Channelopathies in fragile X syndrome

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Channelopathies in fragile X syndrome

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Abstract | Fragile X syndrome (FXS) is the most common inherited form of intellectual disability and the leading monogenic cause of autism. The condition stems from loss of fragile X mental retardation protein (FMRP), which regulates a wide range of ion channels via translational control, protein–protein interactions and second messenger pathways. Rapidly increasing evidence demonstrates that loss of FMRP leads to numerous ion channel dysfunctions (that is, channelopathies), which in turn contribute significantly to FXS pathophysiology. Consistent with this, pharmacological or genetic interventions that target dysregulated ion channels effectively restore neuronal excitability, synaptic function and behavioural phenotypes in FXS animal models. Recent studies further support a role for direct and rapid FMRP–channel interactions in regulating ion channel function. This Review lays out the current state of knowledge in the field regarding channelopathies and the pathogenesis of FXS, including promising therapeutic implications.

Macroorchidism
A condition in which males have abnormally large testes.

Channelopathies
A heterogeneous group of disorders resulting from the dysfunction of ion channels, which can be caused by mutations either in genes encoding channels themselves or in related factors that regulate ion channels.

Hyperexcitability
An abnormal state of a neuron characterized by increased probability of firing action potentials in response to an input.

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Fragile X syndrome (FXS) is the leading monogenic cause of intellectual disability and autism. Affecting both males (~1/4,000) and females (~1/5,000–8,000), FXS is typically associated with cognitive dysfunction (language delay, intellectual disabilities, learning deficits), social and behavioural problems (anxiety, autism spectrum disorders (ASDs)), neurological deficits (seizures, abnormal sleep patterns) and morphological abnormalities (dysmorphic faces and macroorchidism)[1]. The syndrome is caused by loss of fragile X mental retardation protein (FMRP), which most commonly results from the expansion of an unstable triplet CGG-repeat motif (>200 repeats) located within the 5′ untranslated region of the *FMR1* gene on the X chromosome. This expansion causes gene hypermethylation and transcriptional silencing. Rare mutations in *FMR1* that result in 'loss-of-function FMRP' have also been linked to FXS[2].

Increasing evidence suggests that the symptoms of FXS stem from disruptions in neuronal activity[3]. Indeed, FMRP, with its multiple RNA-binding and protein–protein interaction domains (BOX 1), is highly expressed throughout the nervous system[4]. It is involved in a broad range of physiological functions, including genome stabilization, RNA editing, pre-RNA splicing, regulation of cell differentiation and modulation of ion channel function, neuronal excitability and synaptic plasticity. Specific to ion channel function, FMRP works through multiple direct and/or indirect mechanisms to control channel expression and activity[3,5,6] (TABLE 1). Indeed, many types of ion channel are dysfunctional in *Fmr1* knockout (KO) models, including voltage-dependent ion channels (sodium (Na^+) channels, potassium (K^+) channels, calcium (Ca^{2+}) channels and hyperpolarization-activated cyclic nucleotide-gated (HCN) channels), voltage-independent ion channels (small-conductance Ca^{2+}-activated K^+ (SK) channels) and ligand-gated ion channels (ionotropic glutamatergic and GABAergic receptors). Although these abnormalities are not caused by genetic mutations in the ion channel genes themselves, we broadly refer here to these ion channel dysfunctions as channelopathies. At the functional level, these channelopathies contribute to neuronal and network hyperexcitability, cognitive dysfunction and behavioural abnormalities, all of which are part of FXS pathophysiology (FIG. 1). In this Review, we first summarize evidence of hyperexcitability at the behavioural, circuit, cellular and synaptic levels in FXS. We then present findings in support of channelopathies underlying these excitability-related defects, including key molecular mechanisms. Finally, we discuss implications for future therapeutic strategies targeting ion channels in FXS.

Evidence of hyperexcitability in FXS

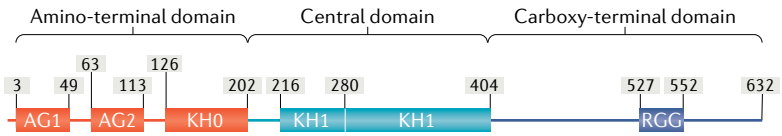
The clinical phenotypes of FXS are quite complex and vary considerably across individuals. However, most exhibit neurological symptoms indicative of network hyperexcitability, including seizures, hyperarousal, hyperactivity and hypersensitivity to sensory stimuli[7]. Neuronal hyperexcitability is also consistently observed in FXS animal models, data from which indicate intrinsic hyperexcitability and/or excitation/inhibition imbalance in neuronal circuits[3,8,9]. We summarize the findings from both human patients and animal models below.

Behavioural abnormalities/symptoms

Epilepsy — an outward expression of circuit-level hyperexcitability — occurs in 10–20% of individuals with FXS. Seizure patterns on electroencephalography

Box 1 | **FMRP functional domains**

The multi-domain structure of fragile X mental retardation protein (FMRP) enables it to modulate the functions of ion channels through multiple mechanisms, including regulation of channel translation, surface expression and/or gating dynamics, or by indirectly regulating channel activity through second messenger signalling pathways. FMRP is a protein of 632 amino acids with a molecular weight of ~80 kDa. The amino-terminal domain (amino acids 1–202) contains two Tudor (Agenet) domains — AG1 (amino acids 3–49) and AG2 (amino acids 63–113), both of which are involved in protein–protein interactions — one nuclear localization signal (amino acids 115–154) and one K homology domain (KH0, amino acids 126–202). The central domain (amino acids 203–404) comprises the KH1 (amino acids 216–280) and KH2 (amino acids 281–404) motifs that mediate RNA binding. The carboxy-terminal domain contains the RGG box (amino acids 527–552), a high-affinity RNA-binding domain that interacts with G-quadruplex RNAs and is essential for FMRP's association with polyribosomes. The C terminus also harbours a low-complexity domain (amino acids 466–632), which can promote dynamic interactions with proteins and RNAs. The low-complexity domain is implicated in the formation of ribonucleoprotein particles. The figure shows the FMRP functional domains[158] (three major domains colour-coded, numbers indicate position within the amino acid sequence).



typically present as benign focal epilepsy of childhood[10], although many affected individuals continue to have seizures after the age of 20 years[11]. Patients with FXS are also hyperresponsive to sensory stimulation and display tactile defensiveness[12,13]. Sensory hyperreactivity can cause a hyperaroused state characterized by disruptions in circadian rhythms, including frequent awakenings from sleep[14,15]. The seizures and hyperresponsivity are recapitulated in FXS models[16]; *Fmr1* KO mice have a lower threshold for audiogenic seizures[17–23], hypersensitivity to sensory stimuli[24–26] and hyperactivity[27–29].

Circuit and network dysfunctions

Increased excitability at the network level has been measured in patients using various non-invasive techniques. For example, electroencephalography abnormalities are commonly observed in individuals with FXS[11,15,30], including centro-temporal spikes, slowed background rhythm and multifocal epileptiform discharges. Sensory hypersensitivity is also apparent in the elevated amplitude of evoked potentials in response to auditory and/or visual stimuli[31], including heightened event-related brain potentials within the auditory cortex of children with FXS[32]. Looking into the mechanisms of cortical network hyperexcitability in individuals with FXS, Morin-Parent et al.[9] used transcranial magnetic stimulation to show that patients with FXS have significantly reduced short-interval intracortical inhibition (mediated by $GABA_A$ receptors ($GABA_A$Rs)), increased long-interval intracortical inhibition (mediated by $GABA_B$ receptors) and increased intracortical facilitation (mediated by glutamatergic receptors) compared with healthy controls. Overall, the data support loss of FMRP leading to network hyperexcitability, hypersensitivity to sensory stimuli and excitation/inhibition imbalance.

Some of the specific neurological symptoms associated with FXS are also indicative of circuit dysfunction in particular brain regions. For example, tactile defensiveness, learning and memory deficits, fear and anxiety — all of which are common in FXS — can be linked to circuit/network abnormalities in the somatosensory cortex, hippocampus or amygdala, respectively. Data from FXS animal models confirm the presence of circuit abnormalities and marked excitation/inhibition imbalance in these regions. In the somatosensory cortex of *Fmr1* KO mice, circuit hyperexcitability manifests as prolonged UP states[33–35]. Studies also find elevated cortical network synchrony in layer 2/3 of the barrel cortex[36], whereas network inhibition during the UP states is less synchronous[33]. Also using this model, Domanski et al. observe diminished feedforward inhibition and increased excitation/inhibition ratio during trains of activity in layer 4 barrel cortex neurons[37]. Similar network excitation/inhibition imbalance is also observed in the primary visual cortex[38] and auditory brain stem[39–41] of FXS animal models. In the hippocampal circuits of *Fmr1* KO mice, hyperexcitability manifests in elevated epileptiform activity[42,43] as well as abnormally high spiking probability and reduced spiking precision in hippocampal feedforward inhibitory circuits[44,45]. These defects are associated with a marked shift in excitation/inhibition circuit balance, primarily a result of reduced activity of feedforward inhibitory hippocampal interneurons. In the amygdala of *Fmr1* KO mice, although the overall excitation/inhibition balance appears to be maintained, the temporal window of peak excitation to peak inhibition is significantly narrower as a result of reduced tonic $GABA_A$R-mediated inhibition[46,47]. This narrowing leads to circuit hyperexcitability and abnormal amygdala functions[46,48–50].

