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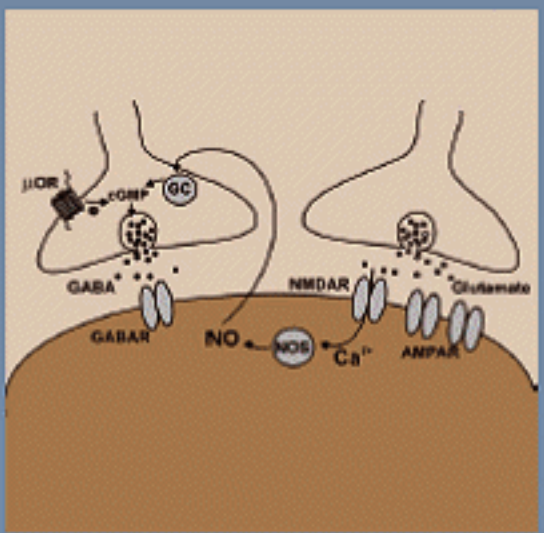

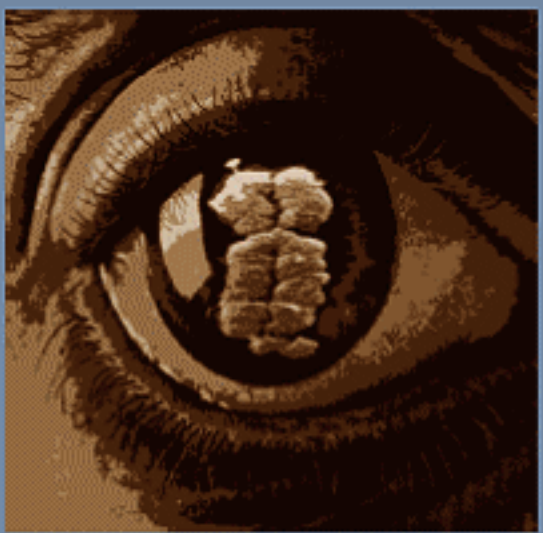

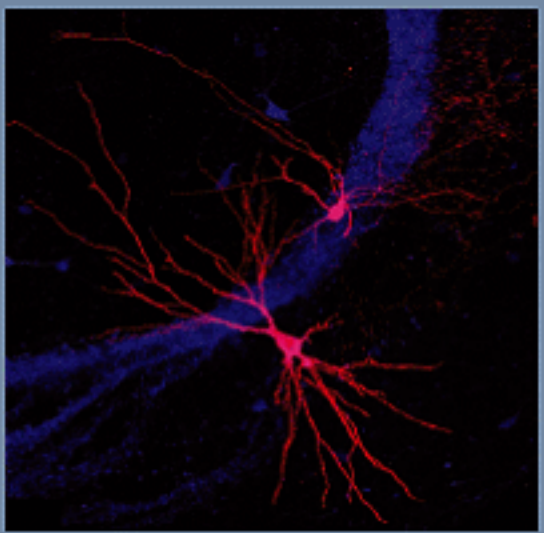

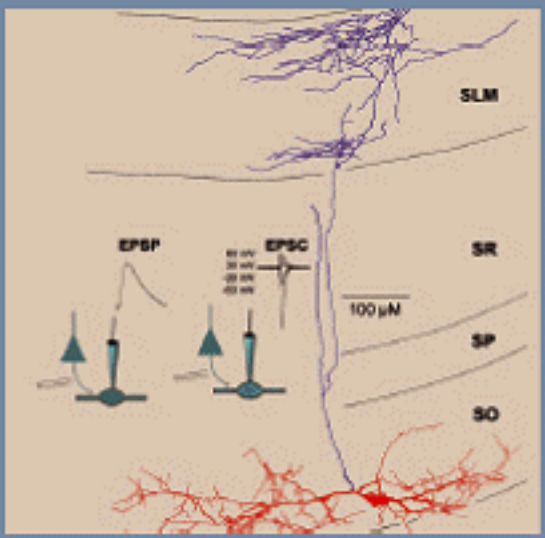

SYMPOSIUM REPORT

Role for metabotropic glutamate receptor 5 (mGluR5) in the pathogenesis of fragile X syndrome

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Metabotropic glutamate receptors (mGluRs) have been implicated in a diverse variety of neuronal functions. Studies reviewed here indicate that exaggerated signalling through mGluR5 can account for multiple cognitive and syndromic features of fragile X syndrome, the most common inherited form of mental retardation and autism. Since a reduction of mGluR5 signalling can reverse fragile X phenotypes, these studies provide a compelling rationale for the use of mGluR5 antagonists for the treatment of fragile X and related disorders.

