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« FMRP and dendritic local translation of α CaMKII mRNA are required for the structural plasticity underlying olfactory learning »

Short title: Local translation and structural plasticity

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ABSTRACT

Background: In the adult brain, structural plasticity allowing gain or loss of synapses remodels circuits to support learning. In Fragile X Syndrome (FXS), the absence of Fragile X Mental Retardation Protein (FMRP) leads to defects in plasticity and learning deficits. FMRP is a master regulator of local translation but its implication in learning-induced structural plasticity is unknown.

Methods: Using an olfactory learning task requiring adult-born olfactory bulb (OB) neurons and cell-specific ablation of FMRP, we investigated whether learning shapes adult-born neuron morphology during their synaptic integration and its dependence on FMRP. We used α CaMKII mutant mice with altered dendritic localization of α CaMKII mRNA as well as a reporter of α CaMKII local translation to investigate the role of this FMRP mRNA target in learning-dependent structural plasticity.

Results: Learning induces profound changes in dendritic architecture and spine morphology of adult-born neurons that are prevented by ablation of FMRP in adult-born neurons and rescued by an mGLUR5 antagonist. Moreover, dendritically translated α CaMKII is necessary for learning and associated structural modifications and learning triggers an FMRP-dependent increase of α CaMKII dendritic translation in adult-born neurons.

Conclusion: Our results strongly suggest that FMRP mediates structural plasticity of OB adult-born neurons to support olfactory learning through α CaMKII local translation. This reveals a new role for FMRP-regulated dendritic local translation in learning-induced structural plasticity. This might be of clinical relevance for the understanding of critical periods disruption in autism spectrum disorder patients, among which FXS is the primary monogenic cause.

INTRODUCTION

In the adult brain, the plasticity involved in learning and memory includes modifications of the strength of existing synapses as well as structural plasticity allowing the gain or loss of synapses (1). Although local dendritic mRNA translation is a major determinant of the synaptic plasticity underlying learning and memory (2), its role in structural plasticity remains unclear. Data pointing to its role in activity-induced structural plasticity stem from animal models deficient for the Fragile X Mental Retardation Protein (FMRP). Fragile X Syndrome (FXS), the most common monogenic form of intellectual disability (3) and autism spectrum disorder (ASD), results from the absence of FMRP due to the silencing of the *FMR1* gene (4). FMRP is an RNA-binding protein expressed in neurons and a key regulator of the local dendritic mRNA translation associated with synaptic plasticity (5). In addition, *Fmr1*^{-/-} mice display disrupted critical periods of experience-dependent plasticity in the somatosensory and visual cortex (6, 7) and increased spine instability and insensitivity to environmental changes (8-10). This suggests a role for FMRP in experience-dependent structural plasticity but direct cell-specific demonstrations of the role of FMRP in learning-induced structural plasticity are lacking.

The olfactory system expresses significant functional and structural plasticity, including the integration of new neurons into the adult olfactory bulb (OB). We previously showed that the morphological differentiation of new neurons in the adult OB is regulated by FMRP (11). We also demonstrated that olfactory activity regulates the dendritic transport and translation of the alpha subunit of the Calcium Calmodulin-dependent Kinase II (α CaMKII) mRNA, one of FMRP's translational mRNA targets and a major player in synaptic plasticity (12). However, how learning shapes the morphology of new neurons in the OB during their integration into circuits and whether FMRP and α CaMKII mRNA local translation play a role in learning-induced structural plasticity is unknown.

Using genetic tools and animal models, we show that olfactory perceptual learning induces profound morphological changes in adult-born neurons with an increase in dendritic complexity, spine density and modifications of spine morphology. FMRP deficiency in new neurons leads to learning deficits and defects in associated structural modifications. Interestingly, these defects are rescued by the mGluR5 antagonist MPEP, tested in clinical trials for FXS (13, 14). In addition, learning induces FMRP-dependent increases of α CaMKII mRNA local translation in dendrites of adult-born neurons, which is necessary for learning and associated structural plasticity.

Collectively, our results reveal a molecular cascade by which FMRP regulation of α CaMKII local translation mediates structural plasticity of adult-born neurons underlying olfactory learning. This highlights a new role of dendritic local translation in learning-induced structural plasticity, necessary for dendrite morphogenesis and spinogenesis, which might be of clinical relevance for understanding disrupted critical periods in ASD patients (15-17).

METHODS AND MATERIALS

Animals

Two-month-old male mice were housed in a 12h light/dark cycle, in cages containing 2 to 6 individuals. Animal care was conducted in accordance with standard ethical guidelines (NIH publication no.85-23, revised 1985 and European Committee Guidelines on the Care and Use of Laboratory Animals 86/609/EEC). The experiments were approved by the local ethic committee «Comité d’Ethique en Expérimentation Animale Charles Darwin C2EA-05 ». All mouse lines were in a C57BL6 background. *Fmr1* knock-out and conditional knock-out mice (1), Nestin::CreERT2 mice (2) and α CaMKII 3'UTR mutant mice (3) were genotyped according to the original protocols.

Perceptual learning

During 10 days, mice were daily exposed to swabs with 100 μ L of pure limonene+ or limonene- in two tea-balls in the home cage for one hour.

Discrimination test

To assess discrimination between limonene+ and limonene-, mice were subjected to a habituation/dishabituation test. After an initial 50-second presentation of mineral oil, the habituation phase consisted in 4 consecutive 50-second presentations of the same odorant, allowing 5-minute intervals (hab1 to hab4), followed by a 50-second presentation of a second odorant (test). Odorants diluted to 1Pa vapor pressure in mineral oil were presented on a swab in a tea-ball. Investigation time was recorded as active sniffing within 1 cm from the tea-ball. The investigator was blind regarding the genotype of animals during the tests.

Supplemental information

See Supplement for stereotaxic injections, histology, image analysis, tamoxifen and MPEP administration, and statistical analysis sections.

RESULTS

FMRP in adult-born neurons is necessary for olfactory perceptual learning

To investigate the role of FMRP in learning-induced structural modifications of new OB neurons, we used an olfactory perceptual learning paradigm, in which mice learn to discriminate two perceptually similar odorants, Limonene + and Limonene - (Lim+, Lim-). Importantly, this learning paradigm depends on OB plasticity (18) and requires adult neurogenesis in the OB (19). Naive WT mice cannot discriminate Lim+ from Lim- as tested by a habituation/dishabituation test (hab/dishab, four habituation trials with one member of the pair followed by a test trial with the other member, Fig.1B and Supplementary Fig.S1A). The significant decrease in investigation times from the first habituation trial to the fourth (Hab1 to Hab4; Supplementary Fig.S1A) shows that the mice habituate to one odorant of the pair. The stable investigation time between Hab4 and Test shows that naive mice cannot discriminate Lim+ from Lim- (Fig.1B). Subsequently, the learning phase includes a 10-day

enrichment period, where mice are exposed one hour per day to both odorants simultaneously (Fig.1A). After this enrichment period, mice are tested again for their capacity to discriminate Lim+ from Lim- through a hab/dish test (Fig.1C, supplementary Fig.S1B). As in the pre-enrichment tests, mice habituate properly (supplementary Fig.S1A). However, the significant difference in investigation times between Hab4 and Test (Fig.1C) shows that WT mice can now discriminate Lim+ from Lim-, as described (19). In contrast, *Fmr1*^{-/-} mice (20) did not learn to discriminate the two odorants after a 10-day enrichment period (Fig.1D and supplementary Fig.S1C). This was not due to a general olfactory discrimination defect of the knocked-out (KO) mice since they spontaneously discriminated two perceptually distinct odorants (Octanal/Carvone, supplementary Fig.S1D) and as previously described (21). This suggests that FMRP is necessary for the perceptual learning underlying discrimination of similar odorants.

