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Commentary

The neurochemical basis for the treatment of autism spectrum disorders and Fragile X Syndrome

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ABSTRACT

Autism spectrum disorders (ASD) and Fragile X Syndrome (FXS) are neurodevelopmental disorders that share overlapping behavioral characteristics. While FXS is known to result from a specific genetic mutation, the causes of the majority of cases of ASD are unknown. Animal models of FXS have revealed new insight into the cellular and biochemical changes that occur in the central nervous system in this disorder, while human genetic studies on individuals with autism have identified sets of genes that may increase susceptibility to the disorder. Together these discoveries suggest overlapping biochemical characteristics and reveal new directions for the potential development of pharmacological therapies that might prove useful in the treatment of both FXS and ASD. In particular, delayed synaptic maturation, abnormal synaptic structure and/or function and alterations in intracellular signaling pathways have been linked to the pathogenesis of FXS and ASD. Aberrations in $GABA_A$ receptor ion channels and the G-protein coupled metabotropic glutamate and $GABA_B$ transmitter systems are also linked to both disorders and these receptors are currently at the forefront of preclinical and clinical research into treatments for both autism and Fragile X Syndrome.

1. Introduction: the clinical features of Fragile X Syndrome and autism spectrum disorders

Fragile X syndrome (FXS) and autism spectrum disorder (ASD) are neurological disorders with overlapping symptoms. Both disorders are generally considered to involve perturbations that occur early in brain development. FXS is a genetic syndrome that is caused by the silencing of the *Fmr1* gene. FXS is the most common inherited form of mental retardation where the presence of the full mutation in the general population has been estimated to occur at a frequency of about 1 in 2500 (see [1] for a discussion on the frequency of FXS). This disorder is considered a ''syndromic form'' of ASD. In addition to FXS, other syndromic forms of ASD include Rett syndrome, tuberous sclerosis, Angelman Syndrome, and Turner syndrome, all of which involve autistic features in a substantial proportion of affected individuals [2]. Clinically, ASD encompasses autism, Asperger's syndrome and pervasive developmental disorder-not otherwise specified. The vast majority of ASD cases are non-syndromic and idiopathic. The frequency of idiopathic ASD is much more common than FXS with estimates in the general population reported as approximately 1 in 100 [3,4].

The core behavioral manifestations of ASD include deficits in social interactions, language and communication, and stereotyped, repetitive and restricted behaviors or interests (for review see [5]). Clinical signs of ASD are usually present by age three and some children may initially show normal development followed by a loss of previously acquired skills and a delay in acquiring new ones. The degree of behavioral and cognitive functioning is highly variable in ASD. For example, an individual with Asperger's syndrome might display some social deficits and preservative behaviors with no cognitive or language delays, while another individual with autism might be completely non-verbal and display severe behavioral and cognitive deficits. Other co-morbidities, including sensory hypersensitivity, gastrointestinal problems, disrupted or abnormal sleep patterns, and epilepsy are also common in ASD [6].

The behavioral features of FXS patients include hyperactivity, mental retardation, learning deficits, developmental delay including delayed speech development, social anxiety and gaze avoidance, sensory hypersensitivity and withdrawal from touch, stereotypic movements and behaviors such as hand flapping and rocking, poor motor coordination, and echolalia [7]. Girls with FXS usually suffer from milder cognitive and behavioral deficits than boys primarily due to the fact females still express some of the

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Fmr1 gene product called fragile X mental retardation protein (FMRP). Most boys with FXS display some autistic-like behavior with approximately a third of the FXS population meeting the formal criteria outlined by the *Diagnostic and Statistical Manual of Mental Disorders*, *Fourth Edition* for ASD. FXS patients diagnosed with autism display more severe social impairments, as well as lower cognitive and language abilities, academic achievement, and adaptive behaviors compared to those without autism [8].

Individuals with ASD or FXS also display an increased susceptibility to epileptic seizures. The incidence of seizures in FXS is estimated to be between 13% and 18% in males and 5% in females [9]. The occurrence of seizures usually begins in early childhood (between 6 months and 4 years of age), but the majority of patients experience a resolution of seizures by adolescence. The reported incidence of seizures in ASD varies widely in different studies and has been reported to be in the range of 3–38% [10]. In some cases there are two peaks of seizure onset, one up to 5 years old and a second in adolescence (>10 years), and a more severe degree of mental retardation is associated with an increased risk of seizures.

Traditional pharmacotherapy for FXS and ASD has been based on treating symptoms. Stimulants are prescribed in FXS to treat attention deficit and hyperactivity while α-adrenergic receptor agonists such as clonidine and guanfacine are used to treat hyperactivity in younger children [7]. Both selective serotonin reuptake inhibitors and antipsychotics are sometimes used to reduce aggression associated with anxiety. For the treatment of seizures, carbamazepine is often used while phenytoin is avoided in children with FXS because of the adverse effects of gum hypertrophy. Phenobarbital and gabapentin are also avoided because they exacerbate behavioral problems in FXS (e.g. hyperactivity). With ASD, the major goal of treatment and management in children has been to maximize function by minimizing ASD core symptoms, decreasing maladaptive behaviors, and supporting child development and learning [11]. These interventions address communication, social and play skills, daily living skills, and academic achievement. Although medical treatments are typically considered an adjunct to educational and behavioral interventions, large proportions of children with ASD receive pharmacologic treatments. Antipsychotics, particularly risperidone, can be efficacious in treating some core symptoms such as maladaptive behavior, hyperactivity, and irritability [11,12]. Stimulants such as methylphenidate are used to treat hyperactivity and antidepressants are prescribed to treat behavioral problems, although the results from clinical trials of selective serotonin reuptake inhibitors in ASD have not been particularly impressive [12]. As discussed below, more specific and efficacious drugs for treating FXS and ASD are now being tested in animal studies and in clinical trials.

2. The known molecular causes of FXS and ASD

2.1. Fragile X Syndrome

FXS is caused by an expansion of a trinucleotide repeat CGG sequence in the 5′ untranslated region of the X-linked *Fmr1* gene [13]. Unaffected individuals generally carry 7–55 repeats; however, when the repeat expands to 200 or greater, hypermethylation and transcriptional silencing of the gene occur. Thus, persons with FXS produce little or no detectable expression of the encoded protein called Fragile X Mental Retardation Protein or FMRP. Individuals with intermediate CGG expansions in the range of 55–200 repeats are known as fragile X premutation carriers and are at increased risk for a related disorder known as Fragile X-Associated Tremor and Ataxia Syndrome (FXTAS) that affects primarily men over the age of 50 [7]. The presence of the premutation in women can also cause premature ovarian failure.

FMRP is an mRNA binding protein that is highly expressed in the brain and testes while lower levels are expressed in other tissues including the ovaries, pituitary, adrenal, and skeletal muscle [14]. Both neurons and astrocytes express FMRP [15]. Multiple mRNA binding sites are present on FMRP and binding of specific mRNAs to the protein regulates their translation, stability and/or dendritic transport [16–18]. The regulation of bound mRNAs (also called "cargoes") is mediated in part by phosphorylation and dephosphorylation of FMRP where phosphorylated FMRP is associated with stalled ribosomes and dephosphorylation of FMRP is associated with actively translating ribosomes [19] (see [20] for a description of the biochemical participants in this process). It is estimated that up to 4% of brain mRNAs may bind FMRP [21]. In the absence of FMRP, the expression of proteins coded for by FMRP cargoes may increase or decrease; these changes in protein expression are thought to underlie the pathogenesis of FXS.

2.2. Genetic studies on ASD

As the name implies, ASD is not a single disorder but has multiple causes and thus it is not surprising that many genes have been associated with ASD. Although most causes of ASD are idiopathic, genetic studies have identified a growing array of genes that are linked to the disorder and several genome wide scans [22,23] have identified dozens of potential "autism genes". Given the diversity of phenotypes, genetic contributions to ASD are likely to be highly heterogeneous and involve a combination of alleles with low and high penetrance.