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Glutamate is the major excitatory neurotransmitter in the mammalian central nervous system. In addition to ligand-gated ion channels, glutamate signals through G-protein-coupled metabotropic receptors (mGluRs). Signalling by group 1 (Gp1) mGluRs occurs postsynaptically via Gq second messenger cascades, and it is now clear that one consequence at many synapses is stimulation of local protein synthesis (Weiler & Greenough, 1993; Weiler *et al*. 1997). The machinery for protein synthesis is localized to the base of dendritic spines, and protein synthesis is thought to be a requirement for stable, enduring modification of the synapse (Steward & Schuman, 2003). It is perhaps not surprising then, that activation of GpI mGluRs can have lasting functional and even structural consequences, and that many of these consequences have been found to be dependent on the translation of new proteins (Merlin *et al.* 1998; Huber *et al.* 2000; Raymond *et al.* 2000; Snyder *et al.* 2001; Vanderklish & Edelman, 2002; Naie & Manahan-Vaughan, 2005).

Gp1 mGluRs have recently been implicated in the pathogenesis of fragile X syndrome (FXS), the leading inherited cause of mental retardation and an identified cause of autism (Dolen *et al.* 2007). FXS is caused by a mutation that leads to transcriptional silencing of the *FMR1* gene, which encodes the fragile X mental retardation protein (FMRP) (Pieretti *et al.* 1991). FMRP functions as a negative regulator of protein synthesis (Li *et al.* 2001; Qin *et al.* 2005*a*; Dolen *et al.* 2007). In the absence of the FMR protein, human patients with FXS have both cognitive (moderate to severe mental retardation), as well as syndromic impairments (behavioural problems, dysmorphic features and seizure disorder) which characterize the disease (Hagerman & Hagerman, 2002).

Since synaptic plasticity is the foundation of most theories of cognitive function in the brain, early studies examined a possible deficit in hippocampal synaptic plasticity in the *Fmr1* knock-out (KO) mouse model of the disease. However, these initial results were disappointing, since both protein synthesis-independent and -dependent forms of hippocampal long-term potentiation (LTP) were normal in the *Fmr1* KO (Godfraind *et al.* 1996; Paradee *et al.* 1999). It was later discovered, however, that a novel form of long-term depression (LTD) in the hippocampus (Huber *et al.* 2000) was exaggerated in the *Fmr1* KO (Huber *et al.* 2002). Unlike the NMDA receptor-dependent forms of plasticity examined previously (Godfraind *et al.* 1996; Paradee *et al.* 1999), this synaptic depression is induced by activation of GpI mGluRs (mGluR-LTD), and is normally protein synthesis dependent. Moreover, this LTD phenotype in the *Fmr1* KO does not represent a general increase in synaptic depression, since the classical NMDA receptor-dependent form of LTD is normal in these mice. These results inspired both excitement and controversy.

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Previous studies had shown that stimulation of synaptic GpI mGluRs with dihydroxyphenylglycine (DHPG) leads to translation of FMRP at synapses *in vitro* (Weiler *et al.* 1997), raising the possibility that FMRP might be necessary for effecting down-stream consequences of GpI mGluR activation. In contrast, the finding that mGluR-LTD is *exaggerated* in *Fmr1* KO mice suggested that instead, FMRP and GpI mGluRs might work in functional opposition, where GpI mGluRs activate protein synthesis and FMRP suppresses it.

Excitement for the latter model grew with (1) the recognition of a number of parallels between phenotypic features of the disease and predicted or known consequences of (over) activation of GpI mGluRs and (2) the proposed mechanistic link between FMRP and GpI mGluRs at the level of protein synthesis regulation. These ideas were outlined in what is now known as 'the mGluR theory of fragile X' (Bear *et al.* 2004) which made the prediction that widespread phenotypic features of FXS could be corrected by down-regulation of GpI mGluRs (see Fig. 1).

