

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/351361728>

# Serotonin 5-HT<sub>7</sub> receptors require Cyclin-Dependent Kinase 5 to rescue hippocampal synaptic plasticity in a mouse model of Fragile X Syndrome

Article in *European Journal of Neuroscience* · May 2021

DOI: 10.1111/ejn.15246

CITATIONS

0

READS

2

5 authors, including:



Lara Costa

Università degli Studi di Messina

26 PUBLICATIONS 423 CITATIONS

SEE PROFILE

# Serotonin 5-HT7 receptors require cyclin-dependent kinase 5 to rescue hippocampal synaptic plasticity in a mouse model of Fragile X Syndrome

Lara Costa<sup>1</sup> | Alessandra Tempio<sup>2</sup>  | Enza Lacivita<sup>3</sup> | Marcello Leopoldo<sup>3</sup> | Lucia Ciranna<sup>2</sup> 

<sup>1</sup>Department of Clinical and Experimental Medicine, University of Messina, Messina, Italy

<sup>2</sup>Department of Biomedical and Biotechnological Sciences, University of Catania, Catania, Italy

<sup>3</sup>Department of Pharmacy, University of Bari, Bari, Italy

## Correspondence

Lucia Ciranna, Department of Biomedical and Biotechnological Sciences, University of Catania, Catania, Italy  
Email: ciranna@unict.it

## Funding information

Università di Catania; Fondazione Telethon, Grant/Award Number: GGP13145

## Abstract

Fragile X Syndrome is a genetic form of intellectual disability associated with autism, epilepsy and mood disorders. Electrophysiology studies in *Fmr1* knockout (KO) mice, a murine model of Fragile X Syndrome, have demonstrated alterations of synaptic plasticity, with exaggerated long-term depression induced by activation of metabotropic glutamate receptors (mGluR-LTD) in *Fmr1* KO hippocampus. We have previously demonstrated that activation of serotonin 5-HT7 receptors reverses mGluR-LTD in the hippocampus of wild-type and *Fmr1* KO mice, thus correcting a synaptic dysfunction typically observed in this disease model. Here we show that pharmacological inhibition of cyclin-dependent kinase 5 (Cdk5, a signaling molecule recently shown to be a modulator of brain synaptic plasticity) enhanced mGluR-LTD in wild-type hippocampal neurons, which became comparable to exaggerated mGluR-LTD observed in *Fmr1* KO neurons. Furthermore, Cdk5 inhibition prevented 5-HT7 receptor-mediated reversal of mGluR-LTD both in wild-type and in *Fmr1* KO neurons. Our results show that Cdk5 modulates hippocampal synaptic plasticity. 5-HT7 receptors require Cdk5 to modulate synaptic plasticity in wild-type and rescue abnormal plasticity in *Fmr1* KO neurons, pointing out Cdk5 as a possible novel target in Fragile X Syndrome.

## KEYWORDS

5-HT7 receptors, Cdk5, Fragile X Syndrome, hippocampus, mGluR-LTD, Serotonin

**Abbreviations:** 5-HT, 5-hydroxy-tryptamine; AMPA,  $\alpha$ -Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; Cdk5, Cyclin-dependent kinase 5; D-AP5, D(-)-2-amino-5-phosphonopentanoic acid; DHPG, dihydroxyphenylglycine; EPSC, excitatory post synaptic current; mGluR-LTD, long-term depression mediated by metabotropic glutamate receptors.

L. Costa<sup>1</sup> and A. Tempio contributed equally.

Edited by: Clive R. Bramham

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2021 The Authors. *European Journal of Neuroscience* published by Federation of European Neuroscience Societies and John Wiley & Sons Ltd.

## 1 | INTRODUCTION

Synaptic plasticity represents the cellular basis for activity-dependent establishment and refinement of nerve circuits underlying learning and memory. Among different forms of synaptic plasticity described in the hippocampus, long-term depression induced by activation of metabotropic glutamate receptors (mGluR-LTD) plays an important role in learning and behaviour (Luscher & Huber, 2010). Alterations of mGluR-LTD have been observed in several animal models of neurological diseases involving learning and behavioral deficits, including Fragile X Syndrome (Luscher & Huber, 2010; Sanderson et al., 2016). Fragile X Syndrome is a genetic form of intellectual disability associated with autistic features, epilepsy and mood disorders (Salcedo-Arellano et al., 2020). In *Fmr1* knockout (KO) mice, a murine model of this disease, metabotropic glutamate receptors (mGluRs) are abnormally coupled to their intracellular signaling machinery, leading to excessive activation of downstream pathways and exaggerated mGluR-LTD (Bear et al., 2004; Huber et al., 2002).

