The trouble with spines in fragile X syndrome: density, maturity and plasticity

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Abstract

Dendritic spines are the principal recipients of excitatory synaptic inputs and the basic units of neural computation in the mammalian brain. Alterations in the density, size, shape, turnover of mature spines, or defects in how spines are generated and establish synapses during brain development, could all result in neuronal dysfunction and lead to cognitive and/or behavioral impairments. That spines are abnormal in fragile X syndrome (FXS) and in the best-studied animal model of this disorder, the *Fmr1* knockout mouse, is an undeniable fact. But the trouble with spines in FXS is that the exact nature of their defect is still controversial. Here, we argue that the most consistent abnormality of spines in FXS may be a subtle defect in ac tivity-dependent spine plasticity and maturation. We also propose some future directions for research into spine plasticity in FXS at the cellular and ultrastructural levels that could help solve a two-decade-long riddle about the integrity of synapses in this prototypical neurodevelopmental disorder.

Keywords

Fragile X syndrome (FXS) is the most common single-gene cause of autism and mental impairment (Hagerman et al., 2010). In this disorder, transcriptional silencing of the *Fmr1* gene causes loss of Fragile X mental retardation protein (FMRP), a synaptic RNA-binding protein with regulatory roles in both neuronal growth and plasticity (Bassell and Warren, 2008, De Rubeis and Bagni, 2010). Despite what are often disabling behavioral problems and profound intellectual dysfunction, the brains of individuals with FXS are normal in appearance, at least at the level of routine neuroimaging or gross inspection at autopsy. In fact, only a rather subtle, microscopic neuropathological abnormality has been identified in FXS: abnormal dendritic spines in the brain. In contrast, other developmental brain disorders such as neurofibromatosis or tuberous sclerosisare characterized by gross neuroanatomical defects that are readily apparent to the naked eye, including brain tumors, atrophy, or

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widespread gliosis, all of which contribute to neuronal dysfunction. Importantly, a similar defect in dendritic spines has been identified in the best-studied animal model of FXS, the *Fmr1* knockout (KO) mice (Dutch-Belgian Fragile X Consortium, 1994). These mutant mice have reproducible problems with learning and memory and anxiety-like behaviors that are reminiscent of what is seen in humans with FXS (Penagarikano et al., 2007). This makes FXS an ideal neurodevelopmental disorder in which to study how altered signaling in certain molecular pathways leads to synaptic defects and dysfunctional circuits. Based on the symptoms of affected individuals, including cognitive impairment, sensory integration deficits, learning disability, anxiety, and autistic traits, scientists have focused their studies on three brain regions: the cerebral cortex, the hippocampus, and the amygdala. Here, we review what is known about *Fmr1* KO mice and humans with FXS in terms of the density, size and shape, and baseline dynamics of dendritic spines, as well as their role in activity-dependent synaptic plasticity. For a discussion of a different dilemma, whether spine defects are directly caused by loss of FMRP or are an epiphenomenon of circuit dysfunction in FXS, please refer to a related review (Portera-Cailliau, 2011).

1. Spine Density

Spine density is an important aspect of network function. As the number of spines increases, so do the number of neuronal connections and the computational power of the brain (Yuste, 2010). It follows, then, that alterations in the numbers of spines would result in significant network dysfunction. This concept prompted neuroscientists, several decades ago, to begin exploring whether the numbers of spines were altered in neuropsychiatric disorders, including those leading to mental retardation and autism (Purpura, 1974).

Thus far, the majority of studies examining dendritic spines in FXS have relied on Golgi staining of pyramidal neurons from the neocortex and hippocampus in either autopsy material from adult individuals with FXS or in *Fmr1* KO mice. The first publications from FXS subjects described an overabundance of immature spines in the dendrites of Layer 3 and Layer 5 pyramidal cells, but did not quantify spine density (Rudelli et al., 1985, Hinton et al., 1991). In a subsequent human FXS autopsy study, William Greenoughand colleagues expanded this hypothesis beyond the apparent spine immaturity, noting that spine density was increased in Layer 5 cortical pyramidal neurons (Irwin et al., 2001). Next, in a series of Golgi studies that systematically investigated dendritic spine pathology in adult *Fmr1* KO mice, the same group reported that in both visual cortex and somatosensory cortices, Layer 5 pyramidal neurons display a significantly elevated spine density (Comery et al., 1997, Galvez and Greenough, 2005, McKinney et al., 2005). Another Golgi study recently reported an elevated spine density in Layer 5 pyramidal neurons of primary somatosensory cortex in KO mice at 1, 7 and 8 weeks of age (though, oddly, not at 2 weeks of age) (Su et al., 2011). Other groups have likewise found a higher than normal spine densityin Layer 2/3 pyramidal neurons from adult *Fmr1* KO mice (Dolen et al., 2007, Hayashi et al., 2007, Liu et al., 2010), suggesting that alterations in spine numbers might be a common defect of all pyramidal neurons across different layers of the neocortex (Figure 1). Because the increase in spine density seen in adult FXS subjects and in mutant mice was unique to this neurodevelopmental disorder (as opposed to the presence of longer, immature-looking spines), this work led to the hypothesis that FXS might result from inadequate synaptic pruning due to "an absence of the [hypothetical and FMRP-dependent] stabilization protein at inactive synapses" (Irwin et al., 2000, Bagni and Greenough, 2005).

