

FRAGILE X SYNDROME: FROM NEUROPLASTICITY TO NEW HOPE

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Introduction

We have entered the era of ‘molecular medicine’ in which it is anticipated that the knowledge of the human genome will reveal causes and treatments for mental illnesses. This process begins with careful clinical identification of patients who can be distinguished by a common set of phenotypic traits, thus defining a syndrome. Molecular genetic studies are then undertaken to test the hypothesis that the syndrome has a shared genetic cause. In the event that disruption of a defined region of the genome causes the disease (a ‘highly penetrant’ mutation in the language of geneticists), then an animal model (usually a mouse) is generated that carries the same genetic disruption. Although the effects of the genetic lesion may (and often do) manifest differently at the behavioral level in animals and humans due to differences in the complexity of the brains, disruptions in elementary neuronal functions are likely to be shared. Understanding this neuronal pathophysiology can lead to identification and validation of potential therapeutic targets. Target discovery drives chemistry to develop molecules that can engage the target and satisfy the pharmacodynamic and pharmacokinetic requirements of a drug. If they can be shown to be safe, drug candidates may then advance to human clinical trials and if successful, become new medicines.

For most major psychiatric disorders, unfortunately, we are still far from fulfilling this promise of molecular medicine. Major disorders such as schizophrenia and bipolar disorder are, despite their simple labels, highly heterogeneous both in presentation and in genetic origin. Disease progression and outcome are also affected by environmental influences that are difficult to study or reproduce in animal models. This daunting phenotypic and etiologic complexity has slowed progress towards developing new therapies.

However, there is a strong sense of optimism that the possibility of substantial progress may soon be realized for autism spectrum disorder (ASD) and associated intellectual disability (ID). First, the genes have been discovered for a number of syndromic disorders that have as prominent features ASD and ID. Second, these gene mutations have been reproduced in animal models that allow detailed examination of the underlying brain

pathophysiology. Third, animal research has converged on altered synaptic function as the likely basis for impaired cognition and possibly ASD. Fourth, insights gained on how synapses function differently in the face of these mutations have suggested novel therapeutic interventions that have been validated in preclinical models and have shown promise in preliminary human clinical trials. Fifth, the fact that ASD and ID can be diagnosed in early childhood maximizes the potential benefit of therapy because it can be started at a time when the brain is most plastic. Finally, animal studies using gene reactivation or pharmacological interventions have suggested that substantial improvements can be seen even when treatments are begun in adulthood.

There have been exciting recent developments in several genetic syndromes associated with ASD and ID, including tuberous sclerosis complex (TSC), neurofibromatosis type 1 (NF1), Rett syndrome, and Down Syndrome. Here I will focus on fragile X syndrome (FXS) where we perhaps are closest to fulfilling the promise of molecular medicine in a psychiatric disorder (Figure 1). FXS was originally called Martin-Bell syndrome, named for the clinicians who recognized it (Martin and Bell, 1943). Features of the syndrome include ID, ASD, hyperactivity and attention deficit, seizures during childhood, and physical differences including a long face, protruding ears, flexible joints, and in males, enlarged testes. Subsequently the disorder was found to associate with an unusual constriction on the X chromosome (Lubs, 1969) and this led researchers to discover the affected gene in 1991 (Verkerk *et al.*, 1991). In FXS, the *FMR1* gene is silenced and the protein product, called FMRP, is not produced. FMRP is an mRNA binding protein that is highly expressed in neurons throughout the brain.

Shortly after the discovery of the gene, a mouse model of the disease was created (Dutch Belgian Fragile X Consortium *et al.*, 1994). The *Fmr1* knockout (KO) mouse has been extensively characterized by neurobiologists motivated not only by an interest in the disease, but also in role of the FMRP protein in synaptic plasticity. Indeed, it was work on synaptic plasticity that led to the discovery of a therapeutic approach that is now in human clinical trials. Here I will briefly trace the history of the neurobiological insights that contributed to these exciting developments. This story teaches the unexpected rewards of fundamental brain research, the importance of sharing data and ideas, and that disease-altering treatments for developmental brain disorders are not only feasible, they are close at hand.

