Small, G.W., Mazziotta, J.C., Collins, M.T., Baxter, L.R., Phelps, M.E., Mandelkern, M.A., Kaplan, A., La Rue, A., Adamson, C.F., Chang, L., et al. (1995). JAMA *273*, 942–947.

Takahashi, R.H., Milner, T.A., Li, F., Nam, E.E., Edgar, M.A., Yamaguchi, H., Beal, M.F., Xu, H., Greengard, P., and Gouras, G.K. (2002). Am. J. Pathol. *161*, 1869–1879.

Yang, A.J., Knauer, M., Burdick, D.A., and Glabe, C. (1995). J. Biol. Chem. *270*, 14786–14792.

DOI 10.1016/j.neuron.2005.02.013

Courting a Cure for Fragile X

Fragile X syndrome is the most common heritable cause of mental retardation. Previous work has suggested that overactive signaling by group I metabotropic glutamate receptors (mGluRs) may be a mechanism underlying many of the disease symptoms. As a test of this theory, McBride et al. show that in a *Drosophila* model for Fragile X syndrome, treatment with mGluR antagonists can rescue short-term memory, courtship, and mushroom body defects.

Fragile X mental retardation syndrome (FXS) is an inherited single-gene disorder. In the afflicted population, the *FMR1* gene is transcriptionally silenced, and the Fragile X mental retardation protein (FMRP) is not made. The consequence in humans is a diverse constellation of psychiatric and neurological symptoms ranging from cognitive impairment to autistic behavior (Hagerman and Hagerman, 2002). Based on research in the *Fmr1* knockout (KO) mouse, the suggestion was made that many of these symptoms could be accounted for by overactive signaling by group I metabotropic glutamate receptors (GpI mGluRs; (Bear et al., 2004; Huber et al., 2002). The implication of this "mGluR theory" is that many symptoms in FXS might respond to treatment with drugs that inhibit GpI mGluRs (Bear, 2005). In this issue of *Neuron*, Tom Jongens, Sean McBride, and colleagues (McBride et al., 2005) describe an audacious test of this notion in fruit flies lacking *dfmr1*, the *Drosophila* homolog of human *FMR1*. Mutant flies exhibit altered courtship behavior, decreased memory in a conditioned courtship assay, and alterations in the structure of the brain (the mushroom bodies). Remarkably, feeding flies drugs that target mammalian mGluR signaling could rescue all three defects. These amazing results fuel a growing sense of optimism that appropriate pharmacological intervention could ameliorate, and possibly even cure, aspects of Fragile X syndrome in humans.

In mammals, mGluRs comprise a family of eight subtypes that are commonly divided into three groups based on their shared signal transduction pathways (Conn and Pin, 1997). Group I mGluRs consist of mGluR1 and mGluR5 and couple to phospholipase C (PLC), which stimulates the turnover of membrane phosphoinositides. Several lines of research led to the mGluR theory of Fragile X (reviewed by Bear et al., 2004). Among them are the findings that (1) activation of GpI mGluRs with a selective agonist stimulates synaptic protein synthesis and trafficking of FMRP, (2) many of the lasting functional consequences of GpI mGluR activation require mRNA translation but not transcription, and (3) when it has been examined, protein synthesis-dependent responses to GpI mGluR activation are exaggerated in the *Fmr1* KO mouse, consistent with a role for FMRP as a translational repressor of selected mRNA transcripts. Considering these findings together with the known consequences of GpI mGluR activation in the brain suggested that many of the symptoms of FXS could be simply accounted for by overactive mGluR signaling (Figure 1A). Because mGluR1 is necessary for proper cerebellar function, mGluR5 has been viewed as the better therapeutic target. There are several drugs that selectively inhibit mGluR5; the most widely used is the noncompetitive antagonist MPEP (2-methyl-6-phenylethynyl-pyridine), with the caveat that at high concentrations it blocks NMDA receptors (Spooren et al., 2001). MPEP was the first food additive used by McBride et al. to treat the behavioral and structural deficits in Fragile X flies.

