

The mGluR theory of fragile X mental retardation

Mark F. Bear¹, Kimberly M. Huber² and Stephen T. Warren³

¹The Picower Center for Learning and Memory, Howard Hughes Medical Institute and Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

²Center for Basic Neuroscience, Department of Physiology, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA

³Department of Human Genetics, Emory University School of Medicine, Atlanta, GA 30322, USA

Many of the diverse functional consequences of activating group 1 metabotropic glutamate receptors require translation of pre-existing mRNA near synapses. One of these consequences is long-term depression (LTD) of transmission at hippocampal synapses. Loss of fragile X mental retardation protein (FMRP), the defect responsible for fragile X syndrome in humans, increases LTD in mouse hippocampus. This finding is consistent with the growing evidence that FMRP normally functions as a repressor of translation of specific mRNAs. Here we present a theory that can account for diverse neurological and psychiatric aspects of fragile X syndrome, based on the assumption that many of the protein-synthesis-dependent functions of metabotropic receptors are exaggerated in fragile X syndrome. The theory suggests new directions for basic research as well as novel therapeutic approaches for the treatment of humans with fragile X, the most frequent inherited cause of mental retardation and an identified cause of autism.

Fragile X is the most common inherited form of human mental retardation. It is typically caused by a trinucleotide repeat expansion in the X-linked *FMR1* gene that prevents expression of the encoded protein, called fragile X mental retardation protein (FMRP) [1]. Brain development in the absence of FMRP gives rise to the major symptoms of fragile X syndrome in humans [2,3]. These include mental retardation in the moderate to severe range, developmental delay, attention deficit and hyperactivity, anxiety with mood lability, and obsessive–compulsive and autistic behaviors. People with fragile X also have poor motor coordination, and an increased incidence of epilepsy. Common peripheral symptoms are heightened sensitivity to tactile irritation and loose bowel movements. Non-neurological symptoms can include a long face, large ears, hyperextensible joints, and enlarged testes in post-pubescent males. Autopsy studies indicate that although the brain is grossly normal, dendritic spines are longer and immature in appearance [4–6]. Spine abnormalities have long been associated with human mental retardation of unknown etiology [7], as well as with Down's and Rett syndromes [8]. Spines, of course, are where excitatory

synaptic transmission and several important forms of synaptic plasticity occur.

A key advance for understanding fragile X was the isolation of the *FMR1* gene and subsequent generation of the *Fmr1* knockout mouse [9]. The phenotype of the *Fmr1* knockout mouse is multifaceted, and generally consistent with the human [3]. The most robust and reproducible behavioral phenotypes are increased locomotor activity and reduced habituation in an open field, and increased susceptibility to audiogenic seizure. Additionally, mild learning deficits have been noted [10]. Importantly, the *Fmr1* knockout has dendritic abnormalities analogous to those in humans – more long, thin spines [11,12]. Thus, there is reason to suspect that many aspects of fragile X can be attributed to altered synaptic development and plasticity.

A study of synaptic plasticity in the hippocampus of the *Fmr1* knockout mouse suggested a novel connection between metabotropic glutamate receptor (mGluR) signaling and the fragile X phenotype [13]. The resulting theory has generated some excitement in the fragile X field because it points to a possible therapeutic approach to the disorder. Here we articulate the origins, assumptions, and potential consequences of the 'mGluR theory'. This is a case study in how basic research can lead in unexpected directions.

From long-term synaptic depression to fragile X

Synaptic activity in the brain can trigger long-lasting changes in synaptic strength called long-term potentiation (LTP) and long-term depression (LTD). In neonates, the mechanisms of LTP seem to be important for retaining nascent synapses, whereas LTD mechanisms seem to be important for activity-guided synapse elimination. These same mechanisms, working in concert, contribute to learning and memory storage throughout postnatal life [14].

Understanding the mechanisms and functional significance of LTP and LTD first required the establishment of paradigms in which they can be reliably elicited. In the case of LTD, the first useful model was developed by Ito in the cerebellar cortex [15,16]. For many years it was believed that homosynaptic LTD might be the exclusive province of the cerebellum, where it was specialized for motor learning, coordination, and balance. However, a reliable method for inducing LTD using low-frequency

synaptic stimulation was eventually established in hippocampus [17], and the study of LTD at synapses throughout the brain has subsequently flourished.

LTD of the parallel fiber to Purkinje cell synapse in the cerebellum is elicited by coincident activation of the parallel fibers and the climbing fibers. Climbing fiber synapses are very powerful, and their activation leads to a large rise in intracellular calcium that is permissive for LTD. However, a key signal that distinguishes active from inactive parallel fiber synapses, and which is required to trigger LTD, is activation of postsynaptic group 1 (Gp1) mGluRs. Gp1 mGluRs, by definition, stimulate phosphoinositide hydrolysis and comprise mGluR1 and mGluR5, which have different tissue and subcellular localization. Induction of cerebellar LTD requires activation of mGluR1 [18,19].

Although the most thoroughly characterized form of LTD in the hippocampus is triggered by activation of postsynaptic NMDA receptors, there is evidence for a second type of LTD that, like in cerebellum, requires activation of postsynaptic Gp1 mGluRs [20]. Interestingly, although both forms of hippocampal LTD can be induced by identical patterns of synaptic stimulation [21,22] and can be expressed as a decrease in the number of postsynaptic AMPA receptors [23,24], they are mechanistically distinct. One of the important distinctions is that LTD triggered by mGluR activation (mGluR-LTD) requires the rapid translation of preexisting mRNA in the postsynaptic dendrites [25]. Although NMDA-receptor-dependent LTD, like LTP, also requires protein synthesis to persist longer than a few hours [26,27], the early expression is protein-synthesis-independent [25,27]. Another distinction is that whereas NMDA-receptor-dependent LTD is readily reversible, mGluR-dependent LTD is not [20]. An irreversible loss of glutamate receptors during mGluR-LTD could be a prelude to synapse elimination [24].

The LTD literature can be confusing because different routes of induction can engage different mechanisms, and these can vary with age and synapse type; so it is important for us to be explicit. Although many details remain to be worked out, particularly the precise role for protein synthesis, the mGluR-LTD in area CA1 that we describe here requires activation of mGluR5 (the major postsynaptic Gp1 receptor in the forebrain) [28], the G_q family of G-proteins [29], and extracellular signal-regulated kinase (ERK), one of the mitogen-activated protein kinases (MAPK) [30]. Although the depression of synaptic transmission and the loss of surface-expressed glutamate receptors occur immediately after mGluR5 activation without new protein synthesis, these changes rapidly revert (within 30 min) if postsynaptic mRNA translation is inhibited (Figure 1a,b). However, new protein synthesis is only required for a finite critical period (<60 min) immediately after activation of mGluRs. A model that captures these features of mGluR-LTD is presented in Figure 1(c).