To assess the role of FMRP selectively in new neurons, we used genetically modified Nestin::CreERT2/*Fmr1*^{flx/y} mice (20, 22)(hereafter called cKO mice, for conditional KO), in which *Fmr1* ablation can be induced in adult-born neuron progenitors and their progeny through CRE activation with tamoxifen (Fig.2A). In our conditions, tamoxifen induces *Fmr1* ablation in 37% of new OB neurons in the cKO mice (Supplementary Fig.S1G-I, $p < 0.0001$ Fischer exact test). cKO mice injected with tamoxifen did not learn to discriminate Lim+ from Lim- after the enrichment period (Fig.1F and supplementary Fig.S1F). In contrast, tamoxifen-injected control cKO mice, not carrying the Cre allele, discriminated Lim+ from Lim- after the 10-day enrichment period (Fig.1E and supplementary Fig.S1E). Decreased neurogenesis in the adult hippocampus of the cKO mice was previously reported (23). While this is unlikely to affect olfactory perceptual learning, which relies on OB mechanisms (18), it was important to verify that *Fmr1* mutation did not alter OB adult neurogenesis, which could explain the observed learning deficit. Control cKO and cKO mice were BrdU-injected and the number of BrdU-positive OB neurons in the granule cell layer after learning was quantified. There was no significant difference in the density of BrdU-positive OB neurons between control cKO and cKO mice (control cKO: 222 ± 27 cells/mm², cKO: 261 ± 28 cells/mm², n=4 mice/group, Kruskal–Wallis test $p = 0.622$) showing that OB neurogenesis is not affected in cKO mice. The learning deficit in cKO mice thus suggests that FMRP in new neurons is necessary for olfactory perceptual learning.

FMRP in adult-born neurons is necessary for learning-induced structural modifications of new neurons

We next asked if the learning deficit in cKO mice could be related to defects in new neuron learning-induced structural plasticity. To analyze this, we labeled a cohort of newly-generated OB neurons in control cKO or cKO mice by injecting a GFP-expressing lentivirus in the SVZ, where new OB neurons originate (Fig.2A,B). Injected mice were subjected, or not, to learning. In the learning paradigm, enrichment took place during integration of the labeled new neurons into the OB synaptic circuits (15 to 24 day post-injection (dpi), Fig.2A). The mice were subsequently sacrificed and the

morphology of OB GFP-labeled granule cells (GCs) was analyzed (11). GCs are anaxonic GABAergic interneurons, whose cell body is localized in the granule cell layer and a long, branching and spiny apical dendrite arborizes in the external plexiform layer (scheme Fig.1C). In our analyses, we considered only GFP-positive GCs with a fully developed dendritic arbor contained entirely within the sections. This allowed us to check FMRP immunoreactivity in cell bodies of analyzed neurons and to avoid underestimating the total dendritic length. The length of the primary dendrite extending from the cell body to the first branching point did not vary in the different conditions analyzed in this study (not shown, *unpaired Student's t test* $p=0.156$ $n=45,72$). The length of the dendritic arbor after the first branching point was $475 \pm 13 \mu\text{m}$ for control cKO mice in basal conditions. After learning, the length increased significantly to $637 \pm 45 \mu\text{m}$ (Fig.2D, F). Learning also induced an increase in the complexity of the apical dendritic arbor, as evidenced by Sholl analysis (Fig.2D,G). In comparison with control cKO mice under basal conditions, apical dendritic arbors of FMRP-depleted (FMRP⁻) new neurons in cKO mice displayed a similar length (Fig.2E, $523 \pm 28 \mu\text{m}$) and dendritic complexity (Fig.2G and H, *repeated-measures ANOVA with two factors*. $F(1,23)=0.985$, $p=0.331$, $n=13-14$). However, in contrast to control cKO mice, the learning paradigm did not induce any change in the dendritic arbor length (Fig.2E, F, $510 \pm 41 \mu\text{m}$) or complexity in FMRP⁻ neurons of cKO mice (Fig.2H). These data strongly suggest that FMRP in new neurons is necessary for learning-induced dendritic remodeling.

We analyzed spine density by counting protrusions in the dendritic arbor of labeled GCs. In control cKO mice, the spine density in basal conditions was $0.45 \pm 0.02 /\mu\text{m}$ and was significantly increased to $0.6 \pm 0.02 /\mu\text{m}$ following learning (Fig.3A,A",B), consistent with the notion that learning induced the formation of new spines and/or increased spine stabilization. As we already observed in similar conditions (11), spine density in FMRP⁻ neurons from cKO mice in basal conditions was significantly increased compared to control cKO mice ($0.55 \pm 0.04 /\mu\text{m}$ versus $0.45 \pm 0.02 /\mu\text{m}$, Fig.3A,A',B, $p=0,02$). However, importantly, learning did not change spine density in FMRP⁻ new neurons ($0.57 \pm 0,05 /\mu\text{m}$, Fig.3A',A",B). This suggests that an increase in spine density does not occur in FMRP⁻ neurons either because FMRP is necessary for an increase in spine density or because the maximum level of spine density is already attained in mutated neurons.

We also analyzed spine length and, as we observed before in similar conditions (11), spines of FMRP⁻ new neurons from cKO mice are significantly longer than spines of neurons from control cKO mice (control cKO $2.84 \pm 0.06 \mu\text{m}$ versus cKO $3.09 \pm 0.09 \mu\text{m}$, $p=0.032$). However, learning had no effect on spine length, independent of the genotype ($p=0.444$).

GCs spines display atypical morphologies with a long neck and variable head diameters (Fig.3A-A"). We thus decided not to follow the typical categorization of mushroom, stubby and thin spines, but to calculate the cumulative frequency distribution of their head diameters. cKO mice were significantly different from control cKO with a shift towards smaller diameters (Fig.3C), consistent with what is

classically described for *Fmr1* mutated neurons. Learning triggered a significant shift towards larger diameters in wild-type new neurons from control cKO mice (Fig.3D and Fig.3A,A"). This increased size of spine heads, in other systems, is interpreted as a reflection of a stabilized synapse consecutive to learning (1). However, learning had no significant effect on spines from FMRP⁻ new neurons of cKO mice (Fig.3E and Fig.3A',A''').

Collectively, the data suggest that FMRP is necessary for olfactory learning-induced structural plasticity of new neurons, by regulating both dendritic and spine structural plasticity.

MPEP rescues learning and dendritic arbor structural plasticity defects in mutated adult-born neurons

In the absence of FMRP, signaling through group-1 metabotropic receptors is increased and insensitive to stimulation (24). Consequently, many therapeutic strategies for FXS are based on targeting the mGluR pathway (13, 14). We thus asked whether FMRP⁻ neuron phenotype could be rescued by a group-1 metabotropic receptor antagonist, MPEP. cKO mice were injected with MPEP (20mg/kg/day) or saline daily during the period of enrichment. Saline-injected cKO mice could not learn the discrimination task (Fig.4A and supplementary Fig.S2A). Remarkably, MPEP injections rescued the learning defects in cKO mice (significant difference between Hab4 and Test, Fig.4B and supplementary Fig.S2B). To test if this was the consequence of a rescued structural plasticity, we analyzed the morphology of new neurons in saline or MPEP-injected cKO mice after learning. In MPEP-injected cKO mice, new FMRP⁻ neurons displayed a lengthened dendritic arbor when compared to saline-injected cKO mice (Fig.4C,D). In addition, FMRP⁻ new neurons from MPEP-injected cKO mice displayed an increased dendritic complexity as compared to neurons from saline-injected cKO mice (Fig.4C,E). Comparison of FMRP⁻ new neurons from MPEP-injected cKO mice after learning with wild-type neurons in control cKO mice after learning (Fig.2E,G) showed that following MPEP injections the dendritic lengths and complexity of mutated neurons was similar to their wild-type counterparts ($p>0.05$). Thus, MPEP can rescue dendritic arbor structural plasticity defects in mutated neurons. In contrast, spine density (Fig.4F) and morphology (Fig.4G) were unchanged in MPEP-injected cKO mice, as compared to saline-injected cKO mice.

This suggests that the rescue of dendritic arbor plasticity defects induced by MPEP treatment is sufficient to rescue the learning phenotype.