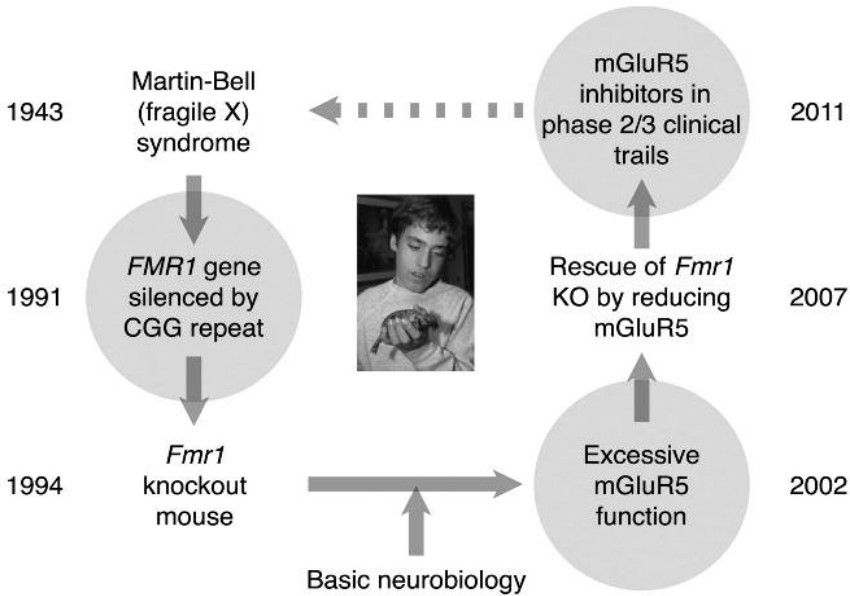


Figure 1. Fulfilling the promise of molecular medicine in FXS. Martin and Bell described in 1943 a group of patients characterized by a common set of features that included intellectual disability and social withdrawal. The causative gene mutation was discovered in 1991. The *FMR1* gene on the X chromosome is silenced, and the protein FMRP is not produced. Shortly thereafter, the *Fmr1* knockout mouse model was generated and has been intensively studied by neurobiologists interested both in the disease and the FMRP protein. In 2002 it was discovered that a form of synaptic plasticity – mGluR-LTD – was exaggerated in the *Fmr1* KO mouse. This led to the mGluR theory of fragile X, which posits that many symptoms of the disease are due to exaggerated responses to activation of the mGluR5 receptor. The theory was validated in 2007 with the demonstration that multiple fragile X phenotypes are corrected in the *Fmr1* KO mouse by genetic reduction of mGluR5 protein production. In addition, numerous animal studies showed that pharmacological inhibition of mGluR5 ameliorates fragile X mutant phenotypes. In 2009, inhibitors of mGluR5 entered into human phase 2 trials. If these are successful, this will represent the first pharmacological treatment for a neurobehavioral disorder that was developed from the bottom-up: from gene discovery to pathophysiology in animals to novel therapeutics in humans. Abbreviations: CGG: cytosine-guanine-guanine, mGluR5: metabotropic glutamate receptor 5, KO: knock out, LTD: long-term synaptic depression. Image courtesy of FRAXA Research Foundation, with permission.

From amblyopia to LTD

The neurobiological thread of discovery originates with the seminal studies of David Hubel and Torsten Wiesel, beginning in the early 1960s. Hubel and Wiesel were the first to systematically explore with microelectrodes the organization of the visual pathway in mammals, from retina to thalamus to visual cortex. They discovered that the primary visual cortex (area 17; striate cortex; V1) is the most peripheral station in the ascending

visual pathway where information from the two eyes is combined. That is, they found in visual cortex neurons that would respond to stimulation of both the right eye and the left eye. This convergence of input from the two eyes is the neurobiological substrate of binocular vision – why we see one world with two eyes. They recognized that the precision with which these connections were established likely required, in addition to genetic instructions, a comparison of the activity patterns arising in the two eyes. As Wolf Singer elegantly describes it, inputs that ‘fire together’ should be those that ‘wire together’ in the visual cortex.

Wiesel and Hubel (1963) tested this idea by temporarily degrading image formation in one eye, a paradigm called monocular deprivation. They found that if this procedure was performed in a young animal, before adolescence, then there was a profound consequence in visual cortex. When normal image formation was restored, the eye that had been deprived no longer was effective in driving robust visual responses in the cortex. This dramatic form of experience-dependent plasticity has fascinated a generation of neuroscientists over the past 50 years. Not only is ocular dominance plasticity a robust example of the role of sensory experience in brain development, it is responsible for a highly prevalent form of childhood visual disability called amblyopia (affecting ~1% of the human population) that results when optical defects are not corrected during infancy or early childhood.

