Dendritic spine instability and insensitivity to modulation by sensory experience in a mouse model of fragile X syndrome

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Fragile X syndrome (FXS) is the most common inherited form of mental retardation and is caused by transcriptional inactivation of the X-linked *fragile X mental retardation 1* (*FMR1*) gene. FXS is associated with increased density and abnormal morphology of dendritic spines, the postsynaptic sites of the majority of excitatory synapses. To better understand how lack of the *FMR1* gene function affects spine development and plasticity, we examined spine formation and elimination of layer 5 pyramidal neurons in the whisker barrel cortex of *Fmr1* KO mice with a transcranial two-photon imaging technique. We found that the rates of spine formation and elimination over days to weeks were significantly higher in both young and adult KO mice compared with littermate controls. The heightened spine turnover in KO mice was due to the existence of a larger pool of "short-lived" new spines in KO mice than in controls. Furthermore, we found that the formation of new spines and the elimination of existing ones were less sensitive to modulation by sensory experience in KO mice. These results indicate that the loss of *Fmr1* gene function leads to ongoing overproduction of transient spines in the primary somatosensory cortex. The insensitivity of spine formation and elimination to sensory alterations in *Fmr1* KO mice suggest that the developing synaptic circuits may not be properly tuned by sensory stimuli in FXS.

autism | imaging | mental retardation | synaptic plasticity | two-photon microscopy

Fragile X syndrome (FXS) is the most common form of inherited mental retardation, affecting about 1 in 4,000 males and 1 in 8,000 females (1). Patients who suffer from FXS exhibit various degrees of cognitive, socio-affective, and sensory-motor abnormalities (2). The syndrome is caused by the expansion of a polymorphic CGG trinucleotide repeat in the 5′ untranslated region of the *fragile X mental retardation 1* (*FMR1*) gene located on the X chromosome (3). The fragile X mental retardation protein (FMRP), which is encoded by the *FMR1* gene, binds to many mRNAs and is believed to regulate protein translation in various subcellular locations, including dendrites and dendritic spines (4, 5).

The *Fmr1* KO mice demonstrate many abnormalities found in FXS patients, such as impairments of learning and memory (6–8), social behaviors (9–11), and sensory processing (12, 13), thus providing an excellent model system to study pathogenic mechanisms underlying FXS. Despite these behavioral abnormalities, the gross structure of the brain is largely intact in FXS patients and in the mouse model of the disorder. The most consistent anatomical finding is an abnormal profile of dendritic spines, postsynaptic protrusions that receive the vast majority of excitatory input in the brains of diverse species (14–17).

In FXS, the adult dendritic spine phenotype includes increases in spine density and spine length and the number of immature-looking spines in the various brain regions examined (15, 16, 18). Similarly, in the visual and somatosensory cortices of adult *Fmr1* KO mice, pyramidal neurons show higher dendritic spine density and more immature, long, and thin dendritic spines than those in WT brains (4, 19, 20). Subsequent studies using younger mice have shown that an increased spine density in the somatosensory cortex is seen in early postnatal life and in adulthood but is not found in mice around 1 mo of age (21, 22). Although these findings suggest that FMRP is important for dendritic spine formation and/or maintenance, the techniques used in previous studies (postmortem tissue and Golgi staining, for example) made it impossible to determine the fate of individual dendritic spines over time. Thus, it remains unclear to what degree the lack of FMRP affects spine formation and elimination at different developmental stages. It is also unknown whether the abundance of immature-looking spines in *Fmr1* KO mice are related to abnormal spine plasticity. Specifically, it is possible that there is a higher degree of spine turnover in *Fmr1* KO mice so that at any point in time there will be more immature-appearing spines. Alternatively, the abundance of immature-looking spines in the *Fmr1* KO mice could be caused by a failure of a subset of spines to mature.

In the present study, we examined dendritic spine development and experience-dependent spine remodeling in *Fmr1* KO mice using a transcranial two-photon imaging technique that allows reimaging of individual spines during different periods of development (23, 24). Our results show that FXS animals have a larger population of transient dendritic spines, compared with WT controls, both during development and in adulthood. Furthermore, transient spines had, on average, smaller head diameter and longer spine neck length compared with persistent spines, suggesting that the population of transient spines contribute in part to the immature spine phenotype previously reported in *Fmr1* KO. Finally, we show that dendritic spine turnover in response to sensory manipulation is abnormal in the KO mice, suggesting that FMRP plays an important role in experience-dependent modification of sensory circuits.