Our research group demonstrated that activation of serotonin 5-HT7 receptors is able to reduce excessive mGluR-LTD in *Fmr1* KO hippocampal neurons (Costa et al., 2012) and rescue learning and behavior in *Fmr1* KO mice in vivo (Costa et al., 2018). We have elucidated the first steps of this 5-HT7 receptor-mediated mechanism of action, which relies on cyclic adenosine monophosphate (cAMP) formation and PKA activation (Costa et al., 2018).

In the present work, we have investigated possible involvement of Cyclin-dependent kinase 5 (Cdk5), a kinase implicated in 5-HT7 receptor-mediated stimulation of axonal and dendritic growth in cortical, hippocampal and striatal neurons (Speranza et al., 2013, 2015, 2017). Cdk5 belongs to a large family of cyclin-dependent kinases, but differs from the other members in several ways: Cdk5 is not involved in the cell cycle, being mostly expressed in post-mitotic neurons, and plays a crucial role in the brain controlling neuronal differentiation and migration during development, cytoskeletal and microtubule regulation and synaptic plasticity (Kawauchi, 2014; Shah & Rossie, 2018). Two specific Cdk5 activators, the intracellular membrane-bound peptides p35 and p39, have been identified and localized exclusively in neurons (Ko et al., 2001). In pathological conditions, p35 is cleaved by calpain (a  $\text{Ca}^{2+}$ -activated protease) into a shorter activator peptide, p25, with a broad cytoplasmic and nuclear localization and a longer half-life, inducing hyperphosphorylation of Cdk5 physiological substrates and abnormal phosphorylation of cytoplasmic and nuclear proteins (Allnutt et al., 2020; Cheung & Ip, 2012; Shah & Rossie, 2018). Aberrant p25/Cdk5 signalling accounts for neuronal damage in mouse models of Alzheimer's disease (Giese, 2014; Liu et al., 2016), Parkinson's disease (He et al., 2020) and traumatic brain injury (Yousuf et al., 2016). Cdk5 downregulation

has been associated with epilepsy (Liu et al., 2020), attention deficit and hyperactivity disorder (Drerup et al., 2010) and schizophrenia (Engmann et al., 2011). In the striatum of postmortem Huntington's disease patients and in a mouse model of this pathology, reduced expression of Cdk5 and p35 was observed (Luo et al., 2005; Paoletti et al., 2008) together with abnormal Cdk5 activation by p25 (Paoletti et al., 2008), indicating a complex dysregulation of Cdk5 signaling in Huntington's disease.

