Neuroscience

FMRP regulates neuronal migration via MAP1B

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Abstract

The Fragile X Syndrome (FXS) is the most common form of inherited intellectual disability, and the first monogenic cause of Autism Spectrum Disorder. FXS is caused by the absence of the RNA-binding protein FMRP (Fragile X Messenger Ribonucleoprotein).

Neuronal migration is an essential step of brain development allowing displacement of neurons from their germinal niches to their final integration site. The precise role of FMRP in neuronal migration remains mostly unexplored. We studied the consequences of FMRP absence on migration, by live-imaging neurons of the postnatal Rostral Migratory Stream (RMS).

In *Fmr1-null* RMS, neurons exhibit a slowed-down migration and an impaired trajectory, associated with defects of their centrosomal movement. RNA-interference-induced knockdown of *Fmr1* shows that these migratory defects are cell-autonomous. Finally, the FMRP mRNA target involved in these defects is MAP1B (Microtubule-Associated Protein 1B), since its knockdown rescues most migratory defects.

Our results thus unveil a new neurodevelopmental role of FMRP, as a crucial actor of neuronal migration linked to MAP1B, potentially important for the understanding of FXS pathophysiology.

eLife assessment

This study presents a **valuable** finding on the role of fragile X messenger ribonucleoprotein (FMRP) and its target the microtubule associated protein 18 (MAP1B) in postnatal tangential migration of neuroblasts. The study combined molecular genetics (FMRP knock-out mice) with acute inactivation of FRMP and MAP1B to conclusively support the notion that FMRP-dependent regulation of MAP1B is necessary for proper neuronal migration toward the olfactory bulb using the rostral migratory stream. The evidence supporting the claims of the authors is **solid**, although inclusion of additional controls for experiments and/or more rigorous description of the finding and conclusions would have strengthened the study. The work improves our knowledge of the molecular mechanisms that control how neurons migrate in the brain to reach their final destinations and confirms that cytoskeleton regulators are key players in this process. will be of interest to developmental neurobiologists.

Introduction

The Fragile X syndrome (FXS) is the most common cause of inherited intellectual disability and a leading cause of autism spectrum disorder. FXS is due to the silencing of the gene *FMR1* and loss of the encoded protein, FMRP (Fragile X Messenger Ribonucleoprotein) (Davis & Broadie, 2017). FMRP is an ubiquitous RNA-binding protein, with high level of expression in the central nervous system (Gholizadeh *et al*, 2015). It is a general regulator of RNA metabolism and especially of mRNA local translation in neurons (Banerjee *et al*, 2018). Its cognate mRNA targets are numerous and diverse, including mRNAs encoding cytoskeletal proteins like MAP1B (Microtubule-Associated Protein 1B) (Ascano *et al*, 2012)(Brown *et al*, 2001)(Darnell *et al*, 2001)(Maurin *et al*, 2018). *Fmr1*-null mice are the murine model of FXS and have allowed characterization of neurodevelopmental and plasticity defects consecutive to the absence of FMRP.

Neuronal migration is a crucial step for the establishment of neuronal circuitry, allowing the displacement of neurons from their site of birth to their final destination of differentiation. Migration defects lead to severe brain pathologies like lissencephaly and cortical heterotopia and might be involved in psychiatric disorders (Romero *et al*, 2018). Interestingly, migration in the human infant brain appears to be even more extended than anticipated from the rodent data (Sanai *et al*, 2011)(Paredes *et al*, 2016). In addition, periventricular heterotopia was described in two FXS patients, suggesting a possible role for FMRP in migration (Moro *et al*, 2006). However, the question of potential migration defects in FXS remains mostly unexplored. In *Fmr1*-null mice, radially migrating embryonic glutamatergic cortical neurons display a defect in the multipolar to bipolar transition (La Fata *et al*, 2014). Additionally, FMRP overexpression of knockdown leads to misplacement of cortical glutamatergic neurons, also suggesting its role in radial embryonic migration (Wu *et al*, 2019). However, to our knowledge, tangential neuronal migration in the absence of FMRP has not been studied so far and, more generally, the dynamics of mutated *Fmr1* neurons have never been analyzed in detail.

Here, using the postnatal rostral migratory stream (RMS) as a migration model as in (Stoufflet *et al*, 2020), we show entirely novel migratory defects induced by the absence of FMRP and the causal role of its mRNA target MAP1B.

Results

FMRP is expressed in migrating neurons of the postnatal RMS

A massive number of neuroblasts are constantly produced in the ventricular/subventricular zone (V/SVZ) and migrate over a long distance along the RMS to the olfactory bulb (OB) (Lim & Alvarez-Buylla, 2016)(Fig. 1A). They display a cyclic saltatory mode of migration, in which the nucleus and centrosome move forward in a “two-stroke” cycle (Bellion *et al*, 2005). The centrosome moves first within a swelling in the leading process, which we call centrokinesis (CK) and the nucleus follows subsequently (nucleokinesis, NK)(Fig. 1B). The neurons then pause and the cycle can reinitiate.