Results

Increased Turnover of Dendritic Spines in *Fmr1* KO Mice. To determine how lack of FMRP affects dendritic spine development, we examined the formation and elimination rates of dendritic spines on apical dendrites of layer 5 pyramidal neurons in the primary somatosensory barrel cortex using a transcranial two-photon imaging approach (Fig. 1 *A–D*). Dendritic spines were imaged twice over a 2-d interval in male *Fmr1* KO mice ($Fmr1^{-/y}$) and littermate controls ($Fmr1^{+/y}$), generated from FVB/C57BL/6J $Fmr1^{+/-}$ × C57BL/6J YFP, and used throughout except where

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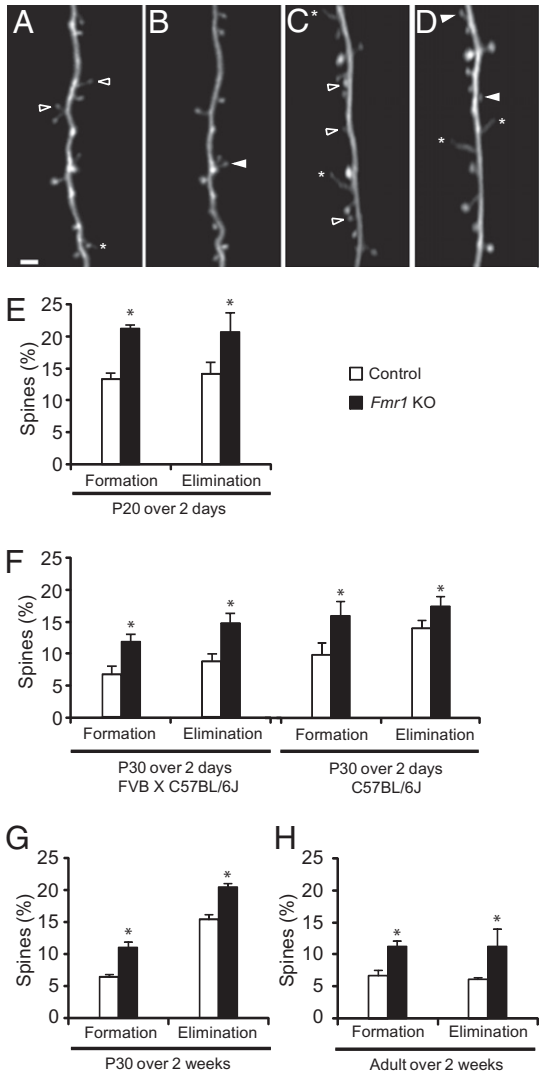

Fig. 1. Increased dendritic spine turnover in the barrel cortex of *Fmr1* KO mice at different developmental stages. (*A–D*) Repeated imaging of dendritic segments in the somatosensory barrel cortex over 2 wk in 1-mo-old WT control mice (*A* and *B*) and *Fmr1* KO mice (*C* and *D*). Open arrowheads indicate spines that were eliminated between the two views, and filled arrowheads indicate new spines that were formed between the two views. Asterisks indicate dendritic filopodia. (*E* and *F*) Spine formation and elimination over 2 d were significantly higher in *Fmr1* KO mice compared with WT control mice at postnatal day 20 (*E*) and postnatal day 30 (*F*). (*G* and *H*) Spine formation and elimination rates over 2 wk were also significantly higher in KO animals than in control mice at postnatal day 30 (*G*) and adult (>4 mo old) (*H*). Data are presented as mean ± SEM (*$P < 0.05$).

noted otherwise. We found that the rates of spine formation and spine elimination over 2 d were significantly higher in the KO animals compared with the WT animals at 3 wk and 1 mo of age (Fig. 1 *E* and *F*; $P < 0.05$). The spine formation and elimination rates over 2 d in 3-wk-old control animals were 13.3 ± 0.6% and 14.1 ± 1.5%, respectively, but in age-matched *Fmr1* KO animals, the spine formation and elimination rates were 21.1 ± 0.3% and 20.7 ± 2.8% (Fig. 1*E*). At 1 mo, spine formation and elimination rates over 2 d were 6.7 ± 1.1% and 8.7 ± 0.8% in control animals and 11.8 ± 0.8% and 14.6 ± 1.2% in KO animals, respectively (Fig. 1*F*). To determine whether this phenotype of increased spine turnover could be detected in animals from a different genetic background, we examined spine dynamics in the *Fmr1* KO and WT control mice on a C57BL/6J background (C57BL/6J × C57BL/6J-YFP F_1 cross). In 1-mo-old animals in this strain, dendritic spines also exhibited significantly higher formation and elimination rates in *Fmr1* KO animals than in WT controls over 2 d (Fig. 1*F*; $P < 0.05$). Neither the percentage of filopodia-like protrusions (headless protrusions) nor their turnover rates were significantly different between KO animals and WT controls, regardless of the ages and genotypes (Fig. S1; $P > 0.05$). Together, these observations suggest that, during development, the loss of *Fmr1* gene function leads to heightened spine turnover over days in the primary somatosensory cortex.