However, some of the more recent morphological analyses of spines in*Fmr1* KO mice have challenged this theory. These new studies differ from previous Golgi studies in two critical parameters: First, *Fmr1* KO pyramidal neurons were now examined during early postnatal development (instead of adulthood, a critical difference considering that FXS presents in

early childhood); and second, these surveys have relied more often on two-photon fluorescence microscopy (rather than using the Golgi method in fixed tissue), allowing investigators to image living neurons. The developmental studies have over whelmingly failed to identify differences in spine density in Layer 2/3, Layer 4, or Layer 5 pyramidal neurons between *Fmr1* KO mice and wild type mice (Meredith et al., 2007, Ruan et al., 2009, Cruz-Martin et al., 2010, Harlow et al., 2010, Pan et al., 2010). Perhaps these contradictory results derive from differences in how the analyses were performed. For instance some investigators may choose to use for their statistics n= number of dendrites (for density) and n= number of spines (for spine length), whereas others use n= number of cells (or n= number of mice). It is also possible that, for unknown reasons, the Golgi impregnation method (or tissue fixation) could by itself bring about an elevated spine density in Fmr1 KO mice, but not wild type mice. Indeed, some have commented that different methodologies of labeling neurons (e.g., Golgi vs. intracellular neurobiotin, DiI, or Lucifer Yellow) might yield different quantitative results for spine density (Nimchinsky et al., 2001, Beltran-Campos et al., 2011). It has also been suggested that Golgi staining may preferentially label certain neuronal populations (e.g., cells with spine abnormalities (Nimchinsky et al., 2001) or cells with higher physiological levels of activity (Benshalom, 1992, Rao et al., 1993). Whether these suggestions are applicable to Fmr1 KO mice as compared to WT mice remains unknown. But in at least one study that compared spine density in fixed tissue vs. living neurons in cultured slices from Fmr1 KO mice, the authors reported a higher spine density in fixed tissue at postnatal day (P) 7 (though not at P14 or P28), but normal spine density in living neurons in organotypic slice cultures of an equivalent age (Nimchinsky et al., 2001). This again implies that the elevated spine density may be a bizarre consequence of fixation, but also that there may be differences in spine density between the first and subsequent postnatal weeks. Indeed, we have also found that in acute brain slices, spine density in Layer 2/3 cortical neurons is transiently elevated in mutant mice at P4-7, but not thereafter (Cruz-Martin & Portera-Cailliau, unpublished). More recently, two *in vivo* imaging studies have reported that spine density is normal in somatosensory cortex of *Fmr1* KO mice at early postnatal stages (Cruz-Martin et al., 2010, Pan et al., 2010), as well as juvenile and adult stages (Pan et al., 2010).

In addition to the possible effects of age and fixation/staining method on spine density, one also wonders whether changes in spine density in *Fmr1* KO mice may vary across different cortical layers, different brain regions, or different mouse strains. After a careful review of the literature, we concluded that the spine density profile in *Fmr1* KO mice is independent of cortical layer (Table 1) or genetic background (Portera-Cailliau, 2011). In contrast, whether a particular spine density phenotype is apparent in the mouse model does depend on the brain region being examined (Figure 1). Given the well-established deficits in mGluR-dependent LTD in the hippocampus of *Fmr1* KO mice (reviewed by (Ronesi and Huber, 2008) and the important role of this region in learning and memory, investigators have also looked for spine alterations in hippocampal pyramidal neurons. Spine density changes appear to be especially variable in the hippocampus, where studies of *Fmr1* KO mice have found either normal (Pfeiffer and Huber, 2007, de Vrij et al., 2008, Levenga et al., 2011b, Su et al., 2011), higher (Antar et al., 2006, Grossman et al., 2006, Gross et al., 2010, Levenga et al., 2011a, Swanger, 2011), or even lower spine densities (Braun and Segal, 2000, Segal et al., 2003). Additional studies of spines in the hippocampus will hopefully resolve these discrepancies. Spine density increases have also been reported in granule cells of the olfactory bulb neurons (Scotto-Lomassese et al., 2011) and in the amygdala (Qin et al., 2011) of *Fmr1* KO mice. The olfactory bulb study provided two important contributions regarding spine density. First, the authors demonstrated that the elevated spine density was cell-autonomous, suggesting that this phenotype may be triggered by loss of FMRP in single neurons and not by some large-scale network effect or by defects in presynaptic axons. Second, the authors showed that spine density in mutant neurons was already elevated at the

earliest time-point examined (14 days *in vitro*), suggesting that the defect resulted from a developmental over production of spines rather than a failure to prune them (Scotto-Lomassese et al., 2011).

