Input-output relationship of CA1 pyramidal neurons reveals intact homeostatic mechanisms in a mouse model of Fragile X Syndrome

Cell Reports

Input-output relationship of CA1 pyramidal neurons reveals intact homeostatic mechanisms in a mouse model of Fragile X Syndrome

Sam A. Booker[1,2,3*], Laura Simões de Oliveira[1,2,3*], Natasha J Anstey[1,2,3,5], Zrinko Kozic[1,2], Owen R Dando[1,2,3,4,5], Adam D Jackson[1,2,3,5], Paul S Baxter[1,4], Lori L Isom[6], Diane L. Sherman[1], Giles E Hardingham[1,4], Peter J. Brophy[1], David J.A. Wyllie[1,2,3,5], Peter C. Kind[1,2,3,5*]

[1] Centre for Discovery Brain Sciences, [2] Simons Initiative for the Developing Brain, [3] Patrick Wild Centre for Autism Research, [4] Dementia Research Institute, University of Edinburgh, Hugh Robson Building, George Square, Edinburgh, EH8 9XD, UK; [5] Centre for Brain Development and Repair, InStem, GKVK Campus, Banglore, 560065, India; [6] Department of Pharmacology, University of Michigan Medical School, Ann Arbor, MI, 48109-5632, USA.

* indicates equal contribution

Correspondence to:

Peter C Kind$
Simons Initiative for the Developing Brain
Hugh Robson Building,
University of Edinburgh,
Edinburgh, UK, EH8 9XD
pkind@ed.ac.uk

Sam A. Booker
Centre for Discovery Brain Sciences
Hugh Robson Building,
University of Edinburgh,
Edinburgh, UK, EH8 9XD
sbooker@ed.ac.uk

Figures: 5 (+5 supplementary)

Tables: 0 (+2 supplementary)

$ - Lead Contact

Summary:

Cellular hyperexcitability is a salient feature of Fragile X Syndrome animal models. The cellular basis of hyperexcitability and how it responds to changing activity states is not fully understood. Here we show increased axon initial segment length in CA1 of the *Fmr1*$^{-/y}$ mouse hippocampus, with increased cellular excitability. This change in length did not result from reduced AIS plasticity, as prolonged depolarisation induced changes in AIS length independent of genotype. However, depolarisation did reduce cellular excitability; the magnitude of which was greater in *Fmr1*$^{-/y}$ neurons. Finally, we observe reduced functional inputs from entorhinal cortex with no genotypic difference in firing rates of CA1 pyramidal neurons. This suggests that AIS-dependent hyperexcitability in *Fmr1*$^{-/y}$ mice may result from adaptive or homeostatic regulation induced by reduced functional synaptic connectivity. Thus, while AIS length and intrinsic excitability contribute to cellular hyperexcitability, they may reflect a homeostatic mechanism for reduced synaptic input onto CA1 neurons.

Key words:

Fragile X Syndrome, ASD/ID, Axon Initial Segment, Hyperexcitability, Homeostasis, Structural Plasticity, Intrinsic Excitability, Whole-Cell Patch-Clamp, 2-Photon Microscopy, Hippocampus

Introduction:

Fragile X Syndrome (FXS) is the leading single gene cause of intellectual disability with co-occurring autism, hyperactivity and epilepsy (Hagerman et al., 1996). FXS results from hypermethylation of the *FMR1* gene and loss of the protein FMRP. Rodent models of FXS have revealed a role for FMRP in directly and indirectly regulating cellular and local circuit excitability (Contractor et al., 2015; Gibson et al., 2008; Zhang et al., 2014). They also display behavioural phenotypes, such as increased susceptibility to audiogenic seizures that have been linked to cellular/circuit hyperexcitability (Dölen et al., 2007; Musumeci et al., 2000; Osterweil et al., 2010). Furthermore, it has been suggested that impairments in learning and memory may result, in part, from these alterations in cellular excitability (Contractor et al., 2015).

Cellular excitability is tightly regulated by, for example: intrinsic mechanisms such as ion channel density and function, synaptic mechanisms that regulate the balance of excitation to inhibition (E:I balance) and the regulation of action potential initiation at the axon initial segment (AIS). FMRP

has been shown to directly regulate excitability through direct FMRP/ion channel interactions (Deng and Klyachko, 2016; Deng et al., 2013). Loss of FMRP also potentially alters the translational regulation, and hence the density and/or activity-dependent expression of ion channels and synaptic proteins critical for regulating excitability (Antoine et al., 2019; Booker et al., 2019; Brager et al., 2012; Bülow et al., 2019; Bureau et al., 2008; Gibson et al., 2008). However, changes in cellular excitability may not be a direct consequence of the loss of FMRP, rather excitability phenotypes may arise as a compensatory or homeostatic change to try to normalise neuronal excitability following altered FMRP-dependent events. Indeed, recent findings indicate that the altered E:I balance observed in the mouse model of FXS serves to normalise neuronal excitability in primary somatosensory cortex (Antoine et al., 2019; Domanski et al., 2019).

The axon initial segment (AIS) plays a key role in regulating neuronal excitability. The AIS originates from the soma or proximal dendrite (Thome et al., 2014) where it is required for the correct integration and dynamic control of action potential (AP) generation (Leterrier (2016)). Although axonal action potentials can still be evoked when the AIS is dissolved (Zonta et al., 2011), spontaneously generated APs are abolished and the waveform of the AP is altered. The AIS is composed of specific cytoskeletal elements (i.e. Ankyrin G and β4-spectrin) and a high density of voltage gated sodium channels (VSGC; (Leterrier, 2016; Ogawa and Rasband, 2008)). Importantly, the AIS is not static, but can regulate its length and position in response to changing neuronal activity states (Grubb and Burrone, 2010; Grubb et al., 2011; Gutzmann et al., 2014; Kuba et al., 2010). Hence, it can act as a means of homeostatic regulation the neuron AP discharge in response to changing activity levels. For example, in cultured dentate granule cells, the AIS shortens in length following prolonged depolarisation (Grubb et al., 2011) while *in vivo*, the AIS has been suggested to increase in length following prolonged sensory deprivation, and modulate VGSC density (Gutzmann et al., 2014; Kuba, 2012; Kuba et al., 2010). However, these homeostatic alterations to AIS length and function require long-term alteration in neuronal activity, which at present have not been observed in CA1 of the hippocampus (Klemmer et al., 2011).

Despite playing a key role in regulating cellular excitability, the AIS has received little attention in FXS, or indeed developmental disorders more generally. A notable exception is an increase in AIS length observed in the Angelman's Syndrome model of intellectual disability (Kaphzan et al., 2011) which shares some excitability features with FXS, as well as a variety of epilepsy mouse models also displaying AIS dependent phenotypes (Wimmer et al., 2010). Here, we directly examine whether the AIS is altered in a mouse model FXS. We focus on pyramidal cells of the

hippocampal area CA1, due to emerging evidence for enhanced cellular excitability of these neurons in *Fmr1*$^{-/y}$ mice (Luque et al., 2017; Talbot et al., 2018). The current study combines immunohistological labelling, whole-cell patch-clamp recordings and live 2-photon imaging to test the hypothesis that AIS pathology contributes to the excitability phenotypes of *Fmr1*$^{-/y}$ mice.

Results:

CA1 PCs are hyperexcitable in the Fmr1$^{-/y}$ *mouse, resulting from increased AIS lengths*

To first assess the excitability of CA1 pyramidal cells (CA1 PCs) we performed whole-cell patch-clamp recordings from acute hippocampal slices of postnatal (P) day 28-35 WT and *Fmr1*$^{-/y}$ littermate mice. We first measured the active properties of CA1 PCs in response to small depolarising steps (25 pA, 500 ms), all from a holding potential of -70 mV to induce well controlled AP discharge (**Figure 1A**). Analysis of AP discharge revealed a leftward shift in the current-frequency response in *Fmr1*$^{-/y}$ mice compared to WT (**Figure 1B**). WT CA1 PCs (33 cells from 15 mice) reliably produced APs with a rheobase current of 156.8 ± 11.6 pA, with a voltage threshold of -42.6 ± 0.6 mV, and peak AP discharge of 36.2 ± 1.1 Hz. In *Fmr1*$^{-/y}$ littermates (29 cells from 14 mice), we observed a significantly lower rheobase current of 111.7 ± 10.8 pA ($\chi^2_{(d.f.60)}$ = 16.6, p = 4.6 x 10^{-5}, GLMM), resulting from a more hyperpolarised voltage threshold of -45.9 ± 1.1 mV ($\chi^2_{(d.f.60)}$ = 13.5, p =0.0002, GLMM). The maximum firing of *Fmr1*$^{-/y}$ neurons was 38.9 ± 1.0 Hz, not significantly higher than WT ($t_{(11,8)}$=1.53, p=0.50, Holm-Sidak test). To confirm that this hyperexcitability was due to altered voltage-threshold, we measured passive membrane properties in *Fmr1*$^{-/y}$ and WT mice. CA1 PCs from WT mice had a resting membrane potential of -60.2 ± 1.4 mV and, in response to small, hyperpolarising steps (-10 pA, 500 ms), an input resistance of 162.1 ± 14.2 MΩ. In *Fmr1*$^{-/y}$ mice input resistance was not different from that of WT (169.4 ± 10.6 MΩ, $\chi^2_{(d.f.60)}$ =0.81, p = 0.37, GLMM), however the resting membrane potential was substantially more hyperpolarised (-64.2 ± 1.9 mV, $\chi^2_{(d.f.60)}$ =8.9, p = 0.003, GLMM). We observed no difference in AP kinetic properties, such as amplitude, half-height duration, or maximum rates of rise or decay, which are detailed in Supplemental Table 1. Further, a full summary of all statistical tests performed in the current study are provided in Supplemental Table 2. Together, these data show that CA1 PCs in *Fmr1*$^{-/y}$ mice appear hyperexcitable due to a reduced threshold for AP discharge.