Collectively, these data support the presence of hyperexcitable circuits and networks in individuals with FXS and animal models, some of which may underlie characteristic FXS symptoms.

Synaptic and cellular defects

Some of the behavioural-level and network-level hallmarks of FXS are driven by cellular and synaptic mechanisms. There is now extensive evidence of changes in synaptic transmission and plasticity as well as dysregulated cellular excitability in FXS. For example, in animal models, neurons across multiple brain regions exhibit an abnormally high action potential firing rate, including in the hippocampus, somatosensory cortex, entorhinal cortex and auditory brainstem[33,36,37,51–54].

In terms of dendritic function and synaptic plasticity, *Fmr1* KO mice have a higher threshold for spike timing-dependent plasticity in the prefrontal cortex[55], and changes in the input resistance of dendritic compartments, which can significantly impact dendritic summation and, thus, cellular excitability[37,56–58]. In line with elevated dendritic excitability, higher single-spine glutamate currents and elevated dendritic gain (resulting in higher action potential output) are observed in somatosensory cortex layer 4 stellate cells of *Fmr1* KO mice[59]. In addition, there are major FMRP-related abnormalities in neurotransmitter release across several types of neuron. For instance, glutamate release is elevated in the axon terminals of dorsal root ganglion neurons[60,61]

Audiogenic seizures
Seizures that are triggered by acoustic stimulation.

UP states
One of the two preferred subthreshold membrane potentials of a neuron, characterized by a more depolarized state during which it is easier for a neuron to fire action potentials.

Feedforward inhibition
A ubiquitous unitary motif in the organization of neural circuits in which an excitatory neuron excites an inhibitory interneuron, which then in turn inhibits a downstream excitatory cell (or cells).

and at the hippocampal CA3–CA1 synapse during spike trains[43,62,63].

Several studies have also observed synaptic deficits in inhibitory interneurons, including reduced GABA release in the hippocampus of *Fmr1* KO mice[44,64], attributable in part to excessive presynaptic $GABA_B$ receptor signalling in inhibitory presynaptic terminals[44]. By contrast, Yang et al. report increased GABA release from basket cells in the cerebellum of *Fmr1* KO mice, resulting in reduced firing frequency among postsynaptic Purkinje neurons[65]. Although contradictory at first glance, Purkinje neurons are inhibitory, and thus the circuit-level effects of these changes are similar — both reduce circuit inhibition and promote hyperexcitability. Similarly, in the amygdala, loss of FMRP causes a marked reduction in the frequency and amplitude of inhibitory postsynaptic currents, reduced tonic inhibition and decreased GABA availability[48]. In the subiculum, loss of FMRP leads to selective reduction of tonic inhibition, but not phasic inhibitory postsynaptic currents, suggesting that specific subtypes of $GABA_A$Rs mediating tonic inhibitory currents are downregulated[66].

Table 1 | **Mechanisms of FMRP regulation of ion channels**

Channel/modulation	Direction of modulation by FMRP	Refs
Translational control		
Kv1.2	⇧ or ⇩	58,65,94
Kv3.1	⇩	67,68
Kv4.2	⇧ or ⇩	94–96
Slack	⇄ or ⇩	72,94
BK	⇧	157
Cav1.3	⇧ or ⇩	88,98
Cav2.1	⇧	89,94
Cav2.3	⇩	90,94
HCN	⇧ or ⇩	56,57,94,101
AMPAR	⇧ or ⇩	59,83,103,104
NMDAR	⇧ or ⇩	59,110–112,115
$GABA_A$R	⇩	64,118–124
Ion channel activity via FMRP–channel interactions		
Slack	⇧	72
BK	⇧	57,62,80
SK	⇧ or ⇩	51,86
Kv1.2	⇧	65
Kv4	⇩	91
Cav3	⇩	91
Ion channel surface expression via FMRP–channel interactions		
Kv1.2	⇧	65
Cav2.1	⇧	60,89
Cav2.2	⇩	60
HCN	⇧ or ⇩	101
AMPAR	⇧ or ⇩	59,107
Second messenger pathways		
I_{NaP}	⇩	52
Unidentified		
NKCC1	⇩	144,145

⇧ or ⇩, FMRP increases or decreases channel function, respectively; ⇄, no significant change in channel function. AMPAR, AMPA receptor; BK, large-conductance voltage and Ca^{2+}-activated K^+ channel; Cav, voltage-gated Ca^{2+} channel; FMRP, fragile X mental retardation protein; $GABA_A$R, GABA type A receptor; HCN, hyperpolarization-activated cyclic nucleotide-gated ion channel; I_{NaP}, voltage-gated Na^+ channel carrying persistent Na^+ current; Kv, voltage-gated K^+ channel; NKCC1, Na^+–K^+–Cl^- co-transporter; NMDAR, NMDA receptor; SK, small-conductance Ca^{2+}-activated K^+ channel.

The roles of channelopathies in FXS

Overview

Most of the pathological changes in excitability associated with FXS can be attributed to ion channel dysfunction that occurs as a result of the loss of FMRP, which regulates multiple types of channel across various neuronal subcompartments (FIG. 1; TABLE 1). The specific functional consequences of FMRP loss include changes in action potential initiation and firing pattern (involving various K^+ and Na^+ channels), neurotransmitter release (involving Ca^{2+} and K^+ channels) and dendritic function (involving K^+, Ca^{2+} and HCN channels, as well as AMPA receptors (AMPARs), NMDA receptors (NMDARs) and $GABA_A$Rs) (BOX 2; TABLE 2). In turn, these functional effects are likely part of the mechanisms underlying cognitive and behavioural hallmarks of FXS. In the following sections, we discuss recent advances in our understanding of channelopathy-based mechanisms of FXS pathophysiology and potential new therapeutic strategies stemming from our growing knowledge of FXS-related ion channel defects. Where applicable, we also review efforts to investigate potential therapeutic interventions that target particular channelopathies (TABLE 3).

Action potential initiation and firing

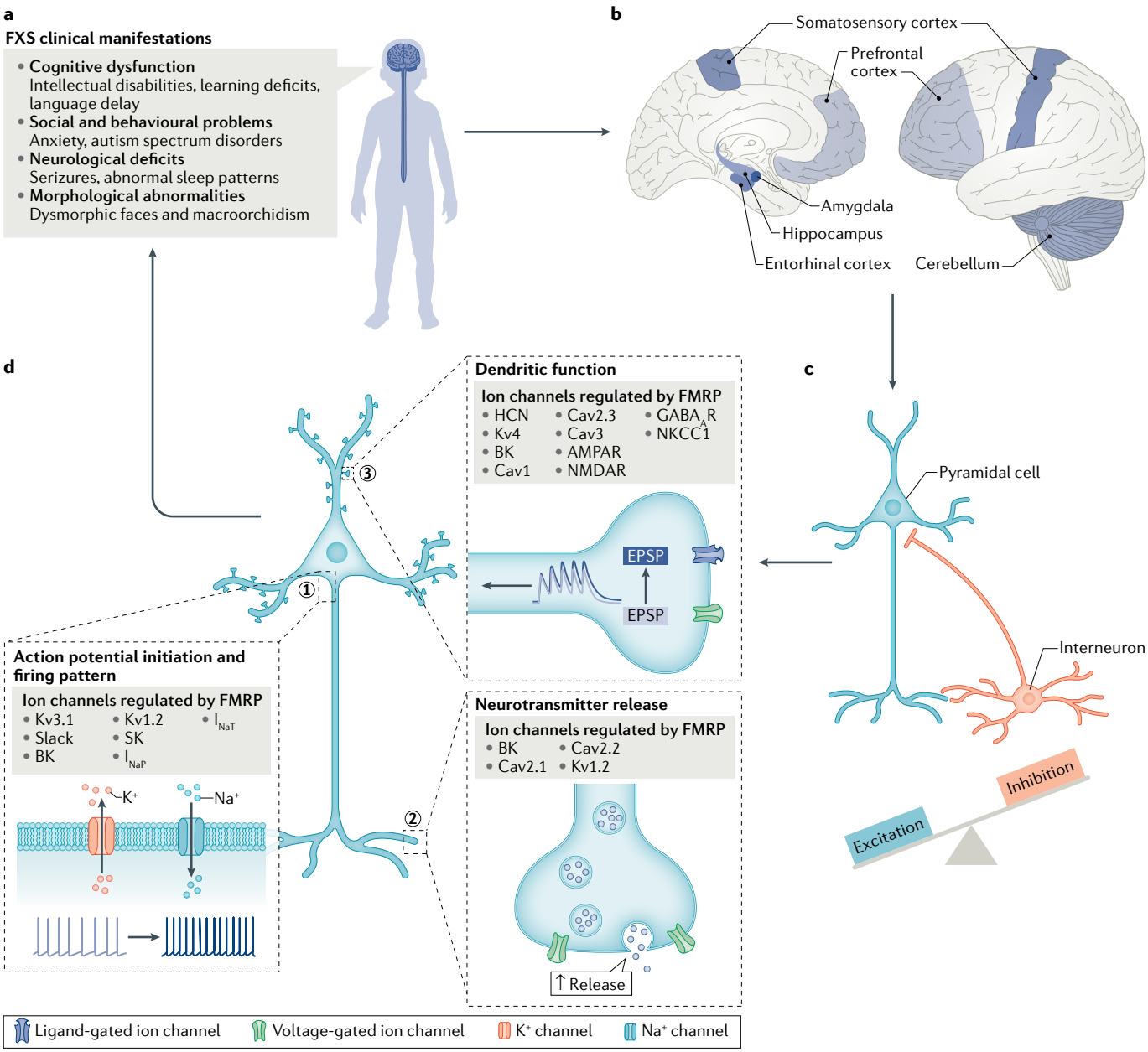
The FXS-related hyperexcitability noted at the behavioural and circuit levels is apparent in the lowered action potential threshold and elevated firing frequency characteristic of *Fmr1* KO animal models. These changes involve functional alterations to both K^+ channels and Na^+ channels.

