

## Reversing the Effects of Fragile X Syndrome

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This column focuses on recent research in which researchers at the Massachusetts Institute of Technology reversed several morphological, neurophysiological, and behavioral consequences of fragile X syndrome in a mouse model of this disorder. In so doing, this column brings together several issues raised in all three previous columns<sup>1-3</sup>: the genetic basis of neuropsychiatric disease, the interaction of genes and the environment in developmental neuropsychiatric disorders, and how the molecular mechanisms underlying such disorders can point the way to effective therapies. Specifically, this issue's column focuses on how synaptic plasticity, which underlies brain development as well as learning and memory, is abnormally regulated in fragile X syndrome and how this abnormality may be modulated by therapeutic interventions.

### FRAGILE X: EXAGGERATED SYNAPTIC PLASTICITY DUE TO UNCHECKED ACTIVITY-DEPENDENT PROTEIN SYNTHESIS

Dölen and colleagues<sup>4</sup> refer to fragile X syndrome as a "synaptopathy," a disorder of synaptic plasticity and synaptic function (see References). Synapses are sites of communication between neurons, and work in the past 2 decades has shown that plastic changes in the efficacy of individual synapses are fundamental to brain development and to learning and memory. Work during the past 5 years has shown that the synaptopathy in fragile X syndrome is a consequence of abnormal protein synthesis occurring at or close to the synapse. The dysregulation

of protein translation is a direct consequence of the mutated fragile X gene. These findings have opened the door to potential neuropharmacological treatments for fragile X syndrome and may eventually guide us to treatments for mental retardation and autism.

In the previous column, we reviewed the genetics of fragile X syndrome.<sup>3</sup> Recall that fragile X results from a mutation in the *FMRI* gene, which encodes the fragile X mental retardation protein (FMRP). The mutation consists of numerous triplet repeats (in this case, the triplet is CGG) in part of the gene. These triplet repeats induce DNA methylation (a process described in previous columns<sup>1-3</sup>) in a region adjacent to the regulatory promoter region. The heavily methylated nucleotide sequence and additional secondary structures that result from the dramatic expansion of triplets effectively "silence" the gene by preventing transcription and the production of FMRP. The absence of FMRP in humans leads to several well-characterized neuropsychiatric problems including mental retardation, developmental delay, gaze aversion, anxiety, attention deficit, hyperactivity, stereotypy, seizures, and impaired social behavior.

The fragile X mutation is linked to an intriguing abnormality in the dendrites of affected neurons (see References). Researchers have duplicated this dendritic abnormality in an animal model of fragile X syndrome, a knockout (KO) mouse that does not express *Fmr1* (the mouse homologue of the human fragile X gene). Figure 1 shows the differences in the dendrites of normal mice (wild type [WT]) and KO mice. Notice the small, thin structures protruding from the dendritic shafts, called dendritic spines. Spines are specialized portions of the dendrite that are postsynaptic to excitatory synapses, usually those using glutamate as a neurotransmitter; hence, they are studded with glutamate receptors where they make contact with presynaptic neurons (not visible in Fig. 1). Note that the spines on the dendrites of the fragile X mice (KO) are more numerous, longer, and thinner than those of normal mice (WT). Researchers