The presence of a high density of sodium channels at the AIS, and its role in controlling voltage threshold (Kole et al., 2008) led us to next ask whether the AIS was structurally altered in the FXS

mouse model, as in the Angelman's Syndrome mouse model (Kaphzan et al., 2011). Immunohistochemical labelling was performed on hippocampal sections from perfusion-fixed mice using AnkyrinG, a specific AIS marker (**Figure 1G**). AnkyrinG reliably labelled AIS emerging from the soma (~80% of AIS) or a proximal dendrite (~20% of AISs), which entered the *str. oriens* of CA1. The average AIS length in WT mice was 25.9 ± 0.3 µm (750 AIS from 8 mice) meanwhile *Fmr1*$^{-/y}$ mice had AISs which on average were 20% longer, with an average length of 31.0 ± 0.3 µm (896 AIS from 9 mice; t-ratio $_{(d.f.10)}$ =-3.67, p = 0.0046, GLMM; **Figure 1H, I**). We confirmed that the number of AIS measured per mouse was sufficient to define the difference we observe using boot-strapping analysis (**Figure S1A and S1B**). We observed similar distances from soma for AIS measured in *Fmr1*$^{-/y}$ CA1 PCs (2.7 ± 0.2 µm from soma) as compared to WT (3.2 ± 0.2 µm, p=0.30, LMM**, Figure S1C**). To determine whether these effects on AIS length were specific to the CA1 or were more widespread across the brain, we next assessed the AIS length in medial prefrontal cortex (mPFC) and hippocampal subfield CA3 (**Figure S1E-H**). In both areas tested, the AIS length was longer in *Fmr1*$^{-/y}$ mice, with 12% and 11% longer AISs from L5 mPFC and CA3 respectively. Analysis of the intrinsic excitability of layer 5 (L5) medial prefrontal cortex PCs revealed that whilst these neurons showed increased excitability, although this was not associated with any change in voltage threshold or rheobase (**Figure S1I-K**). These data suggest that while AIS length may be longer in these other brain areas, it is does not contribute to large physiological changes.

As many factors contribute to the overall excitability of a given neuron, we next asked if this change in AIS length was sufficient to account for the altered voltage threshold. To address this, we produced a single-cell computational model of a CA1 PC based on realistic constraints (Migliore, 2003) and altered AIS length, assuming no change in sodium-channel density (**Figure S2**). Under these conditions, only changing the AIS length results in near identical changes in voltage-threshold and rheobase, without affecting maximum discharge rate. Taken together this data reveals an increase in CA1 PC excitability, primarily due to a voltage threshold change. Furthermore, the region specific increase in AIS length observed in *Fmr1*$^{-/y}$ mice is sufficient to account for the altered excitability.

CA1 PCs in acute hippocampal slices display activity-dependent excitability changes, which is enhanced in Fmr1$^{-/y}$ *mice.*

A key feature of neuronal excitability is its ability to self-modulate in the face of sustained alteration to ongoing activity (Marder and Prinz, 2002). The ability of neurons to undergo such homeostatic

regulation has been shown to be enhanced in the mouse model of FXS, albeit in cultured neurons (Bülow et al., 2019). Therefore, we next asked whether the enhanced excitability we observe in *Fmr1*$^{-/y}$ mice results from underlying changes in cell homeostasis, leading to inappropriate regulation of cell function, using a paradigm previously reported to depolarise neurons over short (Evans et al., 2015; Grubb et al., 2011) and long (Grubb and Burrone, 2010; O'Leary et al., 2010) time scales *in vitro*. To directly assess the effect of depolarisation in a cell-wise manner, we performed whole-cell patch-clamp recordings from the same CA1 PCs before and after 3 hour application of 15 mM KCl, using 15 mM NaCl as an osmotic control. Cells were targeted by inclusion of AlexaFluor 488 (100 µM) in the patch pipette to allow recording from the same neuron and examine intrinsic physiology at both time points (**Figure 2A, B**). From recordings of WT CA1 PCs, 3 hours treatment with 15 mM NaCl did not alter the current-frequency relationship ($t_{1, 11}$ = 0.51, p= 0.49, RM 2-way ANOVA, **Figure 2C, left**) or any other measure physiological property measured. By comparison, 3 hours treatment with 15 mM KCl resulted in a small, but significant decrease in AP discharge in WT CA1 PCs ($t_{1, 18}$ = 6.37, p= 0.02, RM 2-way ANOVA, **Figure 2C, right**), consistent with previous reports (Evans et al., 2015; O'Leary et al., 2010). This change in AP discharge was paired with a 39% increase in rheobase current from 159 ± 13 pA under control to 221 ± 24 pA after KCl (p = 0.002, LMM, **Figure 2E**) and a 23% reduction in input resistance (p = 0.05, LMM), as well as altered K^+ channel function, as the AP decay rate was slowed by 14% (p=0.05, LMM). However, we observed with no change in voltage-threshold (2% depolarised, p = 0.91, LMM, **Figure 2F)**. These findings suggest that AIS structure may be unrelated to the observed physiological changes following such treatment in *ex vivo* brain tissue, which are likely due to multiple, complementary K^+-channel mechanisms (Kole et al., 2007; Kuba et al., 2015; O'Leary et al., 2010).

We next assessed the effect of sustained depolarisation on the function of *Fmr1*$^{-/y}$ CA1 PCs in acute brain slices. 15 mM KCl also appeared to very strongly attenuate the current-frequency relationship in *Fmr1*$^{-/y}$ mice ($t_{1, 17}$ = 21.95, p= 0.0002, RM 2-way ANOVA, **Figure 2B and D**). 15 mM NaCl treatment for 3 hours had no effect on AP discharge ($t_{1, 6}$ = 0.14, p= 0.72, RM 2-way ANOVA, **Figure 2D, left**). Similar to observations from WT mice, we observed a 66% increase in rheobase from 113 ± 11 pA to 188 ± 18 pA; (p = 0.004, LMM, **Figure 2E**) and a tendency to decreased input resistance (-14%, 197 ± 22 MΩ to 167 ± 20 MΩ; p = 0.17, LMM). As for WT mice, we saw no change in voltage threshold following KCl treatment (p = 0.95, LMM, **Figure 2F**). We observed an 18% slowing of the AP decay rate (p = 0.008, GLMM), indicative of a potentially similar role of K^+ channels in this physiological plasticity as in WT neurons. To allow us to compare the change in AP discharge between WT and *Fmr1*$^{-/y}$ mice, we subtracted the number of APs

produced following KCl application from the control measurement, as each current step tested **Figure 2G**). We observed a much larger decrease in the number of APs elicited by *Fmr1*$^{-/y}$ CA1 PCs, compared to WT ($F_{1, 35}$ =23.84, p<0.0001, 2-way ANOVA), which was most apparent over the range of 175 - 300 pA (Interaction: $F_{1, 35}$ = 2.225, p=0.0052; Bonferroni post-tests $T_{17,20}$ = 3.0, 3.0, 3.2, 3.3, 3.3, 3.2; p= 0.05, 0.04, 0.03, 0.02, 0.02, 0.03) In no tested intrinsic parameter did we observe a change following 3 hour treatment with NaCl treatment. These data clearly show that responses to prolonged depolarisation are present in both WT and *Fmr1*$^{-/y}$ mice, however the latter respond more strongly to depolarising stimuli, leading to reduced AP generation.

Short term depolarisation results in AIS shortening in WT and Fmr1$^{-/y}$ *cultured neurons, but lengthening in intact tissue.*

We show above that prolonged depolarisation of neurons in *ex vivo* tissue leads to a reduction in cellular excitability. Previously, it has been shown that application of 15 mM KCl reduces AIS length, over short time-scales in cultured dentate gyrus neurons (Evans et al., 2015; Grubb et al., 2011). Therefore, we next asked whether this form of plasticity was present in *Fmr1*$^{-/y}$ CA1 neurons, perhaps accounting for the differences in AIS length, baseline excitability, and enhanced cellular responses observed.

To address this, we used the same 15 mM KCl or NaCl treatment as we used for the physiological manipulation (Figure 2), but in a subset of acute hippocampal slices. Following slicing and recovery, slices were transferred to chambers containing ACSF, treated for 3 hours with 15 mM KCl or NaCl (as osmotic control) then fixed and immunolabelled for AnkyrinG (**Figure 3A**). Following 15 mM KCl treatment of slices we observed AIS lengths in WT mice of 28.7 ± 1.7 µm, which were 11% longer than that of 15 mM NaCl osmotic controls (25.8 ± 1.4 µm, p<0.001, GLMM **Figure 3B**). In *Fmr1*$^{-/y}$ slices, also we observed a similar increase in AIS length, with AIS lengths following 15 mM KCl treatment of 29.4 ± 1.9 µm, also 11% longer than NaCl controls (26.6 ± 1.6 µm; p<0.001, GLMM; **Figure 2I**). We observed no change in AIS distance from soma following 3 hour 15 mM KCl treatment, as compared to NaCl controls (**Figure S2B**). These data clearly indicate that short-term structural AIS shortening in CA1 neurons, over the timescales as described previously in cultured dentate gyrus neurons, is absent from intact tissue from WT and *Fmr1*$^{-/y}$ mice. In 10-day old mice, we observed a very small shortening of the AIS in WT mice after 3 hours of KCl, which was not present in *Fmr1*$^{-/y}$ neurons (**Figure S3**).

As this form of plasticity has only been previously reported in cell culture, we finally asked whether we could confirm that this form of plasticity was present in our hands, and test whether *Fmr1*$^{-/y}$ neurons are capable of shortening. To examine AIS structure in dissociated hippocampal cultures of WT and *Fmr1*$^{-/y}$ mice (**Figure 3D**) we fixed coverslips at day *in vitro* 10 (DIV10), before and after 15 mM KCl or NaCl treatment, then measured the AIS from AnkyrinG labelling. In WT primary hippocampal neurons, the AIS had an average length of 29.9 ± 1.0 µm. Similar to observations in fixed brains, *Fmr1*$^{-/y}$ neurons had AISs which on average were 7% longer than WT (32.1 ± 0.7 µm, $p < 0.001$, LMM, **Figure 3E**), confirming that hippocampal cultures obey the same relationship as *ex vivo* tissue. Following 15 mM KCl addition to the culture medium for 3 hours WT AIS length in 15 mM KCl was 28.1 ± 1.2 µm, 10% shorter than NaCl controls (31.1 ± 1.2 µm, p<0.001, LMM **Figure 3F**). In *Fmr1*$^{-/y}$ cultures, we observed similarly AIS shortening with 15 mM KCl (30.3 ± 0.8 µm) being 7% shorter than NaCl controls (32.7 ± 1.1 µm, p<0.001, LMM, **Figure 3G**). The degree of AIS shortening between KCl and NaCl was modest, but similar, for both WT and *Fmr1*$^{-/y}$ mice (p=0.41, $T_{(d.f.29)}$=0.82, two tailed Student's T test **Figure 3H**). These data indicate that short-term structural AIS plasticity is present in cultured WT and *Fmr1*$^{-/y}$ neurons, but that different or additional factors may regulate structural plasticity following sustained depolarisation in hippocampal neurons, such as the presence of an intact extracellular matrix. Further, these findings suggest a functional disconnect between AP discharge properties and AIS length following prolonged depolarisation.