The role of K^+ channels. K^+ channels crucially contribute to establishing the resting membrane potential, and setting action potential dynamics and firing patterns. FMRP regulates the translation of numerous K^+ channels, as well as their activity and surface expression (FIG. 2).

FMRP directly regulates the expression of delayed rectifier K^+ channel (Kv3.1) mRNA[67,68]. The rapid voltage-dependent activation/deactivation characteristics of Kv3.1 facilitate high firing rates in cells that express these channels. For example, there is normally a gradient of Kv3.1 expression within the sound localization circuitry of the brainstem (the medial nucleus of the trapezoid body). However, when FMRP is absent, the normal gradient distribution of Kv3.1 is flattened and the levels of Kv3.1b protein are higher overall[69]. Consequently, the high-threshold (Kv3.1-dependent) current is increased in the medial nucleus of the trapezoid body of *Fmr1* KO mice, leading to faster action potential repolarization and higher firing rates (that is, hyperexcitability)[53].

As increased Kv3.1 channel expression contributes to impaired encoding and processing of auditory information in *Fmr1* KO mice[68], these channels are promising therapeutic targets. Indeed, the Kv3.1 channel modulator AUT2 normalizes the action potential firing rate in auditory brainstem neurons and restores the auditory brainstem response in vivo in *Fmr1* KO mice[53]. Targeting Kv3.1 channels may have an even broader therapeutic potential to reduce sensory hypersensitivity across multiple modalities in FXS, as Kv3.1 channels are widely expressed in rapidly firing neurons throughout the nervous system[70]. However, it remains to be determined whether loss of FMRP also increases excitability among these rapidly firing neurons in various brain regions in a Kv3.1-dependent manner.

Slack channels are widely expressed in the brain, including in the brainstem, cerebellum, prefrontal cortex and hippocampus. As a major component of the delayed outward current, Slack channels are critical in regulating action potential firing patterns and temporal precision[71]. FMRP directly modulates Slack channel activity[72]. In a seminal study, Kaczmarek and colleagues show that the amino-terminal domain of FMRP interacts with the Slack channel carboxy terminus to increase the frequency of the channel opening (that is, gating) in a rapid and reversible manner[72,73]. Through this protein–protein interaction, FMRP dynamically and precisely tunes Slack channel activity and, thus, neuronal firing. In the absence of FMRP, Slack currents are significantly reduced, thus increasing neuronal excitability and reducing temporal precision of spiking, as observed in the auditory brainstem of *Fmr1* KO mice[72]. Together with the aforementioned effects on Kv3.1 channels, the FMRP-related decrease in Slack currents likely contributes to auditory hypersensitivity in FXS.



Hyperextensibility
The ability of the finger joints to move beyond their normal range of motion.

Interestingly, an additional 'non-conducting' function for Slack channels in regulating neuronal firing has been reported in *Aplysia* bag cell neurons in which activation of Slack channels is linked to protein synthesis-dependent, prolonged changes in neuronal firing patterns[73]. This function raises the possibility that Slack–FMRP interactions may link changes in neuronal firing to changes in protein translation, known to be disrupted in FXS.

Large conductance voltage and Ca^{2+}-activated K^+ (BK) channels are widely expressed throughout the brain, and in nearly every cellular compartment of neurons[74]. Being both voltage and calcium activated, these channels play critical roles in regulating neuronal excitability, action potential duration, firing and neurotransmitter release[75]. FMRP targets BK channels, regulating translation and function[43,57,62,76–80]. Specifically, the N-terminal domain of FMRP interacts with the channel's regulatory β4-subunit to increase the BK channel's Ca^{2+} sensitivity and, thus, open probability[43,62]. FMRP can also directly bind to the BK channel's pore-forming α-subunit[76,80] to modulate channel gating and increase BK currents[80]. Indeed, studies of both *Fmr1* KO mice and stem cell-derived neurons from patients with FXS report that the loss of FMRP reduces BK channel activity, which in turn leads to a wide range of functional consequences. For example, in hippocampal and cortical neurons these effects include increased firing, excessive action potential broadening, elevated presynaptic Ca^{2+} influx and increased glutamate release[43,62,76,81].

More recent studies support the idea that the loss of FMRP–BK channel interactions contributes to FXS pathophysiology. Particularly interesting is the discovery that a rare FMRP missense mutation (R138Q), which is associated with a partial clinical FXS phenotype (intellectual disability and seizures) in at least one human patient, impairs the protein's translation-independent presynaptic functions, including its ability to interact with BK channels to regulate action potential duration and control glutamate release (that is, excitability)[76]. In *Drosophila*, this mutation impairs the presynaptic-specific function of FMRP to control axonal elaboration in the neuromuscular junction[76]. In mouse cortical neurons, although the R138Q mutation preserves FMRP's canonical mRNA-binding and translational regulation functions — generally associated with (but not limited to) postsynaptic compartments — it impairs interactions with BK channels that ultimately control glutamate release[76]. These results suggest a model in which the effects of R138Q mutation are caused by disruption of FMRP's protein–protein interactions, including FMRP–BK channel interactions, which are important in regulating neuronal excitability and glutamate release, while retaining the translational regulation functions of FMRP.

Despite the compelling alignment of both clinical and preclinical data linking these specific FMRP functions to a specific subset of FXS phenotypes, the story is likely more complicated. Two other individuals with FXS who harbour the same mutation present with different clinical phenotypes: one (male) presents with a more extensive set of FXS features, including intellectual disability, attention deficit hyperactivity disorder, autism, seizures, craniofacial changes and hyperextensibility[2]; the other (unrelated female) presents with mild intellectual disability and attention deficits[82]. Data from a rodent model of FXS specific to the R138Q mutation indicate that at least some postsynaptic functions of FMRP are also impacted by this mutation; surface expression of AMPARs is increased, as is dendritic spine density, hippocampal long-term potentiation (LTP) is impaired and there are socio-cognitive deficits at the behavioural level[83]. These data suggest that the R138Q mutation may disrupt FMRP interactions with other channels as well, resulting in both presynaptic and postsynaptic defects.

Upregulating BK channel activity may be a useful approach to normalize a range of excitability-associated phenotypes in FXS. Indeed, genetically upregulating BK channel activity normalizes multiple synaptic and circuit defects in an FXS mouse model, including action potential broadening, elevated glutamate release and epileptiform activity in the hippocampal circuit[43]. Moreover, the BK channel opener BMS-204352 corrects numerous behavioural phenotypes in *Fmr1* KO mice, including hyperactivity, hypersensitivity to sensory stimuli, social defects, anxiety, deficient spatial memory, impaired nest building and excessive grooming[57,77,84]. Together, these studies suggest that targeting BK channels may