A small number of non-syndromic ASD cases have been linked to specific genetic mutations (for review see [24]). Among these are a number of genes that code for proteins important in synaptic structure and function, including neuroligins, neurexins, SHANK3 and CNTNAP2. This has lead to the hypothesis that the pathogenesis of ASD may be more broadly related to abnormalities at the synapse, and that genetic mutations that result in aberrant synaptic structure and/or function could produce the behavioral and/or cognitive phenotypes associated with ASD [25]. For example, the ASD-associated R451C substitution mutation in the neuroligin-3 gene induces a change in the protein which interferes with cell–surface localization and proper cell–cell interactions [26]. R451C knock-in mice exhibit selective impairment in social interaction accompanied by a significant enhancement in spatial learning abilities – a behavioral phenotype consistent with some cases of autism [27]. Unexpectedly, in contrast to neuroligin-3 knockout mice, R451C knock-in mice showed an increase in inhibitory synaptic transmission in the cortex, with no apparent effect on excitatory synapses, indicating that the R451C substitution likely represents a gain-of-function mutation. These findings demonstrate how identifying known genes associated with autism can provide insight into possible disease mechanisms.

Based on initial reports [28,29], an active area of investigation is the identification of inherited and *de novo* gene copy number variations (CNVs) associated with ASD. It has been suggested that some of the CNVs in the broad phenotypic spectrum of ASD patients may not be ASD-specific, but rather are more generally associated with impaired neurodevelopment [30]. Nevertheless, new emerging themes in CNVs linked with non-syndromic ASD include genes involved in cellular signaling pathways, cell cycling, and proliferation [31]. In a genome-wide analysis of about 1000 ASD cases and 1200 controls, Pinto et al. made the intriguing observation that a number of CNVs in the GTPase/Ras signaling system are significantly associated with ASD. Up-regulation of Ras activity has also been documented in FMR1 mice [32], and abnormalities of the GTPase/Ras systems appear to be a reoccurring theme in other neurodevelopmental and mental

retardation disorders (see [33] for a review). The interest in both ASD and FXS lies in the well established involvement of the GTPase/Ras and related Rho GTPases in mediating dendritic spine development (and synaptic plasticity) which is impaired in FXS and several other mental retardation disorders [33]. Moreover, GTPases, including regulators of G-protein signaling (RGS proteins [34]) are essential proteins in intracellular signal transduction initiated by activation of G-protein coupled receptors such as metabotropic glutamate receptors and $GABA_B$ receptors which are at the forefront of drug discovery in both disorders (see below).

In summary, the identification of genetic abnormalities is a top priority in the study of ASD with the view that common themes will emerge from such studies that will eventually converge on a smaller more manageable number of pathways to pursue for therapeutic drug development.

3. Animal models

Animal models which lack expression of FMRP have been indispensible both in greatly enhancing our understanding of the molecular mechanisms behind FXS and ASD, and in facilitating preclinical drug development. *Drosophila* [35–37], zebrafish [38], and mouse models [39] of FXS have been generated and widely used. The mouse and human *Fmr1* genes and FMRP proteins share a high degree of homology [40] and the tissue specificity and expression profiles of FMRP are generally similar in both species [41], thus making the mouse especially suitable for the study of this disorder. The widely studied FMR1 knockout mice show both morphological abnormalities and changes in behavior consistent with the human disorder [39,42]. As in humans, FMR1 mice display macroorchidism (enlarged testes), which is evident in the mice from postnatal day (PND) 15 onwards and results in a 30–40% increase after about 3 months [39,42]. The dendritic spine abnormalities, a hallmark of human FXS [43], are also recapitulated in FMR1 mice. Long, thin immature spines are observed in these mice, with an overall increase in dendritic spine density indicative of defective pruning in development [44–46].

Another neurological parallel between the mouse model and FXS patients is an increased susceptibility to seizures. However, as opposed to the spontaneous seizures observed in FXS patients, seizures in FMR1 mice are specifically triggered by intense auditory stimuli [47,48]. The ability to induce audiogenic seizures in the FMR1 mouse peaks in the third and fourth weeks of postnatal life [49] and then declines, a pattern that, as noted above, coincides with the resolution of seizures in humans by late adolescence. Several independent studies have also demonstrated hyperactivity in FMR1 mice in the open field test [34,39], and this too is consistent with the high incidence of hyperactivity and attention deficit in children and adolescents with FXS. Finally, FMR1 mice display abnormal social interactions [34,50,51] paralleling the reduced sociability characteristic of FXS and ASD.

Several rodent models of ASD have been developed or proposed as potentially useful paradigms to study ASD. Most models are heavily focused on mimicking the behaviors associated with autism – particularly reduced sociability and communication and stereotyped behaviors [52]. Genetic models harboring mutations known to be associated with human ASD (see discussion above) and rodent strains that replicate some of the behavioral features of ASD, such as low sociability [53] have been used to study the disorder. Several rodent models that entail the use of drugs or hormones have also been proposed; examples include exposure of young mice to valproic acid [54] and neonatal hypothyroidism [55]. Although these paradigms do reproduce some aspects of ASD, the validity and extent of applicability as well as the mechanistic connections to the human disorder requires further investigation. Although the absence of a robust animal model for ASD has been somewhat problematic, the overlapping symptomatology between FXS and ASD suggests the possibility that informative studies on the former may facilitate a better understanding of the latter.

4. Neuroanatomical and neurodevelopmental aspects of FXS and ASD

4.1. Brain imaging studies

The study of changes in brain morphology that occur in FXS and ASD is a well developed field that continues to provide important contributions to our understanding of the CNS pathways affected in these disorders. To date, most of the findings derive from MRI studies conducted on adults. The results of anatomical analyses have pointed to the involvement of several brain regions, including the frontal and temporal cortex, amygdala, and cerebellum [24,56] in both FXS and idiopathic ASD. Many biochemical and behavioral studies on FXS and ASD have focused on changes in forebrain regions while analyses of the midbrain, cerebellum, and brain stem have been relatively neglected. However, neuroanatomical studies of the cerebellum have documented both gross anatomical perturbations and microscopic pathological changes in this structure, and the possibility exists that the cerebellum may be linked with several behavioral manifestations of FXS and ASD [57] such as hyperactivity and impaired social interactions. Additional evidence demonstrating neuroanatomical changes in the cerebellum have been reported by Ellegood et al. who used high resolution MRI imaging to measure volumes in 62 brain regions in young (30 day old) FMR1 mice [58]. The largest change in volume compared to wild-type mice was a reduction in the size of the deep cerebellar nuclei in the FMR1 mice. This observation is interesting because the cerebellar nuclei receive input from Purkinje cells and in turn project to the brainstem and to major forebrain structures via the thalamus. Previous work in the FMR1 mouse has also shown the presence of abnormally long (immature) dendritic spines on cerebellar Purkinje cells, enhanced long-term depression at the parallel fiber-Purkinje cell synapse, and defective eyeblink conditioning [59].

In addition to the early developmental changes seen in FMR1 mice, there is also definitive evidence that neuroanatomical changes occur in the developing brains of children and young adults with FXS and ASD. Hoeft et al. examined gray and white matter volumes in boys with FXS at 1 and again at 3 years of age and compared these findings to age- and developmentally matched controls [60]. Enlarged gray matter volume in the caudate, thalamus, and fusiform gyri and reduced gray matter in the cerebellar vermis was seen at both time points in FXS, suggesting early, possibly prenatal, genetically mediated alterations in neurodevelopment. In contrast, some regions such as the orbital gyri, basal forebrain and thalamus initially showed similar gray matter volumes; however, an altered growth trajectory led to increased size in FXS at 3 years of age, suggesting that delayed or disrupted synaptic pruning occurred postnatally in boys with FXS.