Ocular dominance plasticity is multifaceted, but a key question has concerned the mechanisms responsible for the loss of visual responsiveness wrought by monocular deprivation. The primary modification occurs at excitatory synapses in visual cortex, particularly thalamocortical synapses. Intuition suggests that these synapses simply atrophy due to disuse. However, this is not the case. In fact, inputs from the visually deprived eye are actually protected from disconnection by injecting an anesthetic into the eye (Rittenhouse *et al.*, 1999; Frenkel and Bear, 2004). The data instead support the theoretical suggestion (Bienenstock *et al.*, 1982) that poorly correlated activity that arises in the retina in the absence of crisp image formation is actually the trigger for synaptic depression. This insight led to the search for the mechanisms of homosynaptic long-term depression (LTD) in the cerebral cortex (see Bear, 2003) for review.

Even before the discovery of LTD (Dudek and Bear, 1992) it was recognized that synaptic weakening must result from the release of the neurotransmitter glutamate at excitatory synapses (Bear *et al.*, 1987). The discovery that glutamate could directly activate a class of G protein-coupled receptors – subsequently called metabotropic glutamate receptors (mGluRs) – suggested one potential mechanism for LTD (Bear, 1988). Decades later, we

now understand that there are multiple forms of LTD. In fact, the LTD mechanism that is responsible for amblyopia is dependent on NMDA receptors rather than mGluRs (Yoon *et al.*, 2009). Nonetheless, the mGluR hypothesis was eventually tested in the cerebellum, hippocampus, and elsewhere; and indeed, activation of these receptors is one important trigger for LTD (Luscher and Huber, 2010).

From mGluR-LTD to FXS

There are 8 mGluRs in the genome, and these are divided into three structurally and functionally related groups, numbered 1–3. LTD is triggered in the hippocampus (and elsewhere) by activation of group 1 mGluRs, particularly the receptor designated mGluR5. A simple paradigm to induce LTD is brief application of the selective agonist, DHPG (dihydroxyphenylglycine), but LTD can also be induced by glutamate released in response to patterned electrical activation of synapses (Huber *et al.*, 2001).

The mGluR–LTD resembles homosynaptic LTD triggered by activation of NMDA receptors, which are both expressed by internalization of postsynaptic AMPA-type glutamate receptors (Snyder *et al.*, 2001). However, a distinguishing feature of mGluR–LTD is that it normally requires immediate translation of mRNAs that pre-exist in the dendrites of neurons (Huber *et al.*, 2000). The mGluR–LTD rapidly decays back to baseline if it is induced in the presence of a protein synthesis inhibitor such as cycloheximide.

This requirement for protein synthesis in LTD was surprising, but there were previous indications that mGluRs could regulate protein synthesis. It had been shown biochemically that activation of group 1 mGluRs can stimulate protein synthesis at synapses (Weiler and Greenough, 1993), and some other lasting electrophysiological consequences of mGluR activation had been shown to require protein synthesis (Merlin *et al.*, 1998; Raymond *et al.*, 2000). We now recognize that mGluR5 is part of a molecular machine that ensures that the supply of synaptic proteins keeps up with demand as registered by the release of glutamate at excitatory synapses. Although the mGluR5 receptor triggers several biological responses, one of these is the initiation of new protein synthesis at synapses.

At the turn of the century, the most immediate questions related to how mGluR5 activation regulates protein synthesis at synapses and the identity of the protein species required for LTD. It is here that research on neuroplasticity collided with FXS. Weiler and Greenough had shown in 1997 that one protein synthesized in response to mGluR5 activation was FMRP, the protein missing in FXS (Weiler *et al.*, 1997). We wondered if FMRP might be one of the hypothetical ‘LTD proteins’ and to test this idea we

obtained the *Fmr1* KO mouse. Our hypothesis was that without *Fmr1* mRNA at synapses there would be impaired LTD in the hippocampus. Surprisingly, however, the experiments revealed the opposite phenotype: LTD was exaggerated in the KO (Huber *et al.*, 2002).

Earlier findings *in vitro* suggested that FMRP binds mRNA and inhibits translation (Laggerbauer *et al.*, 2001; Li *et al.*, 2001). Thus to account for our LTD results we proposed that mGluR5 stimulation triggers the synthesis of LTD proteins and in addition, synthesis of FMRP. We imagined that FMRP normally feeds back to *inhibit* further synthesis of LTD proteins, an example of the familiar biochemical principle of end-product inhibition. Without FMRP, protein synthesis proceeds unchecked and, consequently, more LTD is expressed in the *Fmr1* KO.