To further understand how loss of FMRP affects spine development and plasticity, dendritic spines were imaged twice with a 2-wk interval in 1-mo-old mice and adult mice (4.9 ± 0.3 mo of age). We found that spine formation and elimination rates over 2 wk were significantly higher in the KO than in the controls in both age groups (Fig. 1 *G* and *H*; $P < 0.05$). The turnover of dendritic filopodia over 2 wk was comparable between *Fmr1* KO animals and controls ($P > 0.2$). Notably, in 1-mo-old WT mice, the rate of spine elimination over 2 wk was significantly higher than the rate of spine formation (15.4 ± 0.8% vs. 6.4 ± 0.4%; $P < 0.001$), consistent with previously published data showing the existence of a spine "pruning phase" during late postnatal development (23, 25). The KO animals also showed a significantly higher rate of spine elimination than of spine formation at 1 mo of age (20.4 ± 0.5% vs. 11.1 ± 0.9%; $P < 0.001$), suggesting that the spine pruning phase appears to be intact in these mutant animals. Furthermore, the developmental decline in spine elimination proceeded in a similar fashion in both WT and KO mice from 1 to 4 mo of age (Fig. 1 *G* and *H*). Together, these results identified heightened spine turnover as a prominent abnormality of dendritic spine plasticity in *Fmr1* KO mice both during development and in adulthood.

Existence of a Larger Pool of Transient New Spines in KO Mice. The heightened spine turnover observed over 2 d and 2 wk in *Fmr1* KO mice suggests that all dendritic spines in these animals could be more plastic than in WT controls. Alternatively, a subset of spines in the population may be more dynamic, and this pool of dynamic spines may be larger in KO mice than in controls. To distinguish between these two possibilities, we first imaged spines every 2 d for three consecutive sessions in 1-mo-old animals (Fig. 2*A*). We found that 30.0 ± 3.3% and 35.4 ± 2.1% of spines that were formed over the first 2 d persisted within the next 2 d in KO and WT mice, respectively. The survival rate of these newly formed spines over the first 2 d was not significantly different between the two genotypes (Fig. 2*B*; $P > 0.3$). Because a larger percentage of new spines was formed over 2 d in the KO than in the WT mice (Fig. 1*F*), the total fraction of "short-lived" or transient new spines (classified as spines formed during the first 2 d and eliminated within the following 2 d) was larger in *Fmr1* KO mice than in controls (Fig. 2*C*; 8.9 ± 0.6% vs. 4.8 ± 0.9%; $P < 0.05$). Furthermore, because the spine density at this age (1 mo) was comparable in the KO compared with control animals [42.1 and 38.2 spines per 100-μm dendrites in KO and WT ($P = 0.13$ in FVB × C57BL/6J background) and 42.3 and 42.0 spines per 100-μm dendrites in KO and WT ($P = 0.93$ in C57BL/6J background)], the total number of short-lived new spines was also larger in *Fmr1* KO mice than in controls.

In contrast to the low survival rates of newly formed spines, we found that 93.5 ± 1.3% of preexisting spines (those surviving for at least the first 2 d) persisted over the next 2 d in KO mice and that this survival rate was comparable between KO and WT mice (Fig. 2*B*; $P > 0.2$). Thus, not all spines were more plastic in KO than in WT mice. Instead, a larger pool of short-lived newly formed spines mainly account for the heightened spine turnover measured over 2 d in the KO mice.

In addition, we found that 29.8 ± 9.1% and 39.5 ± 11.2% of newly formed spines that appeared during the first 2 wk persisted