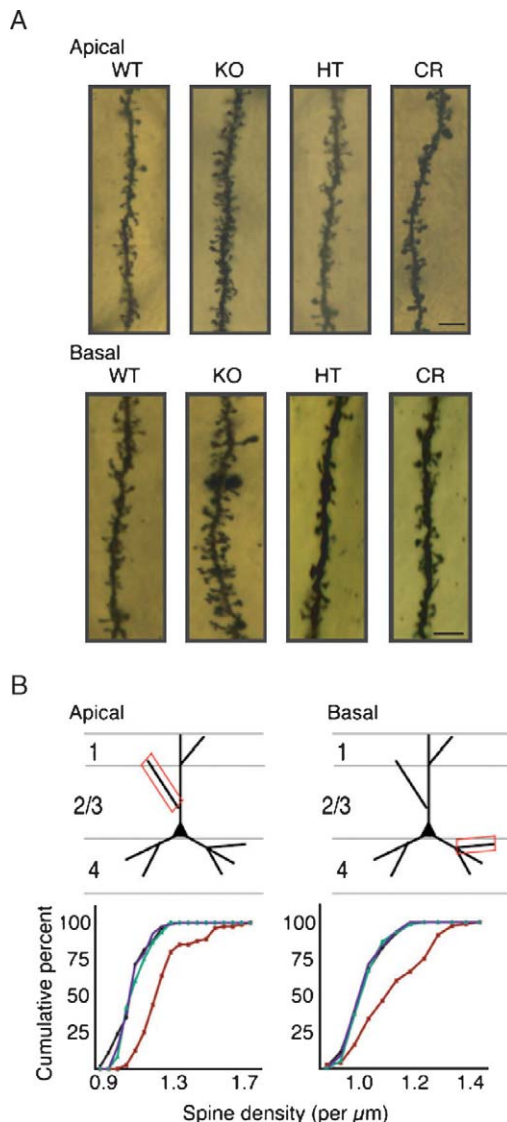
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**Fig. 1** Genetic rescue of dendritic spine phenotype in fragile X syndrome. A, Representative images from apical (A<sub>1</sub>) and basal (A<sub>2</sub>) dendritic segments of layer 3 pyramidal neurons in the binocular region of primary visual cortex of all four genotypes collected at postnatal day 30. B, Cumulative percentage of spines per micron in basal branches. (Note greater spine density in both apical and basal branches in KO mice.) WT = wild type; KO = *Fmr1* knockout; HT = *Grm5* heterozygote; CR = knockout/heterozygote cross. (Reprinted from Dölen G, Osterweil E, Rao BS, et al. Correction of fragile X syndrome in mice. *Neuron*. 2007;56:955–962. Copyright 2007, with permission from Elsevier.)

believe that this morphological abnormality is directly related to the absence of FMRP in fragile X through a disruption of locally translated proteins and abnormal synaptic plasticity.

FMRP is an RNA-binding protein, meaning that its job is to bind specific mRNAs. It is believed that FMRP helps to transport messages along dendrites to sites of the neuron where their protein products are needed for

structural changes to the synapse. It is also believed that FMRP inhibits the translation of these mRNAs until an appropriate signal arrives. This complex of proteins and mRNAs resides in or adjacent to dendritic spines, awaiting incoming signals from excitatory glutamatergic synapses, which initiate local mRNA translation and protein synthesis. This mechanism ensures that the protein synthesis required for synaptic plasticity occurs in response to an incoming signal only in spines that are synaptically active. This activity-dependent protein synthesis is an important cellular mechanism underlying maturational changes in brain development as well as learning and memory. FMRP appears to regulate this process in cooperation with a glutamate receptor known as mGluR5. Here is how it works.

Glutamate receptor activation in the dendritic spines is known to trigger local protein synthesis. Several glutamate receptors are activated at these synapses, but the group I metabotropic glutamate receptor 5 (mGluR5) is believed to be the main one responsible for initiating local mRNA transcription and protein synthesis. These receptors stimulate the synthesis of proteins necessary to stabilize changes in synaptic efficacy and stimulate the synthesis of FMRP as well. FMRP appears to inhibit protein synthesis at this site (because it is a translational repressor), balancing the effect of mGluR5. This process is known as end-product inhibition and is a mechanism for bringing the protein synthesis that has been set in motion by mGluR5 activation to a halt. Accordingly, the absence of FMRP in fragile X leads to a disruption of protein synthesis at the synapse. It turns out that many proteins are inappropriately translated (researchers are also finding other proteins that are downregulated). One consequence of disrupted protein synthesis is the abnormal shape of the dendritic spines. Another is abnormal synaptic plasticity, which is described in more detail in subsequent paragraphs. Researchers call this new hypothesis in fragile X syndrome the mGluR theory of fragile X, suggesting that in the absence of normal FMRP, the response to mGluR5 activation is exaggerated, and the exaggerated response causes the neuropsychiatric symptoms of fragile X syndrome.