One limitation of the above data is that AIS lengths before and after plasticity induction are measured in fixed tissue from different slices, thus precluding direct measurement of potential within-cell shortening of AIS. As such, we next asked if the AIS length of individual CA1 PCs were plastic during the application of 15 mM KCl. To address this, we performed 2-photon live imaging of acute hippocampal slices from a new transgenic mouse expressing GFP fused to the sodium channel β1 subunit (β1-NaV-GFP) which reliably labelled the AIS (**Figure 4A and 4B**), which faithfully reflected AnkyrinG immunolabelling **(Figure S4)**. This transgenic was crossed with the *Fmr1*$^{-/y}$ mouse line and 2-photon z-stacks of images containing *str. pyramidale* and proximal *str. oriens* of CA1 were collected before and after 3 hour treatment with either 15 mM KCl or 15 mM NaCl; AIS lengths were then measured (**Figure 4C**). Comparison of the starting AIS length to the final length showed no difference between either 15 mM NaCl or 15 mM KCl in WT ($F_{1, 158} = 0.62$, $p = 0.43$, sum-of-least-squares F-test, **Figure 4D**) or *Fmr1*$^{-/y}$ mice ($F_{1, 85} = 1.63$, $p = 0.21$, sum-of-least-squares F-test, **Figure 4E**). Comparing the AIS length change between treatments from live imaging in WT mice, we observed that 3 hour 15 mM NaCl treatment produced an average of 1.6 ± 1.0 µm increase in length, not different from the 1.1 ± 1.0 µm increase in length produced

by 15 mM KCl treatment (P=0.67, LMM). NaCl application to brain slices from *Fmr1*$^{-/y}$ mice produced a 1.6 ± 1.3 µm shortening of the AIS, not different from the 2.1 ± 2.0 µm shortening observed in KCl (P=1.0, LMM). Finally, modelling of AIS lengths over the ranges we measured from CA1 PCs reveals that AIS length has the ability to change both AP discharge threshold and rheobase in a non-linear manner. However, small changes in membrane resistance, like those we observe following KCl treatment, may best describe the homeostatic changes in rheobase that we observe (**Figure S2H and S2I**). Taken together, these data further confirm that short-term, depolarisation-induced AIS shortening is absent in *ex vivo* tissue of CA1, even when measured at the single-cell level in real time.

Reduced temporoammonic input to CA1 accounts for homeostatic changes to PCs.

The data we have presented so far show that CA1 PCs in *Fmr1*$^{-/y}$ mice are hyperexcitable, due to increased AP discharge related to increased AIS length, and greater short-term adaptation of membrane excitability. These observations could reflect homeostatic remodelling of CA1 PCs in the *Fmr1*$^{-/y}$ mouse, in response to reduced excitatory tone. To determine whether extrinsic temporoammonic (TA) input to CA1 is reduced in *Fmr1*$^{-/y}$ mice, we performed correlated extracellular field and whole-cell patch-clamp recordings from CA1, combined with electrical stimulation of the TA afferents. Brief trains of electrical stimuli (5 stimuli at 20 Hz, 200 µs stimulus duration) were delivered via a bipolar, twisted NiChrome wire placed in distal *str. lacunosum-moleculare* approximately 1 mm distal to CA1. Input-output relationships were recorded from as both: somatic whole-cell excitatory postsynaptic potentials (EPSP) or field EPSPs (fEPSPs; **Figure 5A**) from *str. lacunosum-moleculare* of CA1. To first assess the degree of TA afferent recruitment, we measured the afferent fibre-volley from fEPSP recordings. We observed that increasing constant-voltage stimuli resulted in a linearly increasing fibre-volley. The relative recruitment of the fibre-volley was similar in *Fmr1*$^{-/y}$ slices, when compared to WT ($F_{1, 348}$ =2.12, p=0.15, Sum-of-least squares F-test), suggesting equivalent recruitment of entorhinal afferents as previously described (Wahlstrom-Helgren and Klyachko, 2015). To determine whether synaptic responses from the TA pathway were altered we measure both the dendritic field and somatic whole-cell responses, measured as a function of fibre-volley amplitude (**Figure 5B**). Despite unchanged fibre-volley amplitudes, the input-output response for fEPSPs in *Fmr1*$^{-/y}$ brain slices was significantly reduced compared to WT, in both peak amplitude ($F_{1, 348}$ = 33.7, p<0.0001, Sum-of-least squares F-test) and area-under-the-curve ($F_{1, 303}$ = 32.1, p<0.0001, Sum-of-least squares F-test). Consistent with this observation, whole-cell EPSPs showed lower input-output

relationships as well ($F_{1, 346}$ = 5.55, p=0.019, Sum-of-least squares F-test, **Figure 5G**) indicating that dendritic filtering is insufficient to overcome reduced synaptic inputs (Brager et al., 2012). As synaptic inhibition and excitation is known to be altered at TA inputs (Wahlstrom-Helgren and Klyachko, 2015) we next asked whether altered inhibition was the cause of the reduced input. Blocking $GABA_A$ receptor-mediated inhibition with picrotoxin was also insufficient to overcome reduced dendritic inputs in *Fmr1*$^{-/y}$ mice both in fEPSP recordings ($F_{1, 370}$ = 23.6, p<0.0001, Sum-of-least squares F-test, Figure 4F) and whole-cell EPSPs ($F_{1, 324}$ = 21.8, p<0.0001, Sum-of-least squares F-test, Figure 4H). Comparing the slopes of individual CA1 PC input-output plots, *Fmr1*$^{-/y}$ cells had lower EPSP recruitment under both control (t_{30}=3.21, p=0.039, LMM; Figure 4I) and in the presence of picrotoxin (t_{30}=3.21, p=0.039, LMM; Figure 4I). Together these data show that TA inputs to the CA1 region of the hippocampus are reduced in strength, providing a plausible explanation for homeostatic compensation of CA1 PC intrinsic physiology and AIS structure, during postnatal development. To test whether to the changes in excitability may represent a homeostatic compensation for the decrease in synaptic input, we examined the probability of spiking of CA1 neurons to a range of stimulation voltages using cell-attached recording, also in the presence of picrotoxin. Despite the decrease in synaptic input to CA1 neurons, no genotypic differences were observed in the output of neurons to electrical stimulation of *str. lacunosum-moleculare* (**Figure 5K and 5L**). Furthermore, the spike output of CA1 PCs was not affected when biologically relevant 20 Hz trains of stimuli were delivered to *str. lacunosum-moleculare* (**Supplementary Figure 5**). Together these data show that homeostatic alterations of CA1 PC intrinsic excitability is sufficient to regulate their spiking output.

Discussion:

In the current study we show that hippocampal CA1 PCs are intrinsically hyperexcitable in a mouse model of FXS, likely due to an underlying increase in AIS length. We show that this genotype specific length change is not due to a lack of plasticity at the AIS, as short-term structural plasticity is present in cultured *Fmr1*$^{-/y}$ neurons. Furthermore, in *ex vivo* tissue we do not observe a depolarisation induced decrease in AIS length in agreement with previous findings in CA1 (Evans et al., 2015) suggesting restriction of AIS length changes in intact tissue on the timescales described. Despite the lack of AIS length decrease (indeed we observe a small but significant increase in AIS length), we do observe a decrease in intrinsic excitability following prolonged depolarisation, the magnitude of which was significantly greater in *Fmr1*$^{-/y}$ mice. We also observed reduced entorhinal input to CA1 PCs, and in the absence of altered inhibition, suggesting a

homeostatic regulation of intrinsic excitability. In support of this activity-dependent homeostatic change in excitability, cell-attached recordings showed no genotypic differences in spike output to entorhinal axon stimulation. In agreement with previous studies, our findings reveal a range of cellular alterations in *Fmr1*$^{-/y}$ mice, some of which are likely compensatory or homeostatic, that result in altered circuit function.

Hyperexcitability in the Fmr1$^{-/y}$ *mouse model and AIS length:*

In the mouse model of Fragile X Syndrome, local microcircuits are hyperexcitable (Contractor et al., 2015; Gibson et al., 2008; Zhang et al., 2014). Indeed, increased intrinsic neuronal excitability has been observed in hippocampal neurons from CA1 (Luque et al., 2017), CA3 (Deng et al., 2013), and entorhinal cortex (Deng and Klyachko, 2016); as well as neocortex (Routh et al., 2017; Zhang et al., 2014). The loss of FMRP has been shown to alter cellular excitability through a range of mechanisms. For example, FMRP directly binds to K^+-channels and the loss of this interaction alters action potential kinetics (Brown et al., 2010; Deng and Klyachko, 2016; Deng et al., 2013). Other studies have suggested altered ion channel expression, resulting from homeostatic regulation or altered mRNA translation (Brager et al., 2012; Routh et al., 2013; Zhang et al., 2014). In this context, we have also observed a decrease in resting membrane potential in CA1 neurons in the *Fmr1*$^{-/y}$ rats. Although difficult to directly relate changes in resting membrane potential in acute slice to neurons *in vivo*, any alteration in resting membrane potential, possibly from an altered K+ leak current, would likely contribute to the altered excitability of these neurons. Changes in cellular excitability, through subsequent effects on circuit dynamics could explain a range phenotypes, from altered cognition to increased sensory reactivity and motor dysfunction (Biane et al., 2015; Breton and Stuart, 2009; Marder and Goaillard, 2006; Ransdell et al., 2012). The change in AIS length shown here in *Fmr1*$^{-/y}$ mice constitutes a further potential mechanism contributing to cellular hyperexcitability in hippocampal CA1 pyramidal neurons. Indeed, the AIS length changes are unlikely related to dendritic complexity (Hamada et al., 2016), as AIS location relative to soma was consistent between genotypes. AIS length modulation and increased intrinsic excitability has also been observed in CA1 of the Angelman's Syndrome mouse model (Kaphzan et al., 2011). This commonality of AIS regulation in another monogenic model of local circuit hyperexcitability suggests a potential convergent mechanism of hyperexcitability between models of ASD/ID.