There were several reasons why it was of interest to investigate the role of FMRP in protein-synthesis-dependent mGluR-LTD. FMRP mRNA is found in dendrites and FMRP protein binds mRNA [31], as we will discuss further. However, the strongest rationale for studying FMRP in

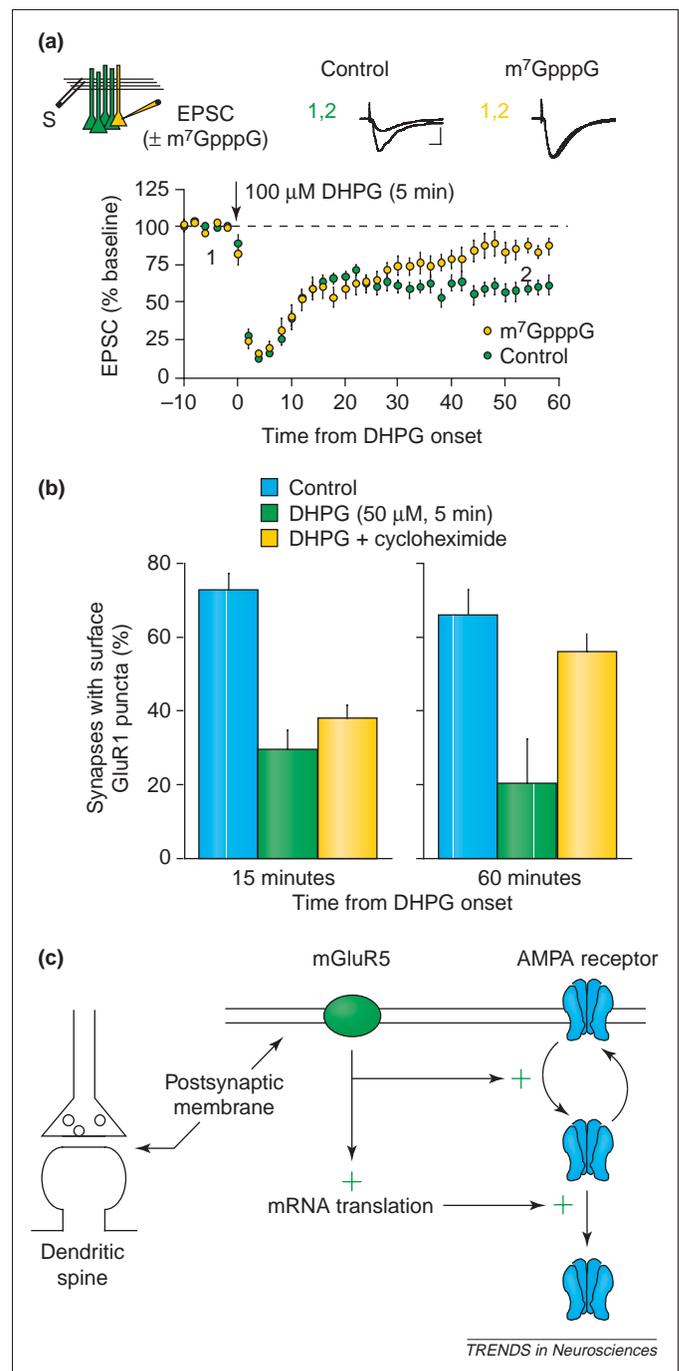


Figure 1. A role for postsynaptic protein synthesis in the stabilization of hippocampal long-term depression (LTD) induced by group 1 (Gp1) metabotropic glutamate receptor (mGluR) activation. **(a)** Summary of experiments in which the effect of activating Gp1 mGluRs in hippocampal slices is compared in the presence or absence of postsynaptic mRNA translation. To activate Gp1 mGluRs, the selective agonist (R,S)-3,5-dihydroxyphenylglycine (DHPG) was briefly applied. To block translation, the mRNA cap analogue (m⁷GpppG) was introduced into CA1 pyramidal neurons through a patch recording pipette. DHPG rapidly depresses synaptic transmission, but this effect persists only if protein synthesis is allowed to occur. Data replotted from Ref. [25]. Inset: schematic of experimental design showing placement of stimulating electrode (S) and the intracellular recording and injection electrode (EPSC). Representative excitatory postsynaptic potentials (EPSCs) are from a control cell and cell containing m⁷GpppG at the times indicated by the numbers on the graph. **(b)** Summary of experiments in which surface expression of synaptic AMPA receptor subunit GluR1 on cultured hippocampal neurons was monitored following DHPG treatment ± the protein synthesis inhibitor cycloheximide. AMPA receptors are rapidly lost from synapses, but are re-expressed on the surface if protein synthesis is inhibited. Data replotted from Ref. [24]. **(c)** Model to account for LTD findings. The loss of surface AMPA receptors is proposed to be at least partially responsible for the depression of synaptic transmission, and this change is stabilized by a process that requires mRNA translation near the synapse. DHPG-induced LTD fails to occur in mGluR5 knockout mice, suggesting this is the key Gp1 mGluR for this response [28].

LTD was that activation of Gp1 mGluRs was reported to stimulate the synthesis of this protein rapidly in synaptoneurosomes [32]. We therefore investigated mGluR-LTD in the *Fmr1* knockout mouse. The anticipated phenotype was defective LTD, so it came as a surprise that mGluR-LTD was actually significantly enhanced in the mutants as compared to wild-type littermates [13]. By contrast, there were no differences in NMDA-receptor-dependent LTD (at least not in the early, protein-synthesis-independent phase), consistent with earlier studies that failed to find any deficits in NMDA-receptor-dependent LTP [33,34]. Thus, the phenotype was specific to the mGluR-dependent form of synaptic plasticity.

Our data showed that one functional consequence of Gp1 mGluR activation – protein-synthesis-dependent LTD – was exaggerated in the absence of FMRP. Based on the evidence that FMRP is normally synthesized following stimulation of Gp1 mGluRs, we proposed a simple model to account for our findings (Figure 2a). According to this model, mGluR activation normally stimulates synthesis of proteins involved in stabilization of LTD and, in addition, FMRP. The FMRP functions to inhibit further synthesis (an example of end-product inhibition), and puts a brake on LTD. Recent research suggests that this ‘black box’ model is actually consistent with the biology of FMRP.

Emerging functions of FMRP

FMRP has been the subject of several recent reviews [3,35–37]. The excitement stems in part from the fact that fragile X syndrome is caused by a defect in a single gene, so understanding the function of the missing protein promises to provide insight into the pathophysiology of mental retardation, as well as cognition in general. However, the other reason for sudden interest is that FMRP has proven to be a fascinating molecule; it has captured the attention of neurobiologists interested in the synaptic control of protein synthesis, and the role of protein synthesis in changing synaptic structure and function.