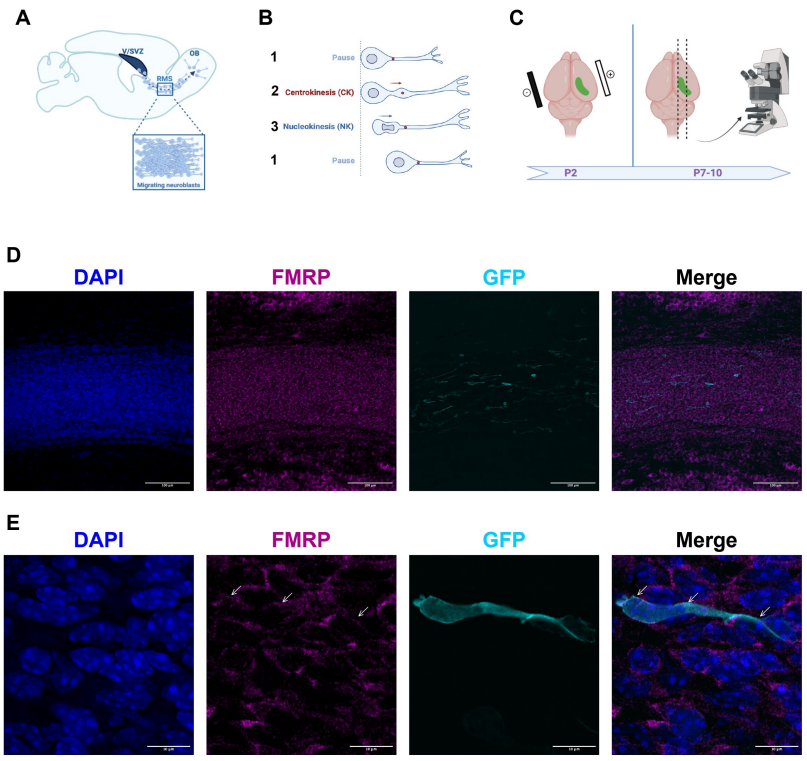

FIGURE 1.

FMRP is expressed in migrating neurons of the murine postnatal RMS.

(A) Scheme of a sagittal section of the postnatal RMS connecting the V/SVZ to the OB. V/SVZ, ventricular/sub-ventricular zone; OB, olfactory bulb; RMS, rostral migratory stream. The inset shows the high density of homotypically migrating neurons in the RMS. (B) Representation of neuroblasts' cyclic saltatory migration. 1. The neuron is in pause. 2. The leading process (LP) extends, and the centrosome moves within a swelling in the LP. 3. The nucleus moves forward. CK, centrokinesis; NK, nucleokinesis. (C) Scheme of the experimental procedure. 2-day old neonates are intraventricularly electroporated with a GFP-expressing plasmid to label a cohort of migrating neurons that can be subsequently visualized in fixed or acute sections of the RMS. (D) Immunohistochemistry of the RMS showing FMRP expression (magenta) along the stream, in which a cohort of GFP-positive neurons (cyan) are visible. Scale bar: 100 µm. (E) Immunohistochemistry of a GFP-positive RMS neuron (cyan) showing FMRP subcellular expression (magenta). The GFP-positive neuron displays a cytoplasmic expression of FMRP around the nucleus (indicated by white arrows), along the leading process and in the growth cone. The surrounding GFP-negative neurons express FMRP as well, according to the same pattern. Scale bar: 10 µm.

After an *in vivo* intraventricular electroporation of a GFP-expressing plasmid in neonate mice, a cohort of GFP-positive neurons can be visualized in the RMS a few days later (Fig. 1.C).

FMRP is expressed in most neurons of the brain (Gholizadeh *et al*, 2015). Accordingly, immunostaining for FMRP reveals that FMRP is strongly expressed in the RMS, where most neurons appear labeled. In individual GFP-positive neurons, FMRP labeling appears as a discrete and punctate staining visible mainly in the cytoplasm both at the rear of the neuron and in the leading process and growth cone (Fig. 1.D,E).

FMRP cell-autonomously regulates neuronal migration

To investigate the involvement of FMRP in RMS migration, we used the *Fmr1-null* mouse line (Fmr1 knockout mice: a model to study fragile X mental retardation. The Dutch-Belgian Fragile X Consortium, 1994). Time-lapse imaging of GFP positive neurons was performed in the control and *Fmr1-null* RMS (movies S.1 and S.2). *Fmr1-null* neurons display a slowed-down migration, an increased pausing time, a more sinuous trajectory, and a defective directionality (Fig. 2.A-D). Additionally, the NK is less frequent and the mean distance per NK is reduced (Fig. 2.E,F).

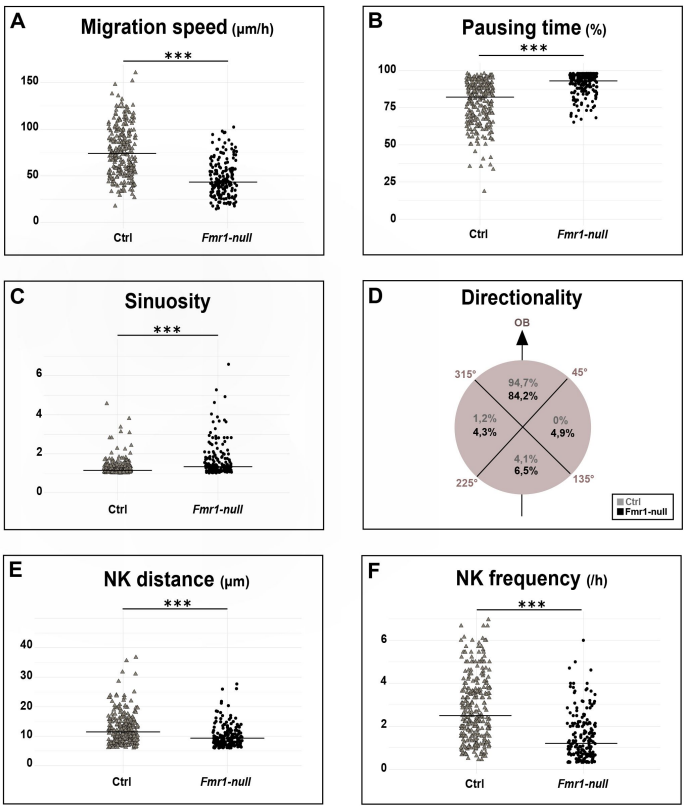

FIGURE 2.