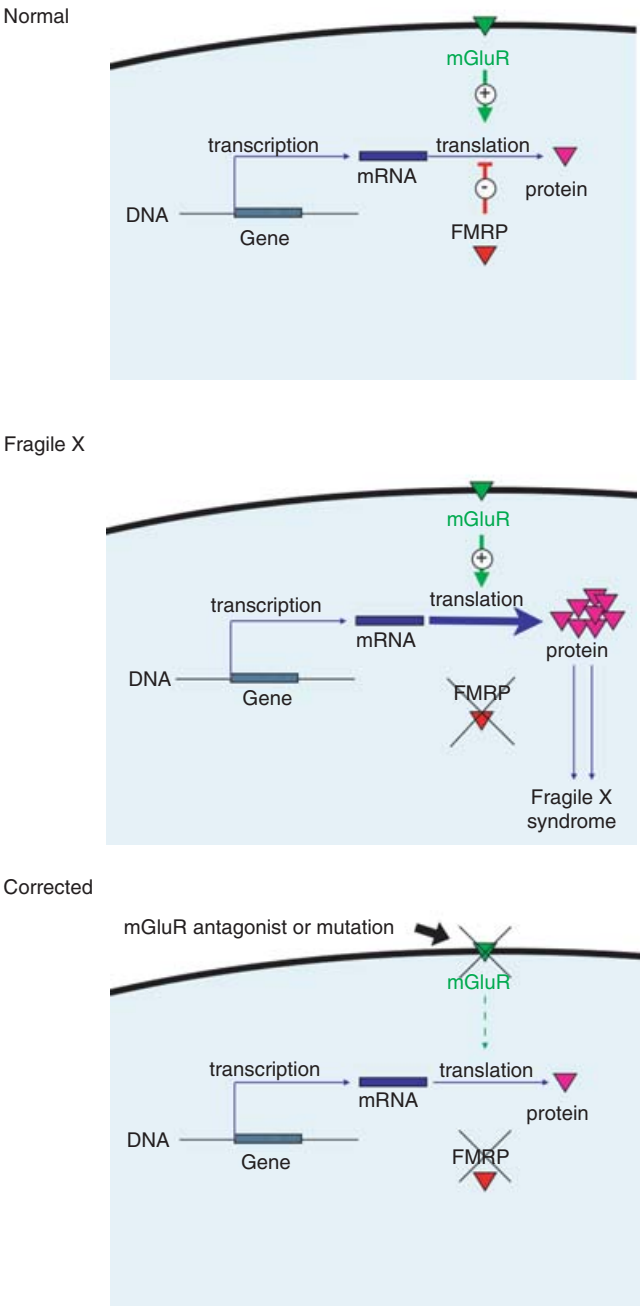

Figure 1. Model for the pathogenesis and correction of FXS
The mGluR theory of fragile X posits that mGluR5 and FMRP regulate translation of mRNA at the synapse in a functionally opponent manner – mGluR5 activation initiates protein synthesis and FMRP suppresses it. In the absence of FMRP, as is the case in FXS, mGluR5-dependent protein synthesis proceeds unchecked, and consequent excessive translation leads to the diversity of clinical features that make up the syndrome. Our results demonstrate that this progression can be corrected by genetic reduction of mGluR5 activity.

Tests of the mGluR theory

In order to test the mGluR theory, we recently made double mutant mice by crossing *Grm5* (encodes mGluR5) mutant mice (Lu *et al.* 1997) with *Fmr1* mutant mice (Consortium, 1994), which yielded male littermates of four different genotypes: wild-type (WT) [*Fmr1* (+/Y) *Grm5* (+/+)], *Fmr1* knockout [*Fmr1* (−/Y) *Grm5* (+/+)], *Grm5* heterozygote [*Fmr1* (+/Y) *Grm5* (+/−)], and the double heterozygote cross [*Fmr1* (−/Y) *Grm5* (+/−)]; these animals are termed WT, KO, HT and CR, respectively. Using these mice we established a number of different *Fmr1* KO phenotypes relevant to the disorder and examined these in the context of mGluR5 knockdown (Dolen *et al.* 2007). The phenotypes examined can be broadly categorized into either cognitive or syndromic features of the disease; cognitive phenotypes include ocular dominance plasticity (ODP), dendritic spine density (DSD), and inhibitory avoidance extinction (IAE). Phenotypes of syndromic features include audiogenic seizure (AGS), body weight (BW), and macroorchidism (MO). In addition, these studies examined the role of FMRP and mGluR5 in the regulation of protein synthesis (PS). As summarized in Fig. 2, in all cases but one (MO), genetic reduction of mGluR5 signalling by 50% returned *Fmr1* KO phenotypes significantly closer to WT, substantiating the opponent regulatory role for mGluR5 and FMRP (Dolen *et al.* 2007).