More work is clearly needed to resolve the ongoing controversies regarding spine density in different brain regions of *Fmr1* KO mice. However, one emerging theme is that the majority of studies to date, and in particular the developmental studies and those that used live imaging in the neocortex, report normal spine density for pyramidal neurons in *Fmr1* KO mice (Table 1).

2. Spine maturity: size, shape and dynamics

The size and shape of dendritic spines are also critical parameters of neuronal function and connectivity. For instance, large spines are associated with bigger presynaptic terminals, and with larger post-synaptic currents (reviewed by Kasai et al., 2010). Likewise, newly formed spines tend to be smaller and therefore establish weaker synapses (Holtmaat et al., 2006). It follows that changes in spine size and turnover (that is, the rate of appearance and disappearance of spines) will also affect network function, and possibly to a greater degree than changes in spine density. For example, sensory deprivation produces dramatic changes in spine turnover, but has a negligible impact on spine density (reviewed by Holtmaat and Svoboda, 2009). Therefore, it is conceivable that loss of FMRP, a protein important for synaptic plasticity, would lead to alterations in spine size, shape or stability.

Size and shape

The original autopsy studies of FXS were the first to raise the issue of dendritic spine immaturity, because of the high prevalence of abnormally long, thin and tortuous spines observed in the dendrites of Layer 3 and Layer 5 pyramidal cells (Rudelli et al., 1985, Hinton et al., 1991). Because these spine morphologies were reminiscent of dendritic protrusions seen during normal cortical development (reviewed by (Portera Cailliau and Yuste, 2001, Yuste and Bonhoeffer, 2004), this led to the hypothesis that FXS could be caused by a failure in spine maturation, much as had been proposed earlier for other types of mental retardation (Purpura, 1974). A decade later, William Greenough conducted the only other human FXS study on spines, and for the first time used a quantitative approach to the analysis. His group reported not only a higher spine density in Layer 5 neurons, but also a higher proportion of longer spines (Irwin et al., 2001). In that study, the authors strived to categorize, for the first time in FXS, different classes of spines based on their morphology along a spine maturity continuum. They found that the proportion of spines exhibiting immature morphologies was greater in the brains of individuals with FXS.

Subsequent studies by the same lab focused on *Fmr1* KO mice, and similarly reported an overabundance of immature spines in the neocortex (Comery et al., 1997, Irwin et al., 2002, Galvez and Greenough, 2005). Independent groups using a similar Golgi-staining approach in fixed tissue also found that pyramidal neurons in the neocortex of adult *Fmr1* KO mice exhibit either unusually long spines (Meredith et al., 2007, Liu et al., 2010), or a preponderance of immature subtypes like dendritic filopodia (Restivo et al., 2005). But not all Golgi studies found changes in average spine length in the mutant mice (Hayashi et al., 2007), and neither did the two recent *in vivo* imaging studies (Cruz-Martin et al., 2010, Pan et al., 2010). Thus, although not all studies agree that spines are abnormally long in *Fmr1* KO mice, most studies that evaluated the relative maturity of spines based on morphological criteria did find too many immature protrusions in the neocortex (Table 1; Figure 1). This immature spine phenotype has also been observed in pyramidal neurons and dentate gyrus granule cells of the hippocampus (Antar et al., 2006, Grossman et al., 2006, de Vrij et al., 2008, Bilousova et al., 2009, Grossman et al., 2010), Purkinje neurons of the cerebellum

(Koekkoek et al., 2005), granule cells of the olfactory bulb (Scotto-Lomassese et al., 2011), and pyramidal-like neurons in the amygdala of *Fmr1* KO mice (Qin et al., 2011). The immaturity of spines may also be subregion-specific, at least in the hippocampus, because it is present in CA1 but not CA3 (Levenga et al., 2011a), which is interesting in light of the different roles that these subregions play in learning and memory.

That said, morphology alone may not be the best way to differentiate between mature and immature spines. Time-lapse imaging studies have revealed that the shape and size of spines is rather dynamic, especially during early brain development. Immature spines undergo rapid changes in size and morphology on a minutes-to-hours scale (Bonhoeffer and Yuste, 2002). In adult mice, spines also display structural transformations, albeit on an hours-to-days scale (Holtmaat et al., 2005). Therefore, we have argued that a snapshot of a dendritic spine in fixed tissue is not the most accurate representation of its actual maturity, and that time-lapse imaging, which provides information on spine dynamics (motility and turnover), is the ideal approach to distinguish between different protrusion subtypes (Portera-Cailliau et al., 2003, Cruz-Martin et al., 2010, Portera-Cailliau, 2011). Dynamic imaging of spines in *Fmr1* KO mice may reveal additional aspects of synaptic dysfunction in FXS.