Enlarged brain regions have also been demonstrated in young children with ASD. By 2.5 years of age, both cerebral gray and white matter have been reported to be significantly enlarged with the most severe enlargement occurring in frontal, temporal, and cingulate cortices [61]. Longitudinal analyses showed that cerebral gray, cerebral white, frontal gray, temporal gray, cingulate gray, and parietal gray developed at an abnormal growth rate in toddlers and females with ASD displayed a more pronounced abnormal growth profile in more brain regions than males with the disorder. White matter may also be compromised. A study conducted on adolescents (a stage at which the human brain has not yet reached maturity) using diffusion tensor imaging showed a reduction of white matter integrity associated with an increase in interstitial

space; this abnormality was observed throughout the CNS in the ASD cases but not matched controls [62]. Interestingly, the authors suggested that the results might indicate a generalized reduction in the number or size of glia in ASD. Glia may also be an important, but as yet under-appreciated factor in FXS. For example, hippocampal neurons exhibit abnormal dendritic morphology and a decreased number of presynaptic and postsynaptic protein aggregates when grown on astrocytes from FMR1 mice, and normal astrocytes could prevent the development of abnormal dendrite morphology in FMR1 mouse neurons [63]. Together, these results indicate that glia likely contribute to abnormal dendrite morphology and synapse development in FXS.

4.2. Microscopic alterations in the CNS

In post-mortem FXS human brains [43] and the FMR1 mouse brain [45,46], the most commonly reported fine structural change has been abnormalities in dendritic spines. Although there is some evidence from human post-mortem brain samples of spine abnormalities in ASD [64], the presence of dendritic spine defects as seen in FXS has not been established and no clear consistent picture of the neuropathological changes in ASD has emerged yet [65]. In FXS, the presence of the spine alterations depends on the brain region and postnatal period examined, and overall, the magnitude of the effect detected with traditional staining methods, such as Golgi stain, is relatively subtle. Nimchinsky et al. reported that during early cortical synaptogenesis, pyramidal cells in somatosensory cortex of FMR1 mice had longer spines than controls [45]. At 1 week, spine length was 28% greater in mutants than in controls. At 2 weeks, this difference was 10%, and at 4 weeks only 3%. Similarly, spine density was 33% greater in mutants than in controls at 1 week of age and at 2 or 4 weeks of age the differences were not detectable. However, spine abnormalities have also been documented in adult (>2 month old) mice [46].

The transient nature of the spine abnormality in the intact animal suggests that FMRP might play a role in the normal process of dendritic spine growth in coordination with the experience-dependent development of cortical circuits. The recent development of transcranial two-photon imaging has allowed for the *in vivo* analysis of spine morphologies over time. Using this technique, Pan et al. demonstrated that the rates of spine formation and elimination, measured over days to weeks, were significantly higher in both young and adult FMR1 mice compared with littermate controls [66]. The heightened spine turnover was due to the existence of a larger pool of short-lived new spines in the knockout mice compared to controls. In addition, the formation of new spines and the elimination of existing ones were less sensitive to modulation by sensory experience in FMR1 mice.

There is also evidence that FMR1 mice show a developmental delay in the reduction of spine turnover and in the transition from immature to mature spine subtypes and that glutamatergic signaling regulates these processes. The results reported by Cruz-Martin et al. [67] showed, surprisingly, that blockade of mGluR signaling, which reverses some adult phenotypes in FMR1 mice (see below), accentuated the immature protrusion phenotype. It was concluded that the absence of FMRP delays spine stabilization and that dysregulated mGluR signaling in FXS may help to partially normalize this early synaptic defect. In further support of a developmental delay in synapse maturation in FXS, Harlow et al. demonstrated that during the perinatal critical period, the lack of FMRP in excitatory thalamocortical synapses in the somatosensory cortex caused dysregulation of glutamatergic signaling maturation (e.g. low ratio of NMDA to AMPA receptors) and the fraction of silent synapses persisting to later developmental times was increased in FMR1 mice [68]. This group indicated that one consequence of these changes was a temporal delay in the window for synaptic plasticity in FMR1 mice, while other forms of developmental plasticity were not altered. Because the precise timing of critical periods during cortical developmental is essential for the proper organization of synaptic connections and circuits, the delayed timing of plasticity windows could contribute to altered refinement of cortical circuits that persist throughout life and contribute to sensory processing deficits in FXS [68].

In summary, work in FMR1 mice has shown that dendritic spine development is delayed and that some spines remain in an immature state in older animals, the latter of which coincides with neuropathological results from human post-mortem studies. The changes in spines are highly likely to be related to defective synaptic plasticity. Finally, aberrant glutamate and GABA receptor mediated signaling are critical factors in the altered neurochemistry and synaptic plasticity and provide an entry point for potential therapeutic intervention.

5. Glutamate and GABA in FXS and ASD

5.1. Metabotropic glutamate receptors

Metabotropic glutamate receptors (mGluR) and associated downstream pathways are at the forefront of basic and clinical research in FXS. The "mGluR hypothesis of FXS" has been a major driver in drug development research since it was first formulated. This theory posits that FMRP normally acts to regulate protein synthesis downstream of Group I mGluRs (mGluR1 and 5) and that in the absence of FMRP, mGluR-dependent protein synthesis is abnormal, accounting for many of the phenotypes associated with FXS [69]. For example, cellular internalization of the AMPA subtype of glutamate receptor channels is induced by activation of Group I mGluRs, and this process is exaggerated in FXS [70,71]. The mGluR theory suggests that FMRP normally inhibits the translation of proteins that mediate AMPA receptor trafficking from the plasma membrane to the intracellular milieu; in the absence of FMRP, over-expression of proteins involved in AMPA internalization decrease cell surface AMPA receptors. This provides an explanation for the consistent observation that synaptic long-term depression is enhanced in FMR1 mice [72]. Overall, animal studies have suggested that many aspects of the FXS phenotype, including behavioral abnormalities, cognitive deficits, and altered dendritic spines may be attributable, at least in part, to excessive signaling by mGluR5.

An obvious prediction of the theory is that reducing Group I mGluR signaling should be beneficial in FXS. This prediction has garnered much support from pharmacological studies carried out using FXS mutant flies [37,73,74] and FMR1 mice [44,49] (see [75] for review), where mGluR5 antagonists have rescued several behavioral, biochemical and anatomical phenotypes in these models. Additional support was revealed through the characterization of FMR1 mice crossed with heterozygous mGluR5 knockout mice where several FXS phenotypes were fully or partially normalized [76].

These studies and other encouraging results derived from animal studies of FXS have led to the initiation of at least three clinical trials of mGluR5 antagonists (www.clinicaltrials.gov). Preliminary findings from a small clinical trial of an mGluR5 antagonist developed by Novartis have been published. The effects of the drug AFQ056 on behavioral symptoms of FXS were examined in a randomized, double-blind, two-treatment, two-period, crossover study of 30 male FXS patients aged 18–35 years [77]. Overall, no significant effects of treatment on the primary outcome measure, the Aberrant Behavior Checklist–Community Edition (ABC-C) score, were seen after treatment for 20 days. However, further analysis of the data showed that a subgroup of 7 of the patients with full FMR1 promoter methylation and no detectable

FMR1 mRNA in blood cells, showed statistically significant improvement on several measures after AFQ056 treatment compared to placebo. No significant response was seen in 18 patients with partial promoter methylation. If confirmed in larger and longer-term studies, these results would suggest that blockade of the mGluR5 receptor in patients with full methylation at the FMR1 promoter may elicit improvement in the behavioral attributes of FXS. It is important to note however, that no estimate of mGluR5 receptor occupancy was given in the Jacquemont et al. study and in future clinical investigations of mGluR5 antagonists, it will be important to establish the relationships between blood and brain drug levels, mGluR5 occupancy, and clinical efficacy. Why subjects lacking FMR1 mRNA expression in peripheral blood cells, and presumably no FMRP expression in the CNS, responded better to treatment than subjects with partial methylation, and presumably some residual FMRP expression, remains unknown. The answer to this question will undoubtedly be vigorously pursued.