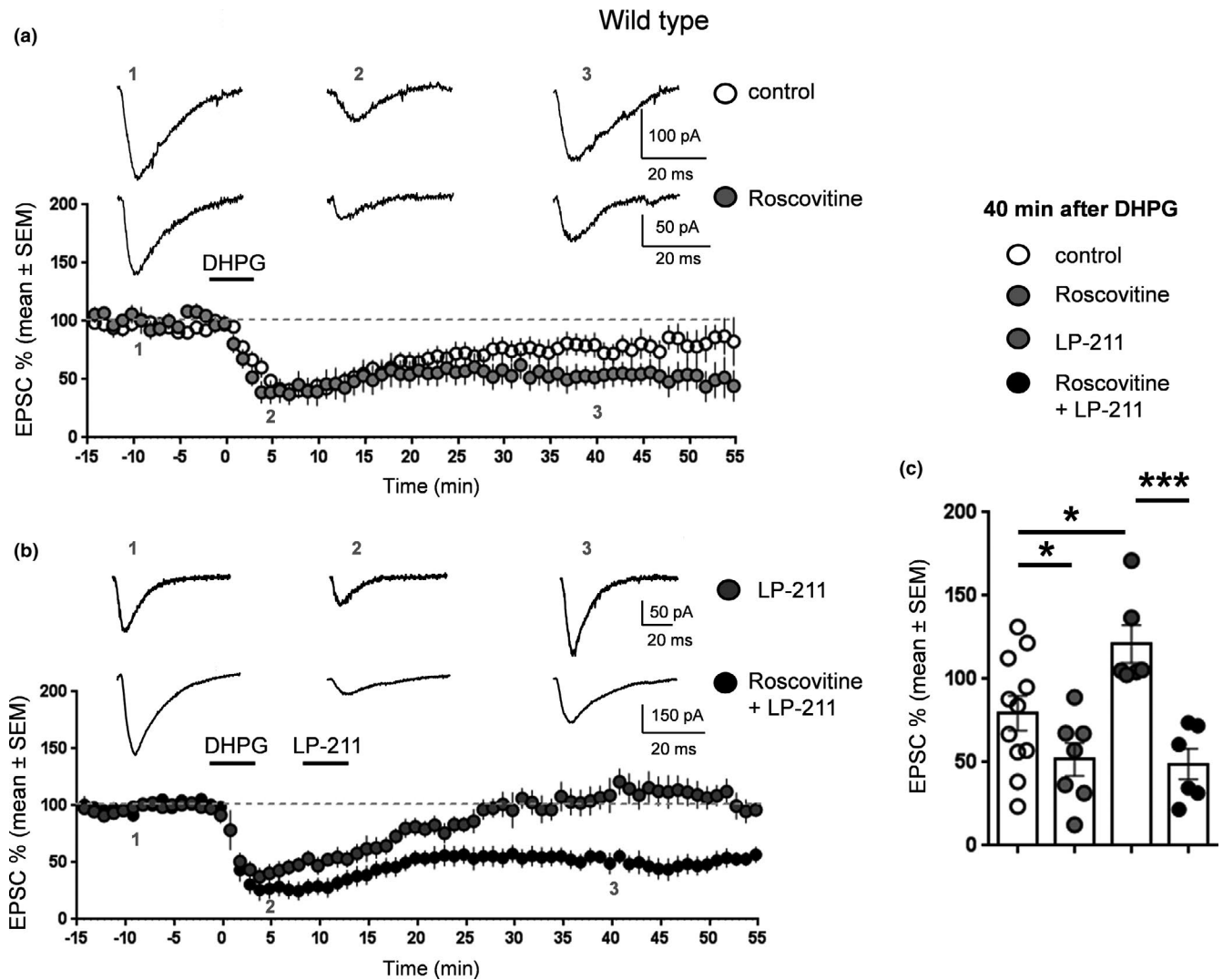
In the present work, we have tested a possible involvement of Cdk5 in 5-HT7 receptor-mediated reversal of mGluR-LTD in the hippocampus of wild-type mice and of the *Fmr1* KO mouse model of Fragile X Syndrome.

## 2 | METHODS

### 2.1 | Electrophysiology recordings

Experiments were performed using patch clamp recording in acute mouse hippocampal slices from wild-type and *Fmr1* KO mice on a C57BL/6J background, obtained from a breeding colony at the University of Catania (Italy). Mice were maintained with a controlled temperature ( $21^{\circ}\text{C} \pm 1^{\circ}\text{C}$ ) and humidity (50%) on a 12 hr light/dark cycle, with ad libitum food and water. All animal experimentation was conducted in accordance with the European Community Council guidelines (2010/63/EU) and was approved by the University Institutional Animal Care and Use Committee (Project # 250 – approval number: 352/2016-PR).