◀ Fig. 1 | **Overview of channelopathies in FXS. a** | Although fragile X syndrome (FXS) clinical manifestations are complex and vary considerably among individuals, most patients with FXS exhibit cognitive dysfunction (intellectual disabilities, learning deficits, language delay), social and behavioural problems (anxiety, autism spectrum disorders) and neurological deficits (seizures, abnormal sleep patterns), indicative of cellular and network hyperexcitability. **b** | Brain regions that are affected in FXS and discussed in this Review (somatosensory cortex, prefrontal cortex, hippocampus, entorhinal cortex, amygdala, cerebellum) in a medial view (left panel, brainstem and cerebellum removed) and a lateral view (right panel). **c** | Cartoon representation of the basic unitary neural circuit showing increased circuit excitability and excitation/inhibition imbalance, a key feature of the circuit changes observed in FXS. **d** | Hyperexcitability defects observed in FXS can be attributed to channelopathy-caused functional abnormalities in action potential initiation and firing, neurotransmitter release and/or dendritic function, which may underlie various FXS phenotypes shown in panel **a** (right-turn arrow from panel **d** to panel **a**). Ion channels contributing to these excitability defects are listed in corresponding inserts. Insert 1: FXS-related hyperexcitability is apparent in elevated neuronal firing frequency. These changes involve functional alterations in both potassium channels and sodium channels, including Kv1.2, Kv3.1, Slack, BK, SK, I_{NaP} and I_{NaT}. Action potential traces in light-blue and blue represent normal and FXS conditions, respectively, indicating that loss of fragile X mental retardation protein (FMRP) increases the action potential firing frequency. Insert 2: transduction of neuronal firing into neurotransmitter release at presynaptic terminals is mediated by Ca^{2+} influx through the voltage-gated calcium channels (VGCCs). Cav2.1 and Cav2.2 channels are the predominant VGCCs supporting neurotransmitter release in central neurons. FMRP can also indirectly regulate neurotransmitter release by controlling the action potential peak and duration, which in turn determine the amplitude and duration of presynaptic calcium influx through VGCCs. This mechanism is mediated by a subset of voltage-gated K^+ channels, including Kv1.2 and BK channels. Insert 3: dendritic excitability is governed, in large part, by ion channels, a number of which are dysregulated in the absence of FMRP, including HCN, Kv4, BK, Cav1, Cav2.3, Cav3, AMPAR, NMDAR, $GABA_AR$ and NKCC1. Excitatory postsynaptic potential (EPSP) waveforms in light-blue and blue denote normal and FXS conditions, respectively, indicating that loss of FMRP enhances EPSP integration and, thus, increases dendritic excitability. AMPAR, AMPA receptor; BK, large-conductance voltage and Ca^{2+}-activated K^+ channel; Cav, voltage-gated Ca^{2+} channel; $GABA_AR$, GABA type A receptor; HCN, hyperpolarization-activated, cyclic nucleotide-gated ion channel; I_{NaP}, voltage-gated Na^+ channel carrying persistent Na^+ current; I_{NaT}, voltage-gated Na^+ channel carrying transient Na^+ current; Kv, voltage-gated K^+ channel; NKCC1, Na^+-K^+-Cl^- co-transporter; NMDAR, NMDA receptor; SK, small-conductance Ca^{2+}-activated K^+ channel.

Box 2 | Mechanisms underlying excitability defects in fragile X syndrome models

Loss of fragile X mental retardation protein (FMRP) dysregulates a large number of ion channels. This, in turn, affects multiple aspects of neuronal excitability, including changes in the action potential initiation and firing rate, presynaptic vesicle release, dendritic properties and synaptic plasticity (TABLE 2).

- The combined activity of voltage-gated Na^+ and K^+ channels determines the action potential shape, firing rate and pattern. For example, channels that are active around threshold potentials, such as small-conductance Ca^{2+}-activated K^+ (SK) channels and Na^+ channels carrying persistent Na^+ current (I_{NaP}), contribute to action potential initiation. Na^+ channels carrying transient Na^+ current (I_{NaT}) determine the action potential rising speed and amplitude. Several families of voltage-gated K^+ channels act in concert to determine action potential repolarization dynamics, and thus the action potential duration.
- Action potential firing patterns are transduced into synaptic vesicle release at synaptic terminals via activation of voltage-gated calcium channels (VGCCs), among which P/Q-type and N-type Ca^{2+} channels are the major source of Ca^{2+} for synaptic vesicle release in central neurons.
- Multiple channels act together to control dendritic excitability and input resistance, and thus spatial and temporal summation, including K^+ channels, VGCCs and hyperpolarization-activated cyclic nucleotide-gated channels. Many of these channels are also involved in the initiation and maintenance of various forms of synaptic plasticity. Ligand-gated ion channels/ionotropic receptors (AMPA, NMDA and $GABA_A$ receptors) function to directly evoke excitatory or inhibitory synaptic currents.
- Many channels are expressed in multiple cellular compartments and be activated by different factors, in cell type-specific and brain region-specific manners.

have therapeutic potential in alleviating numerous excitability-associated FXS phenotypes.

Kv1.2 channels are widely distributed across various cellular compartments of neurons, and play important roles in regulating intrinsic excitability and action potential initiation[85]. In *Fmr1* KO mice, the Kv1-mediated current is downregulated in one population of layer 5 pyramidal cells of the medial prefrontal cortex (called long tract-projecting neurons), leading to increased excitability of these neurons[58]. Interestingly, this defect appears to be cell type-specific, as it is not observed in the neighbouring intratelencephalic projecting pyramidal cells of the same brain region. Mechanistically, FMRP binds Kv1.2 channel mRNA, and interacts directly with the channel through protein–protein interactions to regulate channel activity and surface expression. Specifically, recent studies indicate that the N terminus of FMRP directly binds to a phosphorylated serine motif on the C terminus of Kv1.2 to form a dimer–dimer configuration in which two C-terminal strands of the same Kv1.2 channel bind to one FMRP dimer[65]. This interaction enhances axonal trafficking, membrane expression and gating dynamics of Kv1.2 in axonal terminals of cerebellar basket cells, ultimately impacting presynaptic excitability and Ca^{2+} influx. Consequently, loss of FMRP–Kv1.2 interaction in *Fmr1* KO basket cells increases the duration and amplitude of action potentials, leading to enhanced presynaptic Ca^{2+} influx and excessive GABA release onto Purkinje neurons[65]. As Purkinje neurons are inhibitory, the overall effect of these changes is to promote circuit hyperexcitability.

As Purkinje neurons are the sole output cells from the cerebellar cortex, the Kv1.2 channelopathy is likely to lead to abnormal cerebellar functions. Targeting Kv1.2 may thus help normalize cerebellar circuit hyperexcitability and cerebellar-related abnormalities in FXS. Indeed, the therapeutic potential of one Kv1.2 agonist, docosahexaenoic acid, was recently tested in an FXS mouse model, where it was found to rectify the dysregulated cerebellar inhibition in vitro and to rescue acoustic startle reflex and social interaction phenotypes in vivo[65].

Voltage-independent K^+ channels also play key roles in regulating neuronal excitability by modulating the resting membrane potential, action potential threshold and afterpotentials[85]. Within the context of *Fmr1* KO mice, there is evidence of hypofunction of the SK channel, which is linked to neuronal hyperexcitability (lower action potential threshold, smaller medium afterhyperpolarization, increased firing rate and exaggerated synaptic gain) in CA3 pyramidal cells[51]. The SK current defect stems from the loss of FMRP interactions with the SK2 channel isoform, although channel expression is unaffected. This idea is further supported by the finding that all observed excitability defects in CA3 pyramidal cells of *Fmr1* KO mice are normalized by acute intracellular reintroduction of the N-terminal FMRP fragment (amino acids 1–298) or the genetic reintroduction of an even shorter N-terminal FMRP fragment (amino acids 1–234), both of which lack the KH2 domain and are thus incapable of translational regulation[51]. Interestingly, similar to other FXS-related channelopathies, the SK defects are cell type-specific. Within a single *Fmr1* KO animal, changes to the SK current in CA1 pyramidal cells mirror those apparent in CA3 cells: the SK current increases, as does the spike rate variability and medium afterhyperpolarization, but the action potential rate decreases[86]. Similar cell type-specific changes are also reported for Kv1 (REF.[58]) and HCN channels[56–58] (TABLE 1).

The role of Na⁺ channels. In most neurons, the action potential initiation and rising phase are governed by voltage-gated Na^+ channel availability and activation/inactivation dynamics. Na^+ channels can produce two distinct currents: the fast transient Na^+ current (I_{NaT}) and the non-inactivating persistent Na^+ current (I_{NaP}). I_{NaP} is active at subthreshold voltages, and thus is critical for action potential initiation, whereas I_{NaT} underlies the action potential rising phase[85]. Thus, disruption to either current is likely to impact neuronal excitability.

In *Fmr1* KO mice, I_{NaP} is abnormally increased in the entorhinal cortex layer 3 pyramidal cells, that is, neurons that project directly to the CA1 region. This upregulation of I_{NaP} leads to profound excitability defects, including lower action potential threshold and increased action potential firing[52]. Unlike many K^+ channel defects, which are mediated by FMRP–channel interactions within individual neurons, I_{NaP}-mediated defects are not apparent in neurons isolated from their circuit. This indicates that the defects are not cell autonomous. Indeed, the enhanced I_{NaP} is caused by exaggerated activation of mGluR5 signalling, which in turn acts on Na^+ channels through phospholipase C (PLC) and protein kinase C (PKC)[52]. This signalling mechanism requires circuit activity and is distinct from the well-established mGluR5 signalling cascade affecting local translation in FXS animal models. Thus, Na^+ channel dysregulation is likely a major contributor to neuronal hyperexcitability

Intratelencephalic projecting pyramidal cells
Telencephalic pyramidal cells whose axons project to regions within the telencepalon.

in the FXS mouse model, at least in this subpopulation of cortical neurons.