α CamKII mRNA dendritic local translation is necessary for olfactory perceptual learning and associated structural plasticity

α CaMKII mRNA is one of FMRP's mRNA targets (5) and a major actor in synaptic plasticity. α CaMKII mRNA is dendritically localized and locally translated in different regions of the brain, including the OB (12). We thus asked if α CaMKII local translation is necessary for olfactory perceptual learning and associated structural plasticity. We used mice in which α CaMKII 3'UTR is replaced by the 3'UTR of an unlocalized mRNA (25)(hereafter called Δ 3'UTR). We previously showed that, in these mice, OB dendritic localization and local translation of α CaMKII mRNA are

severely disrupted and that olfactory associative learning is impaired (12). $\Delta 3'$ UTR mice were thus subjected to the non-associative olfactory perceptual learning used here. Contrary to WT littermates (Fig.5A) and similar to *Fmr1* mutated mice, $\Delta 3'$ UTR mice did not learn to discriminate Lim⁺ from Lim⁻ (Fig.5B and supplementary Fig.S3A,B). This failure in perceptual learning is not due to a general olfactory defect (12) and cannot be ascribed to a decreased neuronal production in the OB (density of BrdU-labeled cells in the GCL after learning, WT: 96 ± 7 cells/mm², $\Delta 3'$ UTR: 89 ± 7 cells/mm², n=4 mice per group, *Mann-Whitney rank sum tests* $p=0.343$).

Similar to cKO mice, under basal conditions, new neurons in $\Delta 3'$ UTR mice display normal dendritic arbor length (Fig.5C,D,E) and increased spine density compared to WT (Fig.5G,G',H)($p=0.02$).

Strikingly, after learning, new neurons in $\Delta 3'$ UTR mice display structural plasticity defects comparable to cKO mice, with a lack of dendritic lengthening and a lack of increase in dendritic complexity (Fig.5C-F). Moreover, learning had no effect on $\Delta 3'$ UTR mice spine density (Fig.5G',G'',H) and did not increase spine head diameters (Fig.5J), in contrast to their WT littermates (Fig.5G,G'',I).

Collectively, these data suggest that α CaMKII local translation is necessary for olfactory perceptual learning and associated structural plasticity. It also suggests that α CaMKII mRNA may be the main FMRP mRNA target involved in olfactory learning-induced structural plasticity of new neurons.

Olfactory perceptual learning induces an FMRP-dependent increase of α CaMKII dendritic local translation in adult-born neurons

To directly substantiate a link between α CaMKII local translation and FMRP, we used a previously validated reporter of α CaMKII local translation (26) in WT and *Fmr1* mutated mice. In this reporter, the 3'UTR extremity of α CaMKII mRNA, which mediates its dendritic localization and translational regulation (27) and can be bound by FMRP (28), is associated to a GFP mRNA so that transport and translation of the GFP mRNA reflect the endogenous α CaMKII. The GFP is in an unstable and rapidly degraded membrane-bound form (a destabilized and myristoylated GFP, Fig.6A). Consequently, the presence of GFP is indicative of recently locally translated protein. To label new neurons and to analyze α CaMKII dendritic local translation, we produced an adenovirus (AdV, serotype5) expressing this reporter. Given the relatively low rate of recombination in cKO mice and the low number of cells infected by the AdV, we injected it in the SVZ of WT and *Fmr1*^{-/-} mice. To normalize the staining, we co-injected an mCherry-expressing AdV, whose staining intensity did not vary in the different conditions (Sup Fig.4). We measured the intensity of GFP dendritic labeling over mCherry intensity in new double-infected GCs in basal and learning conditions. Learning induced a significant increase of the translation reporter's dendritic labeling in WT mice (Fig.5B,C,D). In KO mice, this dendritic labeling is increased in basal conditions as compared to WT mice (Fig.6B,C,E). This increased dendritic labeling of the reporter in WT mice is in line with α CaMKII mRNA being a translational target of FMRP, normally acting as a brake on its translation (5). However, in KO mice, the dendritic

labeling in new GCs was not increased after learning (Fig.6B,E,F) and, surprisingly, was even decreased. This might be the result of a destabilization of the reporter's mRNA and/or its decreased translation as a compensatory mechanism consequent to FMRP's absence.

Collectively, our data suggest that olfactory perceptual learning induces an FMRP-dependent increase of α CaMKII local translation in new neurons and that α CaMKII mRNA could be the main FMRP mRNA target involved in the structural plasticity necessary for olfactory learning.

DISCUSSION

Here, we report that new neurons of the adult OB are structurally plastic in response to olfactory learning and that this plasticity is FMRP and α CaMKII local translation dependent. We thus uncover a new and essential role for dendritic local translation in the structural modifications underlying learning.

Structural plasticity is a determinant of learning, allowing spatial modifications of circuits through gain and loss of synapses (1). We show here that perceptual non-associative learning induces an increase of spine density in the apical dendritic arbor of new OB neurons. These spines are synaptically connected to mitral/tufted cells, the OB principal projection neurons. In recent work using associative olfactory learning, an increase in spine density in new OB neurons was observed (29) not in the apical dendritic arbor but in the deep dendritic domain of new GCs, whose spines receive top-down inputs. This raises the interesting possibility that different types of learning might induce different types of structural plasticity in new OB neurons, linking their optimal connectivity to environmental demands. Perceptual learning also induces a lengthening of dendritic arbors accompanied by an increased complexity of new neurons, thus profoundly modifying their morphology, similar to adult-born hippocampal neurons during spatial learning (30). In most regions of the adult brain, the retraction or growth of dendritic branches are limited and rare, once critical periods of development have ended (31). Adult-born OB neurons are thus endowed with unique structural properties, as also suggested by two-photon live imaging studies (32) and monosynaptic tracing (33). As a consequence, remodeling of their geometry upon integration might allow for profound modifications of their connectivity.

We show that FMRP is necessary for olfactory perceptual learning and associated structural modifications. The cKO mouse we used was previously shown to display reduced neurogenesis in the adult hippocampus and spatial learning defects (23). This learning deficit may also be the consequence of a lack of structural plasticity of new hippocampal neurons due to the loss of FMRP, similar to what we observe in the OB. A role for FMRP in activity-dependent dendritic remodeling is consistent with defective critical periods observed in *Fmr1* KO mice (6, 7) and studies of dFMRP null flies reporting activity-dependent pruning deficits (15). Remarkably, we show that antagonizing mGluR signaling in mutated mice through MPEP injections is sufficient to rescue learning and dendritic remodeling. This

MPEP effect on dendritic arbor is in line with the genetic rescue of critical period plasticity defects observed in the visual cortex of *Fmr1* KO mice with reduced mGluR5 expression (7). MPEP had no effect on spine density or morphology defects, which suggests that the rescue of dendritic arbor plasticity defects induced by MPEP treatment is sufficient to rescue the learning phenotype. However, one of the consequence of the dendritic arbor lengthening induced by MPEP is also a corresponding increase in the number of spines, which might participate in the learning rescue.

FMRP has numerous mRNA targets including α CaMKII (5). In addition to regulating synaptic strength, α CaMKII is an important regulator of structural plasticity (34-36). However, its role in structural plasticity has never been correlated with its dendritic local translation. We thus used a reporter of translation to monitor the *in vivo* dendritic translation of α CaMKII in new neurons upon learning. This type of reporter is commonly used in cultured cells (26, 37) and was used in *drosophila* to report increased local synthesis of α CaMKII following olfactory training (38). Even if our analysis of the reporter in fixed tissue did not allow time-lapse analysis of the staining to clearly ascertain that increased fluorescence of the reporter reflects its increased local translation, our data suggest that olfactory perceptual learning increases α CaMKII dendritic synthesis in new neurons. This is consistent with our morphological data showing that learning induces an increased number of spines in new neurons: these supernumerary spines might trigger an elevated glutamatergic input onto these cells, which could lead to increased glutamate-induced dendritic translation of the reporter, as previously observed in cultured hippocampal neurons (39). Interestingly, dendritic synthesis of the reporter was elevated in new neurons of *Fmr1* KO mice in basal conditions, which is in line with α CaMKII being a translational target of FMRP, repressing its translation in basal conditions (5), through binding to its 3'UTR (28). Remarkably, this elevated synthesis could not be further increased by learning and was even decreased. As FMRP has been described as an mRNA stabilizer (40), its absence could lead to an instability of the reporter leading to a reduced translation, particularly visible in learning conditions. Alternatively, this reduction could be the consequence of a compensatory mechanism in *Fmr1* KO mice unveiled in learning conditions through, for example, recruitment of another RNA-binding protein regulating local translation.