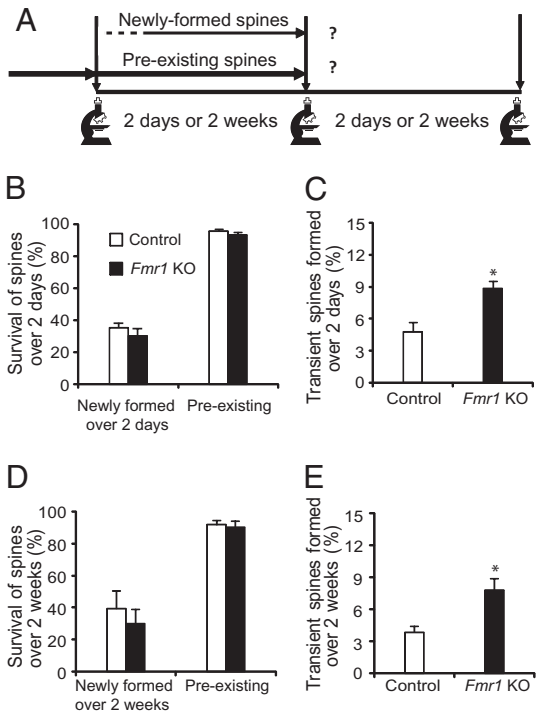

Fig. 2. A larger population of transient spines existed in *Fmr1* KO mice. (*A*) Newly formed and preexisting spines were identified in the first 2 d or 2 wk and reimaged after 2 d or 2 wk. (*B*) Spines formed over 2 d in 1-mo-old mice were largely eliminated over the next 2 d. In contrast, the majority of preexisting spines were maintained for these 2 d. The survival rates of newly formed spines and preexisting spines in the KO mice were not significantly different from those in the WT control mice. (*C*) The percentage of transient spines (formed over 2 d and eliminated over the next 2 d) was higher in *Fmr1* KO animals than in WT mice (*$P < 0.05$). (*D*) Spines formed over 2 wk in 1-mo-old mice were largely eliminated over the next 2 wk, whereas most preexisting spines were maintained during this period. (*E*) The percentage of transient spines that were formed over 2 wk and eliminated over the next 2 wk was also higher in *Fmr1* KO animals than in WT mice (*$P < 0.05$).

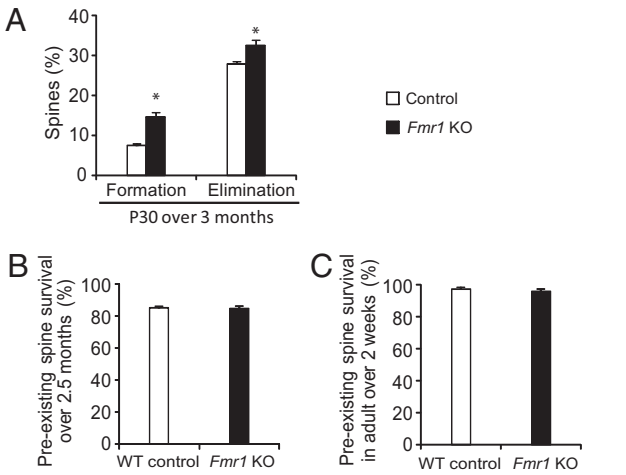

Fig. 3. The majority of dendritic spines have similar long-term stability between KO and WT mice. (*A*) A small fraction of new spines were added in both KO and control mice from 1 mo to 4 mo of age, whereas most of the spines that existed at 1 mo of age survived at 4 mo of age in both KO mice and WT controls. The percentage of spine addition and elimination from 1 mo to 4 mo of age in KO mice was higher than that in control animals (*$P < 0.05$). (*B*) More than 80% of dendritic spines that survived for 2 wk (from postnatal day 30 to 44) persisted into adulthood (4 mo of age) in both *Fmr1* KO and WT animals. (*C*) The majority of adult spines that persisted for at least 2 wk were similarly maintained over the next 2 wk in both WT and *Fmr1* KO mice.

over the next 2 wk in 1-mo-old KO mice and controls, respectively (Fig. 2*D*; $P > 0.2$). On the other hand, 91.7 ± 2.7% and 90.2 ± 3.9% of spines that existed for at least 2 wk continued to be maintained over the next 2 wk in KO and control mice, respectively (Fig. 2*D*; $P > 0.6$). Thus, similar to the experiments covering days, these results reveal two pools of spines with different stability over 2 wk. The pool of spines formed over 2 wk and eliminated in the next 2 wk was significantly larger in KO mice (7.8 ± 1.1%) than in WT animals (3.9 ± 0.6%; $P < 0.05$; Fig. 2*E*). These results further suggest that the higher turnover rate in KO mice is mainly due to a larger population of short-lived new spines rather than reduced overall spine stability.

Majority of Dendritic Spines Have Similar Long-Term Stability in KO and WT Mice. Previous studies have shown that, in WT mice, spines formed early in development and surviving into adulthood are remarkably stable; the majority are maintained through the entire adult life (23, 25, 26). To further understand how lack of FMRP affects spine development and plasticity, we examined spine turnover in WT and KO mice from 1 to 4 mo of age. Regardless of the mouse genotypes, we found that new spines that accumulated from 1 to 4 mo of age accounted for a small (although potentially important) fraction of the total spines (Fig. 3*A*; WT 7.6 ± 0.1% and KO 14.6 ± 1.1%). On the other hand, ~72% and 67% of spines that existed at 1 mo of age were maintained over the next 3 mo in WT and KO mice, respectively (Fig. 3*A*). Notably, in WT mice, 85.3 ± 0.6% of spines that existed at 1 mo of age and persisted for another 2 wk continued to be maintained over the next 2.5 mo. The survival rate of this pool of spines in KO mice was 84.6 ± 1.1%, which was not statistically different from that in WT (Fig. 3*B*; $P > 0.8$). These findings suggest that (*i*) the majority of spines in both WT and KO adult mice (4 mo old) come from spines that are formed early during development (before 1 mo of age) and persist into adulthood and (*ii*) the lack of FMRP does not have a significant effect on the long-term stability of most spines in the adult barrel cortex.

