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Subregion-specific dendritic spine abnormalities in the hippocampus of *Fmr1* KO mice

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ABSTRACT

Fragile X syndrome (FXS) is the most common inherited form of mental retardation and is caused by the lack of fragile X mental retardation protein (FMRP). In the brain, spine abnormalities have been reported in both patients with FXS and *Fmr1* knockout mice. This altered spine morphology has been linked to disturbed synaptic transmission related to altered signaling in the excitatory metabotropic glutamate receptor 5 (mGluR5) pathway. We investigated hippocampal protrusion morphology in adult *Fmr1* knockout mice. Our results show a hippocampal CA1-specific altered protrusion phenotype, which was absent in the CA3 region of the hippocampus. This suggests a subregion-specific function of FMRP in synaptic plasticity in the brain.

1. Introduction

Excitatory synapses on pyramidal neurons in the cerebral cortex and hippocampus terminate at spines, which are short protrusions joined to the dendrite. Their morphology and density are abnormal in several mental retardation syndromes, including fragile X syndrome (FXS) (Fiala, Spacek, & Harris, 2002; Kaufmann & Moser, 2000; Purpura, 1974). FXS is the most common inherited form of intellectual disability and is caused by lack of fragile X mental retardation protein (FMRP). Lack of FMRP is a consequence of a trinucleotide (CGG) repeat expansion in the 5′ UTR of the *FMR1* gene. In unaffected individuals, the CGG repeat in the *FMR1* gene ranges between 5 and 55, while in patients with FXS the repeat exceeds 200 units, which results in hypermethylation of the promoter region. This process leads to silencing of the *FMR1* gene, resulting in lack of FMRP.

FMRP is an RNA-binding protein and plays a crucial role in controlling mRNA translation at the synapse after specific stimulation. It has been shown that FMRP is synthesized after stimulation of group I metabotropic glutamate receptor (mGluR) in synaptosomes (Weiler et al., 1997), and that it acts as a translational repressor in rabbit reticulocyte lysate (Laggerbauer, Ostareck, Keidel, Ostareck-Lederer, & Fischer, 2001). Accordingly, lack of FMRP results in excessive translation of several target mRNAs, including *Map1b*, *SAPAP3* and *PIKE*-S, that are involved in synaptic plasticity (Lu et al., 2004; Narayanan et al., 2008; Sharma et al., 2010). *Fmr1* knockout (KO) mice exhibit enhanced group I mGluR long-term depression (LTD) and it was shown that group I mGluR-LTD is protein-synthesis dependent in hippocampus of wild-type mice, while in hippocampus of *Fmr1* KO mice mGluR-LTD persists in the presence of protein-synthesis inhibitors (Huber, Gallagher, Warren, & Bear, 2002). This led to the hypothesis that owing to the absence of FMRP, proteins that are important for the maintenance of mGluR-LTD are abundantly present in hippocampal slices of *Fmr1* KO mice.

In addition, lack of FMRP has an effect on the protrusion morphology. Neurons of patients with FXS and *Fmr1* KO mice show an increased density of dendritic protrusions, which are on average also longer and thinner, referred as immature protrusions (Comery et al., 1997; Hinton, Brown, Wisniewski, & Rudelli, 1991; Irwin et al., 2002, 2001; McKinney, Grossman, Elisseou, & Greenough, 2005; Rudelli et al., 1985). It has been suggested that this phenotype results in weakening of the synapse through increased levels of AMPA receptor internalization at the postsynaptic membrane (Nakamoto et al., 2007). Pharmacological studies using wild-type neurons showed that prolonged group I mGluR stimulation also results in immature protrusions, resembling the protrusion phenotype in FXS (Vanderklish & Edelman, 2002).

In this report, the protrusion morphology in adult *Fmr1* KO mice in two subregions of the hippocampus, CA1 and CA3, has been studied.

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2. Materials and methods

2.1. Animals

Fmr1 KO mice are generated in our lab as described previously (referred as *Fmr1* KO2 in Mientjes et al., 2006). The *Fmr1* KO line was completely backcrossed in C57Bl/6J mice (>10 generations). Both the *Fmr1* KO mice and the wild-type mice were housed under standard conditions. All experiments were carried out with permission of the local ethical committee. For all experiments, we used male *Fmr1* KO and wild-type littermates.

2.2. Perfusion and tissue preparation

Brains were isolated as described in Shen, Sesack, Toda, and Kalivas (2008). Briefly, mice were deeply anesthetized with ketamine HCl (100 mg/kg,) and xylazine (20 mg/kg) i.p. injected and perfused transcardially with 0.1 M PBS (10 ml), followed by 15 ml 1.5% paraformaldehyde (PFA) in 0.1 M PBS. It has been shown that 1.5% fixation of PFA is important for the dye to fill up small protrusions (Kim, Dai, McAtee, Vicini, & Bregman, 2007). Brains were removed and post-fixed overnight in 1.5% PFA. The next day, brains were coronally (150 μm thick) sectioned using a vibratome at room temperature. Sections were collected in PBS and on the same day diOlistically labeled.

