^2 = 0.15$. Interaction, $F_{(2,26)} = 11.51$, $p = 0.0003$, $\eta^2 = 0.13$. Sidak's test baseline, $p = 0.001$, $\eta^2 = 0.64$; wild type: -36.14 ± 1.09 mV; *fmr1* KO: -29.3 ± 0.79 mV). These results support the hypothesis that A-type K⁺ channels contribute to the depolarized dspsike threshold in *fmr1* KO CA1 neurons. These data also suggest that Ba²⁺ rescued TA-LTP in *fmr1* KO neurons by hyperpolarizing the threshold for dspsike generation.

In *fmr1* KO CA1 pyramidal neurons, dendritic A-type K⁺ channels have a hyperpolarized activation curve and reduced maximal current amplitude (Routh et al., 2013). Are these apparent counteracting changes (easier to activate but reduced amplitude) in dendritic A-type K⁺ channels sufficient to alter dspsike initiation? To answer this question, we performed simulations using an established model of CA1 dendrites and dspsike generation (Gasparini et al., 2004; accession #44050, ModelDB). As in our current-clamp experiments, dendritic input was simulated by a single double exponential current injection sufficient to produce a dspsike. Under control conditions the threshold for simulated dspsikes was -35.3 mV (Fig. 11D, black), consistent with dspsikes recorded from wild-type CA1 dendrites (-36.1 ± 1.09 mV). To simulate the phenotype in *fmr1* KO CA1 dendrites, A-type K⁺ channel V_{1/2} was hyperpolarized to -10.9 mV and $G_{density}$ reduced to 0.0035 pS/cm². Under these knock-out conditions the threshold for dspsikes was -29.4 mV (Fig. 11D, red), consistent with dspsikes recorded from *fmr1* KO CA1 dendrites (-29.3 ± 0.79 mV). These results indicate that the known alterations in dendritic A-type K⁺ channel properties in *fmr1* KO CA1 neurons can depolarize dspsike threshold (Fig. 11E).

Although extracellular Ba²⁺ can be used to test for A-type K⁺ channels (Gasparini et al., 2007; Routh et al., 2013; Kalmbach et al., 2015), Ba²⁺ can also block inwardly rectifying K⁺ channels (Fig. 7; Gasparini et al., 2004; Kim and Johnston, 2015; Ordemann et al., 2019). To explicitly test for the contribution of A-type K⁺ channels to dendritic dspsike threshold, we used the A-type K⁺-channel-specific blocker AmmTx3 (500 nM; Chittajallu et al., 2020; Hu et al., 2020). In agreement with our Ba²⁺ experiments, AmmTx3 significantly reduced dspsike threshold in *fmr1* KO CA1 dendrites but had no effect on wild-type dspsikes (Fig. 11F,G; wild type: $n = 6$ from 3 mice; pre-AmmTx3: -32.56 ± 0.65 mV, post-AmmTx3: -31.91 ± 1.11 mV. Wilcoxon, $W = 5$, $p = 0.69$; *fmr1* KO: $n = 6$ from 3 mice; pre-AmmTx3: -25.52 ± 2.29 mV, post-AmmTx3: -33.84 ± 1.8 mV. Wilcoxon, $W = -21$, $p = 0.03$, $\eta^2 = 0.65$).

Figure 11. Block of A-type K^+ channels hyperpolarizes dspike threshold in *fmr1* KO neurons. **A**, Representative dspike recordings showing recorded voltage (left), first derivative (middle), and second derivative (right). Threshold was determined as 20% of the second peak of the second derivative (arrows). **B**, Representative dspikes during baseline and application of 150 μM Ba^{2+} in wild-type and *fmr1* KO CA1 pyramidal neurons. **C**, Extracellular Ba^{2+} hyperpolarizes dspike threshold in *fmr1* KO but not wild-type dendrites (two-way RM ANOVA, $F_{(1,13)} = 4.9$, $p = 0.045$, $\eta^2 = 0.15$). Interaction, $F_{(2,26)} = 11.51$, $p = 0.0003$, $\eta^2 = 0.13$. Sidak's test, 0 μM Ba^{2+} , $p = 0.001$, $\eta^2 = 0.64$. **D**, Simulation of dspikes under control (black; $V_{1/2} = -0.1$, $G_{\text{density}} = 0.008$ pS/cm 2) and *fmr1* KO-like A-type K^+ channel properties (red; $V_{1/2} = -0.1$, $G_{\text{density}} = 0.0035$ pS/cm 2). **E**, Dspike threshold from current-clamp recordings from wild-type and *fmr1* KO dendrites (0 μM Ba^{2+} condition from **C**) and simulations with normal and *fmr1* KO-like A-type K^+ channel properties. **F**, Representative dspike recordings from wild-type and *fmr1* KO neurons before and after application of 500 nM AmmTx3. Arrows indicate threshold for dspike generation. **G**, AmmTx3 significantly hyperpolarized