Courtship behavior in *Drosophila* is innate and involves a complex set of behaviors that ends in copulation (Figure 2A). Conditioned courtship suppression is an associative learning assay that modifies this set of innate behaviors. Briefly, the conditioning paradigm is as follows. During the training phase, the male is placed with an unreceptive trainer (a previously mated female). Initially, he courts the female vigorously, but over time his courtship activity declines (Figure 2B). In the next phase, his memory is tested with a receptive virgin female. After training, wild-type males will not court this female, even though she is receptive. This suppression of courtship lasts 2–3 hr and constitutes the memory phase (Figure 2C).

As had been previously reported (Dockendorff et al., 2002) *dfmr1* KO flies show diminished innate courtship activity (Figure 2A): KO flies court receptive virgin females less vigorously than wild-types. In the current study, McBride et al. extend the analysis to conditioned courtship suppression. They find that during the training phase (Figure 2B) KO flies show normal courtship suppression, indicating that *learning* is intact in these animals. However, courtship suppression *memory* (Figure 2C) is disrupted: KO flies continue to court tester females at naive levels after exposure to unreceptive trainer females. These behavioral changes correlate with an anatomical defect in the mushroom body, the part of the fly brain believed to be responsible for conditioned courtship learning and memory (Michel et al., 2004).

Pharmacological rescue of all three phenotypes was achieved by feeding KO animals MPEP. Maximal rescue was obtained when treatment began in larvae and continued in adults, suggesting a role for mGluR signaling in both the development and adult expression of the phenotypes. The courtship and conditioned courtship phenotypes could still be rescued when treatment was withheld until adulthood. The mushroom body defect, on the other hand, could only be rescued when treatment began early, suggesting that this structural change is a consequence of an altered developmental

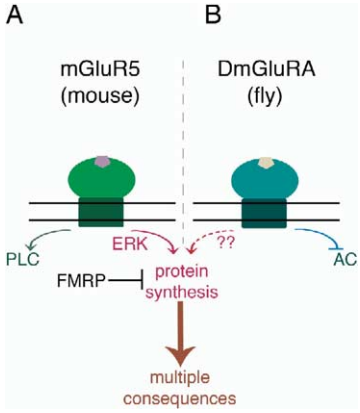

Figure 1. The mGluR Theory of Fragile X

(A) In mammals, many lasting consequences of activating group I mGluRs (mGluRs 1 and 5) require local mRNA translation and appear to be exaggerated in the absence of FMRP. Rather than the "classical" signaling via phospholipase C (PLC), these protein synthesis-dependent actions of mGluR stimulation appear to require activation of extracellular signal-regulated kinase (ERK). (B) In the fly, McBride et al. find that drugs blocking the effect of glutamate at the *Drosophila* mGluR (DmGluRA) can rescue deficits caused by the absence of FMRP. Although DmGluRA resembles vertebrate group II mGluRs that signal via inhibition of adenylyl cyclase (AC), these findings are consistent with the mGluR theory if pathways exist that couple DmGluRA activation to FMRP-regulated protein synthesis.

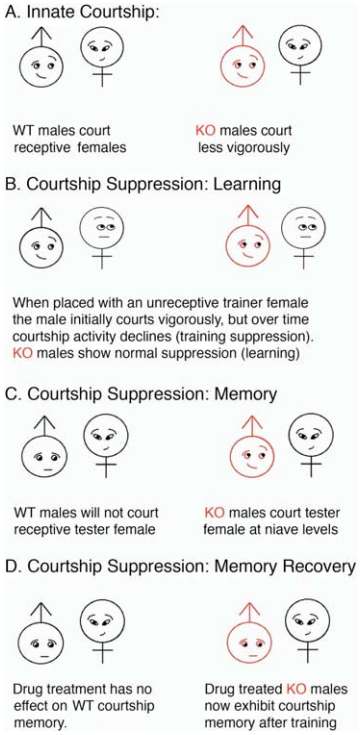

Figure 2. Courtship and Courtship Suppression in Flies

McBride et al. find that courtship suppression memory is impaired in Fragile X flies, but can be completely rescued if the mutants are fed mGluR antagonists during development.

trajectory that cannot be reversed in adults. However, these findings taken together indicate that normal mushroom body anatomy is not required for rescue of the behavioral phenotype. The exciting implication is that pharmacotherapy in early development (which might correspond to a human developmental epoch earlier than the disease is identified) may not be necessary for recovery of a cognitive disruption.