Spine dynamics: Turnover and motility

To date, there have only been three independent studies published in which spine dynamics in *Fmr1* KO mice were investigated using time-lapse imaging. In 2001, a two-photon imaging study of Layer 5 cortical neurons in organotypic slice cultures (P2 + 5 days *in vitro*) reported no changes in spine motility or turnover in the mutant mice (Nimchinsky et al., 2001). Last year, two different studies used *in vivo* two-photon microscopy in GFP-expressing mice to examine spine dynamics in the intact neocortex. In our study, we did not observe differences in spine motility between genotypes, but we did find an abnormally high turnover of spines in Layer 2/3 neurons in the barrel cortex of *Fmr1* KO mice at P10-12 (Cruz-Martin et al., 2010). By P21-24, spine turnover in the mutant mice was normal, as measured at 10 min intervals over a 1 h period, so our results were consistent with a delay in spine stabilization during early postnatal development. The other study examined Layer 5 neurons in primary somatosensory cortex and found higher 2-d and 2-week spine turnover rates at P20 and P30 (Pan et al., 2010). The authors also noted that this phenotype was consistent across C57 and FVB genetic backgrounds. Although it remains to be seen whether Layer 2/3 neurons in KO mice exhibit elevated turnover rates at P30 over 2-d or 2-week intervals (or whether Layer 5 neurons are too unstable over 1 h intervals at P10-12), the results from these two independent groups are very similar. These data suggest that spine instability could be a problem that begins early in brain development and then persists throughout a lifetime in FXS. Incidentally, our finding of increased spine turnover in KO mice at P10-12 (Cruz-Martin et al., 2010) coincides with a critical period in barrel cortex development during which the absence of cortical FMRP also results in significant functional synaptic defects (Harlow et al., 2010). Thus, the increased spine turnover at this early age may be one of the earliest synaptic defects in this disorder.

A variety of spine abnormalities have been reported in *Fmr1* KO mice (Figure 2). But, the most consistent abnormal spine phenotype, the one that matches what has been observed in autopsy material from humans with FXS, is a relative immaturity of dendritic spines, which is manifested by an overabundance of immature morphologies (filopodia-like protrusions) and a higher than normal spine turnover.

3. Future studies: Spine plasticity, synaptic ultrastructure, and molecular pathways

After more than two decades of research into the pathophysiology of fragile X syndrome, we are closer to understanding the molecular pathways regulated by FMRP and the synaptic defects associated with this disorder (Bassell and Warren, 2008). Alterations of dendritic spines, the major recipients of excitatory synapses in the brain, are an important clue to the pathophysiology of FXS. This review underscores the need for additional studies to resolve many outstanding controversies surrounding the various defects in spines that have been reported in *Fmr1* KO mice. In particular, much could be learned from additional surveys of spine density size, shape, and turnover in the amygdala, hippocampus, and cerebellum throughout development. Whenever possible, such studies should be carried out with *in vivo* imaging in GFP-expressing mice, because this methodology preserves all brain connectivity and peripheral inputs. This approach has the added benefit that more homogeneous neuronal populations are labeled (compared to the Golgi method), which might reduce variability in spine analyses.

Perhaps the most exciting insights into spines in FXS will come from work that investigates the contribution of spines to circuit plasticity during learning or sensory processing. How spine plasticity contributes to circuit remodeling - for example, after changes in sensory experience - is one of the most important aspects of spine function (Holtmaat and Svoboda, 2009). In the context of a disease like FXS, characterized by deficits in learning and adaptive behavior, spine plasticity becomes an even more pertinent topic of investigation. Indeed, it has been proposed that FXS is caused by aberrant protein synthesis at the synapse (Bassell and Warren, 2008, Kelleher and Bear, 2008), which leads to alterations in activity-dependent circuit plasticity. Several studies have shown that *Fmr1* KO mice exhibit defects in experience-dependent and other types of synaptic plasticity (such as LTP/LTD; reviewed by Pfeiffer and Huber, 2009), all of which are associated with changes in spine size and/or stabilization. We recently hypothesized that dendritic spines in FXS may be unable to modulate their size or stability in response to changes in neuronal activity, such that the LTP/LTD defects and the alterations of spine stability/maturation are actually different aspects of the same phenotype (Portera-Cailliau, 2011).