Consistent with the above notion, we found that adult spines that persisted for at least 2 wk were maintained at a rate of 97.3 ± 1.0% in control mice and 95.9 ± 0.9% in KO mice over the next 2 wk ($P > 0.2$; Fig. 3*C*). Furthermore, we found that the survival rate of spines formed over 2 wk was low (~45% over the next 2 wk) and comparable between adult WT and KO mice. Together, these results suggest that the higher spine turnover level in the adult KO is also due to a larger pool of newly formed spines, whereas the majority of adult spines are equally and remarkably stable in both genotypes.

Size of Newly Formed and Eliminated Spines Is Smaller than That of Stable Spines. An overabundance of immature-appearing, long, and thin spines are frequently observed in *Fmr1* KO mice and in FXS patients (15, 16). To examine whether the larger pool of transient spines in *Fmr1* KO mice is related to immature-appearing spines, we compared the size of newly formed and eliminated spines to spines that persisted for at least two imaging sessions by measuring several parameters of spine morphology, including adjusted spine head brightness (a measure of volume), head diameter, and spine neck length. We found that, in both WT and KO mice, spines that were newly formed over a 2-d interval in 1-mo-old animals had, on average, smaller head diameter and lower brightness compared with spines persisting for at least 2 d (Fig. 4 *A* and *B*). Although total spine length did not differ significantly between unstable and stable spines (Fig. S2 *A* and *B*), spine neck length was longer in newly formed spines compared with stable spines (Fig. 4*C*, excludes stubby type spines where no neck is present). Similarly, spines that were eliminated

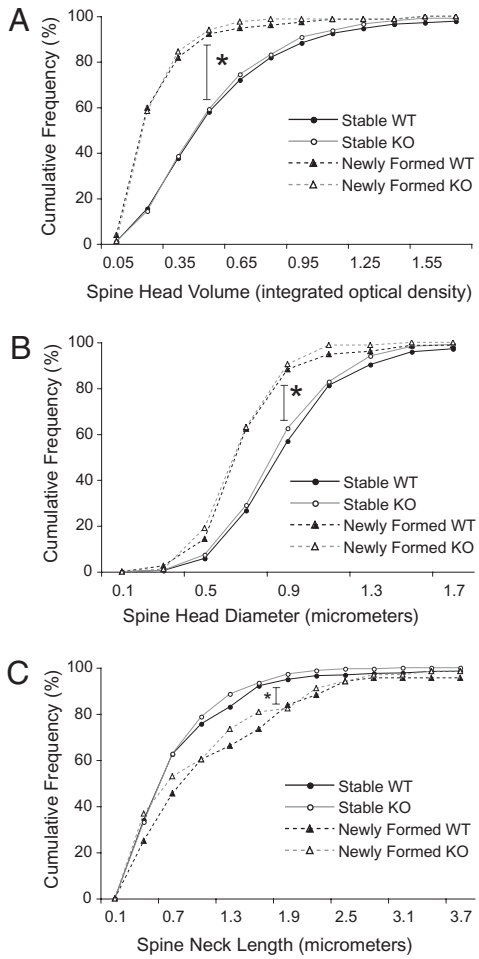

Fig. 4. Newly formed spines were smaller than stable spines. Spine size distribution was examined by plotting the cumulative frequency of sizes of all examined spines and comparing distributions using the Kolmogorov–Smirnov test (*$P < 0.05$). (*A* and *B*) Spines that were newly formed over 2 d in 1-mo-old animals had a smaller head size compared with spines that persisted during both imaging sessions (stable spines) using a measure of spine volume, (*A*) integrated fluorescence intensity of spine head relative to dendritic shaft and (*B*) spine head diameter. (*C*) Spine neck length, excluding stubby (neckless) spines, was significantly longer in newly formed spines compared with stable spines.

over a 2-d period were smaller headed and had longer neck lengths compared with stable spines (Fig. S2 *C–E*). Furthermore, when we compared the rate of turnover in spines grouped by their morphological features, we found that spines with smaller head volume and longer neck length were more likely to be eliminated compared with larger-headed and shorter-necked spines in both genotypes (Fig. S3 *A–D*). Together, these results suggest that the increased population of transient spines in *Fmr1* KO mice may contribute to the immature spine phenotype previously reported in *Fmr1* KO mice.