To investigate the function of α CaMKII local translation in new OB cells, we used mice in which α CaMKII 3'UTR was ablated. These mice were previously shown to display deficits in forms of hippocampal and olfactory memory (12, 27). We show here that these mice are also defective for olfactory perceptual learning, which is accompanied by a lack of new OB neuron structural plasticity. As these mice are constitutively mutated in all α CaMKII expressing cells, it is difficult to circumscribe the cell-autonomous effects of the mutation. However, our reporter of translation data strongly suggest that α CaMKII is locally translated in new GCs upon learning, which lends support to the fact that defects in α CaMKII local translation might be directly related to the defective structural plasticity of new GCs. The similarity between the Δ 3'UTR mice and the *Fmr1* mutated mice is striking: they display the same learning deficits accompanied by similar structural plasticity defects, which point to

α CaMKII as the main FMRP target responsible for these phenotypes. However, paradoxically, the Δ 3'UTR mice display reduced α CaMKII local translation (12), whereas *Fmr1* mutated mice display increased α CaMKII local translation, as seen with our reporter of translation. This suggests that what is important for learning and associated structural modifications is not the absolute quantity of locally translated α CaMKII but rather the possibility of a learning-induced increase in α CaMKII local translation. This FMRP-regulated increase might be essential for a function of locally translated α CaMKII in spinogenesis and dendritogenesis, necessary for learning.

Our work reveals a central role for an FMRP-regulated dendritic translation of α CaMKII in the structural plasticity underlying olfactory learning. This is important in a context where impairment of activity-dependent circuit assembly (15, 17) and defects in critical period plasticity (16) are considered at the center of ASD, among which FXS is the primary monogenic cause.

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REFERENCES

1. Caroni P, Donato F, Muller D (2012): Structural plasticity upon learning: regulation and functions. *Nat Rev Neurosci.* 13:478-490.
2. Sutton MA, Schuman EM (2006): Dendritic protein synthesis, synaptic plasticity, and memory. *Cell.* 127:49-58.
3. Chelly J, Mandel JL (2001): Monogenic causes of X-linked mental retardation. *Nat Rev Genet.* 2:669-680.
4. Bhakar AL, Dolen G, Bear MF (2012): The pathophysiology of fragile X (and what it teaches us about synapses). *Annu Rev Neurosci.* 35:417-443.
5. Bassell GJ, Warren ST (2008): Fragile X syndrome: loss of local mRNA regulation alters synaptic development and function. *Neuron.* 60:201-214.
6. Harlow EG, Till SM, Russell TA, Wijetunge LS, Kind P, Contractor A (2010): Critical period plasticity is disrupted in the barrel cortex of FMR1 knockout mice. *Neuron.* 65:385-398.
7. Dolen G, Osterweil E, Rao BS, Smith GB, Auerbach BD, Chattarji S, et al. (2007): Correction of fragile X syndrome in mice. *Neuron.* 56:955-962.
8. Pan F, Aldridge GM, Greenough WT, Gan WB (2010): Dendritic spine instability and insensitivity to modulation by sensory experience in a mouse model of fragile X syndrome. *Proc Natl Acad Sci U S A.* 107:17768-17773.
9. Cruz-Martin A, Crespo M, Portera-Cailliau C (2010): Delayed stabilization of dendritic spines in fragile X mice. *J Neurosci.* 30:7793-7803.
10. Padmashri R, Reiner BC, Suresh A, Spartz E, Dunaevsky A (2013): Altered structural and functional synaptic plasticity with motor skill learning in a mouse model of fragile X syndrome. *J Neurosci.* 33:19715-19723.
11. Scotto-Lomassese S, Nissant A, Mota T, Neant-Fery M, Oostra BA, Greer CA, et al. (2011): Fragile X Mental Retardation Protein Regulates New Neuron Differentiation in the Adult Olfactory Bulb. *J Neurosci.* 31:2205-2215.
12. Neant-Fery M, Peres E, Nasrallah C, Kessner M, Gribaudo S, Greer C, et al. (2012): A role for dendritic translation of CaMKIIalpha mRNA in olfactory plasticity. *PLoS One.* 7:e40133.
13. Gross C, Berry-Kravis EM, Bassell GJ (2012): Therapeutic strategies in fragile X syndrome: dysregulated mGluR signaling and beyond. *Neuropsychopharmacology.* 37:178-195.
14. Jacquemont S, Berry-Kravis E, Hagerman R, von Raison F, Gasparini F, Apostol G, et al. (2014): The challenges of clinical trials in fragile X syndrome. *Psychopharmacology (Berl).* 231:1237-1250.
15. Doll CA, Broadie K (2014): Impaired activity-dependent neural circuit assembly and refinement in autism spectrum disorder genetic models. *Front Cell Neurosci.* 8:30.
16. LeBlanc JJ, Fagiolini M (2011): Autism: a "critical period" disorder? *Neural Plast.* 2011:921680.
17. Doll CA, Broadie K (2015): Activity-dependent FMRP requirements in development of the neural circuitry of learning and memory. *Development.* 142:1346-1356.
18. Mandairon N, Stack C, Kiselycznyk C, Linster C (2006): Broad activation of the olfactory bulb produces long-lasting changes in odor perception. *Proc Natl Acad Sci U S A.* 103:13543-13548.
19. Moreno MM, Linster C, Escanilla O, Sacquet J, Didier A, Mandairon N (2009): Olfactory perceptual learning requires adult neurogenesis. *Proc Natl Acad Sci U S A.* 106:17980-17985.

20. Mientjes EJ, Nieuwenhuizen I, Kirkpatrick L, Zu T, Hoogeveen-Westerveld M, Severijnen L, et al. (2006): The generation of a conditional Fmr1 knock out mouse model to study Fmrp function in vivo. *Neurobiol Dis.* 21:549-555.
21. Schilit Nitenson A, Stackpole EE, Truszkowski TL, Midroit M, Fallon JR, Bath KG (2015): Fragile x mental retardation protein regulates olfactory sensitivity but not odorant discrimination. *Chem Senses.* 40:345-350.
22. Lagace DC, Whitman MC, Noonan MA, Ables JL, DeCarolis NA, Arguello AA, et al. (2007): Dynamic contribution of nestin-expressing stem cells to adult neurogenesis. *J Neurosci.* 27:12623-12629.
23. Guo W, Allan AM, Zong R, Zhang L, Johnson EB, Schaller EG, et al. (2011): Ablation of Fmrp in adult neural stem cells disrupts hippocampus-dependent learning. *Nat Med.* 17:559-565.
24. Bear MF, Huber KM, Warren ST (2004): The mGluR theory of fragile X mental retardation. *Trends Neurosci.* 27:370-377.
25. Miller S, Yasuda M, Coats JK, Jones Y, Martone ME, Mayford M (2002): Disruption of dendritic translation of CaMKIIalpha impairs stabilization of synaptic plasticity and memory consolidation. *Neuron.* 36:507-519.
26. Aakalu G, Smith WB, Nguyen N, Jiang C, Schuman EM (2001): Dynamic visualization of local protein synthesis in hippocampal neurons. *Neuron.* 30:489-502.
27. Mayford M, Baranes D, Podsypanina K, Kandel ER (1996): The 3'-untranslated region of CaMKII alpha is a cis-acting signal for the localization and translation of mRNA in dendrites. *Proc Natl Acad Sci U S A.* 93:13250-13255.
28. Subramanian M, Rage F, Tabet R, Flatter E, Mandel JL, Moine H (2011): G-quadruplex RNA structure as a signal for neurite mRNA targeting. *EMBO Rep.* 12:697-704.
29. Lepousez G, Nissant A, Bryant AK, Gheusi G, Greer CA, Lledo PM (2014): Olfactory learning promotes input-specific synaptic plasticity in adult-born neurons. *Proc Natl Acad Sci U S A.* 111:13984-13989.
30. Tronel S, Fabre A, Charrier V, Oliet SH, Gage FH, Abrous DN (2010): Spatial learning sculpts the dendritic arbor of adult-born hippocampal neurons. *Proc Natl Acad Sci U S A.* 107:7963-7968.
31. Holtmaat A, Svoboda K (2009): Experience-dependent structural synaptic plasticity in the mammalian brain. *Nat Rev Neurosci.* 10:647-658.
32. Mizrahi A (2007): Dendritic development and plasticity of adult-born neurons in the mouse olfactory bulb. *Nat Neurosci.* 10:444-452.
33. Arenkiel BR, Hasegawa H, Yi JJ, Larsen RS, Wallace ML, Philpot BD, et al. (2011): Activity-induced remodeling of olfactory bulb microcircuits revealed by monosynaptic tracing. *PLoS One.* 6:e29423.
34. Okamoto K, Narayanan R, Lee SH, Murata K, Hayashi Y (2007): The role of CaMKII as an F-actin-bundling protein crucial for maintenance of dendritic spine structure. *Proc Natl Acad Sci U S A.* 104:6418-6423.
35. Jourdain P, Fukunaga K, Muller D (2003): Calcium/calmodulin-dependent protein kinase II contributes to activity-dependent filopodia growth and spine formation. *J Neurosci.* 23:10645-10649.
36. Wu GY, Cline HT (1998): Stabilization of dendritic arbor structure in vivo by CaMKII. *Science.* 279:222-226.
37. Banerjee S, Neveu P, Kosik KS (2009): A coordinated local translational control point at the synapse involving relief from silencing and MOV10 degradation. *Neuron.* 64:871-884.
38. Ashraf SI, McLoon AL, Sclarsic SM, Kunes S (2006): Synaptic protein synthesis associated with memory is regulated by the RISC pathway in *Drosophila*. *Cell.* 124:191-205.