Acute hippocampal slices were prepared as described previously (Costa et al., 2012) from wild-type and *Fmr1* KO mice (postnatal PN age 14–23 days). Briefly, the brains were removed, placed in oxygenated ice-cold artificial cerebrospinal fluid (ACSF; in mM NaCl 124; KCl 3.0;  $\text{NaH}_2\text{PO}_4$  1.2;  $\text{MgSO}_4$  1.2;  $\text{CaCl}_2$  2.0;  $\text{NaHCO}_3$  26; D-glucose 10, pH 7.3) and cut into 300  $\mu\text{m}$  slices with a vibratome (Leica VT 1200S). Slices were continually perfused with oxygenated ACSF and viewed with infrared microscopy (Leica DMLFS). Schaffer collaterals were stimulated with negative current pulses (duration 0.3 ms, delivered every 15 s by A310 Accupulser, WPI, USA). Evoked excitatory post synaptic currents (EPSCs) were recorded under whole-cell from CA1 pyramidal neurons (holding potential  $-70$  mV; EPC7-plus amplifier HEKA, Germany). Stimulation intensity was set to induce half-maximal EPSC amplitude. Series resistance ( $R_s$ ) was continuously monitored by 10 mV hyperpolarizing pulses; recordings were discarded from analysis if  $R_s$  changed by more than 20%. EPSC traces were filtered at 3 kHz and digitized at 10 kHz. Data were acquired and analysed using Signal software (CED, England). The recording micropipette (resistance 1.5–3 M $\Omega$ ) was filled with intracellular solution (in mM: K-gluconate 140; HEPES 10; NaCl 10;  $\text{MgCl}_2$  2;



**FIGURE 1** Inhibition of Cdk5 enhanced mGluR-LTD in CA1 neurons from wild-type mice and prevented 5-HT7 receptor-mediated effect on mGluR-LTD. AMPA receptor-mediated excitatory post-synaptic currents (EPSCs) were recorded in the presence of D-AP5 (50  $\mu$ M) and bicuculline (5  $\mu$ M) under whole-cell patch clamp in the CA3-CA1 synapse in hippocampal slices from wild-type mice. (a) Bath application of the group I mGluR agonist DHPG (100  $\mu$ M, 5 min) induced a long-term depression (mGluR-LTD) of EPSC amplitude (white dots,  $n = 11$ ). When the Cdk5 inhibitor roscovitine (1.6  $\mu$ M) was added to intracellular medium, DHPG-induced mGluR-LTD was enhanced (light grey dots,  $n = 7$ ) with respect to control. (b) When DHPG application was followed by application of the 5-HT7 receptor agonist LP-211 (10 nM, 5 min), mGluR-LTD was completely reversed (dark grey dots,  $n = 6$ ). In the presence of intracellular roscovitine (1.6  $\mu$ M), application of LP-211 did not modify the amount of mGluR-LTD (black dots,  $n = 6$ ). (c) The bar graph shows that the amount of mGluR-LTD measured 40 min after DHPG application (mean EPSC amplitude in all tested neurons, expressed as % of baseline EPSC amplitude; EPSC values of single neurons are displayed for each bar) in the four different experimental conditions (control; roscovitine; LP-211; LP-211 + roscovitine) was significantly different ( $p = 0.0006$  by one-way ANOVA followed by Tukey's multiple comparisons test). \* $p < 0.05$ ; \*\*\* $p < 0.001$

EGTA 0.2; Mg-ATP 3.5; Na-GTP 1; pH 7.3). In a set of experiments, the intracellular solution contained roscovitine, a selective Cdk5 inhibitor, at a concentration (1.6  $\mu$ M) 10-fold higher than the reported  $IC_{50}$  value (0.16  $\mu$ M) of roscovitine on Cdk5/p35 (Meijer et al., 1997). Bath solution (ACSF) was continuously changed at a flow rate of 1.5 ml/min and routinely contained (-)-bicuculline methiodide (5  $\mu$ M, Hello Bio) and D-(-)-2-amino-5-phosphopentanoic acid (D-AP5, 50  $\mu$ M, Hello Bio) to isolate AMPA receptor-mediated EPSCs. S-3,5-dihydroxyphenylglycine (DHPG, 100  $\mu$ M;