5.2. $GABA_A$ and $GABA_B$ receptors

The $GABA_A$ receptor, a heteropentameric GABA-gated chloride channel, and the $GABA_B$ receptor, a heterodimeric G-protein coupled receptor belonging to the same subfamily as the mGluRs, have also received considerable attention in the study of FXS and ASD. Multiple components of the GABA system are deficient in FMR1 mice. The GABA neurotransmitter anabolic enzyme glutamic acid decarboxylase (GAD) [78,79], and the $GABA_B$ receptor R1 subunit but not the R2 subunit [34] have been shown to be under-expressed in FMR1 mice. Interestingly, $GABA_B$ receptor deficient mice are hyperactive and have epileptic seizures [80,81], both of which are phenocopied in FMR1 mice. The under-expression of mRNAs and proteins for several $GABA_A$ receptor subunits relative to wild-type mice has also been documented in FMR1 mice [78,82–84]. Abnormally low expression of the α1 $GABA_A$ receptor subunit occurs as early as postnatal day 5 in the newborn FMR1 mouse brain, while the β2 subunit shows a wild-type level of expression right after birth but then fails to increase further to a normal level of expression and is under-expressed at 2 weeks and later compared to wild-type [84]. Proper expression, trafficking, and stimulation of $GABA_A$ receptors are required for dendritic spine maturation [85] and mice lacking the α1subunit have spines that fail to fully mature [86]. These and other findings prompted Adusei et al. to speculate that the spine abnormalities in FXS might be caused in part by under-expression of GABA receptors and/or enzymes in the developing CNS [84].

It is intriguing that so many of the components of the GABA system are down-regulated or impaired in FMR1 mice although the reason for this is not yet known. One key question that arises is, does the abnormally low expression of GABA receptors and enzymes translate into functional deficits? Recent findings from behavioral studies and brain slices indicate that the answer is yes. We analyzed the behavior of FMR1 mice administered GABAergic agonists. The suppressing effect on locomotor activity in mice given diazepam or loreclezole was reduced in FMR1 compared to wild-type mice (Fig. 1). The effect was greater for the $GABA_A$ receptor β2/3 subunit specific potentiator loreclezole, possibly reflecting the decreased expression of the β2 subunit. A similar explanation can be postulated for results from an experiment investigating the sleep-inducing properties of the $GABA_A$ receptor agonists phenobarbital and etomidate. Etomidate, which is another potent β2/3 subunit specific potentiator, displayed a significantly lower ability to induce sleep in FMR1 mice compared to wild-type mice (Fig. 1). As seen with the reduced locomotor suppressing effects of loreclezole, the reduced sleep-inducing effects of etomidate in adult FMR1 mice may be due to abnormally low expression (approximately 50% of wild-type expression [84]) of the $GABA_A$ receptor β2 subunit.

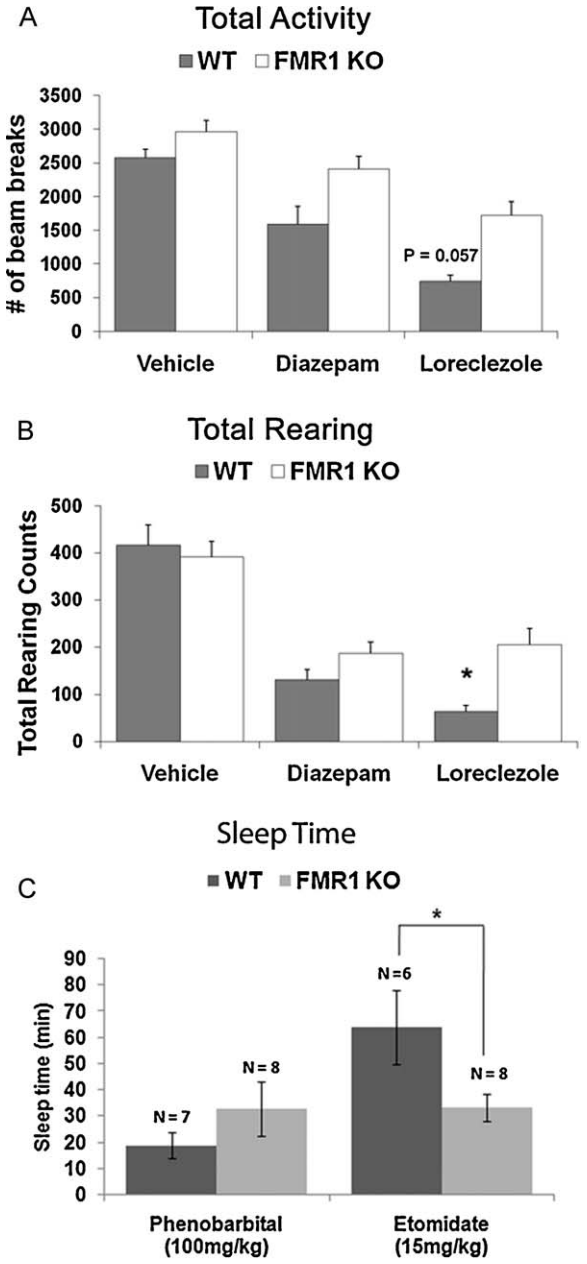

Fig. 1. The effects of $GABA_A$ agonists on locomotor activity and sleep times in wild-type and FMR1 knockout mice. (A–B) Spontaneous locomotor activity was measured over 90 min following i.p. administration of diazepam (1 mg/kg) or loreclezole (30 mg/kg). Drug-treated wild-type (C57BL/6) and FMR1 mice were assessed for total activity (A) and total rearing (B). In the loreclezole treated mice there was a significant genotype × treatment interaction in total rearing (panel B; two-way ANOVA, $^*P < 0.05$) and a strong trend towards significance for total activity (panel A; $P = 0.057$), indicating that FMR1 mice were less susceptible to the sedative effects of loreclezole compared to wild-type controls ($N = 9–11$ for each group). C, The hypnotic effects of phenobarbital (100 mg/kg) and etomidate (15 mg/kg) were assessed in separate sets of adult wild-type C57BL/6 and FMR1 knockout mice by measuring the duration of sleep time as defined by the loss of the righting reflex. FMR1 knockout mice injected with phenobarbital experienced slightly longer sleep times compared with wild-type littermates, but did not reach statistical significance ($P = 0.13$, Student's t-test), while etomidate-injected FMR1 mice slept significantly less than wild-type mice ($^*P < 0.05$). Data represent the mean ± SEM.

Observations made in mouse brain slices also support the notion that GABAergic transmission is impaired in FXS [79,83,87]. The findings of Olmos-Serrano et al. suggest that a lack of ambient GABA, due to deficient action potential-dependent release from inhibitory

interneurons, is a contributor to hyper-excitability in the amygdala of FMR1 mice [79]. A dramatic reduction in the frequency and amplitude of phasic IPSCs and tonic inhibitory currents, as well as the number of inhibitory synapses was seen in FMR1 mice. In addition, neuronal hyperexcitability in principal neurons of the amygdala was rescued by pharmacological augmentation of tonic inhibitory tone using the GABA agonist THIP, a result consistent with a previous study showing impaired GABA tonic current in the hippocampus [83]. A conclusion emanating from these data is that the potential therapeutic value of increasing GABAergic transmission appears to lie in its ability to either compensate for increased excitatory glutamatergic signaling and/or to boost intrinsically deficient inhibitory signaling [48,79,88].

Several important outstanding questions arise from the findings to date. First, do the changes observed in FMR1 mice extend to patients with FXS and ASD, and if so, what is the contribution of impaired GABA production and under-expression of $GABA_A$ and $GABA_B$ receptors to the composite phenotype of these disorders? These questions lead to another key question – would GABAergic drugs be beneficial, and if so which ones?