Migration defects in Fmr1-null neurons.

(A) Migration speed of control (Ctrl) and Fmr1-null neurons. Ctrl: 76 ± 2 µm/h; Fmr1-null: 47 ± 1 µm/h (Mann-Whitney test, p-value < 0.001). (B) Percentage of pausing time of control and Fmr1-null neurons. Ctrl: 78 %; Fmr1-null: 91 % (Mann-Whitney test, p-value < 0.001). (C) Sinuosity index of control and Fmr1-null neurons. Ctrl: 1.32 ± 0.04; Fmr1-null: 1.93 ± 0.16 (Mann-Whitney test, p-value < 0.001). (D) Migration directionality radar represented in four spatial dials. Percentage of cells migrating in each spatial direction in control and Fmr1-null neurons, relatively to the vector going straight from the SVZ to the OB. (Fisher's Exact test, p-value < 0.001). (E) NK mean distance of control and Fmr1-null neurons. Ctrl: 12.5 ± 0.3 µm; Fmr1-null: 10.9 ± 0.8 µm (Mann-Whitney test, p-value < 0.001). (F) NK frequency of control and Fmr1-null neurons. Ctrl: 2.9 ± 0.1 NK/h; Fmr1-null: 1.5 ± 0.1 NK/h (Mann-Whitney test, p-value < 0.001). The black line represents the median. Ctrl: N = 3, n = 275; Fmr1-null: N = 3, n = 184. ***p-value < 0.001.

Given the crucial role of the centrosome in neuronal migration (Higginbotham & Gleeson, 2007), we analyzed its dynamics by performing co-electroporation of GFP and Centrine-RFP in *Fmr1-null* and control neonate mice to co-label migrating neurons and their centrosome (Fig. 3.A). The CK is slowed-down and less frequent in *Fmr1-null* neurons, as compared to controls (Fig. 3.B,C). A CK leading to a subsequent NK was defined as an efficient CK, as opposed to a CK not leading to an NK. CK efficiency is reduced in *Fmr1-null* neurons as compared to controls (Fig. 3.D).

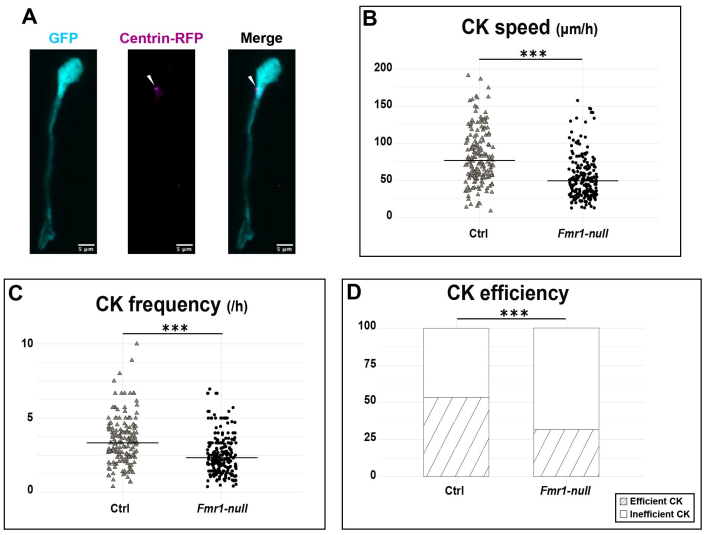

FIGURE 3.

CK defects in Fmr1-null neurons.

(A) Illustration of an RMS-migrating neuron (cyan) co-injected with centrin-RFP (magenta). Scale bar: 5 µm. (B) CK speed of control and Fmr1-null neurons. Ctrl: 82 ± 3 µm/h; Fmr1-null: 54 ± 2 µm/h (Mann-Whitney test, p-value < 0.001). (C) CK frequency of control and Fmr1-null neurons. Ctrl: 3.5 ± 0.1 CK/h; Fmr1-null: 2.6 ± 0.1 CK/h (Mann-Whitney test, p-value < 0.001). (D) Percentage of efficient CKs in control and Fmr1-null neurons. Ctrl: 54 %; Fmr1-null: 33 % (Chi2 = 57.611, p-value < 0.001). The black line represents the median. Ctrl: N = 3, n = 178; Fmr1-null: N = 3, n = 216. *** p-value < 0.001.