Ocular dominance plasticity

It has been known for many years that Gp1 mGluR signalling is highest in visual cortex during the period of maximal ODP (Dudek & Bear, 1989); these studies suggested that postnatal down-regulation of mGluR signalling might be important for the normal maturation and synaptic stabilization of the brain. However,

experiments investigating the role of mGluRs in monocular deprivation (MD)-induced plasticity using drug treatments were inconclusive (cf. Hensch & Stryker, 1996; Huber *et al.* 1998).

Recently, using the molecular genetic approach, we have shown an important role for mGluRs, as well as FMRP, in the regulation of ODP during development (Dolen *et al.* 2007). A 50% reduction in mGluR5 expression prevents ocular dominance plasticity induced by 3 days of MD (which is sufficient to trigger depression of deprived-eye responses in WT mice), suggesting that this receptor normally serves to enable plasticity in the visual cortex. In contrast, in the absence of FMRP, *Fmr1* KO mice show altered ODP that we interpret as 'hyperplasticity.' The response to MD is characterized by both deprived-eye response depression and open-eye response potentiation (which is seen in WT mice only after longer deprivation durations), suggesting that this protein normally serves to restrict plasticity in the visual cortex. This exaggerated plasticity phenotype seen in *Fmr1* KO mice is reversed in CR mice, consistent with opponent regulation of this process by FMRP and mGluR5. Interestingly, ODP is known to be protein synthesis dependent (Taha & Stryker, 2002); it is tempting to speculate that like mGluR-LTD in the hippocampus (Nosyreva & Huber, 2006), ODP in the *Fmr1* KO is protein synthesis independent, due to the mGluR5-mediated overproduction of proteins that support plasticity, and that a 50% reduction in mGluR5 expression rescues the KO phenotype by preventing the overproduction of such proteins (Dolen *et al.* 2007).

Dendritic spine morphology

Changes in dendritic spine shape and number are thought to be the morphological expression of physiological and biochemical synaptic modifications (Harris & Stevens, 1989; Wallace & Bear, 2004). Consistent with this idea, stimulation of Gp1 mGluRs on hippocampal neurons leads to parallel modifications of the synapse including: internalization of AMPA and NMDA receptors (Snyder *et al.* 2001), synaptic depression (Huber *et al.* 2000), and increased density of long thin spines (Vanderklish & Edelman, 2002). Significantly, each of these responses to mGluR activation requires protein synthesis.

Dendritic spine abnormalities have long been associated with mental retardation (Marin-Padilla, 1972). FXS is associated with an increase in the density of long, thin spines (Irwin *et al.* 2000), a phenotype that has been recapitulated in mouse models of the disease (Comery *et al.* 1997). Interestingly, this phenotype exactly parallels dendritic spine changes seen in response to activation of Gp1 mGluRs (Vanderklish & Edelman, 2002). We hypothesized that spine morphology is opponently regulated by FMRP and mGluR5.

To test this hypothesis, visual cortical pyramidal neurons were visualized using the Golgi-Cox staining method. This analysis confirmed that spine density is significantly increased in the KO mice and, further, that the mutant phenotype is rescued by the 50% reduction in mGluR5 expression in CR mice (Dolen *et al.* 2007). Interestingly, the HT mice showed no difference from WT in spine density, suggesting that while the decrease in *Grm5* gene dosage was not sufficient to cause an alteration of spine density by itself, it is sufficient to rescue the increased spine density phenotype seen in the *Fmr1* KO.