Absence of short-term AIS shortening in ex vivo neurons:

In the current study we confirm that AIS undergo short-term structural plasticity in primary dissociated cell-culture following application of a depolarising stimuli, as has been shown previously for dentate granule neurons (Evans et al., 2015). Importantly, this form of plasticity is intact in CA1 *Fmr1*$^{-/y}$ neurons indicating that FMRP in unlikely to play a role in the mechanism by which AIS length is regulated by activity. Furthermore, given that we observed longer AISs in *Fmr1*$^{-/y}$ CA1 neurons, *in vivo* and in a more simplified neuronal circuit present *in vitro*, our data suggest that altered AIS length may be a compensatory or homeostatic mechanism used to regulate neuronal excitability in the mouse model of FXS. Intriguingly, we did not observe a shortening of the AIS in acute CA1 hippocampal slices from 4-5 week old mice in contrast to cell culture from other hippocampal cell types. Instead we observed a small, but consistent increase in AIS length in CA1 following KCl treatment. Given that we observed the length changes in cultured neurons from the same mouse colony we believe that our data casts doubt on the ability of neurons to undergo shortening on the timescales described in cell culture, in *ex vivo* tissue. However, it should be noted that we have only tested one time-point (i.e. 3 hours post stimulation). It is possible that transient changes in AIS length could be taking place over a shorter time-frame, may take longer to manifest, or may be age dependent. Nonetheless, it is clear that the changes in excitability to KCl treatment observed in the current study cannot be explained by changes in AIS length.

Despite an absence of AIS shortening, we did observe a dampening of neuron excitability following 3 hours of 15 mM KCl, as shown in cell culture experiments (Evans et al., 2015) or following longer incubation of KCl (O'Leary et al., 2010), and which also oppose the length changes we observe if the rule of longer AIS equates to increased excitability. Since changes in AIS length cannot explain the changes in excitability, other mechanisms must be involved. One possibility could be altered potassium channel expression or function. Indeed, numerous potassium channels have been linked to pathophysiology in FXS (Contractor et al., 2015; Deng et al., 2013; Zhang et al., 2014). The observed slowing of AP decay kinetics is consistent with altered Kv1.1. function (Kole et al., 2007) and the reduction in input resistance we observe could result from increased leak potassium currents (O'Leary et al., 2010), altered M-channel activity (Wu et al., 2008; Yue and Yaari, 2006; Zhang et al., 2014), or altered tonic $GABA_A$ receptor activation (Curia et al., 2009) . The homeostatic changes in cellular excitability were stronger in *Fmr1*$^{-/y}$ mice, which could result from altered proteostasis (Louros and Osterweil, 2016; Richter et al., 2015) or that neuronal spiking in *Fmr1*$^{-/y}$ mice responds more strongly to stimuli on short-time scales, thus recruiting ion channels that are typically surplus to the required activity state (O'Leary

et al., 2013). However, it is beyond the scope of the current manuscript to identify the precise mechanism underlying the observed changes in cellular excitability.

It has recently been shown that intrinsic homeostasis is altered in cultured *Fmr1*$^{-/y}$ cortical neurons following reduced activity (Bülow et al., 2019), based on neuronal discharge properties not regularly observed in intact brain tissue (Connors and Gutnick, 1990). We now show that increasing activity, results in an exaggerated decrease in neuronal activity in neurons from *Fmr1*$^{-/y}$ mice in *ex vivo* slices. However, consistent with recent findings from somatosensory cortex (Antoine et al., 2019) our finding that CA1 neuronal output is normal despite the decrease in synaptic input from entorhinal cortex suggest that homeostatic mechanisms are able to reset neuronal firing in *Fmr1*$^{-/y}$ neurons.

Network level homeostatic activity in the Fmr1$^{-/y}$ *mouse:*

Altered long-range connectivity has been proposed as a mechanism for cognitive impairment in FXS and *Fmr1*$^{-/y}$ mice (Haberl et al., 2015; van der Molen et al., 2014; Wang et al., 2017), as well as *in vitro* (Bureau et al., 2008; Harlow et al., 2010). Hippocampal function, particularly spatial and episodic memory, has been shown to be deficient in the mouse and rat models of FXS, respectively (Asiminas et al., 2019; D'Hooge et al., 1997; Talbot et al., 2018; Till et al., 2015), however most studies have only examined synaptic function and plasticity at specific synapses, i.e. Schaffer-Collaterals (Huber et al., 2002). The entorhinal cortex is known to drive spatial inputs to CA1 (Fyhn et al., 2004; Miller and Best, 1980), and forms the TA pathway into the CA1 region (Amaral and Witter, 1989). Therefore, the reduced synaptic input we observe in the *str. lacunosum-moleculare* (co-aligning with TA inputs), may serve as a synaptic correlate of reduced spatial performance in FXS mice. The source of this reduced input could originate in the presynaptic domain (Klemmer et al., 2011), as entorhinal input to the dentate gyrus is also reduced (Yun and Trommer, 2011). However presynaptic release properties are not altered at this synapse in *Fmr1*$^{-/y}$ mice (Wahlstrom-Helgren and Klyachko, 2015). An alternative explanation for the altered homeostatic plasticity is altered HCN channel density or function (Shah, 2014), since HCN channels are strongly upregulated in the distal dendrites of CA1 PCs in *Fmr1*$^{-/y}$ mice (Brager et al., 2012). Given the role of HCN channels in reducing dendritic gain (Magee, 1998) and enhancing dendritic supralinearity (Branco and Häusser, 2011), it is plausible that increased HCN channels expression is regulated by reduced TA input. HCN channels may be an additional homeostatic element in *Fmr1*$^{-/y}$ mice, given that their expression is also bidirectional in different cell types (Booker et al., 2019; Kalmbach et al., 2015). In summary, we now postulate that the

increased AIS length and intrinsic excitability in *Fmr1*$^{-/y}$ neurons result from decreased excitation from extrinsic synaptic inputs through altered synaptic strength during development (Booker et al., 2019; Domanski et al., 2019; Harlow et al., 2010).

Acknowledgments**:** The authors would like to thank Olivia Bailey and Naima Eloisgui-Borras for assistance with imaging, Dr. Elizabeth Davenport for E17.5 mouse sexing, and all members of the Kind and Wyllie laboratories for their helpful discussions. We thank our funders: The Simons Foundation Autism Research Initiative (529085), The Patrick Wild Centre, Medical Research Council UK (MR/P006213/1), and the Shirley Foundation and the RS Macdonald Charitable Trust.

Author Contributions:

PCK, DJAW, SAB, LSO - conceptualised and designed the experimental plan; SAB, LSO, NJA – performed and analysed electrophysiology and imaging experiments; ZK, ORD – performed statistical modelling; ADJ – performed computational neuron modelling; LLI, DLS, PJB – generated and validated β1-NaV-GFP mice; PSB, GEH – developed cell culture assays; SAB, LSO, GEH, DLS, PJB, DJAW, PCK – devised experiments; all authors contributed to writing of the manuscript.

Figure Legends:

Figure 1: Increased CA1 PC excitability and AIS length in *Fmr1*$^{-/y}$ mice. A representative voltage responses from WT (black) and *Fmr1*$^{-/y}$ mice (red), in response to depolarising current steps (0 - 400 pA, 25 pA steps, 500 ms duration). **B** current-frequency plot for WT (33 cells from 15 mice) and *Fmr1*$^{-/y}$ (29 cells from 14 mice), indicating increased AP discharge. **C-F** quantification of rheobase, voltage threshold, resting membrane potential and input resistance from both genotypes. **G, upper** overview flattened confocal stacks of CA1 labelled for AnkyrinG (green pseudocolour) and NeuN (blue pseudocolour). **G, lower** high-power magnification of single AIS. The AIS total extent is indicated (dashed line). Scale bars (upper): 20 µm (lower): 5 µm. **H** average cumulative distributions (thick lines) of AIS lengths across all mice examined for each genotype. Cumulative distributions for individual mice shown underlain (thin lines). **I** Quantification of AIS length for each genotype (750 AIS from 8 WT mice, 896 AIS from 9 *Fmr1*$^{-/y}$ mice). All bar chart data is overlain by averages of individual mice, with total mice analysed in parenthesis. Statistics

shown: ns – $p > 0.05$, * - $p < 0.05$ from 2-way RM ANOVA (**B**) and GLMM (**C-I**). All data is shown as mean ± SEM.

Figure 2: Intrinsic physiological plasticity and homeostatic responses in WT and *Fmr1*$^{-/y}$ mice. A, B representative voltage responses from WT (black) and *Fmr1*$^{-/y}$ (red) CA1 PCs to current injections, from -70 mV (0 - 400 pA, 25 pA steps, 500 ms duration). **C** current-frequency plots for the same CA1 PCs from WT mice, when recorded before (top) and after (bottom) 3 hr NaCl (12 cells from 5 mice) or KCl (19 cells from 8 mice) applications. **D** according to the same format as C, but for *Fmr1*$^{-/y}$ mice (NaCl: 7 cells from 4 mice; KCl: 18 cells from 8 mice). Pairwise analysis of rheobase current (**E**) and voltage threshold (**F**) from the same WT and *Fmr1*$^{-/y}$ CA1 PCs. **G** subtracted AP discharge across the range of injected currents given to CA1 PCs. Statistics shown: ns – $p > 0.05$, * $p < 0.05$ from GLMM (E-G) and 2-way RM ANOVA (C,D,H). All data is shown as mean ± SEM.

Figure 3: Short term AIS shortening is absent in acute slices following sustained depolarisation. A Representative flattened confocal stack of AIS labelled in acute hippocampal slices from WT (upper) and *Fmr1*$^{-/y}$ mice, following 3 hour incubation with 15 mM KCl (right) or NaCl osmotic controls (left). AIS were visualised with AnkyrinG (green pseudocolour) and measured in neurons labelled with NeuN (blue pseudocolor). Scale bars: 20 μm. **B** Quantification of AIS length following 3 hour application of 15 mM KCl, compared to NaCl osmotic controls in WT mice. Average AIS length of each mouse tested is shown overlaid. **C** The same analysis but in *Fmr1*$^{-/y}$ mice. **D** AIS measured in primary dissociated hippocampal cell-cultures produced from WT (left) and *Fmr1*$^{-/y}$ (right) mice following 3 hours of 15 mM NaCl or KCl and labelled with AnkyrinG (green) and NeuN (blue). Scale bars: 20 μm (top), 10 μm (bottom). **E** quantification of AIS length under control conditions from WT (black) and *Fmr1*$^{-/y}$ (red) single mouse cultures. Average AIS length per mouse (from 2 coverslips) shown overlain, number of mice indicated in parenthesis. **F** AIS lengths plotted for WT mouse cultured neurons following 3 hours of 15 mM KCl and NaCl. **G** AIS lengths of *Fmr1*$^{-/y}$ neurons following 15 mM KCl and NaCl application. **H** Comparative difference in AIS length (KCl length – NaCl length), plotted for each mouse. Statistics shown: ns – $p > 0.05$, * - $p < 0.05$, from LMM (B-G) and Student's 2-tailed Unpaired t-test (H). All data is shown as mean ± SEM.