#### TESTING THE SYNAPTOPATHY, OR MGLUR THEORY, OF FRAGILE X

Several researchers have used genetically altered strains of mice to test the idea of fragile X as a

**TABLE 1**  
Morphological, Molecular, Neurophysiological, and Behavioral Reversals of the Fragile X Phenotype in Mice

Measure	Result
Spine density	Abnormally high spine density in fragile X mice is rescued in mice with both the fragile X gene and the deficient mGluR5 (CR)
Basal protein synthesis	Abnormal increase in protein synthesis observed in fragile X mice was effectively prevented by reducing the levels of mGluR5, as demonstrated in normal basal protein levels CR mice
Seizure susceptibility	Seizure susceptibility is increased in fragile X mice and was attenuated in the fragile X mice with reduced mGluR5 (CR)
Cortical plasticity	An excess of cortical plasticity in the developing visual cortex of fragile X mice was reversed in the fragile X mice with reduced mGluR5 (CR)
Learning	A behavioral correlate of excess cortical plasticity, demonstrated by exaggerated extinction of inhibitory avoidance in the absence of FMRP, was reversed in the fragile X mice with reduced mGluR5 (CR)

Note: CR = knockout heterozygote cross; mGluR5 = glutamate receptor.  
Data adapted from Dölen et al.<sup>4</sup>

synaptopathy. Bear and coworkers<sup>5,6</sup> reasoned that if they could reduce the signaling through mGluR5 in fragile X mice, they could reduce local protein translation in dendritic spines. The question was whether this would reverse the abnormal spine morphology and abnormal synaptic plasticity. They used FMRP KO mice (*Fmr1* mice), the animal model for fragile X syndrome (the gene for FMRP is absent), to represent what happens in the fragile X phenotype. The researchers also tested mice that produce only half of the normal amount of mGluR5; these mice, called heterozygotes, possess only one of two copies of the gene encoding mGluR5 (removing both copies of the *mGluR5* gene would be too debilitating). The third strain of mice was the most important—a cross between the *Fmr1* mutant mice and the *mGluR5* heterozygotes (CR). These mice were expected to fare better than *Fmr1* KO mice because their defect, the inability to properly regulate protein synthesis in spines, would be compensated for by half the normal amount of mGluR5. All three strains were compared to WT mice as controls.

The results show that a reduction in mGluR5 rescued several deficits normally present in *Fmr1* mutant mice (Table 1). For one thing, the researchers demonstrate a striking finding in spine density. Spine density is greatest in KO mice (in both apical and basal dendritic fields of cortical neurons), confirming previous studies, and is virtually identical in the other three groups (Fig. 1). Although a reduction in mGluR5 (heterozygotes) alone has no effect on spine density, the identical findings in WT controls and mice with both the fragile X gene and the deficiency mGluR5 (CR) shows that abnormally high spine density is rescued by

reducing mGluR5, presumably by restoring more normal levels of protein synthesis.

The researchers also examined differences basal levels of protein synthesis. Earlier work had shown an elevation in basal protein synthesis of a number of synaptic proteins in the hippocampus of fragile X mice, in keeping with the notion that an absence of FMRP represents an absence of end-product inhibition. This abnormal increase in protein synthesis was effectively prevented by reducing the levels of mGluR5, as demonstrated in normal basal protein levels CR mice. Moreover, the researchers showed that a broad range of proteins is overexpressed in fragile X mice, and all of them are returned to normal basal values in CR mice.

Seizure susceptibility is also characteristic of patients with fragile X syndrome and in the mouse model of this disorder. This, too, was attenuated in the fragile X mice with reduced mGluR5 (CR). The researchers used an autogenic seizure paradigm to show significant differences across the four groups of mice. Controls and heterozygotes had a zero incidence of audiogenic seizures compared to 72% of fragile X mice and 33% of CR mice.

The most remarkable finding was the interaction between genes and the environment during visual system development. This was examined by comparing electrical activity in the visual cortex in these mice in response to visual deprivation. Hubel and Weisel won the Nobel Prize in 1981 for their discoveries concerning information processing in the visual system. Among other things, they demonstrated critical periods in the development of the visual cortex of mammals. As described in our first column (March 2008),<sup>1</sup> critical

periods are windows of time in which cortical neurons are particularly sensitive to the input from the environment. Dölen et al.<sup>4</sup> exploited the critical period of visual cortical development in mice to show an excess of cortical plasticity in the visual cortex of *Fmr1* mutant mice. Specifically, normal animals show distinct changes in electrical activity in the visual cortex when one eye is occluded, preventing visual experience. After 3 days, the input from the deprived eye is depressed, and after 7 days, the input from the open eye is potentiated. Together, these events shift the cortical activity in the visual cortex to become dominated by the open eye, and fewer neurons are responsive to the deprived eye. This is known as an ocular dominance shift. A clear demonstration of abnormal synaptic plasticity is demonstrated by differences in how this shift in ocular dominance proceeds in fragile X mice, which show an accelerated pace of cortical plasticity. After a brief period of deprivation (3 days), input from the open eye is potentiated, characteristic of longer periods of deprivation in normal animals. In contrast, mice with half the normal amount of mGluR5 are just the opposite. They show little deprived eye depression at 3 days. Most remarkable of all is the response of the CR mice with both the fragile X gene and reduced mGluR5. Their shift in ocular dominance in the visual cortex resembles that of normal mice, i.e., the fragile X abnormality in synaptic plasticity is reversed.