Thus far, spine plasticity has only been explored in a handful of studies of *Fmr1* KO mice. One paper reported that while chronic stress increases spine density in WT mice, it failed to do so in the mutant mice (Qin et al., 2011). More strikingly, spines in adult *Fmr1* KO mice were insensitive to modulation by sensory experience by whisker trimming for 2 weeks (Pan et al., 2010). In contrast, a different study showed that stimulating neuronal activity with bicuculline affected spines in dissociated hippocampal neurons from wild type and *Fmr1* KO mice to a similar degree (Segal et al., 2003). Thus, future work ought to investigate which activity-dependent spine plasticity events are affected in *Fmr1* KO mice. In addition, studies are needed to evaluate the effects of input deprivation on dendritic spines in various layers of visual or barrel cortex to confirm the initial study by Pan et al. (2010). It would also be interesting to investigate spine plasticity after sensory stimulation or after learning a behavioral task, as was recently done in motor cortex with *in vivo* imaging (Xu et al., 2009). But these studies need not be explored solely *in vivo*, as imaging spine size/turnover in acute slices during LTP/LTD protocols would also yield valuable results.

Another aspect of spines that has been understudied in *Fmr1* KO mice is their appearance at the ultrastructural level, which would inform us on their ability to establish functional synapses. The original study of FXS autopsy material reported a reduction in the length of synapses and in the mean synaptic contact zone area (Rudelli et al., 1985). Indeed, one would predict that abnormally immature and unstable spines would form small, and

therefore weak, synapses; future studies will need to confirm this prediction with electron microscopy in *Fmr1* KO mice. One recent ultrastructural study in the olfactory bulb showed no differences in spine colocalization with PSD95, as well as ostensibly normal dendrodendritic synapsesongranule cells lacking *Fmr1* (Scotto-Lomassese et al., 2011). In contrast, hippocampal neurons lacking FMRP had fewer spines that colocalized with synapsin, a presynaptic marker (Antar et al., 2006), indicating an inability of mutant neurons to establish functional synapses. Another interesting finding is that polyribosomes are scarcer in spines from *Fmr1* KO mice (Weiler et al., 2004). This is intriguing because polyribosomes normally redistribute into spines with enlarged synapses following LTP (Ostroff et al., 2002), again supporting the argument that in the absence of FMRP, dendritic spines fail to undergo the classic signs of synaptic plasticity.

The molecular mechanisms that bring about this inability of spines in *Fmr1* KO mice to play their part in modulating synaptic strength after changes in neuronal activity also merit investigation. Over the last two decades, a variety of signaling pathways have been identified that regulate spine density, turnover size or plasticity (Yuste, 2010, Portera-Cailliau, 2011). Honing in on those pathways that are dysregulated in FXS will be a difficult and time-consuming task, but one that will identify new therapeutic avenues for this disorder (Gross et al., 2011).

4. Spine Pathology: Cause or Consequence of Fragile X Syndrome

The neuropathological defect in fragile X syndrome is the overabundance of immature dendritic spines in cortical pyramidal neurons. In addition, a number of defects in synaptic plasticity have been uncovered with electrophysiology in Fmr1 KO mice (Pfeiffer and Huber, 2009). In theory, such dysfunctional circuits could lead to abnormal spines and vice versa, so it is still not clear which problem comes first. A better understanding of this cause-and-effect relationship will require additional experiments. Given the tight structure-function relationships at the synapse, we have previously argued that the two problems are intimately linked to one another (Portera-Cailliau, 2011). Therefore, as our understanding of the mechanisms that regulate the formation, morphology and lifetime of dendritic spines grows, so will our knowledge of the alterations in functional synaptic plasticity in Fmr1 KO mice. Interestingly, abnormal spines are a common feature of several neurodevelopmental disorders (as is apparent from this Special Issue). In addition, many of the defects in spines and in synaptic/circuit plasticity in these disorders occur during critical periods of early neocortical development. Therefore, further research into experience-dependent synaptic plasticity in sensory cortices will likely uncover candidate signaling pathways that could translate into therapeutic avenues for a variety of clinical syndromes.

The title of this review had what is now an obvious double-entendre. On one hand, dendritic spines are clearly abnormalin FXS. On the other hand, the scientific "trouble" we face is that the exact nature of the spine abnormality is not yet fully understood. The next decade, however, can no doubt find a solution to this puzzle through studies that combine electrophysiology, animal behavior and structural imaging, in order to better define the synaptic defects, both structural and functional, that are triggered by loss of FMRP.