It is important to point out that the increase in transient spines in KO mice (~4%) is smaller than the increase in immature-appearing spines reported in the literature for adult *Fmr1* KO mice and FXS patients. Because there is a substantial overlap in size and length distribution between transient spines and stable spines, some immature-appearing spines likely belong to the stable pool. Furthermore, in 1-mo-old animals, there was a trend toward increased elimination even of larger-headed and short-necked spines in the KO animal (Fig. S3), although these differences did not reach statistical significance. Thus, the larger pool of transient spines in *Fmr1* KO mice may contribute to some, but not all, of the immature-appearing spines seen in KO animals.

Sensory-Dependent Spine Formation and Elimination Are Altered in KO Mice. Because *Fmr1* KO mice have been shown to be hyperactive and hypersensitive to sensory stimuli (6, 27), it is possible that the increased pool of transient spines in *Fmr1* KO mice is related to increased sensory inputs and/or enhanced responses to sensory stimuli. To test this possibility, we used a sensory deprivation paradigm in which all of the whiskers on one side of the facial pad were trimmed daily over 2 wk. We then examined the effect of sensory deprivation on the rates of spine formation and elimination in the barrel cortex contralateral to the whisker trimming side in KO and WT mice at 1 mo of age. We found that sensory deprivation through whisker trimming did not significantly affect spine formation in either WT or KO mice compared with their corresponding untrimmed controls (Fig. 5*A*; $P > 0.4$). In the absence of whisker sensory input from one side of the facial pad, the formation of new spines over 2 wk continued to be higher in KO mice than in the controls (10.4 ± 0.3% in KO and 5.9 ± 0.3% in control; $P < 0.05$), suggesting that it is unlikely that the larger pool of newly formed spines in KO mice is due to enhanced sensory input. Furthermore, in agreement with previous studies (28), we found that whisker trimming over 2 wk reduced the rate of spine elimination in the barrel cortex of WT control mice (15.4 ± 0.8% in nontrimmed and 10.9 ± 0.8% in trimmed mice; $P < 0.01$). Notably, in the KO mice, whisker trimming had no significant effect on the rate of spine elimination (20.4 ± 0.5% in nontrimmed and 19.7 ± 0.9% in trimmed animals; $P > 0.5$), suggesting that regulation of sensory-dependent pruning of existing spines is altered in KO mice.

To further investigate alterations of sensory experience-dependent spine plasticity, we tested the effect of chessboard trimming, which increases the difference in sensory experience coming from adjacent whiskers and has been shown to increase spine formation over a period of days in the barrel cortex (29, 30). First,

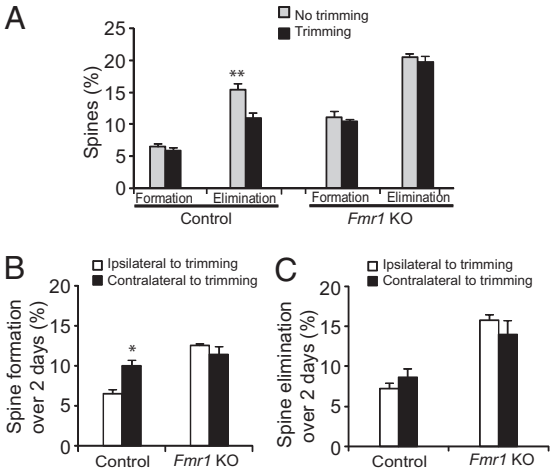

Fig. 5. Sensory manipulation by whisker trimming altered spine elimination or formation rate in WT mice but not in *Fmr1* KO mice. (*A*) Sensory deprivation through whisker trimming on one side of the facial pad reduced spine elimination over 2 wk in WT mice but not in *Fmr1* KO animals (**$P < 0.01$). Sensory deprivation has no significant effect on the rate of spine formation over 2 wk in both WT and KO mice. (*B*) In WT mice, spine formation over 2 d after chessboard trimming was significantly higher in the side contralateral to trimming than on the ipsilateral side. Chessboard trimming over 2 d had no significant effect on spine formation in the barrel cortex contralateral and ipsilateral to the trimming in *Fmr1* KO mice. (*C*) Chessboard trimming over 2 d did not affect spine elimination in the barrel cortex of either the ipsilateral or contralateral brain hemisphere of WT or *Fmr1* KO mice.