Finally, Dölen and colleagues<sup>4</sup> tested a form of learning that is known to require protein synthesis in the hippocampus called inhibitory avoidance extinction. This is a paradigm that requires mice to learn to avoid a certain side of their cage through aversive conditioning, and then to unlearn that avoidance behavior (extinc-

tion). They measured the time it takes, or latency, to enter the dark side of the box at baseline, after avoidance conditioning, and after extinction. Acquisition of the avoidance part of the task is the same for all four groups of mice, but fragile X mice show a shorter latency during the extinction phase, which is regarded by the researchers as exaggerated extinction in the absence of FMRP and is correlated with the increased basal levels of protein synthesis observed in the hippocampus of these mice. Furthermore, as with the other measures, this phenotype is reversed in mice with both the fragile X gene and the reduction in mGluR5.

Each of the measures analyzed here is relevant to human fragile X syndrome, and in each instance, the fragile X phenotype was reversed by a reduction in mGluR5, which apparently restored these mice to normal levels of activity-dependent protein synthesis in the dendritic spines. These findings are remarkable and encouraging. Perhaps the most important aspect of this work is related to visual cortical development because this part of the study directly examines the interaction between genes and experience during development. As the researchers emphasize, their data show that the rate of cortical plasticity is dependent on activity-dependent protein synthesis, which is inhibited by FMRP and stimulated by mGluR5. Too much cortical plasticity apparently contributes to the cognitive and behavioral problems associated with fragile X and probably contributes to the developmental delay apparent in humans with fragile X syndrome.

This hypothesis applies particularly to synapses involved in long-term depression (this will be discussed in more detail in a future column). Changes in synaptic efficacy associated with learning and memory consist of synapses

**TABLE 2**

Functions Related to the mGluRs That Resonate With Symptoms of Fragile X Syndrome and Autism in Humans

Fragile X Phenotype	mGluR5 Involvement
Anxiety and autistic behavior in fragile X	Fear formation and LTP in the amygdala is mGluR5 dependent
Obsessive-compulsive behavior	LTP in corticostriatal synapses involved in habit learning
Heightened behavioral response to sensory stimuli	Excitability of neocortical layer 5 neurons in sensory cortex
Enhanced sensitivity to audiogenic seizures	Antagonists of mGluR5 are anticonvulsants
Hyperalgesia	mGluR5 is expressed in pain fibers innervating the skin
Loose bowels	mGluR5 is present in the intestines; agonists promote and antagonists slow intestinal motility
Disrupted circadian rhythm	mGluRs are involved in circadian rhythmicity in the hypothalamus

Note: LTP = long-term potentiation; mGluR5 = glutamate receptor.

Data adapted from Bear et al.<sup>5</sup>

whose activity is increased or potentiated by activity-dependent protein synthesis (long-term potentiation, LTP) as well as synapses that are decreased or depressed by activity-dependent protein synthesis (long-term depression, LTD). Both increased and decreased synaptic activity constitute important components of learning and memory. Abnormalities in each process can contribute to abnormal development and interfere with normal learning and memory. For example, an increase in LTD may contribute to developmental delays by eliminating activity in too many synapses in individuals with fragile X syndrome. An increase in LTD in the cerebellum (motor control) may contribute to the characteristic deficit in motor coordination. Bear and colleagues<sup>5</sup> speculate about several other functions related to the mGluRs that resonate with symptoms of fragile X syndrome in humans as listed in Table 2; these examples support the mGlu theory of fragile X syndrome and deserve further study.

In summary, the mGluR theory of fragile X syndrome and the results reviewed here suggest that by reducing mGluR5 signaling, it may be possible to decrease local protein synthesis in dendritic spines. In

the presence of the abnormal increases in translation that occur to many synaptic proteins in fragile X, this may be sufficient to restore more normal synaptic activity. The development of antagonists to mGluR5 that may be used therapeutically in humans, such as 2-methyl-6-phebyl-pyridine, is under way.

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