Highlights

1. Defects in the density, shape and turnover of spines occur in FXS

2. How these lead to circuit dysfunction and cognitive-behavioral symptoms is unknown

3. There are still several controversies regarding the exact spine defects in FXS

4. The real problem may be a defect in activity-dependent spine plasticity/maturation

5. Future research should explore further spine integrity and plasticity in FXS

Acknowledgments

Abbreviations

div	days *in vitro*
EM	electron microscopy
FMRP	fragile X mental retardation protein
FXS	fragile X syndrome
GFP	green fluorescent protein
KO	knockout
L	layer
LTD	long-term depression
LTP	long-term potentiation
mGluR	metabotropic glutamate receptor
P	postnatal day
WT	wild type

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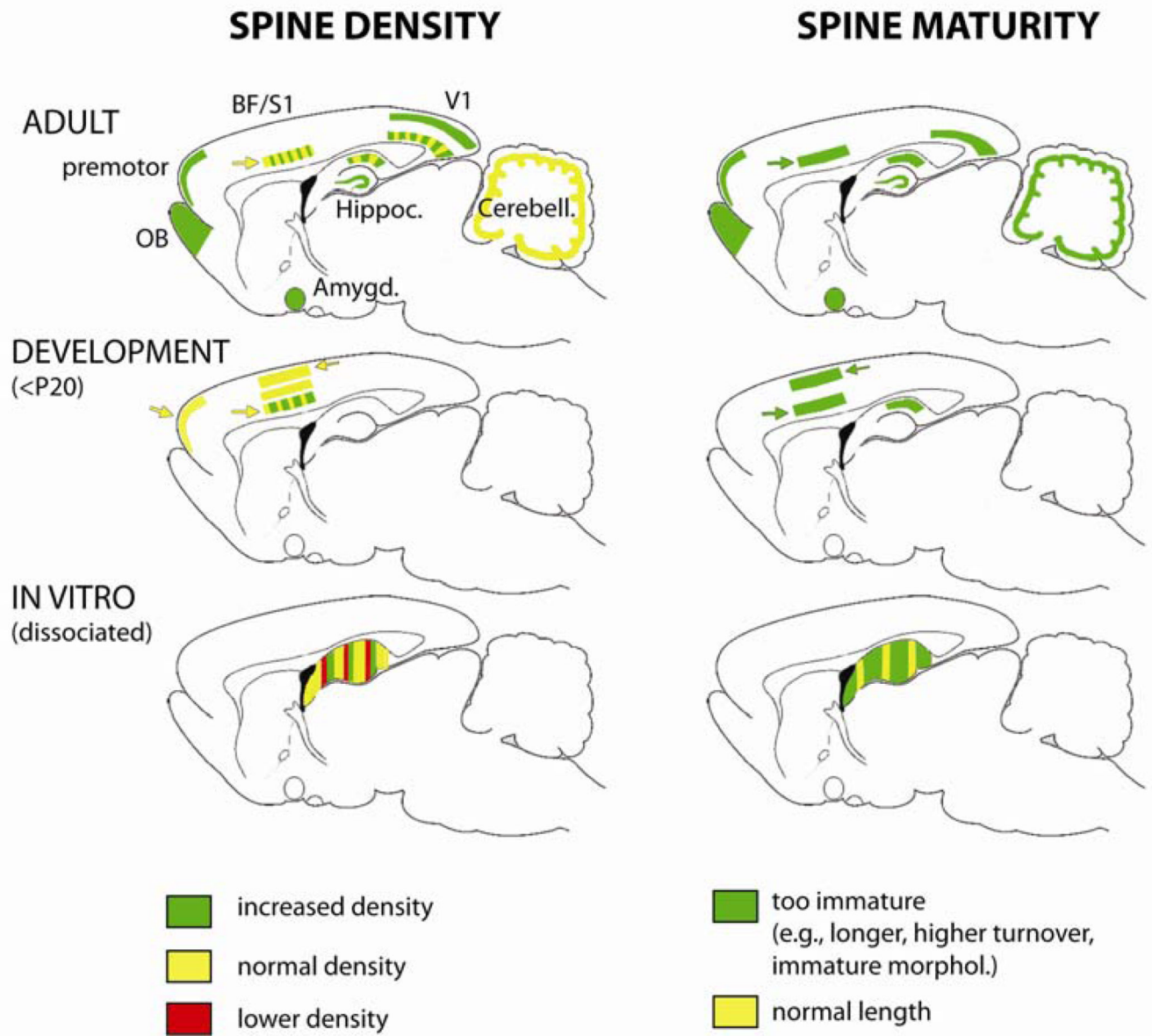
Figure 1. Regional distributions of reported spine defects in the brain of *Fmr1* KO mice
Data on dendritic spines in *Fmr1* KO mice is displayed on sagittal cuts through the mouse
brain (see Table 1). Data from the adult brain, developing brain (P20), and *in vitro* results
from dissociated neurons in culture (hippocampus only) are shown in the top, middle and
bottom rows, respectively. Colored stripes indicate the existence of published studies
reporting different results on either spine density or maturity. The relative thickness of the
colored stripes reflects approximately the relative number of studies supporting one finding
or another. Studies that used imaging in living neurons (*in vivo* or in acute/organotypic brain
slices) are indicated by arrowheads next to the stripes. Note that results of studies are much
more consistent for the spine immaturity phenotype than for the spine density phenotype.
Abbreviations: BF: barrel field; OB: olfactory bulb; S1: somatosensory cortex; V1: visual
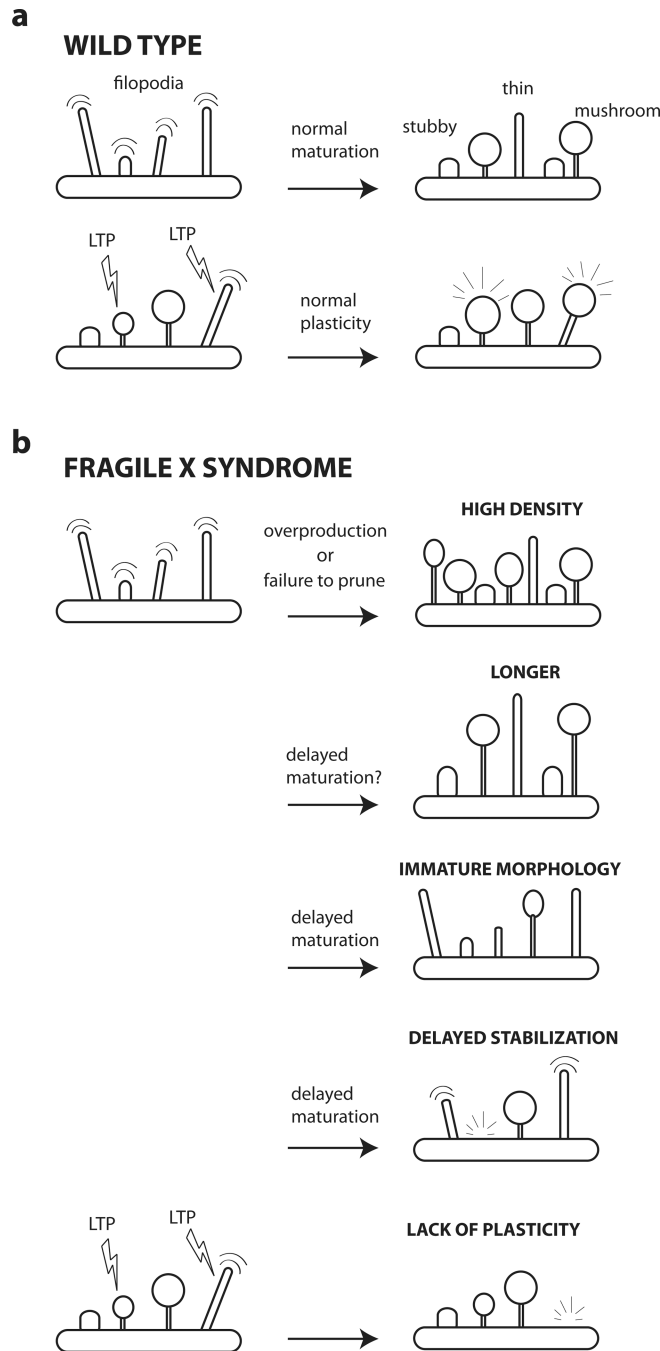cortex.