A-type K^+ channel block restores TA-LTP in *fmr1* KO neurons

To test whether blocking of A-type K^+ channels rescued TA-LTP in *fmr1* KO neurons, we repeated the TBS TA-LTP experiments in the presence of the A-type K^+ channel blocker AmmTx3 (500 nM). Blockade of A-type K^+ channels with 500 nM AmmTx3 rescued TA-LTP in *fmr1* KO CA1 pyramidal neurons (Fig. 12A–C; wild type: $n = 6$ from 3 mice; pre-TBS: 0.13 ± 0.017 mV/ms, post-TBS: 0.23 ± 0.032 mV/ms. Wilcoxon, $W = 21$, $p = 0.03$, $\eta^2 = 0.79$. *fmr1* KO: $n = 6$ from 3 mice; pre-TBS: 0.11 ± 0.027 mV/ms, post-TBS: 0.21 ± 0.048 mV/ms. Wilcoxon, $W = 21$, $p = 0.03$, $\eta^2 = 0.79$). The area under the curve during TBS was not different while AmmTx3 was present in the bath (Fig. 12D,E; wild type: 22.62 ± 3.51 mV $\cdot$ ms; *fmr1* KO: 22.58 ± 4.65 mV $\cdot$ ms. Mann–Whitney, $U = 16$, $p = 0.82$). Together, these results suggest that the hyperpolarized shift in activation of A-type K^+ channels in *fmr1* KO CA1 pyramidal neuron dendrites results in a depolarized threshold for dspikes and a lack of TA-LTP.

Discussion

TBS induces behaviorally relevant LTP of TA inputs in wild-type CA1 neurons, which is critical to hippocampal-dependent learning and memory (Remondes and Schuman, 2004; Bittner et al., 2017). We found that TBS fails to induce TA-LTP in *fmr1* KO CA1 pyramidal neurons. We previously identified an increase in the expression of dendritic h-channels in *fmr1* KO CA1 dendrites (Brager et al., 2012; Brandalise et al., 2020). We thus hypothesized that decreased summation of TA inputs would account for impairments in TA-LTP. However, blocking h-channels with ZD7288 did not rescue TA-LTP in *fmr1* KO neurons. We cannot rule out the contribution of h-channelopathy to reduced synaptic summation leading to impaired TA-LTP. TA-LTP requires Ca^{2+} influx through NMDARs and L-type voltage-gated Ca^{2+} channels (Golding et al., 2002; Remondes and Schuman, 2003). Two-photon Ca^{2+} imaging demonstrated that synaptically evoked dendritic Ca^{2+} signals were smaller in *fmr1* KO neurons. We found that basal TA synaptic transmission was not different between wild-type and *fmr1* KO mice as was described previously (Wahlstrom-Helgren and Klyachko, 2015) but see (Booker et al., 2020). Interestingly, we found no difference in NMDAR EPSPs, dendritic complex spikes, or isolated dendritic Ca^{2+} spikes between wild-type and *fmr1* KO CA1 pyramidal neurons. Dendritic Na^+ -mediated spikes are necessary and trigger the influx of Ca^{2+} necessary for TA-LTP (Golding et al., 2002; Kim et al., 2015). We found that dspike threshold is depolarized in *fmr1* KO dendrites and suggest that an inability of TA TBS to trigger dspikes likely contributes to TA-LTP dysfunction. Indeed, depolarization of the dendritic membrane potential in *fmr1* KO neurons through blockade of K_{IR} channels or direct dendritic current injection restored TA-LTP. Our lab previously demonstrated that A-type K^+ channel expression is reduced in the dendrites of *fmr1* KO CA1 pyramidal neurons, and the activation is shifted to more hyperpolarized potentials (Routh et al., 2013). Despite a reduction in A-type K^+ channel expression, the hyperpolarized shift in activation resulted in a depolarized dspike threshold in *fmr1* KO CA1 dendrites and the rescue of TA-LTP and dspike threshold in *fmr1* KO neurons by the addition of the A-type K^+ channel blocker AmmTx3.