Although little information is available of the status of GABA receptors and enzymes in individuals with FXS, there is evidence from post-mortem studies documenting deficiencies in the GABA system in human ASD. GAD [89] and $GABA_A$ receptor expression are both decreased in ASD [90,91], and similar to FMR1 mice [34], the $GABA_B$ R1 subunit is also down-regulated in human ASD samples [92,93]. The human post-mortem studies conducted to date encompassed relatively small sample sizes so additional studies are needed to provide more detailed information on which receptor subunits are affected, the extent of the deficiency in terms of the degree of receptor down-regulation, and its anatomical and regional extent in the CNS. Functional studies in patients are also required to determine if altered behaviors might be related to GABAergic impairments.

The central question as to the usefulness of GABAergic drugs is beginning to be examined in the FXS field. Increasing GABA exposure during development has been shown to rescue the biochemical, morphological, and behavioral phenotypes of the drosophila model of FXS [37] emphasizing the role of GABA as a regulator of synaptic maturation during critical periods of development. In FMR1 mice, the $GABA_B$ receptor agonist baclofen blocks audiogenic seizures [48]. The fact that baclofen has been used clinically for many years to treat muscle spasticity and pain has fast-forwarded the initiation of a small clinical trial in adults with FXS (see seasidetherapeutics.com).

Finally, on a more speculative note, the manipulation of sex steroids, several of which are known to potently regulate $GABA_A$ receptor function in the brain, could be considered for study in FXS and ASD. Estrogens regulate the expression of GAD in the hypothalamus and therefore can modulate GABA signaling; they also modulate whether GABA is excitatory or inhibitory, via changes in the expression of the potassium–chloride co-transporter KCC2 [94]. Moreover, androgens have been shown to predispose males to $GABA_A$ receptor mediated excitotoxicity [95]. Based on these and other findings, it has been suggested that sex steroids could modulate the excitatory/inhibitory balance, which could sensitize the male brain to ASD and that the modulation of sex-steroid signaling in men with ASD might be therapeutically useful [96]. Further research is needed to determine if alterations in steroid sex hormones in the brain, especially during development, is a factor in FXS and ASD.

6. Intracellular signaling pathways downstream of receptors

Another active area of investigation in FXS and ASD is the analysis of receptor initiated intracellular signaling pathways as these may represent another potential avenue for therapeutic intervention. Several biochemical pathways including phosphoinositide 3-kinase (PI3K), mTOR, and ERK have been shown to be altered in FXS and all three are linked to mGluR mediated signaling. FMRP regulates the synthesis and synaptic localization of p110β, the catalytic subunit of PI3K and consequently, FMR1 mice display excess activity of PI3K, a downstream signaling molecule of multiple cell surface receptors [97]. In wild type mice, agonist stimulation of Group I mGluRs induces both p110β protein expression and PI3K activity, whereas in FMR1 mice p110β protein synthesis and PI3K activity were shown to be elevated and insensitive to mGluR stimulation; this indicates that dysregulated PI3K signaling may underlie the synaptic impairments in FXS [97]. Gross et al. hypothesized that FMRP controls protein synthesis-dependent regulation of synaptic morphology and function through regulation of PI3K signaling [97]. Additional support for this idea was given by the observation that PI3K antagonists rescued FXS-associated phenotypes including dysregulated synaptic protein synthesis, excess AMPA receptor internalization, and increased spine density. Thus elevated signaling in the PI3K-mTOR pathway may provide a functional link between over-activation of Group I mGluRs and aberrant synaptic plasticity and impaired cognition in FXS [97,98]. Although PI3K inhibitors have previously been studied in the context of cancer and inflammation, these new findings suggest that targeting excessive PI3K activity might also be a useful therapeutic strategy for FXS.

mTOR is a signaling molecule situated downstream of PI3K. Phosphorylated mTOR stimulates protein synthesis and as with PI3K, the activity of mTOR is elevated in FMR1 mice, an observation which may be related to the exaggerated long-term depression at CA1 synapses of FMR1 mice [98] (in this study this was demonstrated in the immature hippocampus). The mTOR pathway may also be a viable target for treating tuberous sclerosis. Tuberous sclerosis is a genetic disorder caused in most cases by *de novo* germline mutations in the TSC1 or TSC2 genes. Previous studies reported a diagnosis of ASD in approximately 20–60% of individuals affected by tuberous sclerosis [24]. mTOR inhibitors have been tested as potential therapeutic agents in a mouse model of the disorder where median survival after mTOR inhibition (chronic treatment with rapamycin or RAD001) improved as did weight gain, neurofilament abnormalities, and myelination [99]. As with FXS, there is hope that the findings from the study of tuberous sclerosis may be applicable to the treatment of ASD. Moreover, the findings from the studies cited above and others have indicated that restoration of the underlying molecular defects can improve neurological dysfunction, even if treatment is initiated in adult animals [100]. This suggests the possibility that some of the ongoing pathophysiological processes occurring in the mature brain contribute significantly to the overall neurological phenotype, and that the administration of signaling pathway inhibitors in adults could be therapeutically useful.

Finally, convincing evidence has demonstrated that the ERK signaling system is also aberrant in FMR1 mice. For example, in synaptoneurosomes from wild-type mice, ERK was phosphorylated in response to mGluR1/5 activation. However, in FMR1 mice mGluR stimulation produced rapid dephosphorylation of ERK suggesting that aberrant activation of phosphatases occurs in FMR1 mice in response to synaptic stimulation [101]. In FMR1 synapses, protein phosphatase 2A was over-activated after mGluR1 stimulation, and tyrosine phosphatase is over-activated after mGluR5 stimulation, causing the rapid deactivation of ERK. Thus over-activation of phosphatases in synapses may be impaired in FXS and this may in turn affect synaptic translation, transcription, and synaptic receptor regulation.

Elevated basal protein synthesis and mGluR5 activity in FMR1 mice was shown to be reduced to wild-type levels by acute

inhibition of either mGluR5 or ERK1/2 [102]. Although the mGluR5-ERK1/2 pathway is apparently not constitutively overactive in FMR1 mice, mRNA translation appears to be hypersensitive to basal ERK1/2 activation in the absence of FMRP. This hypersensitivity to ERK1/2 pathway activation was also shown to contribute to audiogenic seizure susceptibility in these mice. These results suggest that the ERK1/2 pathway, and neurotransmitter systems that stimulate protein synthesis via ERK1/2, represent additional therapeutic targets for FXS.

Taken together, the results summarized above and in other studies point to the consistent finding that several intracellular signaling pathways are over-active in the FMR1 mouse. The molecular basis for the enhanced signaling is thought to be related to overactive mGluR activation and loss of FMRP regulation of signaling proteins (e.g. the p110β subunit of PI3K, which is an mRNA substrate of FMRP [97]). Although most authors readily note the possibility of drug development, a more in-depth understanding of the molecular basis for the enhanced signaling could shed light on the most expeditious ways to develop novel treatments.

7. Concluding remarks

In the FXS field, most current efforts in therapeutics are directed towards intervening via glutamate and GABA receptors. Preliminary findings from small clinical trials have revealed promising results with mGluR5 antagonists and a $GABA_B$ receptor agonist. Accumulating evidence from the mouse model of FXS is also shedding light on additional possibilities aimed at inhibiting overactive downstream intracellular signaling pathways, particular the ERK, PI3K, mTOR pathways.

Another less well developed avenue of investigation being pursued in both the FXS and ASD fields is the activation of the immune system. Immune system involvement in ASD has been intermittently proposed in the past but has not been intensively examined until recently. One of several incentives for further study in this area has been the discovery of auto-antibodies targeting brain proteins in both children with ASD and their mothers. These circulating maternal auto-antibodies directed towards fetal brain proteins are highly specific for ASD. Accumulating evidence from children with ASD appears to point to the possibility of defective signaling in pathways that are shared by the immune and central nervous systems (see [103] for a review). Whether or not this may be applicable to FXS remains to be determined, although central nervous system immune activation has been suggested as a possible causative factor in the reactive astrocytosis detected in the FMR1 mouse brain [104]. In addition, the drug minocycline has shown efficacy in a small trial of FXS patients [105]. The rationale for this trial was based on the findings that the absence of FMRP leads to higher levels of matrix metallo-proteinase-9 activity (MMP-9) in the brain and that minocycline inhibits MMP-9 activity and alleviates behavioral and synapse abnormalities in FMR1 mice. However, minocycline also has well known anti-inflammatory properties which may have contributed to the therapeutic effects observed in humans, and in studies conducted on the FMR1 mouse.