2.3. DiOlistic labeling

Tungsten particles (1.3 μm diameter, Biorad) were coated with 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI, Sigma) as previously described (Wu, Reilly, Young, Morrison, & Bloom, 2004). From each mouse, 6–10 hippocampi slices were isolated. The tungsten particles were delivered into the hippocampi using a Helios Gene Gun system (Biorad) at 180 psi. Prior to delivery, a polycarbonate filter with a 3 μm pore size (Falcon 3092; BD Biosciences, Franklin Lakes, NJ) was placed on top of the tissue to filter large clusters of particles. The hippocampi slices were stored at 4 °C for 48 h to allow the dye to diffuse throughout the dendrites and axons. Subsequently, the slices were post-fixed with 4% PFA for 1 h and briefly washed with PBS containing Hoechst. Finally, the slices were mounted onto slides with Mowiol (Mowiol 4-88, Hoechst).

2.4. Confocal imaging

To image the labeled pyramidal neurons in the hippocampal slices, we used a Leica confocal microscope (Leica TCS SP5 Confocal). The fluorescent DiI was visualized using a 543 nm laser line. Images of neurons were acquired using a 63× oil immersion objective, and the image size was 248 μm × 248 μm. The localization of CA1 and CA3 regions was visualized by Hoechst staining (Fig. 1a). The neurons were scanned with an interval of 0.5 μm along the *Z*-axis. The investigator was always blind to the genotype of the mice.

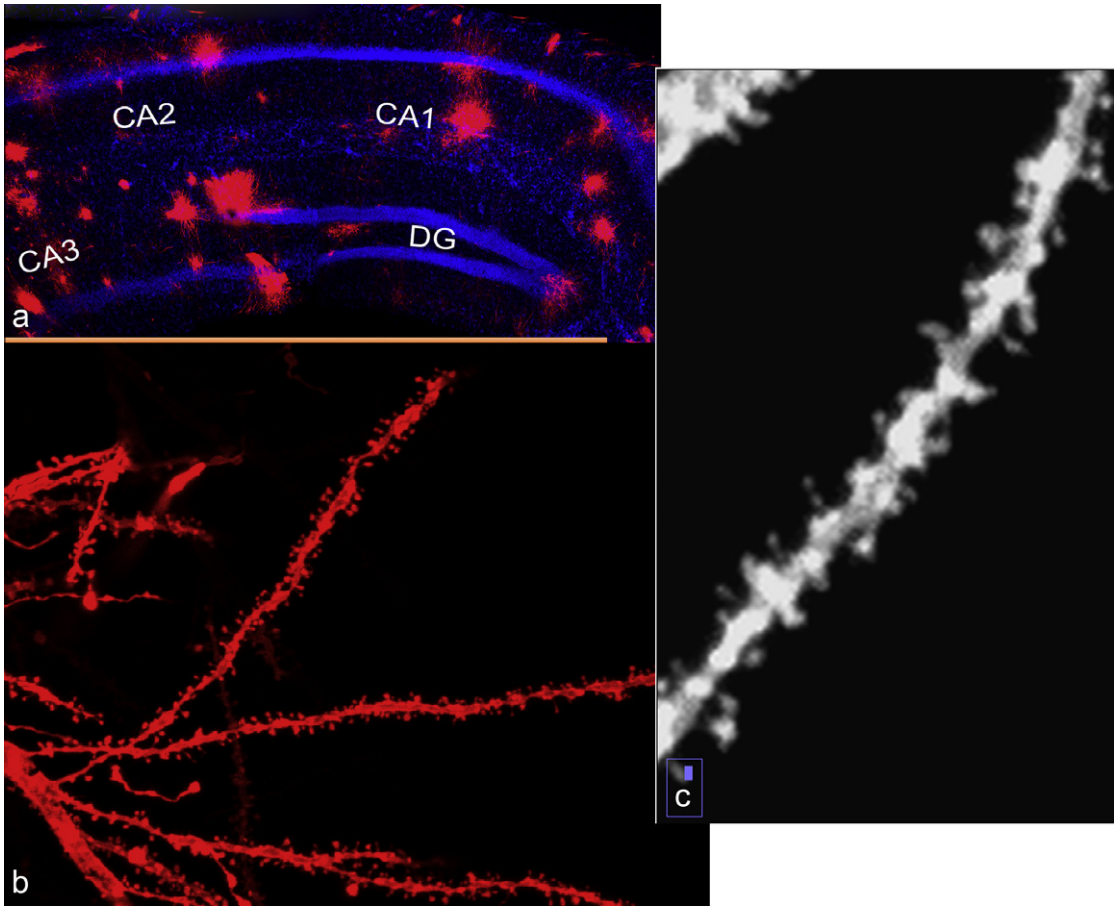

Fig. 1. Representative pictures of diOlistic labeled hippocampal slices. (a) Low magnification of a diOlistic labeled hippocampal slice, including CA1, CA2, CA3 and dentate gyrus (DG) regions. (b) DiOlisitic labeled neuron shows clear dendritic branches with protrusions. (c) Overview of a dendritic branch (zoomed in) which is used to measure the protrusion morphology.