Although the above study did not include direct measures of I_{NaT}, the observation of increased action potential rising speed, an I_{NaT}-dependent parameter, suggests that I_{NaT} is also increased in *Fmr1* KO neurons[52]. Indeed, direct evidence of increased I_{NaT} resulting in larger and faster rising action potentials and a higher gain of somatic excitability is reported in layer 2/3 prefrontal cortex neurons of *Fmr1 KO* mice[54]. This study also finds that the observed excitability changes are accompanied by a large depolarizing shift in the activation of A-type K^+ channels. This shift is expected to decrease the action potential threshold, as is the case when I_{NaP} is higher. However, no such changes were observed[54], indicating that some form of compensatory changes may be involved.

Taken together, the data discussed here reflect a complex interplay of cell type-specific Na^+ and K^+ channelopathies caused by loss of FMRP. In nearly all cases, the deficits contribute to overall neuronal and circuit hyperexcitability. To elucidate further mechanistic detail will require careful consideration of cell type-specific effects, as well as the impact of compensatory changes.

Table 2 | **Mechanisms underlying excitability defects in fragile X syndrome models**

Channel	Changes	Functional abnormality	Refs
Action potential initiation and firing pattern			
Kv3.1	↑	↑ Action potential repolarization speed and firing rate	53,68
Slack	↓	↑ Action potential duration and firing rate	73
BK	↓	↑ Action potential duration, ↓ fAHP	62
Kv1.2	↓	↑ Action potential duration	65
SK	↓	↓ Action potential threshold and mAHP, ↑ action potential firing rate	51
	↑	↑ mAHP, ↓ action potential firing rate	86
I_{NaP}	↑	↓ Action potential threshold, ↑ action potential firing rate	52
I_{NaT}	↑	↑ Action potential rising speed and amplitude ↑ Action potential firing rate, ↓ action potential duration	54
Neurotransmitter release			
Cav2.1	↓	↓ Ca^{2+} influx and vesicle release	89
Cav2.2	↑	↑ Ca^{2+} influx and vesicle release	60,61
BK	↓	↑ Ca^{2+} influx and vesicle release	62
Kv1.2	↓	↑ Ca^{2+} influx and vesicle release	65
Dendritic function			
Kv4	↑	↑ Threshold for LTP, lack of TBS-induced LTP	91,96
Cav3	↑	↑ Ca^{2+} influx	88
BK	↓	↑ Dendritic excitability, ↑ sensory sensitivity, ↑ dendritic spines	57,77
Cav1	↓	↑ Threshold for tLTP	55,98
	↑	↑ Progenitor differentiation to glutamate-responsive cells	88
Cav2.3	↑	↑ Ca^{2+} influx	90
HCN	↓	↑ Input resistance and summation	56,101
	↑	↓ Input resistance and summation	57,59,101
AMPAR	↓	↓ LTP and ↑ LTD	103,104
	↑	↓ LTP, ↑ AMPAR current ↑ Silent dendritic spines	59,83
NMDAR	↓	↓ NMDAR currents	112,115
	↑	↑ NMDAR mEPSC frequency	59
$GABA_AR$	↓	↓ Inhibition	64,119,120
NKCC1	↑	Delayed $GABA_AR$ polarity switch, delayed maturation of glutamatergic synapses	144,145

↑, increase; ↓, decrease. AMPAR, AMPA receptor; BK, large-conductance voltage and Ca^{2+}-activated K^+ channel; Cav, voltage-gated Ca^{2+} channel; fAHP, fast afterhyperpolarization; $GABA_AR$, GABA type A receptor; HCN, hyperpolarization-activated cyclic nucleotide-gated ion channel; I_{NaP}, voltage-gated Na^+ channel carrying persistent Na^+ current; I_{NaT}, voltage-gated Na^+ channel carrying transient Na^+ current; Kv, voltage-gated K^+ channel; LTD, long-term depression; LTP, long-term potentiation; mAHP, medium afterhyperpolarization; mEPSC, miniature excitatory postsynaptic current; NKCC1, Na^+–K^+–Cl^- co-transporter; NMDAR, NMDA receptor; SK, small-conductance Ca^{2+}-activated K^+ channel; TBS, theta-burst stimulation; tLTP, spike-timing-dependent long-term potentiation.

Neurotransmitter release

The transduction of neuronal firing into neurotransmitter release at presynaptic terminals is mediated by Ca^{2+} influx through the voltage-gated calcium channels (VGCCs)[87]. Interestingly, in FXS models, all five types of mammalian VGCC — P/Q (Cav2.1), N (Cav2.2), R (Cav2.3), L (Cav1) and T (Cav3) — are affected[6,60,61,88–91] (FIG. 2; TABLE 2). N-type and P/Q-type channels are the predominant VGCCs supporting neurotransmitter release at most central synapses and are discussed here; those VGCC types that contribute to dendritic excitability changes in FXS are discussed in the next section.

N-type and P/Q-type VGCCs. The N-type VGCC (Cav2.2) plays a prominent role in neurotransmitter release at presynaptic terminals. It was also the first Ca^{2+} channel found to be directly modulated by FMRP through protein–protein interactions[60]. The mechanism of this interaction is distinct from the known FMRP interactions with K^+ channels; in the case of the N-type VGCC, the FMRP C-terminal domain interacts with the N-type channel's synaptic targeting domain[60,61] to suppress the channels' presynaptic localization and surface expression. Consequently, loss of FMRP increases N-type VGCC surface expression and glutamate release at presynaptic terminals of sensory neurons in the dorsal root ganglia[60,61].

The P/Q-type (Cav2.1) channel is also critical for action potential-evoked neurotransmitter release in many types of neuron. It is involved in various forms of synaptic plasticity as well as spatial learning and memory in mice[92]. In cortical neurons, overall levels of P/Q-type channels are unaltered by FMRP loss. However, we know that FMRP can bind to these channels to increase their surface expression[60,89]; this is the opposite of the effect of FMRP interactions with N-type channels. Thus, loss of FMRP results in reduced surface expression of P/Q-type channels, whereas surface expression of N-type channels is simultaneously increased[89]. Interestingly, it has been suggested that N-type and P/Q-type channels have channel type preferred slots in hippocampal synapses, which can be occupied by the competing channel type upon certain disease conditions[93]. Thus, the loss of FMRP may contribute to synaptic transmission deficits

Table 3 | **Manipulations that rescue cellular, circuit and behavioural phenotypes in fragile X syndrome models**

Target	Rescue strategy	Behavioural phenotypes normalized	Refs
Kv1.2	Oral administration of Kv1.2 agonist DHA	Acoustic startle reflex and social interaction	65
Kv3.1	Kv3.1-positive modulator AUT2	MNTB neurons firing rate	53
		Auditory brain response	
Kv4	Kv4 blocker heteropodatoxin	TBS-induced LTP in hippocampus	96
Slack	$FMRP_{1–298}$	Slack open probability	72,73
BK	Intracellular application of $FMRP_{1–298}$	Action potential duration, BK open probability, glutamate release	43,62,76
	Genetic upregulation of BK activity by ablation of BKβ4 subunit	Short-term plasticity	
		Epileptiform activity	
	Intraperitoneal injection of BK channel opener BMS-204352	Acoustic startle reflex, locomotor activity, nest building, grooming, social interactions, anxiety and spatial memory	57,77,84
SK	Intracellular administration of $FMRP_{1–298}$, genetic reintroduction of $FMRP_{1–234}$ or SK channel opener 1-EBIO or NS309	Action potential threshold, mAHP and action potential firing rate	51
		MF CA3 input–output transmission	
NMDAR	Bath application of NMDAR co-agonists glycine or D-serine	NMDAR-dependent LTP in dentate gyrus	112
$GABA_AR$	Intraperitoneal injection of $GABA_AR$ agonist ganaxolone, acamprosate or metadoxine	UP states, locomotor activity, anxiety, marble-burying behaviour and sensory hyperresponsiveness	126,130
NKCC1	Intraperitoneal injection of NKCC1 inhibitor bumetanide	$GABA_AR$ reversal potential of L4 somatosensory neurons	145
		Excitatory synapse development	
		LTP in somatosensory cortex	
		Whisker-evoked responses in the cortex	
FMRP loss	Intravenous injection of $FMRP_{297}$-*tat*	Cav3 and Kv4 currents	91
		Cerebellar MF granule cell LTP	
		Protein levels (APP, αCaMKII and PSD95), locomotor activity	

αCaMKII, α-Ca^{2+}/calmodulin-dependent protein kinase II; AUT2, 4-[(53)oxy)-2-(1-methylethyl) benzonitrile; BK, large-conductance voltage and Ca^{2+}-activated K^+ channel; Cav, voltage-activated Ca^{2+} channel; DHA, docosahexaenoic acid; FMRP, fragile X mental retardation protein; $GABA_AR$, GABA type A receptor; Kv, voltage-gate K^+ channel; LTP, long-term potentiation; mAHP, medium afterhyperpolarization; MF, mossy fibre; NS309, 3-oxime-6,7-dichloro-1*H*-indole-2,3-dione; MNTB, medial nucleus of the trapezoid body; NKCC1, Na^+–K^+–Cl^- co-transporter; NMDAR, NMDA receptor; PSD, postsynaptic density; SK, small-conductance Ca^{2+}-activated K^+ channel; TBS, theta-burst stimulation.

by creating an imbalance between the surface levels of N-type and P/Q-type channels, which differentially contribute to the efficiency of neurotransmitter release and synaptic plasticity[93].