39. Gong R, Park CS, Abbassi NR, Tang SJ (2006): Roles of glutamate receptors and the mammalian target of rapamycin (mTOR) signaling pathway in activity-dependent dendritic protein synthesis in hippocampal neurons. *J Biol Chem.* 281:18802-18815.
40. Bagni C, Oostra BA (2013): Fragile X syndrome: From protein function to therapy. *Am J Med Genet A.* 161A:2809-2821.

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Figure legends

Figure 1

A) Design of the olfactory perceptual learning paradigm. Spontaneous discrimination between Limonene +/- was tested through habituation/dishabituation tests before and after an odor enrichment period. Experimental groups were enriched by introducing Lim+ and Lim- into the home cage for 1h periods over 10 days.

B) Before the enrichment period, WT mice cannot discriminate Lim+ from Lim-. They do not spend more time investigating the test odor than the habituation odor ($p=0.735$ with a *Wilcoxon paired test*, $n = 10$).

C,E) After the enrichment period, WT (C) and control cKO mice (E) can discriminate Lim+ from Lim- indicating perceptual learning. They spend significantly more time investigating the test odor than the habituation odor. ((C) $p = 0.004$ $n = 10$ (E) $p = 0.035$ $n = 9$, with *unilateral paired Student's t test*).

D,F) After the enrichment period, *Fmr1* KO (D) and cKO mice (E) cannot discriminate Lim+ from Lim-. They do not spend more time investigating the test odor than the habituation odor ((D) $p=0.213$, (F) $p = 0.674$ with a *Wilcoxon paired test*, $n = 10$).

Data are expressed as mean values \pm SEM. Hab4: investigation time of the habituation odor during the 4th trial of habituation. Test: investigation time of test odor (the other odorant of the pair).

Figure 2

A) Time-line of the experiment. Adult mice (cKO: Nestin::CreERT2/*Fmr1*^{fllox/y}) were injected with tamoxifen to induce *Fmr1* mutation in Nestin positive progenitor cells and their progeny. Control mice (control cKO) were control littermates lacking the Cre allele. A GFP-expressing lentivirus was injected into their sub-ventricular zone (SVZ). Mice were exposed to the enrichment period during the integration of the labeled new neurons in the olfactory bulb (OB) and tested for perceptual learning before sacrifice.

B) Scheme of a sagittal section of the mouse forebrain. The SVZ of the lateral ventricle (LV) continuously produces new neurons, which migrate along the rostral migratory stream (RMS) and differentiate as interneurons in the olfactory bulb (OB). Subpopulations of young neurons can be labeled through stereotaxic injections of GFP-expressing viruses into the SVZ, which allow their morphological analysis.

C) Scheme of a newly-formed granule cell (GC) of the OB. GCs are anaxonic GABAergic interneurons with a long apical dendrite branching out into a dendritic arbor.

D,E) Representative binarized pictures of the dendritic arbors of GFP labeled new GCs in basal conditions or after perceptual learning in control cKO mice (D) and in cKO mice (F).

Scale Bars: 40 μ m

F) Dendritic arbor length of new GCs in control cKO or cKO mice in basal or learning conditions. Perceptual learning induces a lengthening of the dendritic arbor of new neurons in control cKO mice ($p=0.014$) but not in cKO mice ($p=0.99$). (Two-way ANOVA: Genotype effect, $F(1,47) = 1.247$, $p=0.271$; Learning effect, $F(1,47) = 4.43$, $p=0.039$, Genotype - Learning interaction, $F(1,47) = 6.13$, $p=0.0170$, followed by LSD post hoc test, $n = 11-14-13-13$)

G) Sholl analysis of the dendritic complexity of new GCs in control cKO mice. The origin of the concentric radii was set at the first branching point of the apical dendrite. New GCs display significantly increased complexity after learning compared to basal conditions (repeated-measures ANOVA with two factors. $F_{(1,25)} = 6.92$, $p < 0.001$, followed by Bonferroni post hoc test, $n = 13-17$).

H) Sholl analysis of the dendritic complexity of new GCs in cKO mice. New GCs display similar complexity in basal or learning conditions (repeated-measures ANOVA with two factors. $F_{(1,24)} = 1.324$, $p = 0.261$ $n = 12-15$).

Figure 3

A-A") Representative pictures showing spines of the dendritic arbor of GFP-labeled new GCs in control cKO or cKO mice in basal or learning conditions.

Scale Bars: 5 μ m

B) Spine density in the dendritic arbors of new GCs in control cKO and cKO mice in basal or learning conditions. Perceptual learning induces an increase in spine density in control cKO mice ($p = 0.001$) but not in cKO mice ($p = 0.941$) (Two-way ANOVA: Genotype effect, $F(1,50) = 0.791$, $p=0.378$; Learning effect, $F(1,47) = 4.27$, $p=0.045$, Genotype - Learning interaction, $F(1,50) = 6.13$, $p=0.023$ followed by LSD post hoc test, $n = 14-14-10-17$).

C) Cumulative frequency distribution of spine head diameters of new neurons from control cKO and cKO mice in basal condition. The absence of FMRP in new neurons from cKO mice induces a significant shift towards smaller diameters, as compared to control cKO mice (Kolmogorov-Smirnov test, $p < 0.0001$).

D,E) Cumulative frequency distribution of spine head diameters of new neurons from control cKO (D) and cKO (E) mice with or without learning. Learning induces a significant shift towards larger diameters in control cKO mice (Kolmogorov-Smirnov test, $p=0.009$), which is not significant in cKO mice (Kolmogorov-Smirnov test, $p= 0.0513$).

Figure 4

A) Post-enrichment discrimination test for Lim+ and Lim- in cKO mice injected with saline during the enrichment period of the perceptual learning. They cannot discriminate Lim+ from Lim- (paired Student's t test $p = 0.619$, $n=11$).

B) Post-enrichment discrimination test for Lim+ and Lim- in cKO mice injected with MPEP during the enrichment period of the perceptual learning. They spend significantly more time investigating the

test odor than the habituation odor, indicating perceptual learning (significant difference between investigation times for Hab4 and Test (*paired Student's t test*, $p = 0.001$, $n = 10$)).

C) Representative binarized pictures of the dendritic arbors of GFP labeled new GCs after learning in saline or MPEP-injected cKO mice

Scale Bar: 40 μm

D) Dendritic arbor length of new GCs in cKO mice in learning conditions after injections with saline or MPEP. FMRP⁺ neurons from MPEP-treated cKO mice display longer dendritic arbor than FMRP⁺ neurons from saline-treated cKO mice (*unpaired Student's t test*, $p = 0.37$, $n = 13, 14$).