Figure 2. Possible mechanisms to explain spine defects in fragile X syndrome
These cartoons depict the various dendritic spine defects that have been reported or proposed in patients with FXS or in *Fmr1* KO mice, as well as the theories that may bring about those defects.

(a) Spine maturation and plasticity in wild type mice. During early brain development dendrites are studded with headless protrusions called filopodia. These are highly motile and transient protrusions that play a role in early synaptogenesis. In the adult, dendritic spines can be classified morphologically into three main types: thin, stuffy and mushroom. Thin spines tend to be smaller and have shorter lifetimes (days), whereas mushroom spines tend to be the largest and most stable (weeks to months). Spines are plastic structures such that

changes in network activity (e.g., sensory inputs, learning new tasks) can modify the size, shape and turnover to influence synaptic strength (e.g., experience-dependent plasticity, LTP).

(b) Spine maturation and plasticity in fragile X syndrome. An over-production of spines (or a failure to prune them after development) can lead to a higher spine density as reported in some studies of *Fmr1* KO mice. Alternatively, a delayed maturation of dendritic protrusions results in an overabundance of spines with immature morphologies or higher than normal turnover. In addition, spines in FXS may exhibit defects in activity-dependent plasticity. Potential consequences of a higher spine density on the network might include seizures or an exaggerated response to sensory stimuli due to hyperconnectivity. Potential consequences of having immature spines (delayed stabilization and/or immature morphology) might include problems with learning and memory consolidation, due to a reduction in synapse number or to the formation of weaker, short-lasting synapses. Portential consequences of failure of spines to exhibit normal plasticity (e.g., experience-dependent plasticity) would also include problems with learning and memory, as well as sensory integration defects.