Indirect modulation of calcium influx. In addition to controlling presynaptic surface expression of VGCCs, FMRP can also indirectly regulate neurotransmitter release by controlling the action potential duration (which, in turn, determines the duration of presynaptic calcium influx through VGCCs). This mechanism is mediated by a subset of voltage-gated K^+ channels that regulate action potential duration, and has major implications for glutamate and GABA release, both of which are significantly impacted in *Fmr1* KO mice. As discussed above, reduced BK channel activity in hippocampal and cortical excitatory neurons of *Fmr1* KO mice leads to extensive action potential broadening. This, in turn, elevates presynaptic calcium influx and glutamate release[62]. Similarly, the increased action potential duration apparent in cerebellar basket cells (secondary to reduced Kv1.2 channel activity) leads to excessive GABA release[65]. VGCC–K^+ channel activity interdependence is often reciprocal; for example, VGCCs are the main source of Ca^{2+} to activate BK channels, whereas BK channels can physically couple to pore-forming α-subunits of L-type, P/Q-type and N-type VCGGs[74]. This reciprocal interdependence is present to various extents across different types of central neuron. Thus, it likely contributes to neuronal hyperexcitability within the context of FXS.

Dendritic function

There are numerous reports of disrupted dendritic function in FXS models, including changes in excitability, gain, integration and multiple forms of long-term synaptic plasticity localized to the dendritic compartment[37,55–59]. These functions are governed, in large part, by ion channels, a number of which are dysregulated in the absence of FMRP (BOX 2; TABLE 2).

K^+ channels. The Kv4.2 channel regulates hippocampal and neocortical dendritic excitability and synaptic plasticity. FMRP can bind Kv4.2 mRNA and regulate

its translation[94–96]. FMRP can also directly interact with and regulate Kv4 activity. Specifically, reduced A-type K^+ currents (carried by Kv4 channels) in hippocampal pyramidal cells of *Fmr1* KO mice are associated with hyperpolarizing shifts in channel activation and inactivation kinetics[97], suggesting changes to channel gating properties. Moreover, FMRP constitutively binds to the complex of Kv4 and Cav3 channels in cerebellar

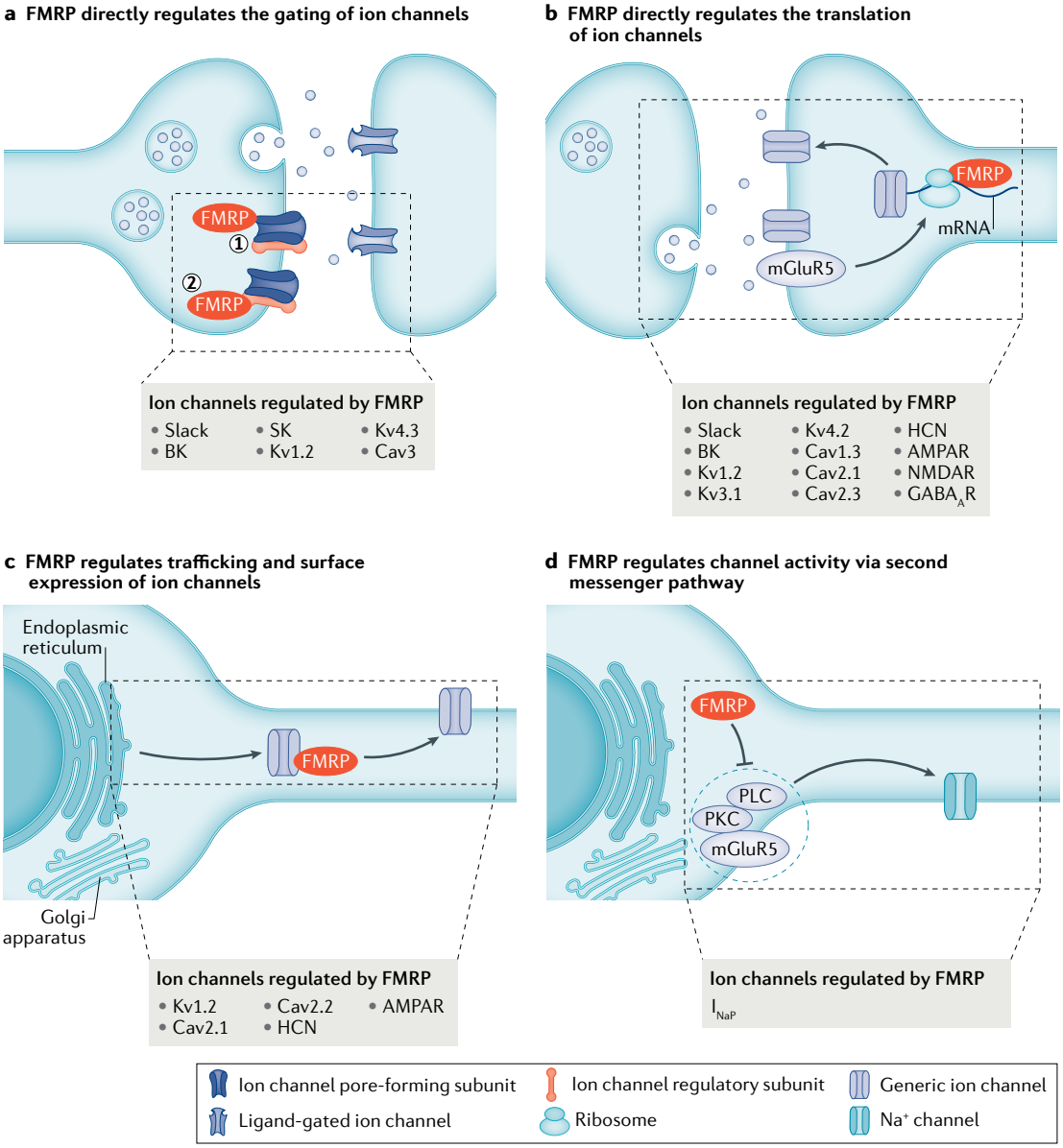


Fig. 2 | **FMRP controls ion channel functions through multiple mechanisms.** Fragile X mental retardation protein (FMRP) regulates channel functions by four major mechanisms. **a** | FMRP regulates ion channel gating. By interacting directly with the pore-forming subunit (1) and/or with the regulatory subunit (2) of ion channels, FMRP modulates the function of the pore-forming subunit and, thus, regulates neuronal excitability and neurotransmitter release. Ion channels regulated by FMRP through this mechanism include Slack, BK, SK, Kv1.2, Kv4.3 and Cav3. **b** | By binding to mRNA of ion channels, FMRP regulates ion channel translation and, thus, modulates neural excitability. Ion channels regulated by FMRP through this mechanism include Slack, BK, Kv1.2, Kv3.1, Kv4.2, Cav1.3, Cav2.1, Cav2.3, HCN, AMPAR, NMDAR and $GABA_AR$. **c** | By binding to ion channels, FMRP modulates ion channel trafficking and surface expression. Ion channels regulated by FMRP through this mechanism include Kv1.2, Cav2.1, Cav2.2, HCN and AMPAR. **d** | FMRP controls ion channel activity via second messenger pathways. By modulating the mGluR5–PKC–PLC pathway, FMRP modulates I_{NaP} and, thus, regulates the action potential threshold and excitability. Note that the same channel can be modulated via multiple mechanisms. Also, the direction of changes, extent and mechanism of channel modulation by FMRP is often brain region, cell type and even subcellular compartment-specific. AMPAR, AMPA receptor; BK, large-conductance voltage and Ca^{2+}-activated K^+ channel; Cav, voltage-gated Ca^{2+} channel; $GABA_AR$, GABA type A receptor; HCN, hyperpolarization-activated, cyclic nucleotide-gated ion channel; I_{NaP}, voltage-gated Na^+ channel carrying persistent Na^+ current; Kv, voltage-gated K^+ channel; mGluR5, metabotropic glutamate receptor subtype 5; NMDAR, NMDA receptor; PKC, protein kinase C; PLC, phospholipase C; SK, small-conductance Ca^{2+}-activated K^+ channel.

granule cells to modulate activity of both channels[91]. In both cases, FMRP decreases channel currents primarily through a strong hyperpolarizing shift in inactivation kinetics, whereas channel density is unchanged[91]. The Cav3–Kv4–FMRP interaction has important functional consequences within the context of FXS. In *Fmr1* KO mice, the theta burst-induced form of LTP is absent in mossy fibre–granule cell synapses of the cerebellar cortex, a change that is attributed to increased Kv4 current[91]. Significantly, acute intracellular administration of the N-terminal FMRP fragment (amino acids 1–297) is sufficient to restore LTP defects; it directly modulates Cav3–Kv4 channel complex activity in the absence of translational changes[91].