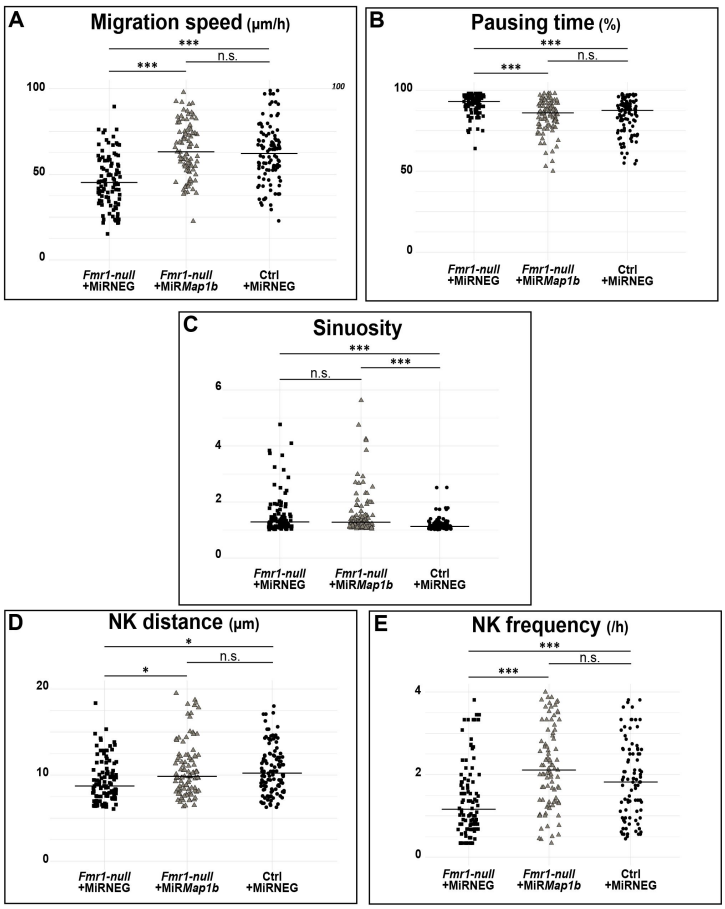

FIGURE 4.

Map1b KD rescues Fmr1-null neurons migration defects.

(A) Migration speed of Fmr1-null neurons expressing MiRNEG and MiRMap1b and control neurons expressing MiRNEG. Fmr1-null neurons + MiRNEG: 47 ± 2 µm/h; Fmr1-null neurons + MiRMap1b: 67 ± 2 µm/h; control neurons + MiRNEG: 64 ± 2 µm/h (Kruskall-Wallis Test: Chi2 = 61.168, p-value < 0.001, df = 2; followed by Dunn's posthoc test). (B) Percentage of pausing time of Fmr1-null neurons expressing MiRNEG and MiRMap1b and control neurons expressing MiRNEG. Fmr1-null neurons + MiRNEG: 92 %; Fmr1-null neurons + MiRMap1b: 84 %; control neurons + MiRNEG: 84 % (Kruskall-Wallis Test: Chi2 = 45.716, p-value < 0.001, df = 2; followed by Dunn's posthoc test). (C) Sinuosity index of Fmr1-null neurons expressing MiRNEG and MiRMap1b and control neurons expressing MiRNEG. Fmr1-null neurons + MiRNEG: 1.77 ± 0.18; Fmr1-null neurons + MiRMap1b: 1.90 ± 0.20; control neurons + MiRNEG: 1.18 ± 0.02 (Kruskall-Wallis Test: Chi2 = 39.807, p-value < 0.001, df = 2; followed by Dunn's posthoc test). (D) NK mean distance of Fmr1-null neurons expressing MiRNEG and MiRMap1b and control neurons expressing MiRNEG. Fmr1-null neurons + MiRNEG: 9.7 ± 0.3 µm; Fmr1-null neurons + MiRMap1b: 11.1 ± 0.4 µm; control neurons + MiRNEG: 10.6 ± 0.3 µm (Kruskall-Wallis Test: Chi2 = 11.573, p-value = 0.003, df = 2; followed by Dunn's posthoc test). (E) NK frequency of Fmr1-null neurons expressing MiRNEG and MiRMap1b and control neurons expressing MiRNEG. Fmr1-null neurons + MiRNEG: 1.5 ± 0.1 NK/h; Fmr1-null neurons + MiRMap1b: 2.4 ± 0.1 NK/h; control neurons + MiRNEG: 2.5 ± 0.2 NK/h (Kruskall-Wallis Test: Chi2 = 39.272, p-value < 0.001, df = 2; followed by Dunn's posthoc test). The black line represents the median. Fmr1-null neurons + MiRNEG: N = 6, n = 102; Fmr1-null neurons + MiRMap1b: N = 3, n = 101; control neurons + MiRNEG: N = 3, n = 78. * p-value < 0.05; *** p-value < 0.001; n.s. (not significant), p-value > 0.05.

Taken together, these results show that FMRP plays a key role in neuronal migration.

To assess whether these migration defects are cell autonomous, we designed an interfering RNA coupled to GFP to cell-autonomously knock-down *Fmr1* mRNA in RMS neurons, similar to (Scotto-Lomassese *et al*, 2011). The interfering miR*Fmr1*-GFP was co-electroporated with centrin-RFP to perform live-imaging (movie S. 3). Analysis of migration and centrosome dynamics (Fig. S1 and S2) showed that *Fmr1* KD is sufficient to recapitulate the entire migratory phenotype described in *Fmr1-null* mutants, demonstrating that FMRP cell-autonomously regulates neuronal migration.

Together, these data show that FMRP is cell-autonomously necessary for proper neuronal migration of RMS neurons, by regulating their speed and directionality as well as centrosome/nucleus coupling.

FMRP regulates neuronal migration through MAP1B

MAP1B is a neuron-specific microtubule-associated protein widely expressed in the developing CNS with crucial roles in diverse steps of neural development including neuronal migration (Yang *et al*, 2012). MAP1B is also a well-know FMRP mRNA target (Zhang *et al*, 2001)(Darnell *et al*, 2001)(Brown *et al*, 2001). It thus appeared as an interesting FMRP target to investigate. As FMRP is a repressor of MAP1B mRNA translation (Brown *et al*, 2001)(Darnell *et al*, 2001)(Lu *et al*, 2004), the overall level of MAP1B usually appears increased in an *Fmr1-null* context (Lu *et al*, 2004)(Hou *et al*, 2006). Accordingly, MAP1B expression is increased in the RMS, as assessed by immunostaining and Western Blot (Fig. S3 A,B)

To assess whether the upregulation of MAP1B is responsible for the migratory phenotype observed in *Fmr1-null* neurons, we cell-autonomously knocked-down *Map1b* in RMS neurons with an interfering RNA. The miR*Map1b*-GFP was electroporated in *Fmr1-null* neonate mice and time-lapse imaging was performed in acute sections of the RMS (movie S. 4). *Fmr1-null* neurons expressing the miR*Map1b*-GFP exhibit a completely restored migration speed, pausing time, NK distance and frequency, which become comparable to miRNeg-GFP control neurons (Fig.6.A,B,D,E). Of note, the sinuosity of *Fmr1-null* neurons expressing miR*Map1b*-GFP was not rescued (Fig.6.C), suggesting that this parameter is MAP1B independent. Overall, our results demonstrate that MAP1B is the main FMRP mRNA target involved in the regulation of neuronal migration.