The findings summarized above appear to provide stunning support for the mGluR theory. However, the audacity of Jongens' study stems from the fact that other than the MPEP binding region, the lone functional mGluR in the *Drosophila* genome (DmGluRA) bears little resemblance to mammalian mGluR5. In fact, DmGluRA is an ortholog of vertebrate group II mGluRs that are negatively coupled to adenyl cyclase (AC). Indeed, McBride et al. were able to show that three different group II-selective, competitive mGluR antagonists and lithium chloride also rescue the Fragile X phenotype. Because all four antagonists showed the same effects, the authors argue that DmGluRA is the relevant target. However, future studies to unequivocally establish the role of mGluR activity in pathogenesis must show that reduction in *DmGluRA* gene dosage (by mutation or RNAi) rescues the *dfmr1*-KO phenotypes and occludes drug effects.

It is possible that DmGluRA in *Drosophila* assumes the full burden of metabotropic glutamate signaling that is divided among the eight different mGluRs in vertebrates. Indeed, the *Drosophila* data are compatible with the mGluR theory as it is currently articulated if there exist pathways that couple DmGluRA activation to FMRP-regulated protein synthesis (Figure 1B). Current data in mammals suggest that Gpl mGluRs stimulate protein synthesis via activation of extracellular signal-regulated kinase (ERK; Gallagher et al., 2004; Zhao et al., 2004), rather than the "classical" pathway involving phosphoinositide turnover (and a known target of lithium). It is obviously of great interest to know if DmGluRA activation stimulates mRNA translation in flies and how this is regulated by mGluR antagonists, lithium, and FMRP. This knowledge will be important in making predictions about how the findings in flies might generalize to humans with Fragile X. In the meantime, however, the current study provides a compelling demonstration that pharmacotherapy has the potential to cure aspects of Fragile X.

Mark Bear has a financial interest in Sention, a company developing mGluR antagonists for treatment of Fragile X.

Gül Dölen and Mark F. Bear
Howard Hughes Medical Institute
The Picower Center for Learning and Memory
Department of Brain and Cognitive Science
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

Selected Reading

Bear, M.F. (2005). Genes Brain Behav. *24*, in press.

Bear, M.F., Huber, K.M., and Warren, S.T. (2004). Trends Neurosci. *27*, 370–377.

Conn, P.J., and Pin, J.P. (1997). Annu. Rev. Pharmacol. Toxicol. *37*, 205–237.

Dockendorff, T.C., Su, H.S., McBride, S.M., Yang, Z., Choi, C.H., Siwicki, K.K., Sehgal, A., and Jongens, T.A. (2002). Neuron *34*, 973–984.

Gallagher, S.M., Daly, C.A., Bear, M.F., and Huber, K.M. (2004). J. Neurosci. *24*, 4859–4864.

Hagerman, R.J., and Hagerman, P.J. eds. (2002). Fragile X Syndrome, Third Edition (Baltimore, MD: The Johns Hopkins University Press).

Huber, K.M., Gallagher, S.M., Warren, S.T., and Bear, M.F. (2002). Proc. Natl. Acad. Sci. USA *99*, 7746–7750.

McBride, S.M.J., Choi, C.H., Wang, Y., Liebelt, D., Braunstein, E., Ferreiro, D., Sehgal, A., Siwicki, K.K., Dockendorff, T.C., Nguyen, H.T., et al. (2005). Neuron *45*, 753–764.

Michel, C.I., Kraft, R., and Restifo, L.L. (2004). J. Neurosci. *24*, 5798–5809.

Spooren, W.P., Gasparini, F., Salt, T.E., and Kuhn, R. (2001). Trends Pharmacol. Sci. *22*, 331–337.

Zhao, W., Bianchi, R., Wang, M., and Wong, R.K. (2004). J. Neurosci. *24*, 76–84.

DOI 10.1016/j.neuron.2005.02.021