The study of ASD and FXS has greatly intensified over the past several years. These efforts have yielded critical new insights into potential new modes of therapeutic intervention. Although the absence of a widely used animal model of ASD has hindered progress on this disorder, the use of the drosophila and mouse models of FXS, along with mouse models of other syndromic forms of autism, have been informative and may be relevant to at least some aspects of ASD. As is often the case in medical research, a key question is how well do the animal models mimic the human disorders? Although the ultimate answer to this question will be determined by clinical trials of drug candidates, more in depth insights gleaned from *in vitro* and animal studies will increase the likelihood that efficacious and safe drugs will ultimately become available for treating ASD and FXS.

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References

[1] Hagerman PJ. The fragile X prevalence paradox. J Med Genet 2008;45:498–9.
[2] Crespi B, Badcock C. Psychosis and autism as diametrical disorders of the social brain. Behav Brain Sci 2008;31:241–61.
[3] Harrington JW. The actual prevalence of autism: are we there yet? Pediatrics 2010;126:e1257–8.
[4] Maenner MJ, Durkin MS. Trends in the prevalence of autism on the basis of special education data. Pediatrics 2010;126:e1018–25.
[5] Ronald A, Hoekstra RA. Autism spectrum disorders and autistic traits: a decade of new twin studies. Am J Med Genet B Neuropsychiatr Genet 2011. doi: 10.1002/ajmg.b.31159.
[6] Bauman ML. Medical comorbidities in autism: challenges to diagnosis and treatment. Neurotherapeutics 2010;7:320–7.
[7] Hagerman RJ, Berry-Kravis E, Kaufmann WE, Ono MY, Tartaglia N, Lachiewicz A, et al. Advances in the treatment of fragile X syndrome. Pediatrics 2009;123:378–90.
[8] Hagerman R, Hoem G, Hagerman P, Fragile X. and autism: Intertwined at the molecular level leading to targeted treatments. Mol Autism 2010;1:12.
[9] Musumeci SA, Hagerman RJ, Ferri R, Bosco P, Dalla BB, Tassinari CA, et al. Epilepsy and EEG findings in males with fragile X syndrome. Epilepsia 1999;40:1092–9.
[10] Levisohn PM. The autism-epilepsy connection. Epilepsia 2007;48(Suppl. 9):33–5.
[11] Huffman LC, Sutcliffe TL, Tanner IS, Feldman HM. Management of symptoms in children with autism spectrum disorders: a comprehensive review of pharmacologic and complementary-alternative medicine treatments. J Dev Behav Pediatr 2011;32:56–68.
[12] Canitano R, Scandurra V. Psychopharmacology in autism: an update. Prog Neuropsychopharmacol Biol Psychiatry 2011;35:18–28.
[13] Verkerk AJ, Pieretti M, Sutcliffe JS, Fu YH, Kuhl DP, Pizzuti A, et al. Identification of a gene (FMR-1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. Cell 1991;65:905–14.
[14] Bakker CE, de Diego OY, Bontekoe C, Raghoe P, Luteijn T, Hoogeveen AT, et al. Immunocytochemical and biochemical characterization of FMRP, FXR1P, and FXR2P in the mouse. Exp Cell Res 2000;258:162–70.
[15] Pacey LK, Doering LC. Developmental expression of FMRP in the astrocyte lineage: implications for fragile X syndrome. Glia 2007;55:1601–9.
[16] Dictenberg JB, Swanger SA, Antar LN, Singer RH, Bassell GJ. A direct role for FMRP in activity-dependent dendritic mRNA transport links filopodial-spine morphogenesis to fragile X syndrome. Dev Cell 2008;14:926–39.
[17] Darnell JC, Fraser CE, Mostovetsky O, Darnell RB. Discrimination of common and unique RNA-binding activities among Fragile X mental retardation protein paralogs. Hum Mol Genet 2009;18:3164–77.
[18] Zalfa F, Eleuteri B, Dickson KS, Mercaldo V, De RS, di PA, et al. A new function for the fragile X mental retardation protein in regulation of PSD-95 mRNA stability. Nat Neurosci 2007;10:578–87.
[19] Ceman S, O'donnell WT, Reed M, Patton S, Pohl J, Warren ST. Phosphorylation influences the translation state of FMRP-associated polyribosomes. Hum Mol Genet 2003;12:3295–305.
[20] Levenga J, de Vrij FM, Oostra BA, Willemsen R. Potential therapeutic interventions for fragile X syndrome. Trends Mol Med 2010;16:516–27.
[21] Brown V, Jin P, Ceman S, Darnell JC, O'donnell WT, Tenenbaum SA, et al. Microarray identification of FMRP-associated brain mRNAs and altered mRNA translational profiles in fragile X syndrome. Cell 2001;107:477–87.
[22] Anney R, Klei L, Pinto D, Regan R, Conroy J, Magalhaes TR, et al. A genome-wide scan for common alleles affecting risk for autism. Hum Mol Genet 2010;19:4072–82.
[23] Lintas C, Sacco R, Persico AM. Genome-wide expression studies in Autism spectrum disorder, Rett syndrome, and Down syndrome. Neurobiol Dis 2010. doi: 10.1016/j.nbd.2010.11.010.
[24] Abrahams BS, Geschwind DH. Advances in autism genetics: on the threshold of a new neurobiology. Nat Rev Genet 2008;9:341–55.
[25] Betancur C, Sakurai T, Buxbaum JD. The emerging role of synaptic cell-adhesion pathways in the pathogenesis of autism spectrum disorders. Trends Neurosci 2009;32:402–12.
[26] Comoletti D, De JA, Jennings LL, Flynn RE, Gaietta G, Tsigelny I, et al. The Arg451Cys-neuroligin-3 mutation associated with autism reveals a defect in protein processing. J Neurosci 2004;24:4889–93.
[27] Tabuchi K, Blundell J, Etherton MR, Hammer RE, Liu X, Powell CM, et al. A neuroligin-3 mutation implicated in autism increases inhibitory synaptic transmission in mice. Science 2007;318:71–6.