Discussion

FMRP is commonly described as a critical regulator of neuronal plasticity and neural development (Richter & Zhao, 2021). However, its role in neuronal migration remains poorly understood.

A role for FMRP in radial embryonic migration was evidenced by (La Fata *et al*, 2014) and (Wu *et al*, 2019). However, to our knowledge, tangential migration in the absence of FMRP has never been analyzed so far, and neither has been the effect of its absence on the dynamics of saltatory migration.

We report strong defects in postnatal migration with a slowed-down and erratic migration. Of importance, even though the mutated neurons are delayed in their movement and lose time finding the proper direction, they ultimately properly arrive in the OB, as we previously showed in the adult (Scotto-Lomassese *et al*, 2011). Live imaging allowed us to perform detailed analysis of both nucleokinesis and centrosome dynamics, which proved both to be deeply perturbed. As microtubules are essential regulators of both processes (Kuijpers & Hoogenraad, 2011) (Tsai & Gleeson, 2005), we suspected MAP1B to be the key FMRP mRNA target involved in the migratory phenotypes. MAP1B is one of the historic targets of FMRP (Brown *et al*, 2001)(Darnell *et al*, 2001)(Zhang *et al*, 2001). It is expressed early in the embryonic brain (Tucker *et al*, 1989) and is essential to different steps of neural development (Gonzalez-Billault *et al*, 2004) and in particular the regulation of embryonic radial migration (González-Billault *et al*, 2005).

We show MAP1B overexpression in *Fmr1*-mutated neurons and rescue the migratory defects through its RNA-interference-induced KD. This identifies MAP1B as the critical FMRP mRNA target involved in the regulation of cyclic saltatory migration.

The importance of FMRP-regulated MAP1B translation was initially evidenced in *drosophila*, where it appeared essential for proper synaptogenesis (Zhang *et al*, 2001). This was later

confirmed in the mouse hippocampus, where increased MAP1B levels in the *Fmr1*-null context also induced defective synaptogenesis (Lu *et al*, 2004). To our knowledge, the importance of the FMRP-MAP1B duo for neuronal migration described here is the only other described neurodevelopmental function of FMRP-regulated MAP1B translation.

In the context of FXS, analyzing migration in the FXS human organoids or assembloïds will be of course of major interest (Levy & Pasca, 2023). It is to be noted that postnatal tangential migration in the infant human brain was recently described to be even more extensive than in mice (Paredes *et al*, 2016)(Sanai *et al*, 2011) so that, if conserved in humans, the defects that we observe might be critical for proper postnatal corticogenesis.

As a conclusion, we report here an entirely new function of FMRP as a regulator of migration linked to microtubules *via* MAP1B. This participates in the fundamental understanding of this multifaceted protein and might also be important for the pathophysiological understanding of FXS.

Material and methods

Mouse lines

Mice were housed in a 12 hours light/dark cycle, in cages containing one or two females and one male. The postnatal mice were housed in their parents' cage. Animal care was conducted in accordance with standard ethical guidelines [National Institutes of Health (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 ethics committee (Comité d'Ethique en Expérimentation Animale Charles Darwin C2EA-05 and the French Ministère de l'Education Nationale, de l'Enseignement Supérieur et de la Recherche APAFIS#13624-2018021915046521_v5). We strictly performed this approved procedure. The mice used were in a C57BL6-J background. *Fmr1-null* mice were genotyped according to the original protocol (Fmr1 knockout mice: a model to study fragile X mental retardation. The Dutch-Belgian Fragile X Consortium, 1994).

MiRNA production

Silencing of *Fmr1* and *Map1b* has been performed using BLOCK-iT Pol II miR RNAi Expression Vector Kits (Invitrogen) and the RNAi Designer (Invitrogen). The sequences of the single-stranded oligos are:

Fmr1 Top: TGCTGTACAAATGCCTTGTAGAAAGCGTTTTGGCCAACTGACTGACGCTTTCTAA GGCATTTGTA,

Fmr1 Bottom: CCTGTACAAATGCCTTAGAAAGCGTCAGTCAGTGGCCAAAACGCTTTCTACAAG GCATTTGTAC,

Map1b Top: TGCTGTGTTGATGAAGTCTTGGAGATGTTTTGGCCACTGACTGACATCTCCAACT TCATCAACA,

Map1b Bottom: CCTGTGTTGATGAAGTTGGAGATGTCAGTCAGTGGCCAAAACATCTCCAAGACT TCATCAACAC.

The double-stranded oligos were inserted in a pcDNA 6.2-GW/EmGFP-miR. The resulting constructions were sequenced before use.

Postnatal electroporation

Postnatal injection and electroporation were performed at postnatal day 2 (P2). Postnatal mice were anesthetized by hypothermia. Pseudo-stereotaxic injection [from lambda medial-lateral (M/L): 0,9; anterior-posterior (A/P): 1,1; dorsal-ventral (D/V): 2] using a glass micropipette (Drummond Scientific Company, Wiretrol I, 5-000-1050) was performed, and 2ul of plasmid (between 5 and 8 μg/ml) was injected. Animals were subjected to 5 pulses of 99.9V during 50ms separated by 950ms using the CUY21 SC Electroporator and 10-mm tweezer electrodes (Harvard Apparatus, Tweezertrode, 10mm, 45-0119). The animals were placed on 37°C plates to restore their body temperature before returning in their parents' cage. Animals were considered as fully restored when moving naturally and their skin color returned to pink.