both hemispheres of each animal were imaged twice (with a 2-d interval) to determine the baseline turnover without whisker trimming. Spine formation over the first 2 d in the barrel cortex did not differ between the hemispheres in either 1-mo-old control WT or KO animals ($P > 0.2$ for both control and KO mice). During the next 2 d, we performed chessboard whisker trimming on one side of the facial pad and imaged spine turnover in both hemispheres again. We found that in control mice, chessboard trimming over 2 d led to a higher rate of spine formation in the hemisphere contralateral to the trimmed side than in the other hemisphere (Fig. 5*B*; 10.0 ± 0.6% contralateral to the trimmed side; 6.5 ± 0.4% ipsilateral to the trimmed side; $P < 0.05$). However, no significant difference in spine formation was found in the barrel cortex of KO mice between the hemispheres contralateral and ipsilateral to chessboard trimming (12.5 ± 0.1% ipsilateral to the trimmed side, 11.4 ± 0.9% contralateral to the trimmed side, $P > 0.25$). Furthermore, we found that chessboard trimming over 2 d had no significant effect on the degree of spine elimination in either WT or KO mice (Fig. 5*C*; $P > 0.2$). These experiments indicate that, unlike in the control mice, the formation rate of spines in KO mice is not altered by chessboard trimming. Together with long-term sensory deprivation experiments, these results suggest that the impact of sensory experience on spine formation and elimination is reduced in KO mice.

Discussion

Abnormalities in the number and morphology of dendritic spines are observed in mental retardation patients with various causes and have been examined using microscopic methodologies for more than 30 y (31). However, until recently, it was not possible to follow the fate of individual dendritic spines in a live animal to determine how changes in spine number occurred in these illnesses (14, 24). In this study, we used intravital two-photon imaging to study how development and plasticity of dendritic spines are altered in a mouse model of FXS. Specifically, we asked how the lack of *Fmr1* gene function affects spine turnover and whether abnormal spine turnover and spine morphology are related to one another in *Fmr1* KO mice. Furthermore, we also examined whether FMRP plays an important role in experience-dependent spine turnover in sensory circuits. Several important conclusions can be drawn from our results. First, spine turnover, including both formation of new spines and elimination of existing spines, is increased in the KO animal compared with age-matched WT controls. This enhanced turnover in the KO is seen early during development and in adulthood. On the other hand, the age-dependent decline in rates of spine turnover and developmental spine pruning are preserved in the KO animal. These findings indicate that increased spine turnover is a major abnormality of dendritic spine development and plasticity in the KO.

Second, we found that the increased spine turnover in the KO mice is due to the existence of a larger pool of transient spines, although the majority of spines in the KO have long-term stability similar to that in the WT. Because spines in the transient pool have, on average, smaller head diameter and longer neck length than stable spines, the increased population of transient spines in *Fmr1* KO mice likely contributes to the immature spine phenotype in *Fmr1* KO mice.

Last, sensory deprivation, as caused by whisker trimming, does not reduce the enhanced spine formation seen in KO mice. Thus, the higher formation rate in KO mice does not stem from a sensory "overload." In two sensory modulation paradigms we used (long-term whisker trimming to reduce spine elimination rates and short-term chessboard trimming to increase spine formation rates), formation and elimination of dendritic spines in KO animals failed to respond to sensory modulation. Thus, synaptic connections in *Fmr1* KO mice appear to be less impacted by sensory stimuli than in WT mice, potentially leading to the establishment of synaptic circuits that are improperly tuned by sensory experience from the outside world.

It is important to point out that previous studies in the visual and somatosensory cortex of *Fmr1* KO mice have shown variable increases (0–20%) in immature-appearing, thin-headed spines on pyramidal cells in KO compared with WT mice, depending on the strain and region examined, as well as the animals' ages. For example, neither Galvez et al. (21) nor Nimchinski et al. (22) found differences in spine morphology or density in 1-mo-old *Fmr1* KO mice, whereas differences were seen at earlier and later periods of development. In the temporal cortex of human patients with FXS, >50% more thin-headed spines were noted (18), whereas in *Fmr1* KO mice, morphological differences were found but to a lesser degree. Thus, the effect of *Fmr1* mutation on spine morphology and turnover could vary, depending on age and on the cell types, cortical layers, cortical regions, and species examined. Although our studies on apical dendrites of layer 5 pyramidal cells have provided several new insights into abnormal development and plasticity in *Fmr1* mutant mice, the generalities of our findings remain to be determined.