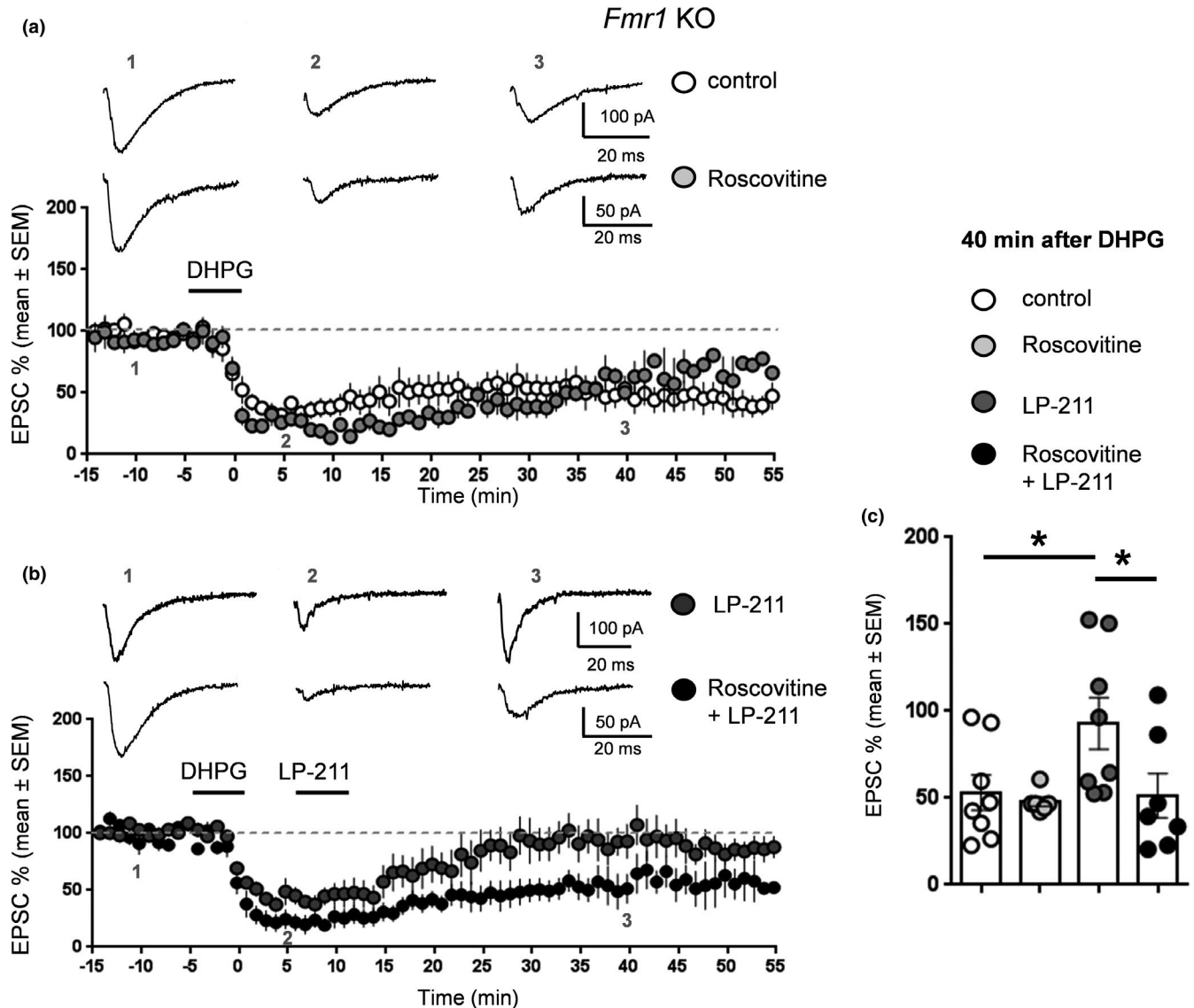
Hello Bio), and LP-211 (10 nM) were dissolved in ACSF and applied by bath perfusion. LP-211 was synthesized and provided by the research group of Prof. Leopoldo (University of Bari, Italy).

## 2.2 | Data analysis

To compare the amount of DHPG-induced LTD in different groups of neurons, EPSC amplitude values were normalized

as follows: peak amplitude values of EPSCs were averaged over 1 min and expressed as % of baseline EPSC amplitude (calculated from EPSCs recorded during at least 15 min before DHPG application). Normalized % EPSC values from each group of neurons were pooled (mean  $\pm$  SEM) and graphically represented as a function of time. The amount of mGluR-LTD was calculated 40 min after LTD induction by DHPG and was normalized as percentage of baseline (% EPSC amplitude; mean  $\pm$  SEM from all tested neurons). Column

graphs indicate normalized % EPSC amplitude (mean  $\pm$  SEM from groups of neurons) 40 min after application of DHPG alone or DHPG with the 5-HT7 receptor agonist LP-211 under different experimental conditions. Single values from each recorded neuron are illustrated for each column. EPSC amplitude values from two groups of neurons were compared using unpaired Student's *t* test, with *n* indicating the number of neurons tested in each condition. Groups of data from four different experimental conditions (Figure 1c and Figure 2c)