[28] Sebat J, Lakshmi B, Malhotra D, Troge J, Lese-Martin C, Walsh T, et al. Strong association of de novo copy number mutations with autism. Science 2007;316:445–9.
[29] Marshall CR, Noor A, Vincent JB, Lionel AC, Feuk L, Skaug J, et al. Structural variation of chromosomes in autism spectrum disorder. Am J Hum Genet 2008;82:477–88.
[30] Rosenfeld JA, Ballif BC, Torchia BS, Sahoo T, Ravnan JB, Schultz R, et al. Copy number variations associated with autism spectrum disorders contribute to a spectrum of neurodevelopmental disorders. Genet Med 2010;12:694–702.
[31] Pinto D, Pagnamenta AT, Klei L, Anney R, Merico D, Regan R, et al. Functional impact of global rare copy number variation in autism spectrum disorders. Nature 2010;466:368–72.
[32] Hu H, Qin Y, Bochorishvili G, Zhu Y, van AL, Zhu JJ. Ras signaling mechanisms underlying impaired GluR1-dependent plasticity associated with fragile X syndrome. J Neurosci 2008;28:7847–62.
[33] Boda B, Dubos A, Muller D. Signaling mechanisms regulating synapse formation and function in mental retardation. Curr Opin Neurobiol 2010;20: 519–27.
[34] Pacey LK, Doss L, Cifelli C, der Kooy DV, Heximer SP, Hampson DR. Genetic deletion of Regulator of G-protein Signaling 4 (RGS4) rescues a subset of fragile X related phenotypes in the FMR1 knockout mouse. Mol Cell Neurosci 2011;46:563–72.
[35] Zhang YQ, Bailey AM, Matthies HJ, Renden RB, Smith MA, Speese SD, et al. Drosophila fragile X-related gene regulates the MAP1B homolog Futsch to control synaptic structure and function. Cell 2001;107:591–603.
[36] Morales J, Hiesinger PR, Schroeder AJ, Kume K, Verstreken P, Jackson FR, et al. Drosophila fragile X protein, DFXR, regulates neuronal morphology and function in the brain. Neuron 2002;34:961–72.
[37] Chang S, Bray SM, Li Z, Zarnescu DC, He C, Jin P, et al. Identification of small molecules rescuing fragile X syndrome phenotypes in Drosophila. Nat Chem Biol 2008;4:256–63.
[38] den Broeder MJ, van der Linde H, Brouwer JR, Oostra BA, Willemsen R, Ketting RF. Generation and characterization of FMR1 knockout zebrafish. PLoS One 2009;4:e7910.
[39] Dutch Belgian Consortium, Fmr1 knockout mice: a model to study fragile X mental retardation. The Dutch-Belgian Fragile X Consortium. Cell 1994;78:23–33.
[40] Ashley CT, Sutcliffe JS, Kunst CB, Leiner HA, Eichler EE, Nelson DL, et al. Human and murine FMR-1: alternative splicing and translational initiation downstream of the CGG-repeat. Nat Genet 1993;4:244–51.
[41] Hinds HL, Ashley CT, Sutcliffe JS, Nelson DL, Warren ST, Housman DE, et al. Tissue specific expression of FMR-1 provides evidence for a functional role in fragile X syndrome. Nat Genet 1993;3:36–43.
[42] Kooy RF, D'Hooge R, Reyniers E, Bakker CE, Nagels G, De BK, et al. Transgenic mouse model for the fragile X syndrome. Am J Med Genet 1996;64:241–5.
[43] Irwin SA, Patel B, Idupulapati M, Harris JB, Crisostomo RA, Larsen BP, et al. Abnormal dendritic spine characteristics in the temporal and visual cortices of patients with fragile-X syndrome: a quantitative examination. Am J Med Genet 2001;98:161–7.
[44] de Vrij FM, Levenga J, van der Linde HC, Koekkoek SK, De Zeeuw CI, Nelson DL, et al. Rescue of behavioral phenotype and neuronal protrusion morphology in Fmr1 KO mice. Neurobiol Dis 2008;31:127–32.
[45] Nimchinsky EA, Oberlander AM, Svoboda K. Abnormal development of dendritic spines in FMR1 knock-out mice. J Neurosci 2001;21:5139–46.
[46] Galvez R, Greenough WT. Sequence of abnormal dendritic spine development in primary somatosensory cortex of a mouse model of the fragile X mental retardation syndrome. Am J Med Genet A 2005;135:155–60.
[47] Chen L, Toth M. Fragile X mice develop sensory hyperreactivity to auditory stimuli. Neuroscience 2001;103:1043–50.
[48] Pacey LK, Heximer SP, Hampson DR. Increased GABA(B) receptor-mediated signaling reduces the susceptibility of fragile X knockout mice to audiogenic seizures. Mol Pharmacol 2009;76:18–24.
[49] Yan QJ, Rammal M, Tranfaglia M, Bauchwitz RP. Suppression of two major Fragile X Syndrome mouse model phenotypes by the mGluR5 antagonist MPEP. Neuropharmacology 2005;49:1053–66.
[50] Spencer CM, Alekseyenko O, Serysheva E, Yuva-Paylor LA, Paylor R. Altered anxiety-related and social behaviors in the Fmr1 knockout mouse model of fragile X syndrome. Genes Brain Behav 2005;4:420–30.
[51] Liu ZH, Smith CB. Dissociation of social and nonsocial anxiety in a mouse model of fragile X syndrome. Neurosci Lett 2009;454:62–6.
[52] Silverman JL, Yang M, Lord C, Crawley JN. Behavioural phenotyping assays for mouse models of autism. Nat Rev Neurosci 2010;11:490–502.
[53] Bolivar VJ, Walters SR, Phoenix JL. Assessing autism-like behavior in mice: variations in social interactions among inbred strains. Behav Brain Res 2007;176:21–6.
[54] Wagner GC, Reuhl KR, Cheh M, McRae P, Halladay AK. A new neurobehavioral model of autism in mice: pre- and postnatal exposure to sodium valproate. J Autism Dev Disord 2006;36:779–93.
[55] Sadamatsu M, Kanai H, Xu X, Liu Y, Kato N. Review of animal models for autism: implication of thyroid hormone. Congenit Anom (Kyoto) 2006;46:1–9.
[56] Lightbody AA, Reiss AL. Gene, brain, and behavior relationships in fragile X syndrome: evidence from neuroimaging studies. Dev Disabil Res Rev 2009;15:343–52.
[57] Allen G, Muller RA, Courchesne E. Cerebellar function in autism: functional magnetic resonance image activation during a simple motor task. Biol Psychiatry 2004;56:269–78.
[58] Ellegood J, Pacey LK, Hampson DR, Lerch JP, Henkelman RM. Anatomical phenotyping in a mouse model of fragile X syndrome with magnetic resonance imaging. Neuroimage 2010;53:1023–9.
[59] Koekkoek SK, Yamaguchi K, Milojkovic BA, Dortland BR, Ruigrok TJ, Maex R, et al. Deletion of FMR1 in Purkinje cells enhances parallel fiber LTD, enlarges spines, and attenuates cerebellar eyelid conditioning in Fragile X syndrome. Neuron 2005;47:339–52.
[60] Hoeft F, Carter JC, Lightbody AA, Cody HH, Piven J, Reiss AL. Region-specific alterations in brain development in one- to three-year-old boys with fragile X syndrome. Proc Natl Acad Sci USA 2010;107:9335–9.
[61] Schumann CM, Bloss CS, Barnes CC, Wideman GM, Carper RA, Akshoomoff N, et al. Longitudinal magnetic resonance imaging study of cortical development through early childhood in autism. J Neurosci 2010;30:4419–27.
[62] Groen WB, Buitelaar JK, van der Gaag RJ, Zwiers MP. Pervasive microstructural abnormalities in autism: a DTI study. J Psychiatry Neurosci 2011;36: 32–40.
[63] Jacobs S, Doering LC. Astrocytes prevent abnormal neuronal development in the fragile x mouse. J Neurosci 2010;30:4508–14.
[64] Hutsler JJ, Zhang H. Increased dendritic spine densities on cortical projection neurons in autism spectrum disorders. Brain Res 2010;1309:83–94.
[65] Amaral DG, Schumann CM, Nordahl CW. Neuroanatomy of autism. Trends Neurosci 2008;31:137–45.
[66] Pan F, Aldridge GM, Greenough WT, Gan WB. Dendritic spine instability and insensitivity to modulation by sensory experience in a mouse model of fragile X syndrome. Proc Natl Acad Sci USA 2010;107:17768–73.
[67] Cruz-Martin A, Crespo M, Portera-Cailliau C. Delayed stabilization of dendritic spines in fragile X mice. J Neurosci 2010;30:7793–803.
[68] Harlow EG, Till SM, Russell TA, Wijetunge LS, Kind P, Contractor A. Critical period plasticity is disrupted in the barrel cortex of FMR1 knockout mice. Neuron 2010;65:385–98.
[69] Bear MF, Huber KM, Warren ST. The mGluR theory of fragile X mental retardation. Trends Neurosci 2004;27:370–7.
[70] Muddashetty RS, Kelic S, Gross C, Xu M, Bassell GJ. Dysregulated metabotropic glutamate receptor-dependent translation of AMPA receptor and postsynaptic density-95 mRNAs at synapses in a mouse model of fragile X syndrome. J Neurosci 2007;27:5338–48.
[71] Nakamoto M, Nalavadi V, Epstein MP, Narayanan U, Bassell GJ, Warren ST. Fragile X mental retardation protein deficiency leads to excessive mGluR5-dependent internalization of AMPA receptors. Proc Natl Acad Sci USA 2007;104:15537–42.
[72] Huber KM, Gallagher SM, Warren ST, Bear MF. Altered synaptic plasticity in a mouse model of fragile X mental retardation. Proc Natl Acad Sci USA 2002;99:7746–50.
[73] McBride SM, Choi CH, Wang Y, Liebelt D, Braunstein E, Ferreiro D, et al. Pharmacological rescue of synaptic plasticity, courtship behavior, and mushroom body defects in a Drosophila model of fragile X syndrome. Neuron 2005;45:753–64.
[74] Choi CH, McBride SM, Schoenfeld BP, Liebelt DA, Ferreiro D, Ferrick NJ, et al. Age-dependent cognitive impairment in a Drosophila fragile X model and its pharmacological rescue. Biogerontology 2010;11:347–62.
[75] Dolen G, Carpenter RL, Ocain TD, Bear MF. Mechanism-based approaches to treating fragile X. Pharmacol Ther 2010;127:78–93.
[76] Dolen G, Osterweil E, Rao BS, Smith GB, Auerbach BD, Chattarji S, et al. Correction of fragile X syndrome in mice. Neuron 2007;56:955–62.
[77] Jacquemont S, Curie A, Des P, Torrioli V, Berry-Kravis MG, Hagerman E, et al. Epigenetic Modification of the FMR1 Gene in Fragile X Syndrome Is Associated with Differential Response to the mGluR5 Antagonist AFQ056. Sci Transl Med 2011;3:64ra1.
[78] D'Hulst C, De GN, Reeve SP, Van DD, De Deyn PP, Hassan BA, et al. Decreased expression of the GABAA receptor in fragile X syndrome. Brain Res 2006;1121:238–45.
[79] Olmos-Serrano JL, Paluszkiewicz SM, Martin BS, Kaufmann WE, Corbin JG, Huntsman MM. Defective GABAergic neurotransmission and pharmacological rescue of neuronal hyperexcitability in the amygdala in a mouse model of fragile X syndrome. J Neurosci 2010;30:9929–38.
[80] Vacher CM, Gassmann M, Desrayaud S, Challet E, Bradaia A, Hoyer D, et al. Hyperdopaminergia and altered locomotor activity in GABAB1-deficient mice. J Neurochem 2006;97:979–91.
[81] Prosser HM, Gill CH, Hirst WD, Grau E, Robbins M, Calver A, et al. Epileptogenesis and enhanced prepulse inhibition in GABA(B1)-deficient mice. Mol Cell Neurosci 2001;17:1059–70.
[82] El Idrissi A, Ding XH, Scalia J, Trenkner E, Brown WT, Dobkin C. Decreased GABA(A) receptor expression in the seizure-prone fragile X mouse. Neurosci Lett 2005;377:141–6.
[83] Curia G, Papouin T, Seguela P, Avoli M. Downregulation of tonic GABAergic inhibition in a mouse model of fragile X syndrome. Cereb Cortex 2009;19: 1515–20.
[84] Adusei DC, Pacey LK, Chen D, Hampson DR. Early developmental alterations in GABAergic protein expression in fragile X knockout mice. Neuropharmacology 2010;59:167–71.
[85] Jacob TC, Wan Q, Vithlani M, Saliba RS, Succol F, Pangalos MN, et al. GABA(A) receptor membrane trafficking regulates spine maturity. Proc Natl Acad Sci USA 2009;106:12500–5.
[86] Heinen K, Baker RE, Spijker S, Rosahl T, van PJ, Brussaard AB. Impaired dendritic spine maturation in GABAA receptor alpha1 subunit knock out mice. Neuroscience 2003;122:699–705.