Interestingly, our findings of spine instability and insensitivity to experience-dependent modulation have striking parallels to the molecular findings in FXS. FMRP is thought to act as a protein translation repressor until a permissive signal causes the release of inhibition, thereby inducing local translation in dendrites (32, 33). Many lines of evidence have shown that local protein translation, via the polyribosomes in dendrites, is an efficient way to rapidly synthesize new proteins involved in synaptic plasticity (34–36). In the *Fmr1* KO neurons, excess protein synthesis is seen under baseline conditions, whereas when a specific stimulus occurs (e.g., DHPG stimulation of mGluR or neuronal activity), there is a failure to induce activity-dependent protein translation, in addition to abnormalities in synaptic plasticity (37–39). Thus, it is possible that excessive basal protein translation and the increased formation of transient spines are intimately related. Furthermore, in the absence of *Fmr1* gene function, activity-dependent spine remodeling may still occur but to a reduced degree because basal translation levels are already high. It has been shown that FMRP interacts with CYFIP1/Sra1 to repress activity-dependent protein translation, and this process is likely regulated by Rac1, a small GTPase important for modulating structural plasticity of dendritic spines (40–42). Additionally, PSD-95 and CaMKII translation after mGluR activity were essentially absent in *Fmr1* KO mice, suggesting a critical function of FMRP in regulating activity-dependent expression of important synaptic proteins (37, 38). Thus, FMRP may directly or indirectly act as a regulatory signal for basal and activity-dependent translation of synaptic proteins, and the disruption of this translation process in the absence of FMRP could underlie abnormal dendritic spine dynamics.

It has been recently shown that, in WT animals, the population of transient spines grows after enriched sensory experience or after behavioral training over days. Furthermore, a small fraction of the newly formed spines can persist over the animal's lifetime, contributing to long-lasting circuit remodeling associated with new sensory or behavioral experience (26). Regardless of sensory experiences and developmental stages, the population of transient spines is always larger in the KO than in the WT mice, suggesting that the pool of transient spines may be less responsive to sensory stimuli in the KO mice. Because the generation of a larger population of transient spines is a prominent deficit in KO mice, it will be important to identify the molecular mechanisms underlying the genesis and plasticity of transient spines to better understand mental retardation pathology in FXS. Furthermore, because abnormal spine density and morphology are found in almost all known causes of mental retardation, it will be important to investigate whether similar abnormalities of spine development and plasticity also occur in other mental disorders.

Materials and Methods

Experimental Animals and in Vivo Transcranial Imaging. *Fmr1* KO mice (FVB) and YFP-H (C57BL/6J) used at New York University were obtained from the Jackson Laboratory. FVB *Fmr1* KO and C57BL/6J YFP-H mice were first crossed to generate F_1 female mice (*Fmr1*$^{+/-}$), which were backcrossed with the YFP-H C57BL/6J males to get littermate WT and KO male mice for experiments. In the second breeding paradigm used at the University of Illinois, either C57BL/6J WT or *Fmr1* KO (from lines maintained at Dr. Greenough's laboratory and recrossed every three to four generations) were bred with YFP-H C57BL/6J mice to obtain either WT or *Fmr1* KO F_1 litters. The procedure of transcranial two-photon imaging and data quantification was described previously (23, 25, 43, 44). All animal procedures were approved by the Institutional Animal Care and Use Committees of University of Illinois and New York University. Detailed breeding paradigm and imaging procedures are provided in *SI Materials and Methods*.

Sensory Manipulation. For sensory deprivation experiments, all whiskers of the right facial pad were trimmed by a pair of scissors under a dissection microscope while the mice were still under anesthesia. The whiskers were then trimmed daily with a small shaver. Whisker trimming in a chessboard pattern was performed with a pair of scissors every other day immediately after each imaging session.

Data Quantification. The percentage of spines eliminated or formed is defined as the number of spines eliminated or formed divided by the number of existing spines at the first view. The change in the total spine number is calculated as 100% plus the percentage of formation and minus the percentage of elimination measured over a given interval. Data throughout the text are presented as mean ± SEM. *P* values were calculated using the Student's *t* test, except where otherwise noted.

Spine density, spine size, and neck length were measured from 3D images using the National Institutes of Health ImageJ software. The spine density was calculated based on the mean spine density of dendritic segments within the imaged regions (200 μm × 200 μm). Spine neck length was measured for spines that remained primarily in one plane. Neck length was measured from the bottom of the head to the shaft of the dendrite for spines with a spine head separate from the dendrite. Spine head size was measured using both spine brightness and head diameter in the plane from which the values were largest. Spine brightness was measured as follows, where Area is the number of pixels in an oval surrounding the head of the spine and mean optical density (MeanOD) is the mean brightness of pixels in that area:

$$\text{Spine Brightness} = (\text{Area (of spine)} \times \text{MeanOD (of spine)} - \text{Area (of spine)} \times \text{MeanOD (of background)})/\text{MeanOD of dendrite.}$$

The MeanOD of both the background and the dendrite was calculated from measurements taken next to each spine, averaged for each dendrite segment.

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