←

dspike threshold in *fmr1* KO but not wild-type CA1 neurons (wild type: Wilcoxon, $W = 5$, $p = 0.69$; *fmr1* KO: Wilcoxon, $W = -21$, $p = 0.03$, $\eta^2 = 0.65$).

Figure 12. Block of A-type K^+ channels restores TA-LTP in *fmr1* KO neurons. **A**, Recording configuration for AmmTx3 TA-LTP experiments. **B**, EPSP slope is significantly increased 30 min after TBS in both wild-type and *fmr1* KO CA1 neurons (wild type: Wilcoxon, $W = 21$, $p = 0.03$, $\eta^2 = 0.79$; *fmr1* KO: Wilcoxon, $W = 21$, $p = 0.03$, $\eta^2 = 0.79$). **C**, Normalized EPSP slope 5 min before and 30 min after TBS of TA inputs. Inset, Representative baseline and post-TBS EPSPs from wild-type and *fmr1* KO CA1 neurons. **D**, Representative traces showing the somatic response during TBS. **E**, Group data of area under the curve during TBS (Mann–Whitney, $U = 16$, $p = 0.82$).

Dendritic nonlinear events in *fmr1* KO CA1 neurons

We provide the first direct comparison of dendritic nonlinear events (complex spikes, Ca^{2+} spikes, and dendritic Na^+ spikes) between wild-type and *fmr1* KO CA1 neurons. Fast Na^+ -dependent spikes are essential for the induction TA-LTP (Kim et al., 2015). We found that the threshold was more depolarized, and the maximum dV/dt was slower for spikes in *fmr1* KO dendrites compared with wild-type. Dendritic complex spikes, which are the combined effect of dendritic Na^+ and Ca^{2+} channels, were not different in frequency or width between wild-type and *fmr1* KO CA1 pyramidal neurons. Recordings of isolated dendritic Ca^{2+} spikes suggest that CA1 dendritic voltage-gated Ca^{2+} channels are not different between wild-type and *fmr1* KO neurons. Previous studies have shown that changes in voltage-gated Ca^{2+} channels in *fmr1* KO mice occur in a brain region- and cell-type-specific manner (Meredith et al., 2007; Danesi et al., 2018; Gray et al., 2019). Our results further illustrate that changes in ion channel function and expression in FXS are not conserved across brain regions.

A-type K^+ channels contribute to spikes in *fmr1* KO but not wild-type CA1 neurons

The threshold for regenerative events is determined by the complement of ion channels and their relative kinetics (Hodgkin and Huxley, 1952a, b; Stafstrom et al., 1984). In particular, there is an interplay between depolarizing sodium conductances and hyperpolarizing potassium conductances. We provide the first investigation of dendritic voltage-gated Na^+ channels in CA1 dendrites of *fmr1* KO mice. The properties of voltage-gated Na^+ currents in our wild-type mouse dendritic recordings were comparable to

previous findings in rats (Magee and Johnston, 1995; Gasparini and Magee, 2002) and were not different between wild-type and *fmr1* KO CA1 pyramidal neurons.

The activation of A-type K^+ channels is hyperpolarized in *fmr1* KO CA1 neuron dendrites (Routh et al., 2013). We hypothesize that the hyperpolarized shift in I_{KA} activation in *fmr1* KO dendrites allows A-type K^+ channels to provide more opposition to Na^+ -dependent depolarization and depolarizes spike threshold. Similar modulation of action potential threshold is known to occur via somatic D-type K^+ channels (Higgs and Spain, 2011; Kalmbach et al., 2015; Ordemann et al., 2019).

Our results support this hypothesis, showing that direct depolarization of dendrites during TBS was sufficient to rescue TA-LTP in *fmr1* KO CA1 neurons. Furthermore, both 25 and 150 μM extracellular Ba^{2+} hyperpolarized spike threshold in *fmr1* KO but had no effect in wild-type CA1 neurons. Simulations of CA1 spikes, in which A-type K^+ channel function was modified to be consistent with the *fmr1* KO phenotype, recapitulated the difference between wild-type and *fmr1* KO spikes. Finally, we confirmed that blockade of I_{KA} hyperpolarized spike threshold and rescued TA-LTP with the specific A-type K^+ channel blocker AmmTx3.