The mGluR theory of FXS

In addition to contributing to LTD, it was known by 2002 that mGluR-dependent protein synthesis has varied effects at different types of synapse (Merlin *et al.*, 1998; Raymond *et al.*, 2000; Vanderklish and Edelman, 2002). This led me to wonder what the consequences might be if group 1 mGluR functions were exaggerated throughout the nervous system in the absence of the negative regulation provided by FMRP. This was a spine tingling moment. It dawned on me that it was possible that many symptoms of FXS could be related to exaggerated responses to mGluR5 (and mGluR1, the other group 1 mGluR). These might include cognitive impairment, anxiety, epilepsy, and even irritable bowel; mGluRs might be a thread that could connect widely varied symptoms of the disease. The exciting and obvious implication was that inhibitors of group 1 mGluR signaling might provide a disease-altering therapy for FXS.

Of course, this was an extremely speculative idea, based on little more than our LTD findings in the *Fmr1* KO mice. The conservative course would have been to keep the idea to ourselves and work quietly to test it before going public. However, we quickly realized that this path would take us years. Because of the tremendous therapeutic possibilities, we were compelled to share this idea immediately with other researchers and enlist their help to test it. Accordingly I presented the ‘mGluR theory’ at a small meeting of fragile X experts in April, 2002 and the next year I helped organize a meeting of mGluR experts to introduce them to FXS (see Bear *et al.*, 2004) for review). These communities accepted the challenge to test the idea, and this has greatly accelerated progress.

Good theories are based on simple, concrete, and testable assumptions, and ours was that in the absence of FMRP exaggerated responses to group

1 mGluR activation (particularly protein synthesis) are pathogenic and responsible for the major neurological and psychiatric symptoms of the disease. This proposal of excessive protein synthesis downstream of mGluR5 has now been confirmed in several studies of the *Fmr1* KO mouse (Aschrafi *et al.*, 2005; Qin *et al.*, 2005; Dolen *et al.*, 2007; Osterweil *et al.*, 2010). Moreover, other electrophysiological and biochemical consequences of mGluR-activation, including epileptogenesis (Chuang *et al.*, 2005), LTP priming (Auerbach and Bear), cerebellar LTD (Koekkoek *et al.*, 2005), and glutamate receptor internalization (Nakamoto *et al.*, 2007) have also been found to be altered in the KO, consistent with increased protein synthesis.

The most important consequence of the theory, of course, is that multiple aspects of FXS should be improved by reducing signaling via mGluR5. This hypothesis has been tested in animal models of fragile X using two approaches, one genetic and the other pharmacological. The genetic approach was to reduce signaling via mGluR5 by crossing a mutant mouse line that expresses only 50% the WT level of mGluR5 (the *Grm5*^{+/-} mouse) with the *Fmr1* KO (Dolen *et al.*, 2007). Remarkably, reducing mGluR5 in the *Fmr1* KO mouse was sufficient to correct 7 of 8 fragile X phenotypes examined, including seizures, hippocampal synaptic plasticity, ocular dominance plasticity, protein synthesis, and dendritic spine density. A similar approach was taken in the fruit fly model of fragile X with similar results (Pan and Broadie, 2007; Pan *et al.*, 2008; Repicky and Broadie, 2009). These experiments validate the theoretical concept that mGluR5 and FMRP act in functional opposition, and that defects caused by the loss of FMRP can be ameliorated by reducing signaling via mGluR5.

The genetic experiments reveal that mGluR5 is indeed a potential therapeutic drug target, and this idea has been extensively tested in animal experiments using a compound called MPEP (2-methyl-6-(phenylethynyl)-pyridine), a negative allosteric modulator of mGluR5 (Gasparini *et al.*, 1999). A dramatic early demonstration of the utility of MPEP was provided by Yan *et al.* (2005), who showed that a particularly severe fragile X phenotype in mice, audiogenic seizure, could be prevented by acute MPEP treatment. In a contemporaneous study McBride *et al.* (2005) showed that chronic drug treatment could correct both neuroanatomical and behavioral defects in the fruit fly model of fragile X. Importantly, they saw improvements even when treatment was begun in adult flies. Subsequent work from a number of laboratories using both mouse and fly models have strongly supported the conclusion that mGluR5 inhibitors can ameliorate many diverse fragile X phenotypes (reviewed by Krueger and Bear, 2011). The fact that this approach works in species as distantly related as flies and mice suggests that

mGluR5 and FMRP have an evolutionarily conserved relationship, which greatly boosts confidence that a similar approach can be successful in humans with FXS.