The fact that FMRP directly modulates Cav3–Kv4 complex activity raises the possibility that this interaction can be targeted as a therapeutic strategy. When the N-terminal fragment of FMRP is modified to facilitate blood–brain barrier crossing (conjugation to *tat*; $FMRP_{297}$-*tat*) and infused into *Fmr1* KO mice, it rapidly improves multiple functions at the cellular and behavioural levels, including restoration of Cav3–Kv4 complex activity, rescue of cerebellar mossy fibre LTP and normalization of hyperactivity in the open field test[91]. Interestingly, $FMRP_{297}$-*tat* also restores levels of several selected proteins (PSD95, CamKII) for at least 24 h. It remains unclear whether the observed changes in synaptic protein levels are a cause or a consequence of normalized LTP, as the $FMRP_{297}$ fragment lacks the ability to associate with polyribosomes and regulate translation. Additionally, there are still no data directly linking improvements in hyperactivity in vivo with restored Cav3–Kv4 complex function or the normalization of mossy fibre LTP. Understanding the mechanisms underlying $FMRP_{297}$-*tat* treatment is important because $FMRP_{297}$-*tat* is likely taken up by a wide range of excitatory and inhibitory neurons throughout the brain, including the cerebellum but also the cortex, hippocampus and other brain areas[91]. However, regardless of the precise mechanisms, a *tat*-conjugate approach is a promising strategy through which to reintroduce a combination of different FMRP segments containing various functional domains, thus wielding broad power to address FXS channelopathies.

In addition to their prominent presynaptic roles in controlling action potential duration and glutamate release, BK channels are also localized to dendrites and regulate dendritic excitability. In *Fmr1* KO mice, BK channel activity is reduced in dendrites of neocortical neurons, leading to exaggerated Ca^{2+} influx accompanying back-propagating action potentials and reduced dendritic spike threshold[57]. The BK channel opener BMS-204352 rescues a wide range of behavioural phenotypes in these mice, which likely reflects the effect of normalizing both presynaptic and postsynaptic functions[57,77,84].

Calcium channels. In addition to the Cav3 (T-type) VGCC discussed above, a large proportion of depolarization-induced Ca^{2+} influx in dendrites is mediated by L-type (Cav1) VGCCs. The L-type channels regulate dendritic excitability, gene expression, synaptic plasticity, and neural differentiation and migration[87]. Cav1.3 mRNA is an FMRP target; channel expression is downregulated in the frontal cortex and cerebellum of *Fmr1* KO mice[98]. In the dendritic spines of prefrontal cortex layer 2/3 neurons from *Fmr1* KO mice, the reduced expression of L-type channels is linked with defects in spike timing-dependent plasticity[55]. Interestingly, in both human patients with FXS and mouse models, Ca^{2+} influx through L-type VGCCs is increased in neural progenitors derived from stem cells[88]. Specifically, the ratio of L-type to T-type channels increases, leading to enhanced progenitor differentiation to glutamate-responsive cells. These findings suggest that there are significant implications of upregulated L-type channel function on differentiation, migration and fate determination in neural progenitor cells within the context of FXS[88].

The R-type calcium current is carried by Cav2.3, whose mRNA is also targeted by FMRP[94]. This channel regulates burst-firing and afterdepolarization in dendrites, and its inhibition disrupts a form of mGluR-dependent long-term depression[87]. Consistent with profound alterations in dendritic excitability and calcium spiking in neurons lacking FMRP, Cav2.3 expression and the R-type Ca^{2+} current are increased in brain synaptosomes and hippocampal cultured neurons from *Fmr1* KO mice[90]. Moreover, loss of FMRP occludes mGluR-dependent Cav2.3 upregulation[90]. We do not yet completely understand how these defects contribute to altered dendritic excitability in FXS models, as other factors are also involved in modulating excitability following mGluR stimulation, including the downregulation of several K^+ channels[99].

HCN channels. HCN channels are active at rest, and therefore play a crucial role in controlling the resting membrane potential, input resistance and, thus, dendritic excitability[100]. They are prominently expressed in the soma and dendrites of excitatory neurons, following an expression gradient that increases towards more distal regions to regulate the integration of synaptic events occurring at different dendritic locations. Numerous cell type-specific alterations in HCN channels are observed in FXS models. For example, in CA1 pyramidal cells of *Fmr1* KO mice, HCN1-subunit expression and dendritic HCN currents are elevated. This, in turn, decreases input resistance, reduces temporal summation and increases membrane resonance frequency[56]. By contrast, in both layer 5 pyramidal cells of the somatosensory cortex and layer 4 stellate cells in these mice, HCN1 subunit expression and HCN current are decreased, leading to opposite changes in excitability, that is, increased dendritic gain[57,59]. Mechanistically, FMRP bidirectionally regulates HCN channels via a protein–protein interaction mechanism in dendrites of hippocampal CA1 pyramidal cells and prefrontal cortex neurons[101]. FMRP associates with the HCN–TRIP8b complex to downregulate (in the hippocampus) or upregulate (in the prefrontal cortex) the number of functional dendritic HCN channels. This previously unrecognized mechanism expands the repertoire of modulatory mechanisms by which FMRP regulates ion channel functions and excitability (FIG. 2), in opposite directions depending on

the cellular milieu. If this type of regulation is observed more broadly in future studies, it may explain many cell type-specific changes in channel expression/activity in neurons lacking FMRP.

Ligand-gated ion channels. The ligand-gated ion channels are a group of postsynaptic neurotransmitter receptors, also known as ionotropic receptors. There are three major ionotropic receptors involved in FXS: AMPARs, NMDARs and $GABA_A$Rs. The AMPARs and NMDARs are non-selective cation channels, whereas $GABA_A$R is a Cl^- channel. In addition to these channels, we discuss the impact of FMRP loss on the chloride transporters that maintain intracellular Cl^- ion homeostasis, as these transporters determine the $GABA_A$R polarity switch early in development involved in synaptogenesis and synapse maturation.

AMPARs mediate fast excitatory synaptic transmission in the brain. Altering the number of postsynaptic AMPARs is a fundamental mechanism of activity-dependent plasticity at excitatory synapses, including some forms of LTP and long-term depression. Local protein synthesis in dendrites is required for stable expression of LTP and long-term depression, and there is now evidence that these newly expressed proteins are regulated by specific RNA-binding proteins, including FMRP[102–107]. Thus, FMRP may indirectly regulate synaptic plasticity through AMPAR-dependent functions[59,83,102–107]. In *Fmr1* KO mice, expression of the AMPAR subunit GluA1 is decreased in the cortex (but not in the hippocampus or cerebellum[103]), as is LTP[103,104]. AMPARs also show brain region/cell type-specific defects in FXS models. Specifically, in the hippocampus, FMRP promotes GluA1 surface expression in adult-born granule cells of the dentate gyrus, but has no effect on total GluA1 protein levels[106,107]. Consequently, loss of FMRP decreases surface expression of GluA1, reduces GluA1-mediated AMPA currents and delays dendritic maturation. By contrast, layer 4 stellate cells of the primary somatosensory cortex in *Fmr1* KO mice show elevated single-spine excitation with normal spine morphology, in part owing to increased AMPAR currents[59]. Consistent with this, studies of a new FXS mouse model (point missense mutation R138Q in FMRP) reveal an increase in both total and surface expression of AMPAR subunits GluA1 and GluA2, decreased thickness of postsynaptic density and reduced LTP[83].

The evidence presented above raises the possibility that positive allosteric modulators of AMPARs could be useful for restoring some of the synaptic deficits associated with FXS. However, a double-blind clinical trial of one such compound, CX516, did not show significant improvements in cognition, language or attention/executive function in individuals with FXS[108]. AMPARs also show brain region/cell type-specific defects in FXS models. Thus, globally targeting AMPARs is unlikely to be effective.