E) Sholl analysis of the dendritic complexity of new GCs in cKO mice injected with MPEP or saline. New GCs display increased complexity in MPEP-injected cKO mice, as compared to saline-injected cKO mice (*repeated-measures ANOVA with two factors*. $F(1, 23) = 4.692$, $p = 0.041$, $n = 14-12$)

F) Spine density in the dendritic arbors of new GCs in cKO mice injected with saline or MPEP during the learning period: the MPEP treatment does not induce any change (*unpaired Student's t test*, $p = 0.37$, $n = 14, 10$).

G) Cumulative frequency distribution of spine head diameters of new neurons from cKO mice injected with saline or MPEP during the learning period: the MPEP treatment does not induce any change (*Kolmogorov-Smirnov test*, $p = 0.1772$).

Figure 5

A,B) Post-enrichment discrimination test for Lim⁺ and Lim⁻ in WT mice (A) or mice ablated for $\alpha\text{CaMKII } 3' \text{UTR}$ ($\Delta 3' \text{UTR}$)(B). WT mice can discriminate the two odorants (significant difference between hab4 and test, ($p = 0.036$ with a Wilcoxon paired test, $n = 8$), whereas $\Delta 3' \text{UTR}$ mice cannot ($p = 0.203$ with a Wilcoxon paired test, $n = 10$).

C,D) Representative binarized pictures of the dendritic arbors of GFP labeled new GCs in WT (C) and ($\Delta 3' \text{UTR}$) mice.

Scale Bar: 40 μm

E) Dendritic arbor length of new GCs in WT and $\Delta 3' \text{UTR}$ mice in basal or learning conditions. Perceptual learning induces a lengthening of neurons in WT mice ($p = 0.018$) but not in $\Delta 3' \text{UTR}$ mice ($p = 0.99$). (*Two-way ANOVA: Genotype effect*, $F(1, 55) = 7.748$, $p = 0.007$; *Learning effect*, $F(1, 55) = 7.53$, $p = 0.008$, *Genotype - Learning interaction*, $F(1, 55) = 2.579$, $p = 0.114$, followed by *LSD post hoc test*, $n = 14-16-16-13$).

F) Sholl analysis of the dendritic complexity of new GCs in $\Delta 3' \text{UTR}$ mice. New GCs display similar complexity in basal or learning conditions (*repeated-measures ANOVA* $F(1, 21) = 0.258$, $p = 0.616$ $n = 13-10$).

G-G") Representative pictures showing spines of the dendritic arbor of GFP-labeled new GCs from WT and $\Delta 3' \text{UTR}$ mice in basal or learning conditions.

Scale Bars: 5 μ m

H) Spine density in the dendritic arbors of new GCs in WT or $\Delta 3'$ UTR mice in basal or learning conditions. Perceptual learning induces an increase of spine density in WT mice ($p=0.008$) but not in $\Delta 3'$ UTR mice ($p=0.865$). (Two-way ANOVA: Genotype effect, $F(1,63) = 8.13$, $p=0.006$; Learning effect, $F(1,55) = 0.955$, $p=0.332$, Genotype - Learning interaction, $F(1,63) = 3.961$, $p=0.05$, followed by LSD post hoc test, $n = 14-21-17-17$).

I,J) Cumulative frequency distribution of spine head diameters of new neurons from WT (I) and $\Delta 3'$ UTR (J) mice with or without learning. Learning induces a significant shift towards larger diameters in WT mice (Kolmogorov-Smirnov test, $p < 0.001$) but not in $\Delta 3'$ UTR mice (Kolmogorov-Smirnov test, $p > 0.05$).

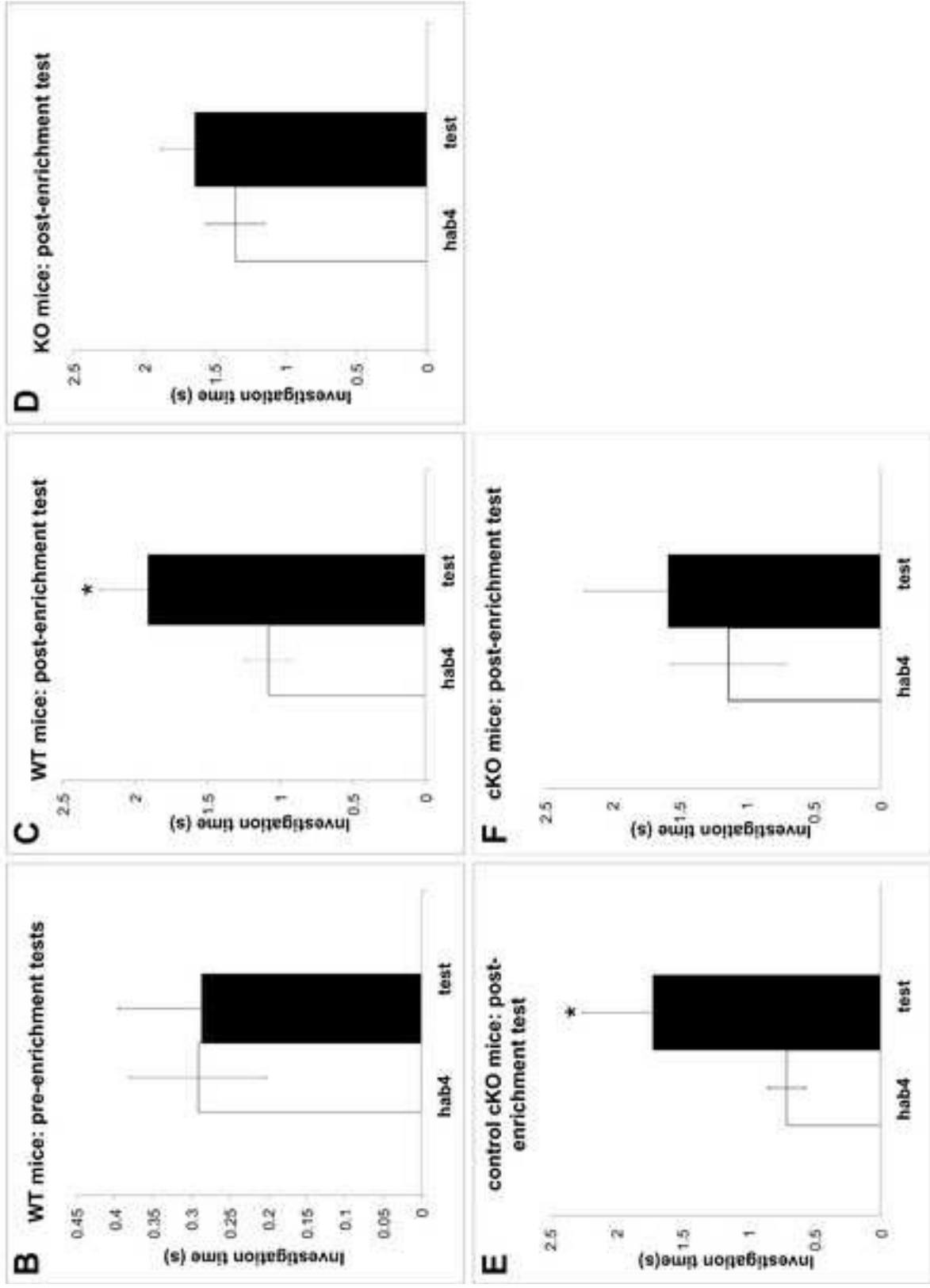
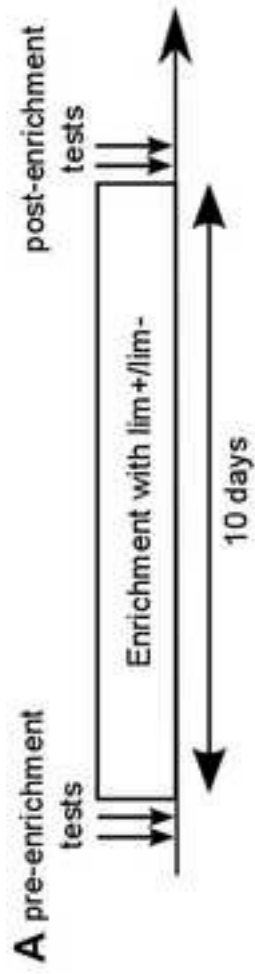
Figure 6