These studies have ushered in a new era in fragile X. Few would have believed it would be possible to develop a small molecule therapy that could substantially improve the outcome of a genetic defect in brain development. Tests of the mGluR theory over the past 10 years have shown beyond question that new, disease-altering treatments are indeed possible. This has inspired a search for additional potential therapeutics in fragile X and some very interesting new targets have emerged, including enzymes ‘downstream’ from mGluR5 (*e.g.*, Bilousova *et al.*, 2009; Min *et al.*, 2009) and ‘upstream’ neurotransmitter receptors that regulate the release of glutamate in the brain (*e.g.*, Chang *et al.*, 2008).

Clinical trials

2011 is an auspicious moment. Exploratory ‘phase 2’ human clinical trials have now been completed using compounds designed to dampen mGluR5 activation or signaling (Berry-Kravis *et al.*, 2008a; Berry-Kravis *et al.*, 2008b; Berry-Kravis *et al.*, 2009; Erickson *et al.*, 2010; Jacquemont *et al.*, 2011). These include fenobam and AFQ056 (mGluR5 inhibitors), lithium (inhibitor of enzymes downstream of mGluR5), and arbaclofen (agonist of GABA-B receptors that reduce glutamate release). The results have been sufficiently encouraging that two compounds, AFQ056 and arbaclofen, have advanced into larger phase 3 trials. If successful, these studies could lead to regulatory approval of these drugs for the treatment of fragile X syndrome in children and adults. Needless to say, we await the outcome of these studies with great anticipation. Results should be available by the end of 2012.

Discussions of clinical trials often lead to two questions: (1) when must treatment begin to be effective, and (2) what aspects of human FXS do we hope to improve. These issues are critical because they can mean the difference between success and failure of a clinical trial, even if the approach is fundamentally correct. If treatment must begin in infancy to alter the trajectory of brain development, then trials initiated in young adults may fail simply because a ‘critical period’ has been missed. This presents a particular risk for compounds that are entering human trials for the first time, because regulatory agencies are appropriately cautious about allowing treatment in young children before there is a thorough understanding of potential toxicity. Fortunately the good news emerging from animal studies is that it does appear that measurable improvements still occur when treatments are begun after adolescence.

The other risk, applying to all new treatments, is that the wrong ‘end-points’ are chosen to assess drug efficacy. The endpoints that lead to regulatory approval are those that improve the quality of life for the affected individuals and their families, which are not easily measured reproducibly. Although we take great pride in the rescue of various synaptic defects in animals, it remains an open question precisely how these findings will translate to behavior in humans. Mouse behavior, in my opinion, does not provide much guidance because of dramatic differences in brain and behavioral complexity, and in cognitive capability. We hope that the measures that have appeared to respond in the exploratory trials will show robust, statistically significant improvements in the phase 3 trials.

Based on the strength of the animal research, I am confident that if we can start the correct treatment(s) at the correct time, treat for the correct duration and at the correct dose, and if we measure the correct outcome, clinical trials in fragile X will be successful and we will be able to provide substantial benefit to the affected individuals. Of course, that is a lot of ‘ifs’ so we must be prepared to tolerate some failure before we triumph. But triumph we will.

New hope for developmental brain disorders

In this brief review I have highlighted the explosive progress in FXS that occurred when the streams of genetics and neurobiology mixed together. This is only one example, however. The study of genetically engineered animal models of several other human syndromes associated with ASD and ID have also yielded insights that suggest the possibility of meaningful drug therapy, with benefits even when that therapy is begun in adulthood (Silva and Ehninger, 2009). Moreover, it has also become apparent that many genetic mutations associated with ASD and ID may affect common biochemical signaling pathways (Kelleher and Bear, 2008). Thus, there is reason to hope that a treatment developed for a ‘rare’ cause of ASD and ID like FXS may be beneficial for others, even before we fully understand what these other causes are.

It is important to add, however, that while drug therapies might correct disruptions in synaptic biochemistry, they will never substitute for quality sensory experience and education. We imagine the drug treatment will unlock the potential for substantial gains in cognitive and social behaviors. But this potential will only be realized when pharmacotherapy is combined with appropriate cognitive and behavioral therapies that exploit life-long neuroplasticity.

Acknowledgements

The research described in this article has been supported by the National Eye Institute, the National Institute for Child Health and Human Development, the National Institute of Mental Health, the National Institute for Neurological Disease and Stroke, the Howard Hughes Medical Institute, the FRAXA Research Foundation, and the Simons Foundation Autism Research Initiative.

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