Of particular importance for our thesis is the role of FMRP in mRNA translation regulation. FMRP is associated with actively translating polysomes in an RNA-dependent manner via messenger ribonucleoprotein (mRNP) particles [38]. A missense mutation (I304N) in the RNA-binding domain of the protein prevents the polysome association and results in severe mental retardation, suggesting that this interaction is key to the function of the protein [39]. Several innovative approaches have been taken to identify the mRNA targets of FMRP, in the hope of identifying which proteins are misregulated in fragile X [40–43]. This story has taken a very interesting twist recently with the discovery that FMRP specificity can be conferred by binding RNAs that are untranslatable. In one recent study, FMRP was shown to bind *BC1* [44], an untranslated message abundant in dendrites that functions as a translation repressor [45]. It was reported that *BC1* can specifically repress translation of the mRNAs for the synaptic proteins Arc and α -Ca²⁺/calmodulin-dependent protein kinase II (α CaMKII), and the dendritic microtubule associated protein 1b (MAP1b) [44]. Furthermore, FMRP has been shown to be a part of the machinery

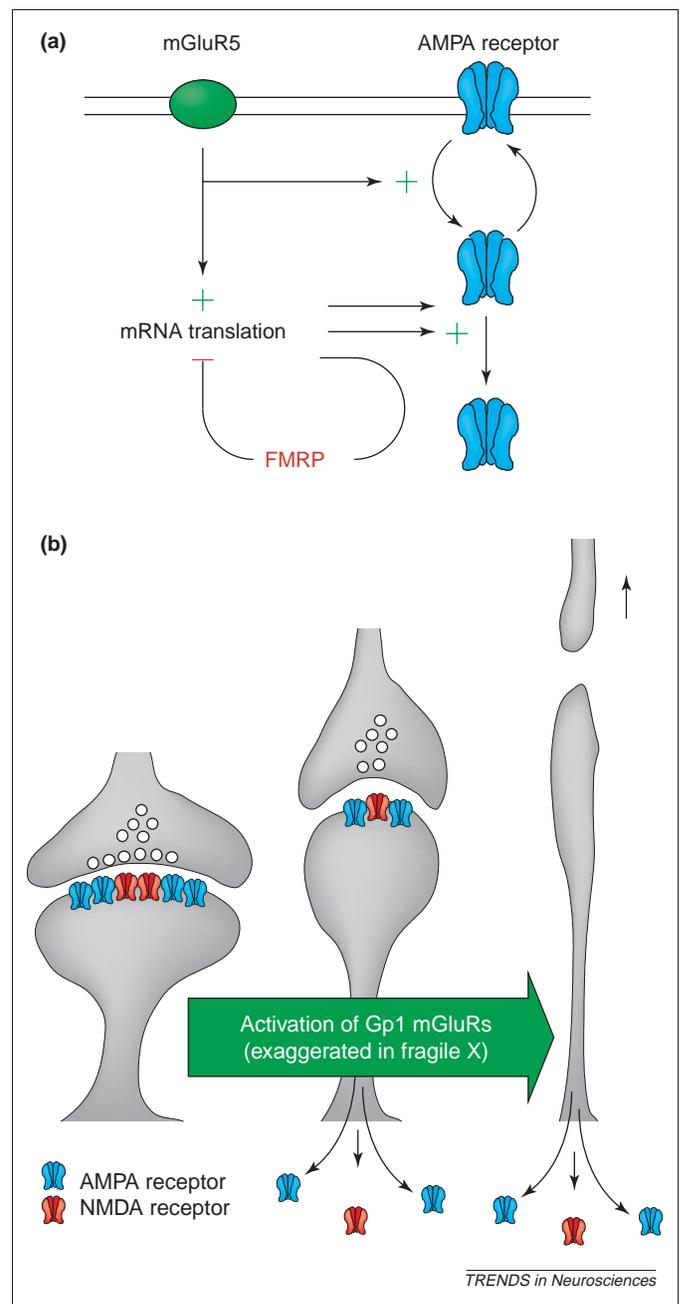


Figure 2. Models of protein synthesis-dependent, functional and structural consequences of group 1 (Gp1) metabotropic glutamate receptor (mGluR) activation at hippocampal synapses, and the role of FMRP. **(a)** Model to account for exaggerated mGluR-long-term depression (mGluR-LTD) in the *Fmr1* knockout mouse, based on the assumption that the fragile X mental retardation protein (FMRP) is synthesized in response to mGluR activation and functions as a translational repressor (modified from Ref. [13]). **(b)** Model relating the net loss of synaptic AMPA and NMDA receptors [24] and elongation of dendritic spines [67] observed following Gp1 mGluR activation in cultured hippocampal neurons. We propose that these responses are indicative of increased synapse loss and/or turnover following Gp1 mGluR activation. Both responses require mRNA translation and, if exaggerated in the absence of FMRP, could account for the delay in synaptic maturation and elongated spines in fragile X. According to this view, elongated spines in fragile X are weakened synapses en route to elimination, and/or filopodial extensions of dendrites seeking to replace lost synapses.

for translation regulation by RNA interference (RNAi) [46]. Specifically, FMRP is part of a RISC nuclease complex that represses translation by directing small interfering RNAs (siRNA) to their mRNA targets [47,48].

The case for FMRP as a translational repressor seems particularly strong for MAP1b. First, genetic evidence in

flies *in vivo* shows that the *Drosophila* FMRP homolog (DFXR) represses translation of Futsch, ortholog of the mammalian MAP1b [49]. Second, although anatomical variation has been noted [43], the protein is significantly increased in total brain lysates from *Fmr1* knockout mice [44]. Third, MAP1b mRNA is increased on polysomes in cells derived from fragile X patients, consistent with FMRP negatively regulating translation of this transcript [40]. Finally, the absence of FMRP has recently been shown to directly interfere with the developmentally programmed MAP1b decline in the mammalian brain, with the increased MAP1b leading to increased microtubule stability (Y. Feng *et al.*, unpublished).

Experiments *in vitro* initially suggested the possibility that FMRP is a general repressor of translation [50,51]. However, there are now several studies suggesting this role might be restricted to specific messages. Indeed, synthesis and/or subcellular localization of several proteins appears to be disrupted in the absence of FMRP [42,43,52]. We will return to this point later in the review.

Emerging functions of Gp1 mGluR-stimulated protein synthesis

It has been recognized for many years that the machinery for protein synthesis is present in the dendrites of cortical neurons near synapses [53,54]. Translation of pre-existing mRNAs can be activated in different ways by different signals (e.g. TrkB and NMDA receptor activation), but it is now very clear that activation of Gp1 mGluRs is a potent stimulus for local protein synthesis [52,55,56]. Moreover, in cases where it has been specifically examined, many functional consequences of Gp1 mGluR activation are – like LTD – protein-synthesis-dependent (Table 1).

The first study to show that a lasting effect of Gp1 mGluR activation requires protein synthesis was performed by Merlin *et al.* using hippocampal slices. The phenomenon under investigation was the gradual and persistent prolongation of epileptiform bursts in area CA3 following activation of Gp1 mGluRs with the selective agonist (R,S)-3,5-dihydroxyphenylglycine (DHPG). This action of DHPG on network excitability was blocked by inhibitors of mRNA translation, but not transcription [57–59].