**FIGURE 2** Inhibition of Cdk5 did not modify mGluR-LTD in CA1 neurons from *Fmr1* KO mice and prevented 5-HT7 receptor-mediated effect on mGluR-LTD. AMPA receptor-mediated excitatory post-synaptic currents (EPSCs) were recorded from CA1 neurons in the presence of D-AP5 (50  $\mu$ M) and bicuculline (5  $\mu$ M) in hippocampal slices from *Fmr1* KO mice. (a) Bath application of DHPG (100  $\mu$ M, 5 min) induced mGluR-LTD (white dots; *n* = 8). In the presence of intracellular roscovitine (1.6  $\mu$ M) the amount of mGluR-LTD was not modified (grey dots, *n* = 6) with respect to control conditions. (b) Application of LP-211 (10 nM, 5 min) completely reversed mGluR-LTD in control conditions (dark grey dots, *n* = 8) but had no effect on mGluR-LTD in the presence of intracellular roscovitine (black dots, *n* = 7). (c) The bar graph shows the amount of mGluR-LTD measured 40 min after DHPG application (mean EPSC amplitude in all tested neurons, expressed as % of baseline EPSC amplitude; EPSC values of single neurons are displayed for each bar). The amount of mGluR-LTD in the four experimental conditions (control; roscovitine; LP-211; LP-211 + roscovitine) was significantly different ( $*p = 0.0331$  by one-way ordinary ANOVA followed by Tukey's multiple comparisons test)



were compared by one-way ANOVA followed by Tukey's multiple comparisons test (GraphPad Prism 6, USA)

### 3 | RESULTS

Excitatory post synaptic currents (EPSCs) mediated by  $\alpha$ -Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors for glutamate were evoked every 15 s by stimulation of Schaffer collaterals and were recorded from single CA1 pyramidal neurons under whole-cell patch clamp. In wild-type hippocampal slices, application of DHPG (100  $\mu$ M, 5 min), an agonist of group I metabotropic glutamate receptors (mGluRs), induced a long-term depression (mGluR-LTD) of AMPA receptor-mediated EPSCs (EPSC amplitude 40 min after DHPG:  $79 \pm 10\%$  with respect to baseline EPSC amplitude prior to DHPG application,  $n = 11$ ; Figure 1a). In a series of experiments, the Cdk5 inhibitor roscovitine (1.6  $\mu$ M) was included in the intracellular pipette solution, thus was present since the beginning of recording: in this condition the amount of DHPG-induced mGluR-LTD was significantly enhanced with respect to control conditions (EPSC amplitude:  $51 \pm 9\%$ ,  $n = 7$ , versus  $79 \pm 10\%$ ,  $n = 11$ , wild-type DHPG + roscovitine versus wild-type DHPG,  $p = 0.04$ ,  $t = 1.821$ ,  $df = 16$ ; unpaired  $t$  test; Figure 1a and c).

We have previously demonstrated that activation of 5-HT7 receptors reverses mGluR-LTD in wild-type and in *Fmr1* KO hippocampal neurons (Costa et al., 2012, 2015, 2018). Confirming our previous data, application of the selective 5-HT7 receptor agonist LP-211 (10 nM, 5 min) 5 min after DHPG application significantly reversed mGluR-LTD (EPSC amplitude:  $121 \pm 1\%$ ,  $n = 6$ , versus  $79 \pm 10\%$ ,  $n = 11$ , wild-type DHPG + LP-211 versus wild-type DHPG,  $p = 0.011$ ,  $t = 2.513$ ,  $df = 15$ ; unpaired  $t$  test; Figure 1b and c).