Figure 4: Live imaging of the AIS fails to reveal short term structural plasticity. A low power flattened confocal stack of CA1 of the hippocampus showing β1-NaV-GFP (β1-GFP, green pseudocolour) expression compared to AnkyrinG (red pseudocolour), showing an overlapping distribution. Scale bar: 100 μm. **B** high magnification of a β1-NaV-GFP labelled PC and AIS, demonstrating faithful overlap of GFP with AnkG labelling. Scale bar: 20 μm. **C** representative 2-photon images of CA1 showing β1-NaV-GFP labelling under control conditions (0 hrs) compared to 3 hours of treatment with 15 mM NaCl (top) or KCl (bottom). Scale bar: 10 μm. **D** comparison of AIS length at the before (x-axis) to the AIS length 3 hours later for 15 mM NaCl (filled circles) and 15 mM KCl (open circles), in WT CA1 PCs. Data is shown for 99 AIS treated with NaCl and 65 AISs treated with KCl from 7 WT mice and fitted with linear regression (solid line – NaCl, dashed line – KCl). **E** the same data but plotted for 44 AIS treated with NaCl and 45 AISs treated with KCl from 5 *Fmr1*$^{-/y}$ mice. All data is shown as individual cell replicates with, where appropriate, fitted of linear relationship.

Figure 5: Reduced temporoammonic inputs to the CA1 region. A slice recording configuration showing the stimulus electron (**Stim**) placed in the perforant path, extracellular field electrode (**Field**) placed in *str. L-M* (**SLM**) and the whole-cell patch-clamp electrode (**Patch**) in *str. pyramidale* (**Pyr**). Scale bar: 100 μm. **B** Representative extracellular field EPSP (fEPSP, top) and whole-cell EPSP (bottom), recorded in response to increasing voltage stimulation (0 – 100 V DC), from WT (black) and *Fmr1*$^{-/y}$ (red) mice. **C** Input-output relationship for the afferent fibre volley amplitude in WT (black, 14 slices from 6 mice) and *Fmr1*$^{-/y}$ (red 18 slices from 7 mice) mouse slices. Number of slices indicated. **D, E** Field EPSP amplitude and integral recorded in *SLM*, plotted as a function of afferent fibre volley amplitude from the same slices as above. **F** field EPSP plotted against fibre volley following 50 μM picrotoxin bath application (WT: 12 slices from 5 mice; *Fmr1*$^{-/y}$: 18 slices from 7 mice). **G** Whole-cell patch-clamp recorded EPSP against fibre volley amplitude (WT: 14 cells from 6 mice; *Fmr1*$^{-/y}$: 18 cells from 7 mice). **H** Whole-cell EPSP amplitude in the presence of picrotoxin (WT: 10 cells from 5 mice; *Fmr1*$^{-/y}$: 20 cells from 7 mice). All data (D-H) is plotted with linear regression (straight lines). **I** quantification of the slope of input-output relationships for all EPSPs measured from whole-cell recordings. **J** Representative cell attached recordings from CA1 PCs following stimulation of *SLM*, overlaid and showing cell spiking for WT (black) and *Fmr1*$^{-/y}$ (red) neurons. **K** Quantification of the % of CA1 PCs that responded to TA stimulation with a spike, in cell attached mode, at any stimulation voltage (light shading), compared to those that did not spike (dark shading). **L** Measured spike probability of CA1 PCs at

each stimulus strength for WT (black, 26 cells from 7 mice) and *Fmr1*$^{-/y}$ CA1 PCs (red, 30 cells from 9 mice). Number of cells tested shown in parenthesis. Statistics shown: * - $p<0.05$, from 2-way RM ANOVA (**C**), Sum-of-least-squares F-test (**D-H**), GLMM (**I**). Data is shown as mean ± SEM.

STAR METHODS:

RESOURCE AVAILABILITY

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Peter C Kind (pkind@ed.ac.uk).

Lead Contact:

Peter C Kind (pkind@ed.ac.uk).

Materials Availability:

All materials will be made available upon reasonable request.

Data and Code Availability:

All data generated in this study will be made available upon reasonable request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Mouse models

All procedures were performed according to Home Office (ASPA, 2013) and The University of Edinburgh Ethical Board. Mice were maintained on a C57/Bl6J background and housed on a 12hr light/dark cycle with *ad libitum* access to food and water. For cell-cultures full litters of male mice were taken at embryonic day 17.5 (E17.5). For 2P imaging experiments, double transgenic mice were bred with β1-NaV-GFP male mice (see below) crossed with *Fmr1*$^{+/-}$ mice.

Dissociated hippocampal culture preparation

Hippocampal neuronal cell culture were prepared as described previously (Baxter et al., 2015). Briefly, hippocampi from embryonic day 17.5 mice were dissected, neurons dissociated in 36000 USP units/ml papain, and then plated on glass-coverslips coated with poly-L-lysine at a density of 1315 cells/mm^2. Only male mice were selected and a sample of cells from each mouse was

kept for later genotyping. Cells were grown in Neurobasal A culture medium supplemented with B-27 (Invitrogen, Carlsbad), 1 mM glutamine, 1% rat serum (Harlan Laboratories) and antibiotic/antimycotic (Life Technologies Ltd). Neurons were treated with mitotic inhibitor Cytosine β-D-arabino-furanoside hydrochloride (4.8 µM) at Div 4 to limit astrocyte proliferation and grown in Neurobasal A media until 10 days in vitro (DIV10).

METHOD DETAILS

Generation of β1-NaV-GFP mice:

Transgenic mice expressing the beta1 subunit of the sodium channel (b1-Nav) fused to GFP at the C-terminus under the control of the Thy1.2 promoter (Caroni, 1997) were generated by pronuclear injection. The β1-Na_V-GFP cDNA (McEwen et al., 2009) was cloned into the blunted XhoI site of the pTSC21k vector (Lüthi et al., 1997), released using Not I as previously described (Zonta et al., 2011) and used for pronuclear injection (Sherman and Brophy, 2000). Transgenic mice were backcrossed to the C57BL6J/Ola strain. Male b1-Nav-GFP were then backcrossed with female *Fmr1*$^{+/-}$ C57BL6J/Crl mice for at least six generations before data collection.

Acute slice preparation

Acute brain slices were prepared similarly to previously described (Booker et al., 2017). Briefly, mice were anesthetised with isofluorane, decapitated and their brain rapidly dissected into ice-cold carbogenated (95 % O2/5 % CO2) sucrose-modified artificial cerebrospinal fluid (ACSF; in mM: 87 NaCl, 2.5 KCl, 25 $NaHCO_3$, 1.25 NaH_2PO_4, 25 glucose, 75 sucrose, 7 $MgCl_2$, 0.5 $CaCl_2$). 400 µm brain slices were cut on an oscillating blade vibratome (VT1200S, Leica, Germany). Slices were cut in both the coronal and horizontal planes; in which prelimbic prefrontal cortex (PL-mPFC) or dorsal hippocampus (coronal) and ventro-medial hippocampus (horizontal) were present. Slices were transferred to a submerged chamber in sucrose-ACSF at 35°C for 30 min and then stored at room temperature until needed.

Whole-cell patch-clamp recordings

For electrophysiological recordings, slices were transferred to a submerged recording chamber perfused with pre-warmed carbogenated ACSF (in mM: 125 NaCl, 2.5 KCl, 25 $NaHCO_3$, 1.25 NaH_2PO_4, 25 glucose, 1 $MgCl_2$, 2 $CaCl_2$) at a flow rate of 4-6 mL.min^{-1} at 31± 1 °C).Slices were

visualised under infrared differential inference contrast microscopy with a digital camera (Orca 2, Hamamatsu, Japan) mounted on an upright microscope (BX61-WI, Olympus, Japan) and a 20x water-immersion objective lens (1.0 N.A., Olympus, Japan). Whole-cell patch-clamp recordings were performed with a Multiclamp 700B (Molecular Devices, CA, USA) amplifier. Recording pipettes were pulled from borosilicate glass capillaries (1.7 mm outer/1mm inner diameter, Harvard Apparatus, UK) on a horizontal electrode puller (P-97, Sutter Instruments, CA, USA). For recordings, pipettes were filled with a K-gluconate based internal solution (in mM 142 K-gluconate, 4 KCl, 0.5 EGTA, 10 HEPES, 2 $MgCl_2$, 2 Na_2ATP, 0.3 Na_2GTP, 1 Na_2Phosphocreatine, 2.7 Biocytin, pH=7.4, 290-310 mOsm), resulting in 3-5 MΩ tip resistance. Cells were rejected if: they were more depolarised than -50 mV, series resistance >30 MΩ, or series resistance changed by more than 20% over the course of the recording. For recordings, coronal slices were used to assess baseline intrinsic excitability to match histological findings, while horizontal slices were used for 2-photon imaging, acute plasticity, and temporoammonic stimulation.

Stimulation of the temporoammonic pathway was made with a bipolar twisted Ni:Chrome wire electrode placed in *str. lacunosum-moleculare* at the border of CA1 and subiculum (See Figure 5A) in slices which CA3 was severed to prevent recurrent activation. Field EPSPs were recorded with a second electrode (patch pipette filled with ACSF) placed in *str. L-M* and a CA1 PC recorded in first cell-attached or whole-cell configurations. Increasing stimuli of 200 μs duration were given to *str. L-M* at 5 or 10 second intervals from constant-voltage stimulation box (Digitimer, Cambridge, UK).