NMDARs are involved in synaptic transmission, plasticity and experience-dependent synaptic refinement during development. FMRP regulates transport and stabilization of NMDAR subunits GluN1, GluN2B (REF.[109]) and GluN2A (REF.[110]). In the dentate gyrus of *Fmr1* KO mice, NMDAR currents are decreased and LTP is reduced[111]. The reduction in NMDAR-dependent synaptic plasticity in this region is accompanied by lower levels of GluN1, GluN2A and GluN2B subunits. Indeed, the NMDAR co-agonists glycine and D-serine can restore NMDAR-dependent LTP in these mice to wild-type levels[112,113]. At the behavioural level, NMDAR hypofunction is also linked to impaired context discrimination in adult *Fmr1* KO mice[114]. Most recently, Yau et al. found that lower NMDAR currents in the dentate gyrus of *Fmr1* KO mice are associated with a significant decrease in dendritic complexity (that is, reduced dendritic length and number of intersections)[115]. Yet, again, evidence points to cell type-specific changes in NMDAR levels and functions as a result of FMRP loss. NMDAR signalling is elevated in stellate cells of somatosensory cortex layer 4, and dendritic morphology is normal[59]. In these cells, the increase in dendritic gain and enhanced summation appear to be caused by a threefold increase in the number of polysynaptic spines and an increase in intrinsic excitability. Interestingly, although the loss of FMRP causes abnormal synaptogenesis, there is little correlation between spine structure and function, at least at the age of the animals used in this study[59].

Fast inhibitory synaptic neurotransmission in the brain is mostly mediated by GABA and its ionotropic target, $GABA_A$Rs[116]. The $GABA_A$R is a pentameric ion channel that is selectively permeable to Cl^- ions. Animal models of FXS show abnormalities in $GABA_A$R expression and function, as do human patients with FXS (for a review see REF.[117]). Kooy and colleagues initially found decreased expression of $GABA_A$R δ-subunit mRNA in *Fmr1* KO mice[118]. Further studies from multiple groups found that the protein levels of more than half of the $GABA_A$R subunits (including α1–α4, β1–β3, γ1, γ2 and δ) are lower in FXS mouse models[48,64,119–124] and throughout the brain of patients with FXS[125]. In addition, the amount of GABA itself is also lower across multiple brain regions in FXS mouse models[126,127]. Such hypofunction of the GABAergic system is likely to cause profound circuit hyperexcitability. Indeed, Morin-Parent et al. demonstrate that reduced $GABA_A$R function causes impaired short-interval intracortical inhibition, which contributes to cortical hyperexcitability in individuals with FXS[9].

Given the critical role of the GABAergic system in network excitability, $GABA_A$R agonists have the potential to ameliorate excitability-associated deficits in FXS[128,129]. The $GABA_A$R agonists acamprosate, ganaxolone and metadoxine can improve multiple defects in *Fmr1* KO models, including neuromuscular junction overgrowth and locomotor defects[130], prolonged cortical UP state and anxiety[131], and marble-burying behaviour and sensory hyperresponsiveness[126]. However, when these compounds were tested in clinical trials of individuals with FXS, there were no significant improvements in outcome measures[132–135]. Another strategy is to target metabotropic $GABA_B$ receptors to alleviate FXS deficits by normalizing the GABAergic system. However, again, although the $GABA_B$ receptor agonist baclofen restores cognitive and behavioural deficits in animal models[136–139], it did not impact primary outcome

Critical period
A time period during early postnatal life when the development and maturation of functional properties of the brain is strongly dependent on experiences or environmental influences.

measures in clinical trials[140,141]. These results highlight the limitations of targeting a single aspect of excitability defects in FXS. Ultimately, the most effective treatment strategies are likely to be multifactorial.

Chloride transporters. In addition to wielding fine control over circuit excitability, GABAergic transmission plays a critical trophic role in cortical development through its early depolarizing action. During the first few postnatal weeks (the critical period) in rodents, activating $GABA_AR$ depolarizes neurons, increasing their excitability and promoting Ca^{2+} entry[142]. This in turn regulates cell migration, proliferation and synaptogenesis. The early depolarizing effect of GABA results from a transient high intracellular Cl^- concentration that is only present early in development and is determined by the expression pattern of two Cl^- co-transporters: Na^+–K^+–Cl^- co-transporter (NKCC1) and K^+–Cl^- co-transporter (KCC2). NKCC1, which transports Cl^- into neurons, is expressed at high levels early after birth, whereas the levels of KCC2, which extrudes Cl^- from neurons, gradually increase over the course of development[143]. In the FXS mouse model, NKCC1 expression stays high through the end of the critical period, thus delaying the $GABA_AR$ polarity switch and disrupting the normal trophic function of GABA[144–146].

Systemic administration of bumetanide to inhibit NKCC1 during the critical period rectifies the intracellular Cl^- imbalance in layer 4 somatosensory cortex neurons and corrects the development of thalamocortical excitatory synapses in *Fmr1* KO mice[145]. Inhibiting NKCC1 during early development also leads to broad remodelling of the proteome in the barrel cortex, and restores adult tactile response maps in *Fmr1* KO mice[145]. These findings provide a rationale for considering NKCC1 as a novel therapeutic target in FXS.

Caveats of targeting channelopathies

As we have presented above, channelopathies play wide and varied roles in FXS pathophysiology. This makes ion channel-targeting therapies of particular interest. Indeed, multiple ion channel modulators show promise based on their ability to normalize cellular-level, circuit-level and behaviour-level abnormalities in FXS animal models (TABLE 3). We note above many cases in which specific ion channel-targeting therapies have already been tested in both animals and human patients. However, in most cases, clinical trials fail to show efficacy. In fact, recent studies emphasize that loss of FMRP can have diverse effects on the same ion channel depending on the brain region, cell type or even subcellular compartment[56,58,97]. This will make targeting any given ion channel for therapeutic intervention in FXS particularly challenging. Recent modelling studies also indicate that many cellular and synaptic pathologies in *Fmr1* KO mice are antagonistic at the circuit level, and hence may be compensatory to the primary pathology[37]. Thus, although there has been major progress towards identifying the molecular dysfunctions at synaptic and cellular levels, we need to better understand the overall effects of various channelopathies on circuit functions to design more effective therapeutic strategies. Moreover, because no single channelopathy can account for all, or even most, of the defects in FXS, a combinatory approach is almost certainly necessary. Alternatively, a strategy that targets the primary causes of ion channel dysfunctions (such as loss of interactions with, or translational regulation by, FMRP) might prove more efficient. For example, a *tat*-conjugate approach to reintroduce the N-terminal FMRP fragment via intravenous injection restores ion channel and synaptic functions in at least part of the brain, as well as locomotion activity at the behavioural level in *Frm1* KO mice[91]. Finally, a role for astrocytes in modulating neuronal excitability and plasticity in FXS is beginning to emerge[147–149]. This may represent another potential target through which to normalize excitability deficits in FXS.

Implications for ASD

FXS is one of the many causes of ASD and has comorbidities with other genetic causes and also with idiopathic ASD. FXS and FMRP loss-independent ASD, although genetically distinct, share significant clinical phenotypes. Indeed, their similarities suggest some level of common pathophysiology, including similar ion channel-based deficits[150]. There are multiple types of ion channel and transporter that are affected, in similar ways, in both FXS and FMRP loss-independent ASD[151], including Kv4.2 (REFS[152,153]), BK channels[154], Na^+ channels[155], Ca^{2+} (L-type, R-type and T-type) channels, HCN1 (REF.[156]), $GABA_AR$ and NKCC1 (REF.[151]). Moreover, channelopathies responsible for hyperexcitability of somatosensory neurons are part of the core developmental pathology in ASD models, leading to region-specific brain abnormalities during the critical period[156]. Peripherally restricted $GABA_AR$ agonists, which reduce hyperexcitability within peripheral sensory circuits, improve numerous ASD-associated behaviours in mouse models, including tactile over-reactivity, anxiety-like behaviours and certain social impairments[156]. Together, these findings indicate that what we learn about the role of channelopathies in FXS pathogenesis is likely to have important implications for ASD stemming from other causes.

Concluding remarks

We now understand that FMRP wields control over neuronal and circuit excitability by regulating ion channels (FIG. 1; TABLES 1 and 2). Indeed, FMRP modulates the physiological functions of many different types of ion channel, each of which contributes uniquely to controlling intrinsic excitability, action potential properties, firing patterns, synaptic transmission, synaptic plasticity and dendritic integration. FMRP can regulate ion channel function via protein–protein interactions with pore-forming or regulatory subunits, or channel expression, and some channels are regulated via both mechanisms (FIG. 2). FMRP can also indirectly modulate channel activity through second messenger pathways. The various mechanisms through which FMRP regulates channel function may be brain region-specific, cell type-specific or even subcellular compartment-specific. Given that direct FMRP–channel interactions are rapid and reversible, this mechanism means that ion channels can be precisely and dynamically tuned, which is critical

for normal excitability, synaptic plasticity and information processing, and, ultimately, normal behavioural and cognitive functions. Consequently, loss of FMRP, as occurs in FXS, triggers a series of channelopathies that contribute to circuit hyperexcitability-related abnormalities, which in turn underlie core symptoms of FXS. Focusing on the role of channelopathies in such neuronal and circuit-level defects will be crucial in understanding the mechanistic basis of FXS pathophysiology and in designing novel therapeutic strategies.

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