In the presence of intracellular roscovitine, (1.6  $\mu$ M) application of LP-211 (10 nM, 5 min) was unable to reverse mGluR-LTD in wild-type slices (EPSC amplitude:  $51 \pm 9\%$ ,  $n = 7$ , versus  $49 \pm 9\%$ ,  $n = 6$ ; wild-type DHPG + roscovitine versus wild-type DHPG + roscovitine + LP-211,  $p = 0.42$ ,  $t = 0.1895$ ,  $df = 11$ , Figure 1b and c). LP-211 reversed mGluR-LTD in control conditions but not in the presence of roscovitine (EPSC amplitude:  $121 \pm 1\%$ ,  $n = 6$ , versus  $49 \pm 9\%$ ,  $n = 6$ , wild-type DHPG + LP-211 versus wild-type DHPG + LP-211 + roscovitine,  $p = 0.0003$ ,  $t = 4.912$ ,  $df = 10$ ; unpaired  $t$  test; Figure 1b and c). Ordinary one-way ANOVA followed by Tukey's multiple comparisons test was performed to compare the amount of mGluR-LTD in the four different conditions (control; roscovitine; LP-211; LP-211 + roscovitine, Figure 1c), confirming a highly significant difference ( $***p = 0.0006$ ).

In *Fmr1* KO slices, application of DHPG (100  $\mu$ M, 5 min) induced mGluR-LTD in control conditions and in the presence of intracellular roscovitine (1.6  $\mu$ M) and the amount

of mGluR-LTD was similar in the two conditions (EPSC amplitude:  $53 \pm 10\%$ ,  $n = 8$  versus  $50 \pm 3\%$ ,  $n = 6$ ; *Fmr1* KO DHPG versus *Fmr1* KO DHPG + roscovitine;  $p = 0.39$ ,  $t = 0.2670$ ,  $df = 12$ ; Figure 2a and c). When comparing data obtained in the presence of intracellular roscovitine, the amount of mGluR-LTD in wild-type was not significantly different from *Fmr1* KO (EPSC amplitude  $51 \pm 9\%$ ,  $n = 7$  versus  $50 \pm 3\%$ ,  $n = 6$ ; wild-type DHPG + roscovitine versus *Fmr1* KO DHPG + roscovitine;  $p = 0.78$ ,  $t = 0.2817$ ,  $df = 11$ ; compare the grey dots columns in Figure 1c and Figure 2c).

In *Fmr1* KO neurons, application of LP-211 (10 nM, 5 min) significantly reversed mGluR-LTD in control conditions (EPSC amplitude:  $53 \pm 10\%$ ,  $n = 8$ , versus  $93 \pm 14\%$ ,  $n = 8$ , *Fmr1* KO DHPG versus *Fmr1* KO DHPG + LP-211,  $p = 0.0219$ ,  $t = 2.216$ ,  $df = 14$ ; unpaired  $t$  test; Figure 2b and c) but had no effect in the presence of roscovitine, (EPSC amplitude:  $51 \pm 12\%$ ,  $n = 7$ , versus  $50 \pm 3\%$ ,  $n = 6$ ; *Fmr1* KO DHPG + roscovitine + LP-211 versus *Fmr1* KO DHPG + roscovitine;  $p = 0.47$ ,  $t = 0.07344$ ,  $df = 11$ ; Figure 2b and c). With intracellular roscovitine, the effect of LP-211 on mGluR-LTD was significantly reduced with respect to control (EPSC amplitude:  $93 \pm 14\%$ ,  $n = 8$ , versus  $51 \pm 12\%$ ,  $n = 7$ , *Fmr1* KO DHPG + LP-211 versus *Fmr1* KO DHPG + LP-211 + roscovitine,  $p = 0.0286$ ,  $t = 2.087$ ,  $df = 13$ ; unpaired  $t$  test; Figure 2b and c). The amount of mGluR-LTD in the four different experimental conditions (control; roscovitine; LP-211; LP-211 + roscovitine, Figure 2c) was significantly different ( $*p = 0.031$ , one-way ANOVA followed by Tukey's multiple comparisons test). LP-211-mediated reversal of mGluR-LTD was completely abolished by roscovitine in wild-type and in *Fmr1* KO to a comparable extent (EPSC amplitude:  $49 \pm 9\%$ ,  $n = 6$ , versus  $51 \pm 12\%$ ,  $n = 7$ , wild-type DHPG + LP-211 + roscovitine versus *Fmr1* KO DHPG + LP-211 + roscovitine,  $p = 0.896$ ,  $t = 0.1336$ ,  $df = 11$ ; unpaired  $t$  test; compare Figures 1c and 2c).