Behaviour: learning and memory

Ultimately, changes in the machinery of synaptic plasticity must be related to behavioural output. A role for mGluR5 in behavioural measures of learning and memory has been established using the *Grm5* KO mice. These mice show impairments in spatial learning, contextual fear conditioning, and reward based learning (Lu *et al.* 1997; Chiamulera *et al.* 2001), consistent with mechanistic studies implicating these receptors in various forms of synaptic plasticity. Furthermore, studies using the mGluR5 receptor antagonist, MPEP, have shown that mGluR5 is necessary for reference and working memory performance as well as hippocampal LTP (Naie & Manahan-Vaughan, 2004), and that this effect is protein synthesis dependent (Naie & Manahan-Vaughan, 2005).

Establishing behavioural learning phenotypes in the *Fmr1* KO mouse has been surprisingly difficult, particularly in light of the profound cognitive impairments seen in human patients with the disease. For example, strain-specific variation has confounded attempts to

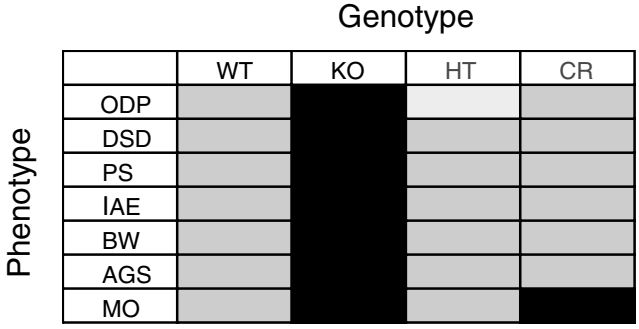

Figure 2. Genetic rescue of FXS phenotypes
Seven measures of cognitive and syndromic function were assayed in animals of four genotypes as a test of the mGluR theory of FXS. In each of the seven measures (ocular dominance plasticity (ODP), dendritic spine density (DSD), inhibitory avoidance extinction (IAE), audiogenic seizure (AGS), body weight (BW) and macroorchidism (MO)) *Fmr1* KO mice showed exaggerated responses (dark grey). Six out of 7 fragile X phenotypes were brought significantly closer to wild type levels (light grey) by Grm5 knockdown in the CR mice. Mice with a 50% reduction in mGluR5 signalling were not significantly different from WT in all cases, except for ODP where they showed reduced levels of plasticity (white). These results suggest that fragile X is a syndrome of excess, which can be corrected by decreasing mGluR5 signalling.

identify learning and memory phenotypes in the *Fmr1* KO, since phenotypes established on the FVB clonal background have been absent in C57Bl6/J strains (Bernardet & Crusio, 2006), raising the possibility that in the FVB clonal strain, background polymorphisms aggravate *Fmr1* KO phenotypes.

We discovered a learning and memory phenotype that is robustly expressed in *Fmr1* KO mice on the C57Bl6/J background. Previous studies had examined inhibitory avoidance (IA) learning in *Fmr1* KO mice on the FVB background (Qin *et al.* 2005*b*), but consistent with earlier reports using the Morris water maze (Dobkin *et al.* 2000), the IA phenotype is absent on the C57Bl6/J background (Consortium, 1994; Dolen *et al.* 2007). However, we went on to examine IA extinction, since this form of hippocampal learning and memory is also protein synthesis dependent (Power *et al.* 2006). IA extinction is exaggerated in *Fmr1* KO mice, and restored to WT levels in CR mice, consistent with an opponent role for mGluR5 and FMRP in regulating learning and memory at the behavioural level (Dolen *et al.* 2007).

A recent study showed that IA induces long-term potentiation (LTP) of Schaffer collateral synapses in area CA1 of the hippocampus (Whitlock *et al.* 2006). Although neither NMDA-dependent LTP in CA1 (Godfraind *et al.* 1996; Paradee *et al.* 1999) nor IA learning (Consortium, 1994; Dolen *et al.* 2007) is altered in the *Fmr1* KO, the increased IA extinction in *Fmr1* KO mice could be due, at least in part, to excessive mGluR-dependent synaptic weakening. Indeed, like IA extinction, mGluR-LTD in the fragile X mice was reduced by reducing mGluR5 expression by 50%.

It is noteworthy that no behavioural phenotype was detected in the mGluR HT mice. Thus, while the 50% reduction of mGluR5 expression is below the threshold to disrupt IA extinction or dendritic spine density, it is sufficient to correct the fragile X phenotype. This finding suggests that partial down-regulation of mGluR5 can have therapeutic benefit without disrupting normal function (see below).