All intrinsic membrane properties were measured in I-clamp. Passive membrane properties, including membrane time constant, input resistance, were measured small hyperpolarising steps (10 pA, 500 ms duration), from resting membrane potential. Active properties were determined from a series of depolarising current steps (0 to +400 pA, 500 ms) from a holding potential of -70mV. All AP properties were determined from the first AP elicited at rheobase. For recordings before 15 mM NaCl or KCl treatment, the intracellular solution included 100 μM AlexaFluor 594 hydrazide (Invitrogen, Dunfermline, UK), which allowed later visual identification of the cell. All recordings were filtered online at 10 kHz with the built-in 4-pole Bessel Filter and digitized at 20 kHz (Digidata1440, Molecular Devices, CA, USA). Traces were recorded in pCLAMP 9 (Molecular Devices, CA, USA) and stored on a personal computer. Analysis of electrophysiological data was performed offline using the open source software package Stimfit (Guzman, Schlögl, and Schmidt-Hieber 2014), blind to both genotype and treatment condition.

2-photon imaging

To measure the AIS length in real time, we performed live imaging of β1-NaV-GFP mice. Live imaging was performed on 400 µm thick horizontal, hippocampal slices, as described above. For imaging we used a custom built galvanometric scanning 2-photon microscope (Femto2D-Galvo, Femtonics, Budapest, Hungary) fitted with a tuneable wavelength Ti:Sapphire laser (Chameleon, Coherent, CA, USA), with laser power controlled by a Pockels cell (Conoptics, CT, USA). Signals were detected with photomultiplier tubes through the MES microscope software (Femtonics, Hungary). Following a whole-cell patch-clamp recording being obtained, neurons were dye filled and baseline intrinsic physiology recordings collected. Then a small region of interest (ROI), which comprised the dye filled cell and proximal *str. oriens* of CA1, thus covering the full extent of CA1 PC AIS. Following baseline recordings, a Z-stack (1 µm steps) was taken in the top 50 µm of the slice. Then slices were transferred to back to a holding chamber containing recording ACSF with either 15 mM NaCl or 15 mM KCl added 3 h. At the end of the 3 h treatment, slices were then transferred back to the recording chamber and the same ROI (using the filled cell as a landmark) was imaged, under the initial conditions.

Dissociated hippocampal culture preparation

Hippocampal neuronal cell culture were prepared as described previously (Baxter et al., 2015). Briefly, hippocampi from embryonic day 17.5 mice were dissected, neurons dissociated in 36000 USP units/ml papain, and then plated on glass-coverslips coated with poly-L-lysine at a density of 1315 cells/mm^2. Only male mice were selected and a sample of cells from each mouse was kept for later genotyping. Cells were grown in Neurobasal A culture medium supplemented with B-27 (Invitrogen, Carlsbad), 1 mM glutamine, 1% rat serum (Harlan Laboratories) and antibiotic/antimycotic (Life Technologies Ltd). Neurons were treated with mitotic inhibitor Cytosine β-D-arabino-furanoside hydrochloride (4.8 µM) at Div 4 to limit astrocyte proliferation and grown in Neurobasal A media until 10 days in vitro (DIV10).

Short term plasticity

Induction of short term AIS plasticity was performed according to previous studies (Evans et al., 2015; Grubb and Burrone, 2010). In dissociated cell-culture, Neurobasal A media was supplemented with 15 mM KCl or NaCl, from 1 M stocks and coverslips returned to the incubator for 3 hours at 37 °C. For acute slice plasticity, following recovery at 35 °C (as above) slices were

transferred to a holding chamber containing recording-ACSF, with 15 mM KCl or NaCl added on top of baseline ionic concentrations. Slices were then incubated for 3h at 35 °C, and carbogenated throughout. Following incubation with KCl or NaCl, coverslips or slices were immediately immersion fixed with 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer (PB), pH 7.35 for 20 minutes (coverslips) or 1 hour (slices) at room temperature. For intrinsic physiology plasticity and 2-photon imaging, slices were transferred from the recording chamber into 15 mM KCl or NaCl (as above). After 3 hour incubation, slices were returned to the recording chamber circulating with fresh ACSF and further recordings performed.

Histological processing and imaging

For immunohistochemistry, mice were perfusion fixed. Briefly, mice were sedated with isofluorane, followed by terminal anaesthesia with sodium pentobarbital (27.5 mg/kg body weight) via intraperitoneal injection. Mice were then transcardially perfused with 20 mL PB with 0.9% saline (PBS), followed by 20 mL of 4% PFA in PB (pH 7.4). The brain was removed and post-fixed in 4% PFA for 1h. 50 µm coronal sections were cut using either an oscillating blade vibratome (VT1000S, Leica, Germany) or a freezing microtome (HM430, Thermo Scientific, UK). Immunocytochemistry was then performed on free floating sections. Sections were washed in PBS, and then blocked for 1 hour at room temperature in 10% normal goat serum (NGS), 0.3% TritonX-100, 0.05% NaN_3 in PBS. Slices were then incubated in primary antibodies raised against Ankyrin G (mouse, 1:500, clone-N106/36 NeuroMab, UNC Davis, CA, USA) and NeuN (rabbit, 1:500, ABN78, Millipore) diluted in PBS containing 5% NGS, 0.3% TritonX-100 and 0.05% NaN_3, for 24 to 72 hours at 4°C. Slices were thoroughly washed in PBS, then secondary antibodies (anti-mouse and anti-rabbit AlexaFluor488 and AlexaFluor 568, Invitrogen, UK) applied diluted in PBS containing 3% NGS, 0.1% TritonX-100 and 0.05% NaN3, for 3 hrs at room temperature or 24 hrs at 4°C. Slices were rinsed in PBS, then PB and mounted on glass slides with Vectashield hard-set mounting medium (H1400, Vector Labs, UK). For cell-culture blocking time was reduced to 10 minutes; primary antibodies incubated over-night at 4°C and secondary antibody incubation for 1 hour at room temperature. Both primary and secondary antibody solutions were identical to those used in slices, but lacking Triton-X.

Confocal image stacks were collected with an AxiovertLSM 510 (Zeiss, Germany) invert scanning-confocal microscope equipped with a 63x (N.A. 1.4, Zeiss, Germany) oil-immersion

objective lens. Z-stacks (1 µm steps, 1024x1024 pixels) containing ROIs were collected either through the entire 50 µm section (perfusion fixed tissue) or the top 20-30 µm of acute slices. Two stacks of each brain region were collected per experimental condition for each animal. For cell culture experiments, z-stacks (1 µm steps, 1024 x 1024 pixels) were taken from the top to bottom of the monolayer of cells and 2 images per coverslip were collected with a 40x (N.A. 1.3, Zeiss, Germany) oil-immersion objective lens.

Image analysis

All image analysis was performed with the FIJI package of ImageJ. Based on AnkyrinG or β1-NaV-GFP labelling, AIS were manually traced from their distal tip to either the base of AnkyrinG labelling or the soma (β1-NaV-GFP images) through the 3D image stack using the segmented line tool in FIJI. For fixed tissue, up to 50 AIS were measured for each image, giving a total of up to 100 AIS for each mouse per brain region. For coverslips, 10 to 15 AIS were measured per coverslip. When measuring distance from soma, each AIS was measured, then the distance from the base, to the soma surface measured. For the β1-NaV-GFP labelling validation, 25 AISs per mouse were measured. Independent confirmation of the methodology for AIS measurement was performed by three experimenters, all blind to genotype, and demonstrated a high degree of consistency.

Computational modelling:

Simulations were performed in NEURON 7.6 software (Carnevale and Hines, 2006) using a realistic CA1 pyramidal neuron morphology (Migliore, 2003). Four additional voltage-dependent currents were added: fast Na^+ (I_{Na}), fast K^+ (I_{Kv}), slow non-inactivating K^+ (I_{Km}) and high voltage activated Ca^{2+} (I_{Ca}) and a Ca2+ -dependent K^+ current (I_{KCa}) (Mainen and Sejnowski, 1996). Cytoplasmic resistance (R_i) was set to 150 Ω.cm, membrane capacitance (C_M) was set to 1 µF/cm^2 and membrane resistance (R_M) was set to 30 MΩ.cm^2. The electrical impact of dendritic spines in realistic neuron morphologies was simulated by doubling dendritic C_M and halving dendritic R_M (Holmes, 1989). The axon consisted of a variable length axon initial segment (AIS) followed by twenty 100 µm long segments with low membrane capacitance (C_M = 0.1 µF/cm^2) and high resistance (R_M = 150 kΩ.cm^2) representing myelinated regions, interspersed with 1 µm long Nodes of Ranvier (R_M = 50 Ω.cm^2). Conductance densities (in pS.µm^{-2}) were as follows. Dendrites: g_{Na} = 20, g_{Ca} = 0.3, g_{KCa} = 3, and g_{Kv} = 0.1. Soma: as dendrites but g_{Kv} = 200. Axon

initial segment: g_{Kv} = 2000, g_{Na} = 30,000. Nodes of Ranvier: g_{Na} = 30,000. Reversal potential for different ionic currents were E_{Leak} = -70 mV, E_K = -90 mV, E_{Na} = 60 mV, E_{Ca} = 140 mV). For input resistance changes, R_M was scaled uniformly throughout all compartments. Simulations were performed at 40 kHz. 500 ms somatic current steps were applied and the rheobase determined to the nearest 1 pA. AP threshold was classified as the voltage at which the speed of membrane depolarisation first exceeded 20 mV/ms. Simulations were performed at 31 ºC.

QUANTIFICATION AND STATISTICAL ANALYSIS

All experiments were performed blind to genotype, age and treatment (where applicable. Throughout, all data is shown as mean ± SEM. Where applicable, data was analysed with either a linear mixed-effects model (LMM), or its generalised form (GLMM), whereby the variability due to random effects (animal, slice) was taken into account, allowing for direct measurement of genotype and/or treatment effects. Mixed-effects models were fitted using the Lme4 R package (Bates et al., 2014), where the tested variable is a fixed-effect parameter (i.e. genotype, age or treatment) and random effects (animal, slice) are modelled vectors. All data was tested for normality, with *p*-values reported as the output of ANOVA tests. Additionally, repeated measures 2-way ANOVA, with Holm-Sidak post-tests (Figure 1B, Figure 3C, D, H) and Sum-of-least-squares F-tests (Figure 4D-H) were employed. For boot-strapping analysis, the proportions of AIS (% of 100 AIS) from the dataset, in an animal dependent manner, were randomly sampled 1000 times and LMM ran on the subsampled data. *P* values were calculated from these repeated LMM tests and plotted for each percentage of the data sampled and plotted as the X-axis. As some animals had >95 AIS measured, but not 100, a percentage was used instead of absolute AIS number. Where reported, statistical significance was assumed if $p < 0.05$.