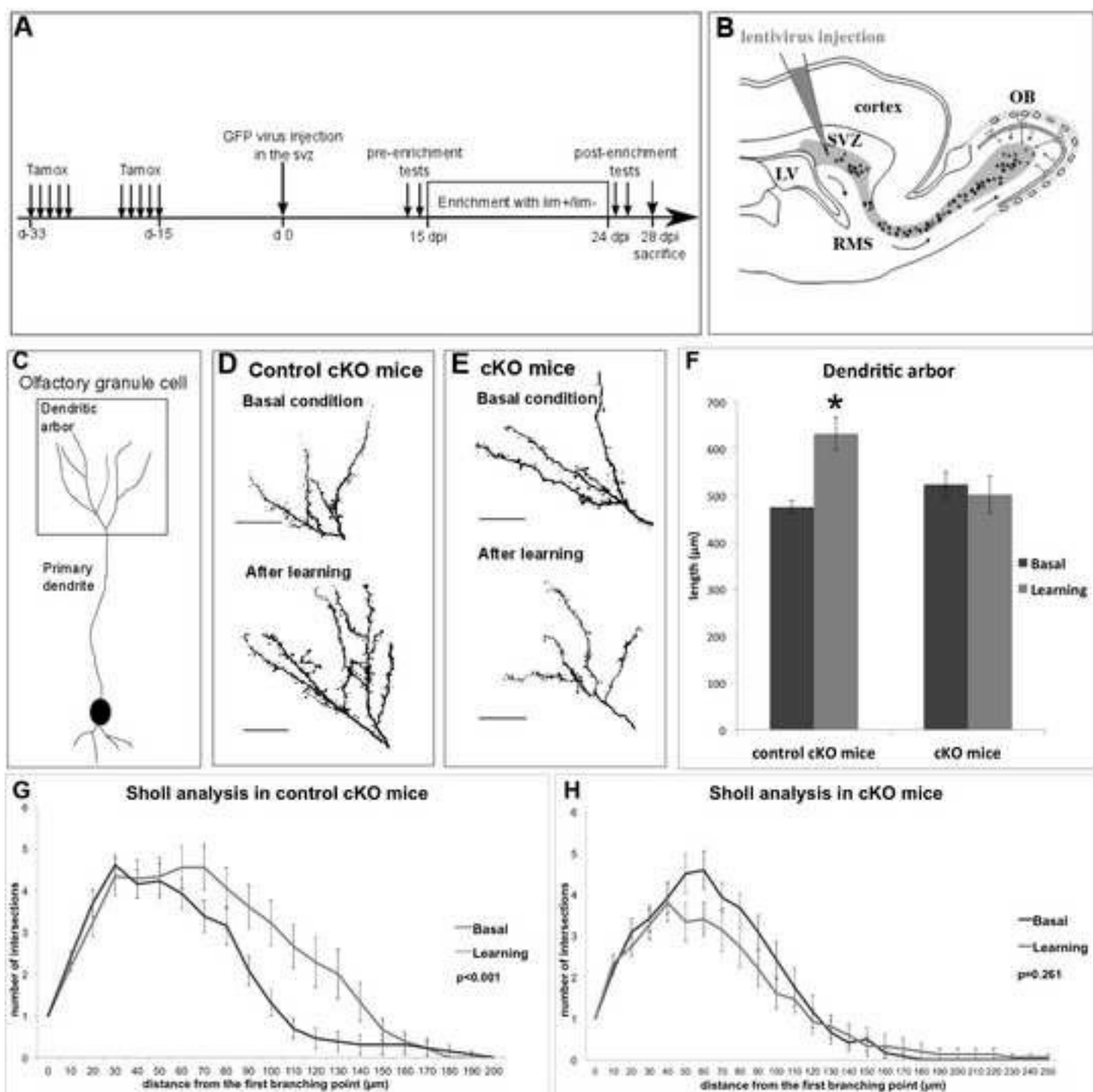
A) Scheme of the α CaMKII reporter of translation. Myr-d-eGFP: myristoylated destabilized enhanced GFP. UTR: untranslated region. PA: polyadenylation sequence.

B) Ratio of the dendritic labeling of new GCs by the GFP translation reporter over an mCherry normalizer in WT and *Fmr1*^{-/-} mice in basal or learning conditions (Two-way ANOVA: Genotype effect, $F(1,68) = 9.39$, $p=0.247$; Learning effect, $F(1,68) = 1.36$, $p=0.257$, Genotype - Learning interaction, $F(1,68) = 1.30$, $p < 0.0001$ followed by LSD post hoc test, $n = 28-44-38-34$). Learning induces an increase in the dendritic labeling of new GCs in WT mice ($p < 0.01$). In basal conditions, new GCs from *Fmr1* KO mice display increased dendritic labeling as compared to WT mice ($p=0.012$). Learning does not increase this labeling and even reduces it ($p=0.031$).

C-F') Representative pictures of the dendritic labeling of new GCs doubly infected with the Myr-d-eGFP reporter of translation (C-F) and an mCherry normalizer (C'-F') in WT and *Fmr1* KO mice in basal or learning conditions.