In hippocampal area CA1, brief activation of Gp1 mGluRs can facilitate the induction of LTP without altering baseline responses [60]. However, stronger activation of Gp1 mGluRs can reverse previously induced LTP [61] and, as reviewed

above, induce LTD *de novo* [20,62]. All these effects are blocked by protein synthesis inhibitors [25,63,64].

Transient activation of Gp1 mGluRs in hippocampal slices and cultures stimulates the loss of surface-expressed synaptic AMPA and NMDA receptors [24,65] and reduces presynaptic release of glutamate [66]. Prolonged treatment of hippocampal neurons with DHPG also increases the proportion of long, thin dendritic spines [67]. These changes are likely to be related, because synapses on thin spines have a smaller postsynaptic density, fewer AMPA receptors, and a reduced number of synaptic vesicles docked at the presynaptic active zone [68–70]. Again, all these effects require mRNA translation, but not transcription.

Finally, it is noteworthy that the findings on mGluR-LTD in hippocampus inspired a re-examination of cerebellar LTD, which was also found to require rapid protein synthesis [71]. This result suggests that linkage between Gp1 mGluRs and protein synthesis is not restricted to mGluR5, or to hippocampal synapses.

The mGluR theory of fragile X mental retardation

Our studies in the *Fmr1* knockout mouse led us to suggest that exaggerated LTD could slow net synaptic maturation (by tipping the balance away from synapse gain towards synapse loss during the critical period of synaptogenesis), and therefore contribute to the developmental delay and cognitive impairment associated with fragile X (Figure 2b). However, FMRP is widely expressed in the brain, including most, if not all, neurons that express Gp1 mGluRs. We therefore considered the possibility that all functional consequences of Gp1 mGluR-dependent protein synthesis might be exaggerated in the absence of FMRP. An intriguing picture began to emerge. From the literature already reviewed here, overactive or inappropriate Gp1 mGluR signaling might lead to epilepsy, cognitive impairment, developmental delay, an increased density of long, thin dendritic spines, and loss of motor coordination – key features of fragile X syndrome (Table 1).

The picture becomes even more complete when we consider other functions of Gp1 mGluRs not yet tied to protein synthesis. Suspicious coincidences include the following:

- Fear memory formation and LTP in amygdala are mGluR5-dependent [72], and mGluR5 antagonists are anxiolytic [73]. Anxiety and autistic behavior are common in fragile X, and the *Fmr1* knockout mice display abnormal contextual and conditional fear responses [34].

Table 1. The functional consequences of Gp1 mGluR activation that have been shown to require mRNA translation, listed in the order in which they were discovered, and their possible relevance to fragile X syndrome^a

Effect of Gp1 mGluR-stimulated protein synthesis	Related fragile X phenotype in mouse or human	Refs
Prolongation of epileptiform bursts in hippocampal area CA3	Childhood epilepsy (human) Audiogenic seizure (mouse)	[57,59]
Priming of LTP in hippocampal area CA1	Cognitive impairment, developmental delay	[63]
LTD in hippocampal area CA1	Cognitive impairment, developmental delay	[25]
Internalization of postsynaptic glutamate receptors on cultured hippocampal neurons	Cognitive impairment, developmental delay	[24]
LTD in cerebellar cortex	Loss of motor coordination	[71]
Elongation of dendritic spines on cultured hippocampal neurons	Elongated, immature dendritic spines	[67]
Reversal of LTP (depotentialization) in hippocampal area CA1	Cognitive impairment, developmental delay	[64]

^aAbbreviations: Gp1 mGluR, group 1 metabotropic glutamate receptor; LTD, long-term depression; LTP, long-term potentiation.

- LTP of the corticostriatal synapse, believed to be important for habit formation [74], requires activation of mGluR1 and mGluR5 [75]. Fragile X syndrome is characterized by obsessive–compulsive behaviors.

- The mGluR5-specific antagonist 2-methyl-6-phenylethynyl-pyridine (MPEP) is anticonvulsant, and raises the threshold for audiogenic seizure in sensitive strains of mice [76]. Enhanced sensitivity to audiogenic seizures is a robust phenotype in *Fmr1* knockout mice in several genetic backgrounds [77].

- mGluR5 activation induces a long-term increase in the excitability of neocortical layer 5 neurons [78]. Fragile X is characterized by heightened behavioral responses to sensory stimuli, and larger sensory evoked potentials [79].

- mGluR1 and mGluR5 knockout mice show impaired pre-pulse inhibition of auditory startle [80]. Pre-pulse inhibition is enhanced in *Fmr1* knockouts [77,81].

- mGluR5 is expressed in C-fiber innervation of the skin [82] where it has been implicated in the mechanisms of hyperalgesia [83,84]. Individuals with fragile X exhibit heightened sensitivity to tactile irritation.

- mGluR5 is expressed in the enteric innervation of the ileum [85,86]. Agonists promote, and antagonists slow, intestinal motility. Loose bowels are a common complaint in fragile X.

- Translation of the circadian rhythmicity of the molecular clock in the mouse suprachiasmatic nucleus into neural firing requires activation of Gp1 mGluRs [87]. Disrupted circadian rhythm is a striking phenotype in *Drosophila* lacking *dFMR* [88–90].

Putting these pieces together, it appeared that over-active signaling by group 1 mGluRs could contribute to many of the symptoms of fragile X, not just exaggerated LTD and slowed synaptic development. This synthesis suggested a theory – the psychiatric and neurological aspects of fragile X syndrome are a consequence of exaggerated responses to mGluR1/5 activation – that was based on the following assumptions:

(i) Proteins are synthesized in response to activation of mGluR1/5 near synapses in many brain regions, where they contribute to diverse neuronal functions.

(ii) FMRP negatively regulates responses triggered by mGluR-stimulated protein synthesis.

This theory, portrayed in Figure 3(a), was first presented to a collection of experts at a Cold Spring Harbor Laboratory Banbury Meeting in April 2002.

Predictions and progress

A theory can be tested in two ways: (i) assessing the validity of the underlying assumptions, and (ii) spinning out their consequences [91]. The follow-up Banbury meeting in 2003 revealed that such tests are underway in several laboratories, in addition to our own. It is premature to report on these studies, but we can make some explicit predictions.