Syndromic features: epilepsy, growth, macroorchidism

In addition to synaptic plasticity, Gp1 mGluRs have been implicated in a variety of other functions. For example, agonists of Gp1 mGluRs act as convulsants in rodents (Conn & Pin, 1997) and selective Gp1 mGluR antagonists block seizures in a range of rodent models of epilepsy (Chapman *et al.* 2000; Yan *et al.* 2005). In addition, mGluR5 is a regulator of feeding behaviour, and mGluR5 antagonists are known to be appetite suppressants (Bradbury *et al.* 2005). Finally, GpI mGluR RNAs are abundantly expressed in the testicles, with high levels of both mGluR5 and mGluR1 expression in the seminiferous tubuli and germ cells (Storto *et al.* 2001); their function there is not known.

The most common neurological abnormality in FXS is epilepsy, occurring in approximately 20% of children with the disease, and presenting as seizure and EEG abnormalities (Musumeci *et al.* 1988). This phenotype has been robustly replicated in *Fmr1* KO mice using the audiogenic seizure paradigm (Musumeci *et al.* 2000; Yan *et al.* 2005; Dolen *et al.* 2007). In the *Fmr1* KO mice, increased seizure susceptibility is attenuated by reducing expression of mGluR5 (Dolen *et al.* 2007) and by receptor antagonism with MPEP (Yan *et al.* 2005). Interestingly, studies have shown that the generation of epileptiform bursts is both mGluR and protein synthesis dependent (Merlin *et al.* 1998), consistent with the opponent regulation of translation-dependent epileptogenesis by FMRP and mGluR5.

Children with FXS show accelerated prepubescent growth (Loesch *et al.* 1995). We were able to recapitulate this phenotype in the *Fmr1* KO mouse, and showed that this phenotype is also rescued by selective reduction in mGluR5 expression (HT mice did not have altered growth compared to WT) (Dolen *et al.* 2007). Although it is currently not known how Gp1 mGluRs regulate growth, it is interesting to note that these receptors are highly expressed in the lateral and ventromedial hypothalamus (van den Pol *et al.* 1995), brain regions that are thought to be important for the endocrine regulation of growth. While it is not clear how the reduction in mGluR5 gene dosage leads to a rescue of the *Fmr1* KO growth phenotype, it is clear that at the cellular level, FMRP and mGluR5 work in an opponent fashion to regulate body weight.

Finally the macroorchidism phenotype (large testicles) has been recognized for over 20 years, and occurs in over 80% of adult males with FXS (Nielsen *et al.* 1982). This phenotype is also robustly recapitulated in the *Fmr1* KO model of the disease (Consortium, 1994; Dolen *et al.* 2007); however, the pathogenesis of this phenotype is unlikely to be related to mGluR5, since we were not able to correct this phenotype by either mGluR5 knockdown or knockout strategies (Dolen *et al.* 2007). Still the possibility remains that FMRP interacts with the other GpI mGluR, mGluR1, in the pathogenesis of this phenotype.

Therapeutic implications

Current medications used for FXS are aimed at treating symptoms of the disease without regard to underlying pathophysiology. This treatment regimen includes the usual battery of neuropsychiatric pharmocopia, including mood stabilizers, antipsychotics, anticonvulsants, antidepressants, anxiolytics and psychostimulatants. While this symptomatic approach can ameliorate certain features, often medication used for one symptom can exacerbate others. Most importantly, none of these

treatments is effective in correcting cognitive impairment, arguably the most debilitating feature of the disease.

The data reviewed here provide the first real hope of global therapy for FXS – increased mGluR5 signalling provides a thread that connects diverse manifestations of the disease. Of course this model awaits validation in forthcoming clinical trials in humans. As the signalling cascade between mGluR5 and FMRP is delineated, and the theory is broadened to include other relevant neurotransmitter systems, additional targets may be identified. For now, it is important to note that metabotropic glutamate receptors are particularly amenable to pharmacological manipulation (Marino & Conn, 2006), and these studies provide compelling evidence that these receptors if targeted appropriately (i.e. with antagonists), will have significant therapeutic value for the treatment of FXS and related disorders.

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