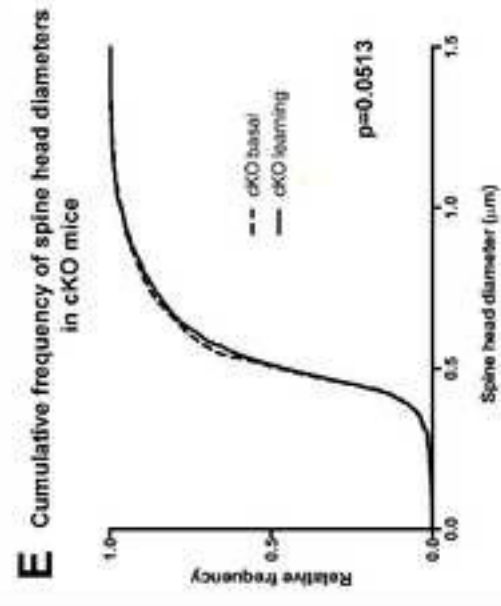
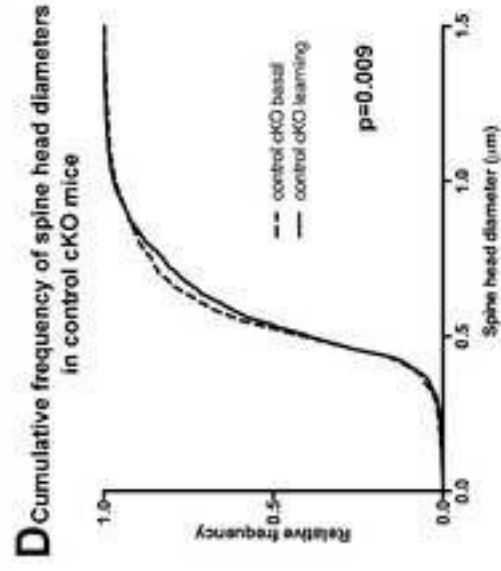
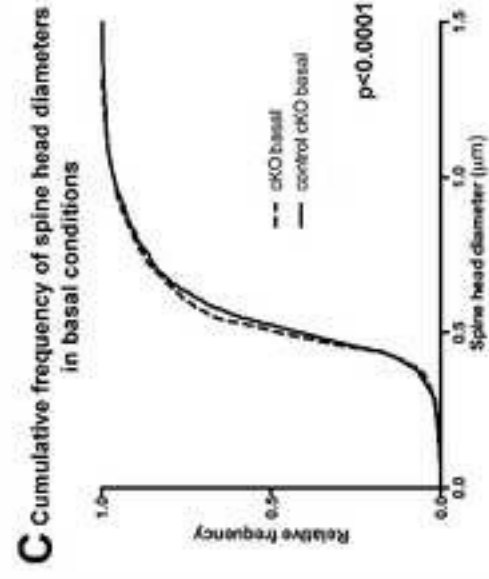
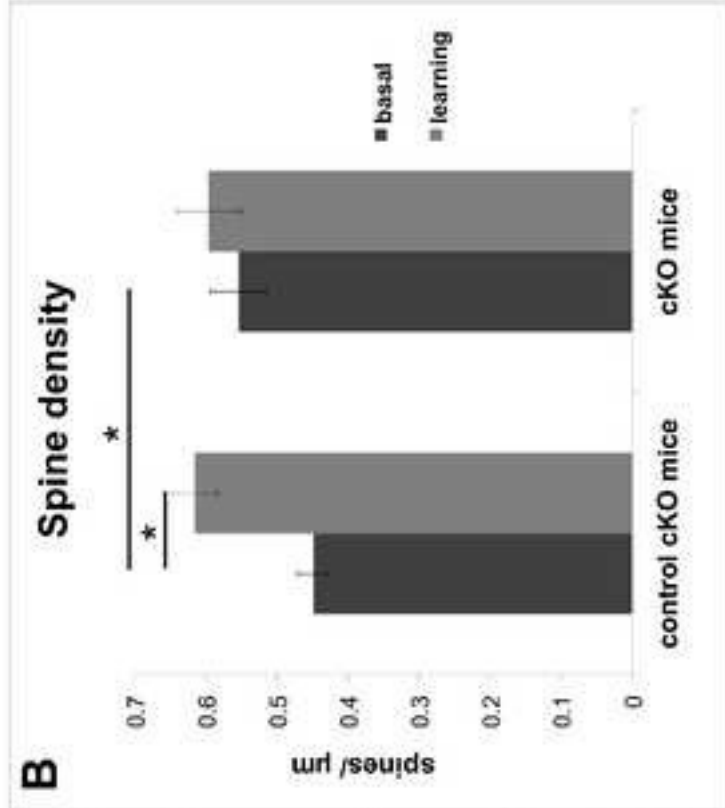
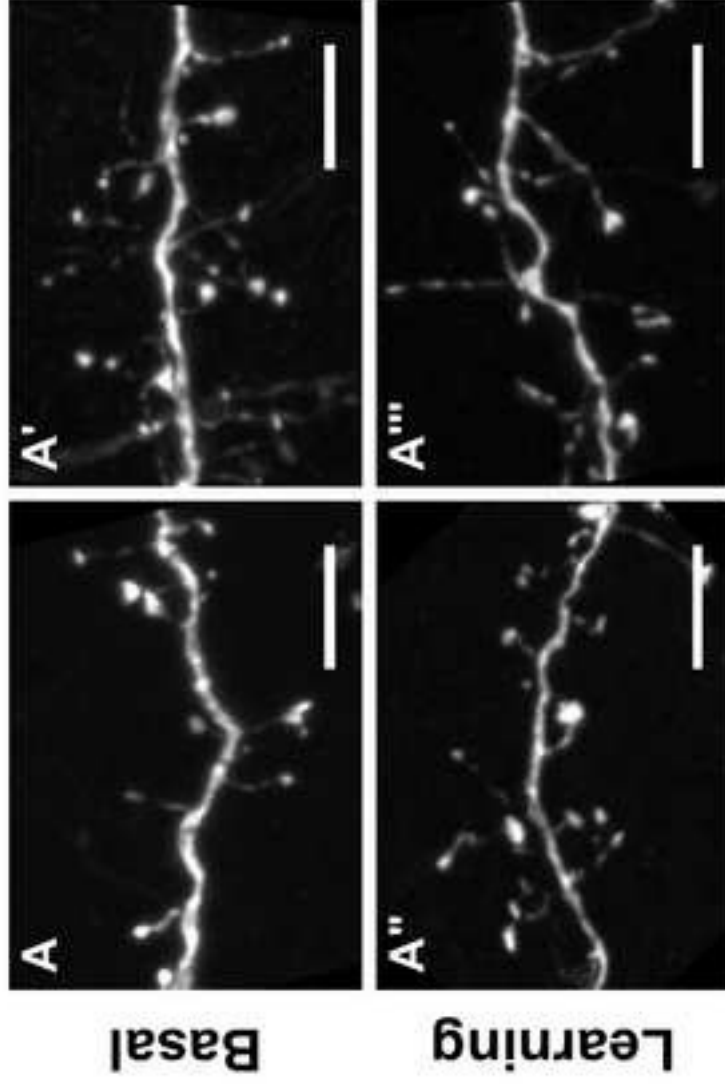
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cKO

Control cKO



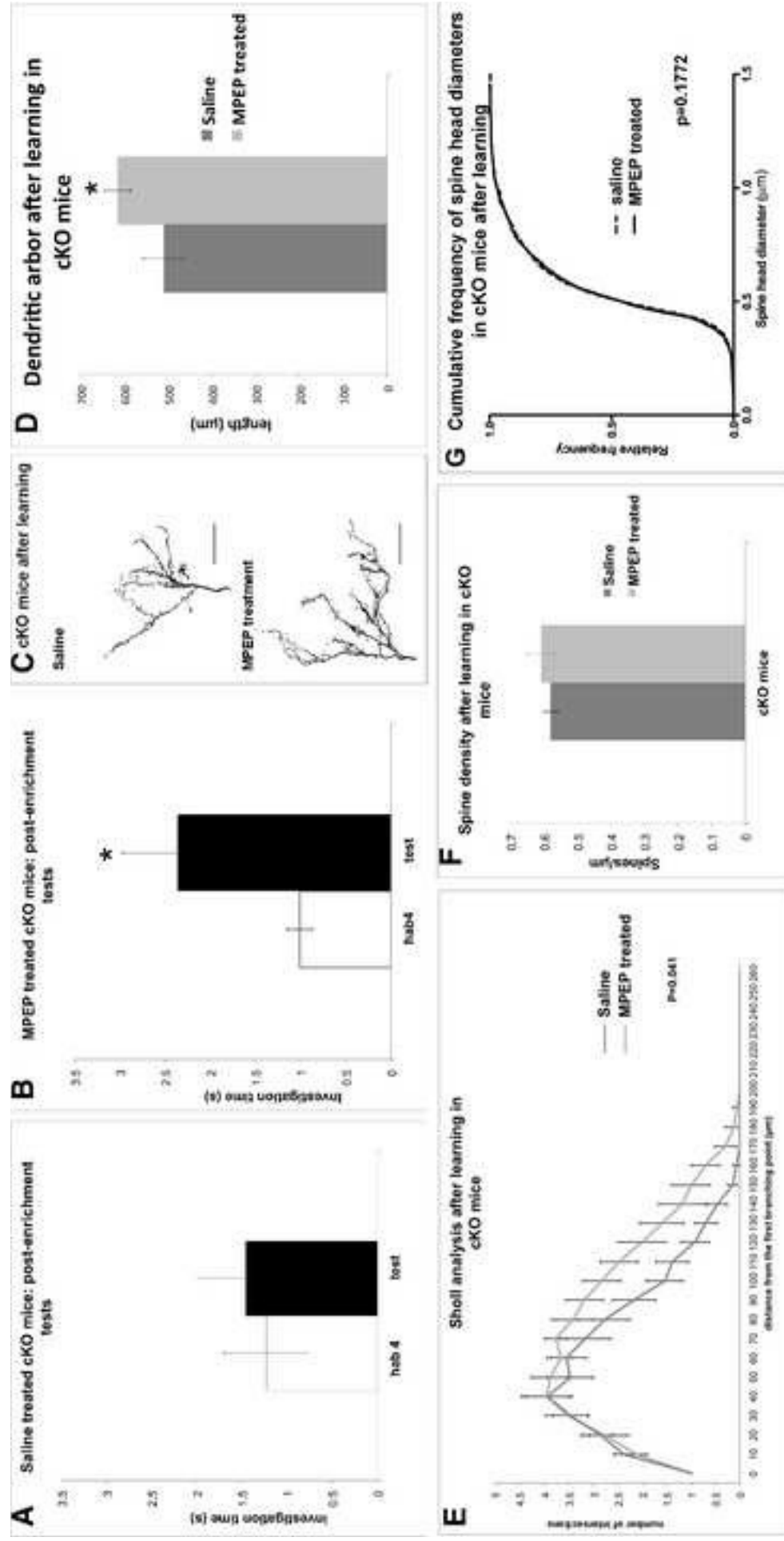


Figure 4