The first assumption suggests that many of the long-lasting responses to Gp1 mGluR activation will prove to be protein synthesis dependent. In the case of cerebellar LTD, this assumption was tested and validated [71]. We predict that Gp1 mGluR-dependent corticostriatal and amygdala

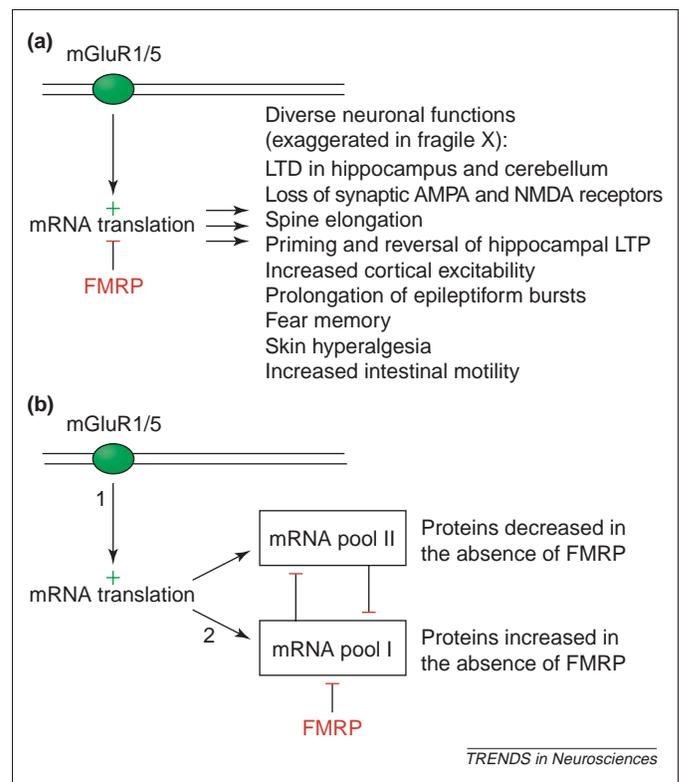


Figure 3. Group 1 (Gp1) metabotropic glutamate receptors (mGluRs) and fragile X: beyond LTD (a) The mGluR theory of fragile X. (b) Model to explain heterogeneity of translational responses in *Fmr1* knockouts. Activation of Gp1 mGluRs stimulates translation of two pools of mRNA that are in competition for the translational machinery. Repression of pool I by the fragile X mental retardation protein (FMRP) allows efficient translation of pool II. Although step 1 is the therapeutic target suggested by the mGluR theory, this model suggests that step 2 could be a better alternative. Abbreviations: LTD, long-term depression; LTP, long-term potentiation

LTP share this requirement for rapid mRNA translation (without new transcription). We also speculate that experience-dependent priming of audiogenic seizures will prove to be an mGluR5- and protein-synthesis-dependent form of synaptic or cellular plasticity.

A prediction that derives from the second assumption is that other known consequences of Gp1 mGluR-dependent protein synthesis will be exaggerated in the *Fmr1* knockout mouse. These should include increased cerebellar LTD, prolonged epileptiform bursts in hippocampal area CA3, and greater LTP priming and depotentiation in area CA1; and we expect that new behavioral phenotypes could emerge based on these findings. We also predict a greater response to DHPG in cultured hippocampal neurons – longer, thinner spines and an exaggerated loss of glutamate receptors. It is noteworthy that a paper recently appeared showing that one biochemical consequence of Gp1 mGluR activation, *de novo* synthesis of the synaptic protein PSD95, fails to occur in cultured cortical neurons from the *Fmr1* knockout mouse [52]. Thus, in future iterations of the theory, more precision will be required in specifying the protein-synthesis-dependent responses negatively regulated by FMRP (see following discussion).

The most important consequence of the theory, obviously, is that aspects of the fragile X phenotype should be rescued by reducing signaling through Gp1 mGluRs. Partial rescue might be accomplished genetically, for example, by crossing the *Fmr1* knockout mice with mice

lacking one or both genes for mGluR5 and mGluR1. Although less definitive, an even more exciting possibility is pharmacological rescue, for example, with Gp1 mGluR antagonists. First indications are positive: very recent data from Bauchwitz and colleagues indicates that the robust audiogenic seizure phenotype in *Fmr1* knockout mice is prevented by systemic administration of the mGluR5 antagonist MPEP [92].

Prospects for treatment of fragile X syndrome with Gp1 mGluR antagonists

The theory portrayed in Figure 3(a) suggests that it might be possible to overcome the loss of FMRP by dampening the protein synthesis triggered by activation of Gp1 mGluRs – this is the conceptual basis for the use of mGluR antagonists to reverse the fragile X phenotype. However, two caveats must be considered.

First, as mentioned previously, recent research suggests that although some proteins are overexpressed in the absence of FMRP (e.g. Arc and MAP1b) [44], others appear to be under-expressed or misexpressed [42,43,52]. A revision of our model to account for these recent findings is shown in Figure 3(b). According to this scheme, mGluR activation stimulates the translation of two pools of mRNA, those that are negatively regulated by FMRP (pool I) and those that are not (pool II). Competition between the pools for the translation machinery leads to a yin–yang, or push–pull, type of regulation. By inhibiting translation of messages in pool I, FMRP promotes translation of messages in pool II. Conversely, in the absence of FMRP increased translation of pool I inhibits translation of pool II. Such a model might be a better fit to available data, but it does raise concern about the quality of mGluRs as a target for treatment of fragile X. If aspects of the fragile X phenotype are attributable to decreased translation of mGluR-stimulated synthesis of proteins in pool II, it is difficult to see how an mGluR antagonist would be useful (selective blockers of pool I translation would be an alternative). The second (possibly related) caveat is that animals lacking mGluR5 [93] show cognitive deficits. Thus, blocking mGluR5 could potentially exacerbate the cognitive impairments in fragile X.

Despite these potential concerns, the known actions of Gp1 mGluR antagonists clearly suggest considerable therapeutic potential in fragile X. Most attention has been directed to mGluR5 antagonists, because mGluR1 blockers cause ataxia by disrupting cerebellar function. The prototypical mGluR5-selective antagonist is MPEP [94]. In animal models, systemically administered MPEP has been shown to have broad and potent anticonvulsant and anxiolytic actions without causing overt effects on locomotor activity. MPEP can reverse inflammation-induced mechanical hyperalgesia by inhibiting mGluR5 receptors in the C-fibers of the skin. And, by inhibiting mGluR5 receptors in the gut, MPEP can reduce bowel motility. Even the most skeptical would agree it is astonishing that a single compound could target such disparate symptoms of human fragile X syndrome as epilepsy, anxiety, hyperalgesia, and loose bowels.

As for the first caveat raised above, it is possible that we are correct about the utility of mGluR5 antagonists in

fragile X for the wrong reasons, or this concern might simply be unwarranted. Regarding the second caveat, proper cognitive function appears to require synaptic plasticity within a finite dynamic range. Mutations that cause this range to be exceeded in either direction (e.g. by too much or too little LTP) impair learning and memory [95]. Antagonists of mGluR5 might correct the mild cognitive deficits seen in the *Fmr1* knockout by bringing synaptic plasticity back into its proper range. Thus, two wrongs (cognitive impairment in *Fmr1* and *mGluR5* knockout mice) could make a right.