KEY RESOURCES TABLE (KRT)

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Mouse monoclonal anti-AnkyrinG	NeuroMAB	N106/36
Rabbit polyclonal anti-AnkyrinG	Santa Cruz	sc-28561
Rabbit polyclonal anti-NeuN	Millipore EMD	ABN78
Mouse monoclonal anti-NeuN	Millipore EMD	MAB377

Alexa 488 goat anti-mouse	Thermo Fisher Scientific	A-11001
Alexa 568 goat anti-mouse	Thermo Fisher Scientific	A-11004
Alexa 568 goat anti-rabbit	Thermo Fisher Scientific	A-11011
Alexa 633 goat anti-rabbit	Thermo Fisher Scientific	A-21071
To-Pro 3 Iodide	Thermo Fisher Scientific	T3605
Chemicals, Peptides, and Recombinant Proteins		
Picrotoxin	Hellobio	HB0506
Alexafluor 594 Hydrazide	Thermo Fisher Scientific	A10438
Vectashield mounting medium	Vector Labs	H-1400
Experimental Models: Organisms/Strains		
Mouse: C57/Bl6J, B6.129P2-Fmr1tm1Cgr/J (Dutch Belgian Consortium)	Jackson Labs	003025
β1-NaV-GFP	Newly generated	n/a
Software and Algorithms		
Graphpad Prism 7	GraphPad Software	https://www.graphpad.com
R/ R studio	R Core Team (2013), RStudio Team (2015)	https://www.r-project.org; https://rstudio.com
pClamp	Molecular devices	https://www.moleculardevices.com/products/axon-patch-clamp-system/acquisition-and-analysis-software/pclamp-software-suite#gref
Stimfit	Guzman, Schlögl, and Schmidt-Hieber 2014	https://github.com/neurodroid/stimfit
ImageJ	Schneider et al., 2012	https://imagej.net/Fiji/Downloads
NEURON 7.6	(Carnevale and Hines, 2006)	https://neuron.yale.edu/neuron/

Supplementary Table 2: Details of statistical tests performed in the current study. Related to Figures 1 – 5 and Supplementary Figures 1, 3, 4, 5. Details of all statistical tests with reference to specific location in article body, mean ± SEM, number of replicates, replicate tested, test performed, data model, and test outputs; for both main article and supplementary materials.

Declarations of Interests:

The authors declare that they have no competing interests.

References:

Amaral, D., and Witter, M. (1989). The three-dimensional organization of the hippocampal formation: a review of anatomical data. Neuroscience *31*, 571-591.

Antoine, M.W., Langberg, T., Schnepel, P., and Feldman, D.E. (2019). Increased excitation-inhibition ratio stabilizes synapse and circuit excitability in four autism mouse models. Neuron.

Asiminas, A., Jackson, A.D., Louros, S.R., Till, S.M., Spano, T., Dando, O., Bear, M.F., Chattarji, S., Hardingham, G.E., and Osterweil, E.K. (2019). Sustained correction of associative learning deficits after brief, early treatment in a rat model of Fragile X Syndrome. Science translational medicine *11*, eaao0498.

Bates, D., Mächler, M., Bolker, B., and Walker, S. (2014). Fitting linear mixed-effects models using lme4. arXiv preprint arXiv:14065823.

Baxter, P.S., Bell, K.F., Hasel, P., Kaindl, A.M., Fricker, M., Thomson, D., Cregan, S.P., Gillingwater, T.H., and Hardingham, G.E. (2015). Synaptic NMDA receptor activity is coupled to the transcriptional control of the glutathione system. Nature communications *6*, 6761.

Biane, J.S., Scanziani, M., Tuszynski, M.H., and Conner, J.M. (2015). Motor cortex maturation is associated with reductions in recurrent connectivity among functional subpopulations and increases in intrinsic excitability. Journal of Neuroscience *35*, 4719-4728.

Booker, S.A., Campbell, G.R., Mysiak, K.S., Brophy, P.J., Kind, P.C., Mahad, D.J., and Wyllie, D.J. (2017). Loss of protohaem IX farnesyltransferase in mature dentate granule cells impairs short-term facilitation at mossy fibre to CA3 pyramidal cell synapses. The Journal of physiology *595*, 2147-2160.

Booker, S.A., Domanski, A.P., Dando, O.R., Jackson, A.D., Isaac, J.T., Hardingham, G.E., Wyllie, D.J., and Kind, P.C. (2019). Altered dendritic spine function and integration in a mouse model of Fragile X Syndrome. Nature communications *10*, 1-14.

Brager, D.H., Akhavan, A.R., and Johnston, D. (2012). Impaired dendritic expression and plasticity of h-channels in the fmr1−/y mouse model of fragile X syndrome. Cell reports *1*, 225-233.

Branco, T., and Häusser, M. (2011). Synaptic integration gradients in single cortical pyramidal cell dendrites. Neuron *69*, 885-892.

Breton, J.D., and Stuart, G.J. (2009). Loss of sensory input increases the intrinsic excitability of layer 5 pyramidal neurons in rat barrel cortex. The Journal of physiology *587*, 5107-5119.

Brown, M.R., Kronengold, J., Gazula, V.-R., Chen, Y., Strumbos, J.G., Sigworth, F.J., Navaratnam, D., and Kaczmarek, L.K. (2010). Fragile X mental retardation protein controls gating of the sodium-activated potassium channel Slack. Nature neuroscience *13*, 819-821.

Bülow, P., Murphy, T., Bassell, G.J., and Wenner, P. (2019). Homeostatic Intrinsic Plasticity Is Functionally Altered in Fmr1 KO Cortical Neurons. Cell Reports *26*, 1378-1388. e1373.

Bureau, I., Shepherd, G.M., and Svoboda, K. (2008). Circuit and plasticity defects in the developing somatosensory cortex of FMR1 knock-out mice. Journal of Neuroscience *28*, 5178-5188.

Carnevale, N.T., and Hines, M.L. (2006). The NEURON book (Cambridge University Press).

Caroni, P. (1997). Overexpression of growth-associated proteins in the neurons of adult transgenic mice. Journal of neuroscience methods *71*, 3-9.

Connors, B.W., and Gutnick, M.J. (1990). Intrinsic firing patterns of diverse neocortical neurons. Trends in neurosciences *13*, 99-104.

Contractor, A., Klyachko, V.A., and Portera-Cailliau, C. (2015). Altered neuronal and circuit excitability in fragile X syndrome. Neuron *87*, 699-715.

Curia, G., Papouin, T., Séguéla, P., and Avoli, M. (2009). Downregulation of tonic GABAergic inhibition in a mouse model of fragile X syndrome. Cerebral cortex *19*, 1515-1520.

D'Hooge, R., Nagels, G., Franck, F., Bakker, C., Reyniers, E., Storm, K., Kooy, R., Oostra, B., Willems, P., and De Deyn, P. (1997). Mildly impaired water maze performance in maleFmr1 knockout mice. Neuroscience *76*, 367-376.

Deng, P.-Y., and Klyachko, V.A. (2016). Increased persistent sodium current causes neuronal hyperexcitability in the entorhinal cortex of Fmr1 knockout mice. Cell reports *16*, 3157-3166.

Deng, P.-Y., Rotman, Z., Blundon, J.A., Cho, Y., Cui, J., Cavalli, V., Zakharenko, S.S., and Klyachko, V.A. (2013). FMRP regulates neurotransmitter release and synaptic information transmission by modulating action potential duration via BK channels. Neuron *77*, 696-711.

Dölen, G., Osterweil, E., Rao, B.S., Smith, G.B., Auerbach, B.D., Chattarji, S., and Bear, M.F. (2007). Correction of fragile X syndrome in mice. Neuron *56*, 955-962.

Domanski, A.P., Booker, S.A., Wyllie, D.J., Isaac, J.T., and Kind, P.C. (2019). Cellular and synaptic phenotypes lead to disrupted information processing in Fmr1-KO mouse layer 4 barrel cortex. Nature communications *10*, 1-18.

Evans, M.D., Dumitrescu, A.S., Kruijssen, D.L., Taylor, S.E., and Grubb, M.S. (2015). Rapid modulation of axon initial segment length influences repetitive spike firing. Cell reports *13*, 1233-1245.

Fyhn, M., Molden, S., Witter, M.P., Moser, E.I., and Moser, M.-B. (2004). Spatial representation in the entorhinal cortex. Science *305*, 1258-1264.

Gibson, J.R., Bartley, A.F., Hays, S.A., and Huber, K.M. (2008). Imbalance of neocortical excitation and inhibition and altered UP states reflect network hyperexcitability in the mouse model of fragile X syndrome. Journal of neurophysiology *100*, 2615-2626.

Grubb, M.S., and Burrone, J. (2010). Activity-dependent relocation of the axon initial segment fine-tunes neuronal excitability. Nature *465*, 1070-1074.

Grubb, M.S., Shu, Y., Kuba, H., Rasband, M.N., Wimmer, V.C., and Bender, K.J. (2011). Short- and long-term plasticity at the axon initial segment. Journal of Neuroscience *31*, 16049-16055.

Gutzmann, A., Ergül, N., Grossmann, R., Schultz, C., Wahle, P., and Engelhardt, M. (2014). A period of structural plasticity at the axon initial segment in developing visual cortex. Frontiers in neuroanatomy *8*, 11.

Haberl, M.G., Zerbi, V., Veltien, A., Ginger, M., Heerschap, A., and Frick, A. (2015). Structural-functional connectivity deficits of neocortical circuits in the Fmr1−/y mouse model of autism. Science advances *1*, e1500775.

Hagerman, R.J., Staley, L.W., O'conner, R., Lugenbeel, K., Nelson, D., McLean, S.D., and Taylor, A. (1996). Learning-disabled males with a fragile X CGG expansion in the upper premutation size range. Pediatrics *97*, 122-126.

Hamada, M.S., Goethals, S., de Vries, S.I., Brette, R., and Kole, M.H. (2016). Covariation of axon initial segment location and dendritic tree normalizes the somatic action potential. Proceedings of the National Academy of Sciences *113*, 14841-14846.

Harlow, E.G., Till, S.M., Russell, T.A., Wijetunge, L.S., Kind, P., and Contractor, A. (2010). Critical period plasticity is disrupted in the barrel cortex of FMR1 knockout mice. Neuron *65*, 385-398.