Potential mechanism for the hyperpolarized activation of A-type K^+ channels

The voltage dependence of A-type K^+ channels in CA1 pyramidal dendrites is modulated by multiple signaling cascades. Stimulation of protein kinase C (PKC), by activation of metabotropic glutamate and muscarinic acetylcholine receptors, and cAMP-dependent protein kinase A (PKA), by dopaminergic and β -adrenergic receptors, produce a depolarizing shift in the activation of A-type K^+ channels (Hoffman and Johnston, 1998, 1999).

Reduction in PKC and/or PKA activity could account for the hyperpolarization of A-type K^+ channel activation in *fmr1* KO CA1 neurons. There is evidence from human and animal models of FXS exhibiting lower cAMP levels (Berry-Kravis and Huttenlocher, 1992; Choi et al., 2015). Lower cAMP levels could reduce basal PKA activity and lead to a hyperpolarized shift of A-type K^+ channel activation. A study on cortical synaptosomes found that basal PKC activity was not different between wild-type and *fmr1* KO mice (Weiler et al., 2004). A more recent study, however, found that PKC ϵ expression was lower in the hippocampus of *fmr1* KO mice (Marsillo et al., 2021). PKC ϵ is abundant in the nervous system and activated by G protein-coupled receptors (Akita, 2002). Furthermore, PKC ϵ is activated by phorbol esters, which were used to investigate PKC-dependent modulation of A-type K^+ channels (Hoffman and Johnston, 1998). Thus, PKC ϵ is a potential candidate for regulating the voltage dependence of A-type K^+ channel activation. Additionally, FMRP is a positive regulator of both PKA (Sears et al., 2019) and

PKC (Zhao et al., 2015); therefore, loss of FMRP in FXS could result in reduced PKA and PKC activity. Thus, changes in the basal activity of PKA and/or PKC could contribute to the hyperpolarized shift in A-type K^+ channel activation that alters dsipike threshold and impairs TA-LTP in *fmr1* KO CA1 neurons.

Consequences for hippocampal learning and memory

There are two major changes in A-type K^+ channel function in *fmr1* KO CA1 dendrites—lower maximum current and hyperpolarized activation. LTP induction of excitatory inputs to CA1 pyramidal neurons requires active dendritic events. Schaffer collateral LTP requires backpropagating action potentials to provide the depolarization necessary for NMDA receptor activation (Magee and Johnston, 1997). In the SLM region of CA1 however, where the TA inputs synapse onto the distal dendrites of CA1 pyramidal neurons, backpropagating action potentials are unreliable and often fail to propagate into the distal apical dendrites. Rather, it is locally generated dsipikes that provide the necessary depolarizing signal for the induction of LTP. Alterations in dendritic A-type K^+ channel function in *fmr1* KO CA1 pyramidal neurons causes changes to the active events affecting LTP of both excitatory pathways. Reduced maximal A-type K^+ current allows the amplitude of back propagating action potentials to be larger, which results in a reduced threshold for Schaffer collateral LTP (Routh et al., 2013). By contrast, the hyperpolarized shift in the activation of A-type K^+ channels provides a small, but effective, hyperpolarizing influence on the dendritic membrane potential that depolarizes dsipike threshold and impairs TA-LTP. The coordinated plasticity of TA inputs, which convey current information about the external environment, and Schaffer collateral inputs, which convey stored information from prior experiences, is critical for hippocampal-dependent memory tasks. The combined changes in A-type K^+ channel function alter the dendritic processing of these two critical excitatory pathways. Our findings show an inability to induce LTP in TA synapses in a pathway critical for the induction of long-term memory (Remondes and Schuman, 2004). This finding provides critical new information for the understanding of the FXS disease phenotype.

FXS is marked by deficits in learning and memory, making the discovery of specific therapeutic targets necessary for the understanding and future treatment of the disorder. Our previous work, along with other studies, showed how changes in the expression of A-type K^+ channels affect Schaffer collateral LTP in FXS (Gross et al., 2011; Lee et al., 2011; Routh et al., 2013). Here, we show that changes in the properties of A-type K^+ channels have an impact on TA-LTP. Therefore, although A-type K^+ channels represent a potential therapeutic target for FXS interventions, manipulations must take into account both the change in expression and the shift in gating.

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