We believe mGluR5 antagonists have great promise as a potential treatment for the neurological and psychiatric symptoms of fragile X expressed in adults. However, if the syndrome is a lasting consequence of brain development with exaggerated Gp1 mGluR signaling, it is possible that early intervention with receptor antagonists could prevent some symptoms from occurring altogether.

Beyond fragile X

There is a great deal left to be learned about how protein synthesis is regulated by, and in turn influences, synaptic transmission in the brain. However, two things are certain: (i) FMRP is only one of many proteins and signaling pathways involved in the synaptic regulation of protein synthesis, and (ii) where there is biology, there is pathology. If we are correct that key aspects of fragile X are due to unregulated synaptic protein synthesis, it seems reasonable to anticipate that other disorders with similar symptoms might be traced to defects elsewhere in the same molecular pathways. It is interesting to note that other types of human developmental disorder, including autism, have many of the same core characteristics as fragile X. These include developmental delay and cognitive impairment, increased incidence of childhood epilepsy, a higher proportion of long, thin dendritic spines, reduced motor coordination, heightened anxiety, and altered gastrointestinal function. Thus, the mGluR theory could have broader applicability than just to fragile X.

Acknowledgements

Supported in part by grants from FRAXA Research Foundation and the National Institute for Child Health and Human Development. We thank Mike Tranfaglia and Gül Dolen for helpful comments on the manuscript.

References

- 1 O'Donnell, W.T. and Warren, S.T. (2002) A decade of molecular studies of fragile X syndrome. *Annu. Rev. Neurosci.* 25, 315–338
- 2 Hagerman, R.J. (2002) The physical and behavioral phenotype. In *Fragile X Syndrome: Diagnosis, Treatment, and Research* (Hagerman, R.J. and Hagerman, P., eds), pp. 3–109, The Johns Hopkins University Press
- 3 Bakker, C.E. and Oostra, B.A. (2003) Understanding fragile X syndrome: insights from animal models. *Cytogenet. Genome Res.* 100, 111–123
- 4 Rudelli, R.D. *et al.* (1985) Adult fragile X syndrome. Clinico-neuropathologic findings. *Acta Neuropathol. (Berl.)* 67, 289–295
- 5 Hinton, V.J. *et al.* (1991) Analysis of neocortex in three males with the fragile X syndrome. *Am. J. Med. Genet.* 41, 289–294
- 6 Irwin, S.A. *et al.* (2001) Abnormal dendritic spine characteristics in the temporal and visual cortices of patients with fragile-X syndrome: a quantitative examination. *Am. J. Med. Genet.* 98, 161–167
- 7 Purpura, D.P. (1974) Dendritic spine 'dysgenesis' and mental retardation. *Science* 186, 1126–1128