Holmes, W.R. (1989). The role of dendritic diameters in maximizing the effectiveness of synaptic inputs. Brain research *478*, 127-137.

Huber, K.M., Gallagher, S.M., Warren, S.T., and Bear, M.F. (2002). Altered synaptic plasticity in a mouse model of fragile X mental retardation. Proceedings of the National Academy of Sciences *99*, 7746-7750.

Kalmbach, B.E., Johnston, D., and Brager, D.H. (2015). Cell-type specific channelopathies in the prefrontal cortex of the fmr1-/y mouse model of fragile X syndrome. eNeuro, ENEURO. 0114-0115.2015.

Kaphzan, H., Buffington, S.A., Jung, J.I., Rasband, M.N., and Klann, E. (2011). Alterations in intrinsic membrane properties and the axon initial segment in a mouse model of Angelman syndrome. Journal of Neuroscience *31*, 17637-17648.

Klemmer, P., Meredith, R.M., Holmgren, C.D., Klychnikov, O.I., Stahl-Zeng, J., Loos, M., van der Schors, R.C., Wortel, J., de Wit, H., Spijker, S.*, et al.* (2011). Proteomics, Ultrastructure, and Physiology of Hippocampal Synapses in a Fragile X Syndrome Mouse Model Reveal Presynaptic Phenotype. Journal of Biological Chemistry *286*, 25495-25504.

Kole, M.H., Ilschner, S.U., Kampa, B.M., Williams, S.R., Ruben, P.C., and Stuart, G.J. (2008). Action potential generation requires a high sodium channel density in the axon initial segment. Nature neuroscience *11*, 178.

Kole, M.H., Letzkus, J.J., and Stuart, G.J. (2007). Axon initial segment Kv1 channels control axonal action potential waveform and synaptic efficacy. Neuron *55*, 633-647.

Kuba, H. (2012). Structural tuning and plasticity of the axon initial segment in auditory neurons. The Journal of physiology *590*, 5571-5579.

Kuba, H., Oichi, Y., and Ohmori, H. (2010). Presynaptic activity regulates Na+ channel distribution at the axon initial segment. Nature *465*, 1075.

Kuba, H., Yamada, R., Ishiguro, G., and Adachi, R. (2015). Redistribution of Kv1 and Kv7 enhances neuronal excitability during structural axon initial segment plasticity. Nature communications *6*, 1-12.

Leterrier, C. (2016). The axon initial segment, 50 years later: a nexus for neuronal organization and function. In Current topics in membranes (Elsevier), pp. 185-233.

Louros, S.R., and Osterweil, E.K. (2016). Perturbed proteostasis in autism spectrum disorders. Journal of neurochemistry *139*, 1081-1092.

Luque, M.A., Beltran-Matas, P., Marin, M.C., Torres, B., and Herrero, L. (2017). Excitability is increased in hippocampal CA1 pyramidal cells of Fmr1 knockout mice. PloS one *12*, e0185067.

Lüthi, A., Van Der Putten, H., Botteri, F.M., Mansuy, I.M., Meins, M., Frey, U., Sansig, G., Portet, C., Schmutz, M., and Schröder, M. (1997). Endogenous serine protease inhibitor modulates epileptic activity and hippocampal long-term potentiation. Journal of Neuroscience *17*, 4688-4699.

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

Mainen, Z.F., and Sejnowski, T.J. (1996). Influence of dendritic structure on firing pattern in model neocortical neurons. Nature *382*, 363.

Marder, E., and Goaillard, J.-M. (2006). Variability, compensation and homeostasis in neuron and network function. Nature Reviews Neuroscience *7*, 563-574.

Marder, E., and Prinz, A.A. (2002). Modeling stability in neuron and network function: the role of activity in homeostasis. Bioessays *24*, 1145-1154.

McEwen, D.P., Chen, C., Meadows, L.S., Lopez-Santiago, L., and Isom, L.L. (2009). The voltage-gated Na+ channel β3 subunit does not mediate trans homophilic cell adhesion or associate with the cell adhesion molecule contactin. Neuroscience letters *462*, 272-275.

Migliore, M. (2003). On the integration of subthreshold inputs from perforant path and Schaffer collaterals in hippocampal CA1 pyramidal neurons. Journal of computational neuroscience *14*, 185-192.

Miller, V.M., and Best, P.J. (1980). Spatial correlates of hippocampal unit activity are altered by lesions of the fornix and entorhinal cortex. Brain research *194*, 311-323.

Musumeci, S.A., Bosco, P., Calabrese, G., Bakker, C., De Sarro, G.B., Elia, M., Ferri, R., and Oostra, B.A. (2000). Audiogenic seizures susceptibility in transgenic mice with fragile X syndrome. Epilepsia *41*, 19-23.

O'Leary, T., Williams, A.H., Caplan, J.S., and Marder, E. (2013). Correlations in ion channel expression emerge from homeostatic tuning rules. Proceedings of the National Academy of Sciences *110*, E2645-E2654.

O'Leary, T., van Rossum, M.C., and Wyllie, D.J. (2010). Homeostasis of intrinsic excitability in hippocampal neurones: dynamics and mechanism of the response to chronic depolarization. The Journal of physiology *588*, 157-170.

Ogawa, Y., and Rasband, M.N. (2008). The functional organization and assembly of the axon initial segment. Current opinion in neurobiology *18*, 307-313.

Osterweil, E.K., Krueger, D.D., Reinhold, K., and Bear, M.F. (2010). Hypersensitivity to mGluR5 and ERK1/2 leads to excessive protein synthesis in the hippocampus of a mouse model of fragile X syndrome. Journal of Neuroscience *30*, 15616-15627.

Ransdell, J.L., Nair, S.S., and Schulz, D.J. (2012). Rapid homeostatic plasticity of intrinsic excitability in a central pattern generator network stabilizes functional neural network output. Journal of Neuroscience *32*, 9649-9658.

Richter, J.D., Bassell, G.J., and Klann, E. (2015). Dysregulation and restoration of translational homeostasis in fragile X syndrome. Nature Reviews Neuroscience *16*, 595.

Routh, B.N., Johnston, D., and Brager, D.H. (2013). Loss of functional A-type potassium channels in the dendrites of CA1 pyramidal neurons from a mouse model of fragile X syndrome. Journal of Neuroscience *33*, 19442-19450.

Routh, B.N., Rathour, R.K., Baumgardner, M.E., Kalmbach, B.E., Johnston, D., and Brager, D.H. (2017). Increased transient Na+ conductance and action potential output in layer 2/3 prefrontal cortex neurons of the fmr1−/y mouse. The Journal of physiology *595*, 4431-4448.

Shah, M.M. (2014). Cortical HCN channels: function, trafficking and plasticity. The Journal of physiology *592*, 2711-2719.

Sherman, D.L., and Brophy, P.J. (2000). A tripartite nuclear localization signal in the PDZ-domain protein L-periaxin. Journal of Biological Chemistry *275*, 4537-4540.

Talbot, Z.N., Sparks, F.T., Dvorak, D., Curran, B.M., Alarcon, J.M., and Fenton, A.A. (2018). Normal CA1 place fields but discoordinated network discharge in a Fmr1-null mouse model of fragile X syndrome. Neuron *97*, 684-697. e684.

Thome, C., Kelly, T., Yanez, A., Schultz, C., Engelhardt, M., Cambridge, S.B., Both, M., Draguhn, A., Beck, H., and Egorov, A.V. (2014). Axon-carrying dendrites convey privileged synaptic input in hippocampal neurons. Neuron *83*, 1418-1430.

Till, S.M., Asiminas, A., Jackson, A.D., Katsanevaki, D., Barnes, S.A., Osterweil, E.K., Bear, M.F., Chattarji, S., Wood, E.R., and Wyllie, D.J. (2015). Conserved hippocampal cellular pathophysiology but distinct behavioural deficits in a new rat model of FXS. Human molecular genetics *24*, 5977-5984.

van der Molen, M.J., Stam, C.J., and van der Molen, M.W. (2014). Resting-state EEG oscillatory dynamics in fragile X syndrome: abnormal functional connectivity and brain network organization. PloS one *9*, e88451.

Wahlstrom-Helgren, S., and Klyachko, V.A. (2015). GABAB receptor-mediated feed-forward circuit dysfunction in the mouse model of fragile X syndrome. The Journal of physiology *593*, 5009-5024.

Wang, J., Ethridge, L.E., Mosconi, M.W., White, S.P., Binder, D.K., Pedapati, E.V., Erickson, C.A., Byerly, M.J., and Sweeney, J.A. (2017). A resting EEG study of neocortical hyperexcitability and altered functional connectivity in fragile X syndrome. Journal of neurodevelopmental disorders *9*, 11.

Wimmer, V.C., Reid, C.A., So, E.Y.W., Berkovic, S.F., and Petrou, S. (2010). Axon initial segment dysfunction in epilepsy. The Journal of physiology *588*, 1829-1840.

Wu, W.W., Chan, C.S., Surmeier, D.J., and Disterhoft, J.F. (2008). Coupling of L-type Ca2+ channels to KV7/KCNQ channels creates a novel, activity-dependent, homeostatic intrinsic plasticity. Journal of neurophysiology *100*, 1897-1908.

Yue, C., and Yaari, Y. (2006). Axo-somatic and apical dendritic Kv7/M channels differentially regulate the intrinsic excitability of adult rat CA1 pyramidal cells. Journal of neurophysiology *95*, 3480-3495.

Yun, S.H., and Trommer, B.L. (2011). Fragile X mice: Reduced long-term potentiation and N-Methyl-D-Aspartate receptor-mediated neurotransmission in dentate gyrus. Journal of neuroscience research *89*, 176-182.

Zhang, Y., Bonnan, A., Bony, G., Ferezou, I., Pietropaolo, S., Ginger, M., Sans, N., Rossier, J., Oostra, B., and LeMasson, G. (2014). Dendritic channelopathies contribute to neocortical and sensory hyperexcitability in Fmr1−/y mice. Nature neuroscience *17*, 1701.

Zonta, B., Desmazieres, A., Rinaldi, A., Tait, S., Sherman, D.L., Nolan, M.F., and Brophy, P.J. (2011). A critical role for Neurofascin in regulating action potential initiation through maintenance of the axon initial segment. Neuron *69*, 945-956.