Table 1

Summary of abnormal dendritic spine phenotypes reported in *Fmr1* KO mice

The spine phenotypes reported in different brain regions of *Fmr1* KO mice have been grouped according to three main results: increased density, normal density, and relative immaturity. Studies that used imaging in living neurons (*in vivo* or in acute/organotypic brain slices) are highlighted in gray.

Region / Phenotype	Cortex	Hippocampus	Cerebellum	Olfactory bulb	Amygdala
Increased spine density	*Layer 2/3:* Dolen et al., 2007 (P30) Hayashi et al., 2007 (2 months) Liu et al., 2010 (12 weeks) *Layer 5:* βSu et al., 2011 (1, 7, 8 weeks) Nimchinsky et al., 2001 (P7) Comery et al., 1997 (16 weeks) McKinney et al., 2005 (P60-90) Galvez & Greenough, 2005 (P73)	*Pyramidal cells in CA1:* Levenga et al., 2011a (25 weeks, CA1) *Granule cells in dentate gyrus:* Grossman et al., 2010 (P15-60) *Whole hippocampus dissociated:* Antar et al., 2006 (P0+16div) Swanger et al., 2011(E17+18div)	*Parkinje neurons:* Koekkoek et al., 2005 (adult)	*Granule cells:* Scotto-Lomassese et al., 2011 (2 months)	*Pyramidal-like neurons:* Qin et al., 2011 (~3 months)
Normal spine density	*Layer 2/3:* Cruz-Martin et al., 2010 (P7–P21) Meredith et al., 2007 (P14-23) *Layer 4:* Harlow et al., 2010 (P7, P14) *Layer 5:* Nimchinsky et al., 2001 (P14, P28) Galvez & Greenough, 2005 (P25) Restivo et al., 2005 (12 weeks)	*Pyramidal cells in CA1:* Grossman et al., 2006 (P60–P90) *Whole hippocampus dissociated:* Braun & Segal, 2000 (P0+7div) Su et al., 2011 (P0+8 div) †Levenga et al., 2011b (E16+14div) *Segal et al. 2003 (P1+14div) de Vrij et al., 2008 (E18+21div)			

Region	Cortex	Hippocampus	Cerebellum	Olfactory bulb	Amygdala
Phenotype					
	Pan et al., 2010 (P30)				
	Irwin et al., 2002 (60–90 days)				
Relative immaturity of spines	*Layer 2/3:*	*Pyramidal cells in CA1:*	*Purkinje neurons:*	*Granule cells:*	*Pyramidal-like neurons:*
	Cruz-Martin et al., 2010 (P10-12; higher turnover; more filopodia / fewer mushroom spines)	Bilousova et al., 2009 (P7; more filopodia / fewer mushroom)	Koekkoek et al., 2005 (adult; longer)	Scotto-Lomassese et al., 2011 (2 months; longer)	Qin et al., 2011 (~3 months; longer)
	Meredith et al., 2007 (P14-23; longer)	Grossman et al., 2006 (P60–P90; longer, more immature morphology)			
	Liu et al., 2010 (13 weeks; longer)	¥Levenga et al., 2011a (25 weeks; more filopodia / fewer mushroom)			
	Layer 5:	*Granule cells in dentate gyrus:*			
	Su et al., 2011 (1, 2, 7, 8 weeks; longer)	Grossman et al., 2010 (P15–P60; more immature morphology)			
	Nimchinsky et al., 2001 (P7–P28)	*Whole hippocampus dissociated:*			
	Pan et al., 2010 (P20, P30; high turnover)	Antar et al., 2006 (P0+16div; longer, more filopodia / fewer spine synapses)			
	Comery et al., 1997 (16 weeks; longer)	de Vrij et al., 2008 (E18+21div; longer, more filopodia / fewer mature spines)			
	Restivo 2005 (12 weeks; more immature morphology)	Su et al., 2011 (P0+8div; longer)			
	Irwin et al., 2002 (60–90 days; more immature morphology)	‡Levenga et al., 2011b (E16+14div; longer)			
	Galvez & Greenough, 2005 (P73; longer, more immature morphology)	Bilousova et al., 2009 (E15+14div; longer)			
	McKinney et al., 2005 (P60–P90; longer, more immature morphology)	Swanger et al., 2011 (E17+18div; longer, more filopodia / fewer mushroom)			

℘ In this study, spine density in somatosensory cortex was elevated at 1, 7 and 8 weeks of age, but not at 2 weeks.

† This is the only study that has reported a decrease in spine density in neocortical neurons (dissociated, E16+20div).

* This study actually reported a lower than normal spine density in Fmr1 KO mice.

¥ This study found that spines were not immature in CA3; also, they found no change in average spine length.

‡ This study found that spine length was normal in neocortical neurons (dissociated, E16+20div).