- 8 Kaufmann, W.E. and Moser, H.W. (2000) Dendritic anomalies in disorders associated with mental retardation. *Cereb. Cortex* 10, 981–991
- 9 Bakker, C.E. *et al.* (1994) Fmr1 knockout mice: a model to study fragile X mental retardation. The Dutch–Belgian fragile X consortium. *Cell* 78, 23–33
- 10 Kooy, R.F. (2003) Of mice and the fragile X syndrome. *Trends Genet.* 19, 148–154
- 11 Nimchinsky, E.A. *et al.* (2001) Abnormal development of dendritic spines in FMR1 knock-out mice. *J. Neurosci.* 21, 5139–5146
- 12 Irwin, S.A. *et al.* (2002) Dendritic spine and dendritic field characteristics of layer V pyramidal neurons in the visual cortex of fragile-X knockout mice. *Am. J. Med. Genet.* 111, 140–146
- 13 Huber, K.M. *et al.* (2002) Altered synaptic plasticity in a mouse model of fragile X mental retardation. *Proc. Natl. Acad. Sci. U. S. A.* 99, 7746–7750
- 14 Bear, M.F. (1998) The role of LTD and LTP in development and learning. In *Mechanistic Relationships between Development and Learning* (Carew, T.J. *et al.*, eds), pp. 205–225, Wiley
- 15 Ito, M. *et al.* (1982) Climbing fibre induced depression of both mossy fibre responsiveness and glutamate sensitivity of cerebellar Purkinje cells. *J. Physiol.* 324, 113–134
- 16 Ito, M. (1989) Long term depression. In *Annual Review of Neuroscience* (Vol. 12) (Cowan, M.W. *et al.*, eds), pp. 85–102, Annual Reviews
- 17 Dudek, S.M. and Bear, M.F. (1992) Homosynaptic long-term depression in area CA1 of hippocampus and effects of *N*-methyl-D-aspartate receptor blockade. *Proc. Natl. Acad. Sci. U. S. A.* 89, 4363–4367
- 18 Aiba, A. *et al.* (1994) Deficient cerebellar long-term depression and impaired motor learning in mGluR1 mutant mice. *Cell* 79, 377–388
- 19 Shigemoto, R. *et al.* (1994) Antibodies inactivating mGluR1 metabotropic glutamate receptor block long-term depression in cultured Purkinje cells. *Neuron* 12, 1245–1255
- 20 Oliet, S.H. *et al.* (1997) Two distinct forms of long-term depression coexist in CA1 hippocampal pyramidal cells. *Neuron* 18, 969–982
- 21 Lee, H.K. *et al.* (2003) Phosphorylation of the AMPA receptor GluR1 subunit is required for synaptic plasticity and retention of spatial memory. *Cell* 112, 631–643
- 22 Kemp, N. and Bashir, Z.I. (1999) Induction of LTD in the adult hippocampus by the synaptic activation of AMPA/kainate and metabotropic glutamate receptors. *Neuropharmacology* 38, 495–504
- 23 Carroll, R.C. *et al.* (1999) Rapid redistribution of glutamate receptors contributes to long-term depression in hippocampal cultures. *Nat. Neurosci.* 2, 454–460
- 24 Snyder, E.M. *et al.* (2001) Internalization of ionotropic glutamate receptors in response to mGluR activation. *Nat. Neurosci.* 4, 1079–1085
- 25 Huber, K.M. *et al.* (2000) Role for rapid dendritic protein synthesis in hippocampal mGluR-dependent long-term depression. *Science* 288, 1254–1257
- 26 Manahan-Vaughan, D. *et al.* (2000) Requirement of translation but not transcription for the maintenance of long-term depression in the CA1 region of freely moving rats. *J. Neurosci.* 20, 8572–8576
- 27 Sajikumar, S. and Frey, J.U. (2003) Anisomycin inhibits the late maintenance of long-term depression in rat hippocampal slices *in vitro*. *Neurosci. Lett.* 338, 147–150
- 28 Huber, K.M. *et al.* (2001) Chemical induction of mGluR5- and protein synthesis-dependent long-term depression in hippocampal area CA1. *J. Neurophysiol.* 86, 321–325
- 29 Kleppisch, T. *et al.* (2001) G(alpha)q-deficient mice lack metabotropic glutamate receptor-dependent long-term depression but show normal long-term potentiation in the hippocampal CA1 region. *J. Neurosci.* 21, 4943–4948
- 30 Huber, K.M. *et al.* (2001) Evidence for a novel signal transduction pathway in hippocampal mGluR-dependent LTD. Program number 388.7. In *Abstract Viewer/Itinerary Planner*, Society for Neuroscience Online
- 31 Antar, L.N. *et al.* (2004) Metabotropic glutamate receptor activation regulates fragile X mental retardation protein and FMR1 mRNA localization differentially in dendrites and at synapses. *J. Neurosci.* 24, 2648–2655
- 32 Weiler, I.J. *et al.* (1997) Fragile X mental retardation protein is translated near synapses in response to neurotransmitter activation. *Proc. Natl. Acad. Sci. U. S. A.* 94, 5395–5400
- 33 Godfraind, J.M. *et al.* (1996) Long-term potentiation in the hippocampus of fragile X knockout mice. *Am. J. Med. Genet.* 64, 246–251
- 34 Paradee, W. *et al.* (1999) Fragile X mouse: strain effects of knockout phenotype and evidence suggesting deficient amygdala function. *Neuroscience* 94, 185–192
- 35 Chiurazzi, P. *et al.* (2003) Understanding the biological underpinnings of fragile X syndrome. *Curr. Opin. Pediatr.* 15, 559–566
- 36 Jin, P. and Warren, S.T. (2003) New insights into fragile X syndrome: from molecules to neurobehaviors. *Trends Biochem. Sci.* 28, 152–158
- 37 Antar, L.N. and Bassell, G.J. (2003) Sunrise at the synapse: the FMRP mRNP shaping the synaptic interface. *Neuron* 37, 555–558
- 38 Feng, Y. *et al.* (1997) Fragile X mental retardation protein: nucleocytoplasmic shuttling and association with somatodendritic ribosomes. *J. Neurosci.* 17, 1539–1547
- 39 Feng, Y. *et al.* (1997) FMRP associates with polyribosomes as an mRNP, and the I304N mutation of severe fragile X syndrome abolishes this association. *Mol. Cell* 1, 109–118
- 40 Brown, V. *et al.* (2001) Microarray identification of FMRP-associated brain mRNAs and altered mRNA translational profiles in fragile X syndrome. *Cell* 107, 477–487
- 41 Darnell, J.C. *et al.* (2001) Fragile X mental retardation protein targets G quartet mRNAs important for neuronal function. *Cell* 107, 489–499
- 42 Miyashiro, K.Y. *et al.* (2003) RNA cargoes associating with FMRP reveal deficits in cellular functioning in Fmr1 null mice. *Neuron* 37, 417–431
- 43 Chen, L. *et al.* (2003) The fragile X mental retardation protein binds and regulates a novel class of mRNAs containing U rich target sequences. *Neuroscience* 120, 1005–1017
- 44 Zalfa, F. *et al.* (2003) The fragile X syndrome protein FMRP associates with BC1 RNA and regulates the translation of specific mRNAs at synapses. *Cell* 112, 317–327
- 45 Wang, H. *et al.* (2002) Dendritic BC1 RNA: functional role in regulation of translation initiation. *J. Neurosci.* 22, 10232–10241
- 46 Jin, P. *et al.* (2004) Biochemical and genetic interaction between the fragile X mental retardation protein and the microRNA pathway. *Nat. Neurosci.* 7, 113–117
- 47 Caudy, A.A. *et al.* (2002) Fragile X-related protein and VIG associate with the RNA interference machinery. *Genes Dev.* 16, 2491–2496
- 48 Ishizuka, A. *et al.* (2002) A *Drosophila* fragile X protein interacts with components of RNAi and ribosomal proteins. *Genes Dev.* 16, 2497–2508
- 49 Zhang, Y.Q. *et al.* (2001) *Drosophila* fragile X-related gene regulates the MAP1B homolog Futsch to control synaptic structure and function. *Cell* 107, 591–603
- 50 Lagerbauer, B. *et al.* (2001) Evidence that fragile X mental retardation protein is a negative regulator of translation. *Hum. Mol. Genet.* 10, 329–338
- 51 Li, Z. *et al.* (2001) The fragile X mental retardation protein inhibits translation via interacting with mRNA. *Nucleic Acids Res.* 29, 2276–2283
- 52 Todd, P.K. *et al.* (2003) The fragile X mental retardation protein is required for type-I metabotropic glutamate receptor-dependent translation of PSD-95. *Proc. Natl. Acad. Sci. U. S. A.* 100, 14374–14378
- 53 Steward, O. and Schuman, E.M. (2001) Protein synthesis at synaptic sites on dendrites. *Annu. Rev. Neurosci.* 24, 299–325
- 54 Steward, O. and Schuman, E.M. (2003) Compartmentalized synthesis and degradation of proteins in neurons. *Neuron* 40, 347–359
- 55 Weiler, I.J. and Greenough, W.T. (1993) Metabotropic glutamate receptors trigger postsynaptic protein synthesis. *Proc. Natl. Acad. Sci. U. S. A.* 90, 7168–7171
- 56 Job, C. and Eberwine, J. (2001) Identification of sites for exponential translation in living dendrites. *Proc. Natl. Acad. Sci. U. S. A.* 98, 13037–13042
- 57 Merlin, L.R. *et al.* (1998) Requirement of protein synthesis for group 1 mGluR-mediated induction of epileptiform discharges. *J. Neurophysiol.* 80, 989–993
- 58 Lee, A.C. *et al.* (2002) Role of synaptic metabotropic glutamate receptors in epileptiform discharges in hippocampal slices. *J. Neurophysiol.* 88, 1625–1633
- 59 Stoop, R. *et al.* (2003) Activation of metabotropic glutamate 5 and