[87] Centonze D, Rossi S, Mercaldo V, Napoli I, Ciotti MT, De C, et al. Abnormal striatal GABA transmission in the mouse model for the fragile X syndrome. Biol Psychiatry 2008;63:963–73.
[88] D'Hulst C, Kooy RF. The GABAA receptor: a novel target for treatment of fragile X? Trends Neurosci 2007;30:425–31.
[89] Fatemi SH, Halt AR, Stary JM, Kanodia R, Schulz SC, Realmuto GR. Glutamic acid decarboxylase 65 and 67 kDa proteins are reduced in autistic parietal and cerebellar cortices. Biol Psychiatry 2002;52:805–10.
[90] Oblak AL, Gibbs TT, Blatt GJ. Reduced GABA(A) receptors and benzodiazepine binding sites in the posterior cingulate cortex and fusiform gyrus in autism. Brain Res 2011;1380:218–28.
[91] Fatemi SH, Reutiman TJ, Folsom TD, Rooney RJ, Patel DH, Thuras PD. mRNA and protein levels for GABAAalpha4, alpha5, beta1 and GABABR1 receptors are altered in brains from subjects with autism. J Autism Dev Disord 2010;40:743–50.
[92] Oblak AL, Gibbs TT, Blatt GJ. Decreased GABA(B) receptors in the cingulate cortex and fusiform gyrus in autism. J Neurochem 2010;114:1414–23.
[93] Fatemi SH, Folsom TD, Reutiman TJ, Thuras PD. Expression of GABA(B) receptors is altered in brains of subjects with autism. Cerebellum 2009;8:64–9.
[94] Galanopoulou AS. GABA receptors as broadcasters of sexually differentiating signals in the brain. Epilepsia 2005;46(Suppl. 5):107–12.
[95] Nunez JL, McCarthy MM. Androgens predispose males to GABAA-mediated excitotoxicity in the developing hippocampus. Exp Neurol 2008;210:699–708.
[96] Rubenstein JL. Three hypotheses for developmental defects that may underlie some forms of autism spectrum disorder. Curr Opin Neurol 2010;23:118–23.
[97] Gross C, Nakamoto M, Yao X, Chan CB, Yim SY, Ye K, et al. Excess phosphoinositide 3-kinase subunit synthesis and activity as a novel therapeutic target in fragile X syndrome. J Neurosci 2010;30:10624–38.
[98] Sharma A, Hoeffer CA, Takayasu Y, Miyawaki T, McBride SM, Klann E, et al. Dysregulation of mTOR signaling in fragile X syndrome. J Neurosci 2010;30:694–702.
[99] Meikle L, Pollizzi K, Egnor A, Kramvis I, Lane H, Sahin M, et al. Response of a neuronal model of tuberous sclerosis to mammalian target of rapamycin (mTOR) inhibitors: effects on mTORC1 and Akt signaling lead to improved survival and function. J Neurosci 2008;28:5422–32.
[100] Ehninger D, Silva AJ. Rapamycin for treating Tuberous sclerosis and Autism spectrum disorders. Trends Mol Med 2010.
[101] Kim SH, Markham JA, Weiler IJ, Greenough WT. Aberrant early-phase ERK inactivation impedes neuronal function in fragile X syndrome. Proc Natl Acad Sci USA 2008;105:4429–34.
[102] Osterweil EK, Krueger DD, Reinhold K, Bear MF. Hypersensitivity to mGluR5 and ERK1/2 leads to excessive protein synthesis in the hippocampus of a mouse model of fragile X syndrome. J Neurosci 2010;30:15616–27.
[103] Goines P, Van de Water J. The immune system's role in the biology of autism. Curr Opin Neurol 2010;23:111–7.
[104] Yuskaitis CJ, Beurel E, Jope RS. Evidence of reactive astrocytes but not peripheral immune system activation in a mouse model of Fragile X syndrome. Biochim Biophys Acta 2010;1802:1006–12.
[105] Paribello C, Tao L, Folino A, Berry-Kravis E, Tranfaglia M, Ethell IM, et al. Open-label add-on treatment trial of minocycline in fragile X syndrome. BMC Neurol 2010;10:91.