Postnatal acute brain slices

Brain slices of mice aged from P6 to P10 were prepared as previously described in (Stoufflet *et al*, 2020). Pups were sacrificed by decapitation and the brain was removed from the skull. Sagittal brain sections (250 μm) were cut with a VT1200S microtome (Leica). Slices were prepared in an ice-cold cutting solution of the following composition: 125 mM NaCl, 0.4 mM $CaCl_2$, 1 mM $MgCl_2$, 1.25 mM NaH_2PO_4, 26 mM $NaHCO_3$, 5 mM sodium pyruvate, 20 mM glucose and 1 mM kynurenic acid, saturated with 5% CO_2 and 95% O_2. Slices were incubated in this solution for 30 minutes at room temperature and then placed in recording solution (identical to the cutting solution, except that the $CaCl_2$ concentration is 2 mM and kynurenic acid is absent) for at least 30 minutes at 32°C before image acquisition.

Time-lapse video microscopy of postnatal slices

To analyze neuronal migration and centrosome dynamics, images were obtained with an inverted SP5D confocal microscope (Leica) using a 40x/1.25-numerical aperture (N.A.) objective with 1.5 optical zoom or an upright SP5 MPII two-photon microscope (Leica) using a 25x/0.95-N.A. objective with 1.86 optical zoom. Images were acquired every 3 minutes for 2 to 3 hours. The temperature in the microscope chamber was maintained at 32°C during imaging and brain slices were continuously perfused with heated recording solution (see above) saturated with 5% CO_2 and 95% O_2.

Analyses of neuronal migration and centrosome movement

Analyses were performed using ImageJ (NIH Image; National Institutes of Health, Bethesda, MD) software and MTrackJ plugin (Meijering, Dzyubachyk, et Smal 2012). The nucleus and the centrosome (when centrin-RFP was co-injected) were tracked manually on each time frame during the whole movie. We considered a NK as a movement superior to 6 μm between two consecutive time points (3 minutes-interval). For cell migration, calculation of speed, percentage of pausing time, sinuosity, directionality, NK distance and frequency was performed using the x,y,t coordinates of the nucleus of each cell. Cells tracked for less than 30 minutes and cells that did not perform any NK during the whole tracking were excluded. A CK was defined as a forward movement superior to 2 μm followed by a backward movement superior to 2 μm. For centrosome movement, calculation of CK speed, frequency and efficiency was performed using the x,y,t coordinates of the centrosome of each cell and the x,y,t coordinates of each corresponding nucleus.

Immunohistochemistry

P7 to P10 mice were lethally anesthetized using Euthasol. Intracardiac perfusion with 4% paraformaldehyde was performed. The brain was post-fixed overnight in 4% paraformaldehyde and then rinsed three times with phosphate-buffered saline (PBS) 1x (Gibco, 1400-067). 50 μm sagittal sections were made with VT1200S microtome (Leica). Slices were placed 1 hour in a saturation solution (10% fetal bovine serum; 0.5% Triton X-100 in PBS). Primary antibodies used in this study are: GFP (Aves; GFP-1020; 1/1000), FMRP (Developmental Studies Hybridoma Bank; 2F5-1; 1/200), MAP1B (Santa Cruz Biotechnology; sc-365668; 1/300). The antibodies were diluted in saturation solution. Slices were incubated for 48 to 72 hours at 4°C under agitation with the antibodies and then rinsed three times with PBS 1x. Secondary antibodies used are: anti-chicken immunoglobulin Y (IgY) Alexa Fluor 488 (1/1000; Jackson ImmunoResearch; 703-545-155) against anti-GFP, anti-mouse immunoglobulin G2b (IgG2b) Alexa Fluor 555 (1/2000; Jackson ImmunoResearch; 703-545-155) against anti-FMRP and anti-MAP1B. The antibodies were diluted in saturation solution. Slices were incubated with secondary antibodies for 1 hour at room temperature under agitation, protected from light. After rinsing three times with PBS 1x, slices were counter-colored with Hoechst and mounted in Mowiol.

Tissue collection and Western blotting

RMS were manually micro-dissected from 5 to 6 postnatal mouse brains and pooled in a PBS 1x (0.5% glucose) solution. After centrifugation, protein extraction was performed on the tissue pellet. Samples were homogenized in a lysis buffer with the following composition: 25 mM Tris HCl pH 7.5, 150 mM NaCl, 1% NP40, 0.5% NaDeoxycholate, 1 mM EDTA, 5% glycerol, 1X EDTA-free protease inhibitor cocktail (Sigma, 4693132001). After centrifugation, samples were loaded and run in NuPAGE 3-8% Tris-Acetate Gel (Invitrogen, EA0378BOX) at 120V for 15 minutes then 180V for 40 minutes. Transfer to nitrocellulose Immobilon-PVDF-P membrane (Millipore, IPVH00010) was performed at 40V overnight at 4°C. The membrane was then saturated for 1 hour in TBSt containing 5% powder milk. Primary antibodies used are: MAP1B (Santa Cruz Biotechnology, sc-365668, 1/100), Vinculin (Cell Signaling Technology, 13901S, 1/1000). The antibodies were diluted in TBSt containing 5% powder milk. Secondary antibodies used are: ECL anti-mouse immunoglobulin G (IgG) horseradish peroxidase linked whole antibody (1/10 000; Fisher Scientific; NXA931V) for anti-MAP1B, Peroxidase-conjuguated AffiniPure F(ab')2 Fragment Donkey Anti-Rabbit IgG (H+L) (1/5 000; Jackson ImmunoResearch; 711-036-152) for anti-Vinculin. The antibodies were diluted in TBSt containing 5% powder milk. Labeling was visualized using Pierce ECL Western Blotting Substrate (Thermo Scientific; 32209) and luminescent image analyzer LAS-3000.