- NMDA receptors underlies the induction of persistent bursting and associated long-lasting changes in CA3 recurrent connections. *J. Neurosci.* 23, 5634–5644
- 60 Cohen, A.S. and Abraham, W.C. (1996) Facilitation of long-term potentiation by prior activation of metabotropic glutamate receptors. *J. Neurophysiol.* 76, 953–962
- 61 Bashir, Z.I. and Collingridge, G.L. (1994) An investigation of depotentiation of long-term potentiation in the CA1 region of the hippocampus. *Exp. Brain Res.* 100, 437–443
- 62 Palmer, M.J. *et al.* (1997) The group I mGlu receptor agonist DHPG induces a novel form of LTD in the CA1 region of the hippocampus. *Neuropharmacology* 36, 1517–1532
- 63 Raymond, C.R. *et al.* (2000) Metabotropic glutamate receptors trigger homosynaptic protein synthesis to prolong LTP. *J. Neurosci.* 20, 969–976
- 64 Zho, W.M. *et al.* (2002) The group I metabotropic glutamate receptor agonist (S)-3,5-dihydroxyphenylglycine induces a novel form of depotentiation in the CA1 region of the hippocampus. *J. Neurosci.* 22, 8838–8849
- 65 Xiao, M.Y. *et al.* (2001) Metabotropic glutamate receptor activation causes a rapid redistribution of AMPA receptors. *Neuropharmacology* 41, 664–671
- 66 Zakharenko, S.S. *et al.* (2002) Altered presynaptic vesicle release and cycling during mGluR-dependent LTD. *Neuron* 35, 1099–1110
- 67 Vanderklish, P.W. and Edelman, G.M. (2002) Dendritic spines elongate after stimulation of group 1 metabotropic glutamate receptors in cultured hippocampal neurons. *Proc. Natl. Acad. Sci. U. S. A.* 99, 1639–1644
- 68 Harris, K.M. and Stevens, J.K. (1989) Dendritic spines of CA 1 pyramidal cells in the rat hippocampus: serial electron microscopy with reference to their biophysical characteristics. *J. Neurosci.* 9, 2982–2997
- 69 Nusser, Z. *et al.* (1998) Cell type and pathway dependence of synaptic AMPA receptor number and variability in the hippocampus. *Neuron* 21, 545–559
- 70 Schikorski, T. and Stevens, C.F. (1997) Quantitative ultrastructural analysis of hippocampal excitatory synapses. *J. Neurosci.* 17, 5858–5867
- 71 Karachot, L. *et al.* (2001) Induction of long-term depression in cerebellar Purkinje cells requires a rapidly turned over protein. *J. Neurophysiol.* 86, 280–289
- 72 Rodrigues, S.M. *et al.* (2002) The group I metabotropic glutamate receptor mGluR5 is required for fear memory formation and long-term potentiation in the lateral amygdala. *J. Neurosci.* 22, 5219–5229
- 73 Tatarczynska, E. *et al.* (2001) Potential anxiolytic- and antidepressant-like effects of MPEP, a potent, selective and systemically active mGlu5 receptor antagonist. *Br. J. Pharmacol.* 132, 1423–1430
- 74 Graybiel, A.M. (1998) The basal ganglia and chunking of action repertoires. *Neurobiol. Learn. Mem.* 70, 119–136
- 75 Gubellini, P. *et al.* (2003) Corticostriatal LTP requires combined mGluR1 and mGluR5 activation. *Neuropharmacology* 44, 8–16
- 76 Chapman, A.G. *et al.* (2000) Anticonvulsant activity of two metabotropic glutamate group I antagonists selective for the mGlu5 receptor: 2-methyl-6-(phenylethynyl)-pyridine (MPEP), and (E)-6-methyl-2-styryl-pyridine (SIB 1893). *Neuropharmacology* 39, 1567–1574
- 77 Chen, L. and Toth, M. (2001) Fragile X mice develop sensory hyperreactivity to auditory stimuli. *Neuroscience* 103, 1043–1050
- 78 Sourdet, V. *et al.* (2003) Long-term enhancement of neuronal excitability and temporal fidelity mediated by metabotropic glutamate receptor subtype 5. *J. Neurosci.* 23, 10238–10248
- 79 Castren, M. *et al.* (2003) Augmentation of auditory N1 in children with fragile X syndrome. *Brain Topogr.* 15, 165–171
- 80 Brody, S.A. *et al.* (2003) Disruption of prepulse inhibition in mice lacking mGluR1. *Eur. J. Neurosci.* 18, 3361–3366
- 81 Nielsen, D.M. *et al.* (2002) Alterations in the auditory startle response in Fmr1 targeted mutant mouse models of fragile X syndrome. *Brain Res.* 927, 8–17
- 82 Tachibana, T. *et al.* (2003) Immunohistochemical expressions of mGluR5, P2Y2 receptor, PLC-beta1, and IP3R-I and -II in Merkel cells in rat sinus hair follicles. *Histochem. Cell Biol.* 120, 13–21
- 83 Walker, K. *et al.* (2001) mGlu5 receptors and nociceptive function II. mGlu5 receptors functionally expressed on peripheral sensory neurones mediate inflammatory hyperalgesia. *Neuropharmacology* 40, 10–19
- 84 Neugebauer, V. *et al.* (1999) Role of metabotropic glutamate receptor subtype mGluR1 in brief nociception and central sensitization of primate STT cells. *J. Neurophysiol.* 82, 272–282
- 85 Hu, H.Z. *et al.* (1999) Functional group I metabotropic glutamate receptors in submucous plexus of guinea-pig ileum. *Br. J. Pharmacol.* 128, 1631–1635
- 86 Liu, M. and Kirchgessner, A.L. (2000) Agonist- and reflex-evoked internalization of metabotropic glutamate receptor 5 in enteric neurons. *J. Neurosci.* 20, 3200–3205
- 87 Park, D. *et al.* (2003) Translation of clock rhythmicity into neural firing in suprachiasmatic nucleus requires mGluR-PLCbeta4 signaling. *Nat. Neurosci.* 6, 337–338
- 88 Morales, J. *et al.* (2002) *Drosophila* fragile X protein, DFXR, regulates neuronal morphology and function in the brain. *Neuron* 34, 961–972
- 89 Dockendorff, T.C. *et al.* (2002) *Drosophila* lacking dfmr1 activity show defects in circadian output and fail to maintain courtship interest. *Neuron* 34, 973–984
- 90 Inoue, S. *et al.* (2002) A role for the *Drosophila* fragile X-related gene in circadian output. *Curr. Biol.* 12, 1331–1335
- 91 Bear, M.F. and Cooper, L.N. (1998) From molecules to mental states. *Daedalus* 127, 131–144
- 92 Yan, Q. *et al.* (2003) Audiogenic seizures and effects of mGluR5 antagonist MPEP in the fMR1-tm1cgr Fragile X mouse. Program number 634.5. In *Abstract Viewer/Itinerary Planner*, Society for Neuroscience Online
- 93 Lu, Y.M. *et al.* (1997) Mice lacking metabotropic glutamate receptor 5 show impaired learning and reduced CA1 long-term potentiation (LTP) but normal CA3 LTP. *J. Neurosci.* 17, 5196–5205
- 94 Spooren, W.P. *et al.* (2001) Novel allosteric antagonists shed light on mglu(5) receptors and CNS disorders. *Trends Pharmacol. Sci.* 22, 331–337
- 95 Migaud, M. *et al.* (1998) Enhanced long-term potentiation and impaired learning in mice with mutant postsynaptic density-95 protein. *Nature* 396, 433–439

Reproduction of material from Elsevier articles

Interested in reproducing part or all of an article published by Elsevier, or one of our article figures? If so, please contact our *Global Rights Department* with details of how and where the requested material will be used. To submit a permission request on-line, please visit:

http://www.elsevier.com/wps/find/obtainpermissionform.cws_home/obtainpermissionform

Alternatively, please contact:

Elsevier
Global Rights Department
PO Box 800,
Oxford OX5 1DX, UK.
Phone: (+44) 1865-843830
Fax: (+44) 1865-853333
permissions@elsevier.com