Statistics

All manipulations and statistical analyses were implemented with R (4.2.1, R Foundation for Statistical Computing, Vienna, Austria). Normality in the variable distributions was assessed by the Shapiro-Wilk test. Furthermore, the Levene test was performed to probe homogeneity of variances across groups. Variables that failed the Shapiro-Wilk or the Levene test were analyzed with non-parametric statistics using the one-way Kruskal-Wallis analysis of variance on ranks followed by Nemenyi test post hoc and Mann-Whitney rank sum tests for pairwise multiple comparisons. Variables that passed the normality test were analyzed by means of one-way ANOVA followed by Tukey post hoc test for multiple comparisons or by Student's *t* test for comparing two groups. Categorical variables were compared using Pearson's c^2 test or Fisher's exact test. A *p*- value of <0.05 was used as a cutoff for statistical

significance. Results are presented as the means ± SEM. The statistical tests are described in each figure legend.

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Reviewer #1 (Public Review):

This work investigated Fragile X Messenger Ribonucleoprotein (FMRP) protein impact on neuroblast tangential migration in the postnatal rostral migratory stream (RMS). Authors conducted series of live-imaging on organotypic brain slices from Fmr1-null mice. They continued their analysis silencing Fmr1 exclusively from migrating neuroblasts using electroporation-mediated RNA interference method (MiRFmr1 KD). These impressive approaches show that neuroblasts tangential migration is impaired in Fmr1-null mice RMS and these defects are mostly recapitulated in the MiRFmr1neuroblasts.This nicely supports the idea that FMRP have a cell autonomous function in tangentially migrating neuroblasts. It is an important part of this work as migrating neuroblasts are in contact with each other and surrounding glial cells while migrating towards the olfactory bulb. The authors also confirm that FMRP mRNA target Microtubule Associated Protein 1B (MAP1B) is overexpressed in the Fmr1-null mice RMS. They successfully use electroporation-mediated RNA interference method to silence Map1b in the Fmr1-null mice neuroblasts. This discreet and elaborate experiment rescues most of the migratory defects observed both in Fmr1-null and MiRFmr1neuroblasts. Altogether, these results strongly suggest that FMRP-MAP1B axis has an important role in regulation of the neuroblasts tangential migration in RMS. Neurons move forward in cyclic saltatory manner which includes repeated steps of leading process extension, migration of the cell organelles and nuclear translocation. Authors reveal by analyzing the live-imaging data that FMRP-MAP1B axis is affecting movement of centrosome and nucleus during saltatory migration. An important part of the centrosome and nucleus movement is forces mediated by microtubule dynamics. Authors propose that FMRP regulate tangential migration via microtubule dynamics regulator MAP1B. This work provides valuable new information on regulation of the neuroblasts tangential saltatory migration. These findings also increase and improve our understanding of the issues

involved in Fragile X Syndrome (FXS) disorders. The conclusions of this work are mostly supported by the data. However, methods and data analysis would benefit from more careful and comprehensive scrutiny.

1.) It would be beneficial for the detail-oriented readers to have a more comprehensive section of neuronal migration analysis. It would help to understand better the details of the results and analysis. For example, percentage of pausing time. Is neuroblast migration speed and pausing time (%) separated from each other? Does this mean that migration speed is measured from time of the nucleus movement, and it excludes pausing time? Sometimes migration speed refers to the total distance that cells have moved divided by the time between images (e.g., Nam et al. 2007, J Comp Neurol 505: 190-208). This is also important as neuroblasts migration speed fluctuate during their migration in RMS (e.g., Belvindrah et al. 2017, J Cell Biol 216: 2443-2461). It could be useful, for example, to show a plot of total migration distance distribution between controls, Fmr1-null, MiRFmr1 KD and MiRMap1b KD neuroblasts.

2.) The author's claim that Fmr1 interfering RNA (MiFmr1 KD) model "recapitulates the entire migratory phenotype described in Fmr1-null mutants". This is evident from the data analysis for the migration speed, pausing time percentage and NK distance. Parallel, but slightly weaker effect is seen in, NK frequency, CK frequency and CK efficiency. However, interpretation of the directionality analysis causes some concerns.
a) Sinuosity index
Fmr1-null mice (ctr: 1.32{plus minus}0.04; Fmr1-null: 1.93{plus minus}0.16; Figure 2C) and MiFmr1 KD neurons (MiRNEG: 1.5{plus minus}0.12; MiRFmr1 KD 1.62 {plus minus}0.08; Figure S1C). The latter results are significant, but standard error of means (SEM) seems to overlap. In addition, there is only a minor difference between control and MiRFmr1 KD cells sinuosity index.
b) Directionality radar
Migration directionality radar seems to be considerably different between Fmr1-null (Figure 2D) and MiFmr1 KD results (Figure S1D).
It would be beneficial for this article to fully disclose, how these analyses were performed. For example, how sinuosity index was calculated and what does it precisely measure. It would greatly help to understand better the directionality analysis. To make these results more solid authors could have used original migration trajectories in the rose and/or trajectory plots. These plots visualize better the migration directionality results and clarify the changes in the directionality during migration.

3.) Authors claim that "Overall, our results demonstrate that MAP1B is the main FMRP mRNA target involved in the regulation of neuronal migration". Results and analysis show that migration speed, pausing time percentage, NK distance and NK frequency migratory defects are all rescued in Fmr1-null mice when MiRMap1b KD was introduced to the neuroblasts (Figure 4). These results are very interesting, linking FMRP-MAP1B axis to the microtubule dynamics.

4.) Authors could refine in discussion what is known about FRMP in neuronal migration. For example, La Fata et al. 2014 found that N-cadherin protein levels were lower in Fmr1-null mice and reintroducing N-cadherin rescued embryonic radial migration defects. N-cadherin is also expressed in the RMS and its deficiency affects negatively to the neuroblast migration (e.g., Porlan et al. 2014, Nat Cell Biol (7):629-38). This relationship of FMRP and N-cadherin could be discussed and considered in the article more closely. Overall, the article will benefit from clearer writing and more comprehensive discussion.

Reviewer #2 (Public Review):

In this manuscript, the authors conducted a straightforward molecular approach to link FMRP and MAP1B proteins functionally. Both proteins are connected since FMRP is a translational regulator of the MAP1B protein, a microtubule-stabilizing factor.

The results combined molecular genetics (FMRP knock-out mice) with acute inactivation of FRMP and MAP1B to conclusively support the notion that FMRP-dependent regulation of MAP1B is necessary for proper neuronal migration toward the olfactory bulb using the rostral migratory stream.

Overall, these results increase our knowledge of the molecular mechanism that controls how neurons migrate in the brain to reach their final destinations and confirms that cytoskeleton regulators are key players in this process.

Reviewer #3 (Public Review):

Neuronal migration is one of the key processes for appropriate neuronal development. Defects in neuronal migration are associated with different brain disorders often accompanied by intellectual disabilities. Therefore, the study of the mechanisms involved in neuronal migration helps to understand the pathogenesis of some brain malformations and psychiatric disorders.

FMRP is an RNA-binding protein implicated in RNA metabolism regulation and mRNA local translation. FMRP loss of function causes fragile X syndrome (FXS), the most common form of inherited intellectual disability. Previous studies have shown the role of FMRP in the multipolar to bipolar transition during the radial migration in the cortex and its possible relation with periventricular heterotopia and altered synaptic communication in humans with FXS. However, the role of FMRP in neuronal tangential migration is largely unknown. In this manuscript, the authors aim to decipher the role of FMRP in the tangential migration of neuroblasts along the rostral migratory stream (RMS) in the postnatal brain. By extensive live-imaging analysis of migrating neuroblasts along the RMS, they demonstrate the requirement of FMRP for neuroblast migration and centrosomal movement. These migratory defects are cell-autonomous and mediated by the microtubule-associated protein Map1b.

Overall, the manuscript highlights the importance of FMRP in neuronal tangential migration. They performed an analysis of different aspects of migration such as nucleokinesis and cytokinesis in migrating neuroblasts from live-imaging videos.
However, the work is quite incomplete. The role of FMRP and Map1b in neuronal migration is not well introduced and discussed. In the cortex, FMRP is mainly implicated in the multipolar to bipolar transition of immature neurons, but not in the migration itself (la Fata et al., 2014). In fact, Fmr1 KO mice do not show impairment in cortical lamination. On the other hand, very less is mentioned about the role of Map1b in neuronal migration. It is not shown whether overexpression of Map1b alters neuroblast migration and recapitulates the Fmr1 KO phenotype.

Moreover, it is unclear to me which are the anatomical consequences of aberrant migration of neuroblasts in the Fmr1 KO mice. Authors mention that neuroblasts properly arrive at the OB and they refer to a previous publication (Scotto-Lomassese et al., 2011). However, this study does not show the distribution of neuroblasts in the SVZ, along the RMS or in the olfactory bulb (OB) in mutant mice. On the contrary, they said that there is no delay in the

migration or maturation of granular cells arriving at the OB (Scotto-Lomassese et al., 2011). In summary, the authors do not show the functional consequences of aberrant neuroblast migration in the Fmr1 KO mice, making weaker the assumption that the study is important for the understanding of FXS pathophysiology.

Author Response:

We are grateful to the three referees for their overall positive evaluation of our work and valuable constructive suggestions. We will address their public reviews with utmost care, as well as their private recommendations.

To Reviewer #1: thanks for the positive comments and for the appreciation of our « impressive approaches »

- We will add a more comprehensive section of neuronal migration analysis in the Material and Methods section. Sorry for that regrettable lack of precision.
- We will address the comments about the sinuosity index definition and interpretations.
- We will enhance the clarity of our writing and delve deeper into the discussion. As mentioned to Reviewer #3, the brevity of the text was influenced by the Short report format.

To Reviewer #2 : thanks for the overall positive appreciation.

We will also consider the recommendations for authors with care.

To Reviewer #3 : thanks a lot for the feedback.

- We will further develop the introduction and discussion sections as suggested. Regrettably and as mentioned to Reviewer #1, we had to significantly condense them due to the space constraints imposed by the Short Report format.
- We will attempt to overexpress Map1B in order to assess the potential phenotypic similarity to the Fmr1 null condition, as suggested. However, it is important to acknowledge that this experiment may not yield a definitive answer due to potential differences in the level of Map1B expression driven by a CMV promoter compared to its endogenous expression in Fmr1 null neurons, as well as variations in the subcellular distribution of the overexpressed Map1B.
- Regarding the anatomical consequences of aberrant migration, we acknowledge that neither our present work nor our previous study by Scotto-Lomassesse et al. provide evidence in this regard, as pointed out by the reviewer. Indeed, the delayed neurons do reach the olfactory bulb based on our findings. However, other studies have demonstrated that a delay in migration can have important functional consequences (eg Bocchi et al, 2017 doi: 10.1038/s41467-017-01046-w). Accordingly, we will revise and moderate our conclusions on this specific point.