These results together show that Cdk5 inhibition prevented 5-HT7 receptor-mediated reversal of mGluR-LTD both in wild-type and in *Fmr1* KO neurons.

### 4 | DISCUSSION

Our data show that Cdk5 inhibition in wild-type hippocampal CA1 neurons enhanced mGluR-LTD to a level comparable to *Fmr1* KO neurons. This result differs from control conditions, in which the amount of mGluR-LTD in wild-type neurons is significantly lower than that observed in *Fmr1* KO neurons (Choi et al., 2011; Costa et al., 2012; Gomis-Gonzalez et al., 2016; Huber et al., 2002; Zhang et al., 2009). Enhancement of mGluR-LTD in wild-type neurons following Cdk5 inhibition suggests that, in physiological conditions, Cdk5 exerts a negative control on mGluR-LTD. Our results also suggest that either the expression or the function

of Cdk5 in *Fmr1* KO neurons might be reduced compared to wild-type and that reduced Cdk5 function might account for enhanced mGluR-LTD. In accordance with our hypothesis, a recent study shows a reduced expression of Cdk5 in the hippocampus of *Fmr1* KO mice (Zhang et al., 2020). In future studies, it might be interesting to measure the activation level of Cdk5 and of its physiological activators p35 and p39 in neurons from *Fmr1* KO mice and, possibly, in human neurons derived from Fragile X Syndrome patients using induced pluripotent stem cell (iPSC) differentiation strategies.

We further show that activation of 5-HT7 receptors was unable to reverse mGluR-LTD in both wild-type and *Fmr1* KO neurons following Cdk5 inhibition, showing that 5-HT7 receptors recruit Cdk5 to modulate mGluR-LTD.

Roscovitin has a similar affinity for Cdc2 (also known as Cdk1), Cdk2, Cdk5 and Cdk7, with reported  $IC_{50}$  values of 0.65, 0.7, 0.16 and 0.45  $\mu$ M respectively (Meijer et al., 1997; Schang et al., 2002). However, published data suggest that in our experimental conditions roscovitin acted primarily on Cdk5. Indeed, Cdc2 and Cdk2 play a key role in the cell cycle and are expressed exclusively by dividing cells during embryonic development: their maximal expression in mouse forebrain was found between embryonic day 1 and 11 (E1-E11), was barely detectable by E16-17 and remained very low throughout adult life. Conversely, an opposite pattern of expression and activity was described for Cdk5, which is expressed in mouse forebrain and hippocampus exclusively in post-mitotic neurons, with a growing level of expression from embryonic to adult ages (Tsai et al., 1993). Another study showed a weak expression of Cdk1 and Cdk2 in mouse hippocampal pyramidal neurons, but at PN 11 (very close to the age of mice used in our study) they were detected at low levels only in the nucleus and not in the cytoplasm; cytoplasmic expression of Cdk1 and Cdk2 in hippocampal neurons was found only in adults (9 months PN) (Schmetsdorf et al., 2005). Very little information is presently available about Cdk7 expression in the brain. In mouse cortical neurons, Cdk7 levels were very low before PN 30 (He et al., 2017). In the present work, we have studied fully differentiated (non-dividing) mouse hippocampal pyramidal neurons at a post natal age (PN 14–23) when Cdk5 is highly expressed whereas Cdk1, Cdk2 and Cdk7 expression levels are very low. Therefore, we believe that in our experimental conditions roscovitin acted primarily through Cdk5 inhibition.

In our experiments, roscovitin was included in the intracellular pipette solution, thus Cdk5 inhibition was exclusively exerted in the CA1 neuron under recording, indicating a post-synaptic role of Cdk5 in 5-HT7 receptor-mediated effect.

In the last decade, interesting publications have indicated a connection between 5-HT7 receptors and Cdk5, showing that 5-HT7 receptors require Cdk5 to stimulate axonal elongation and dendrite formation in cultured neurons from rodent brain cortex, hippocampus and striatum (Speranza et al., 2013,

2015, 2017). The intracellular pathway linking 5-HT7 receptors to Cdk5 activation remains to be clarified. A plausible link might be the cAMP pathway, since increases in cAMP levels were shown to stimulate p35 expression and Cdk5 activity in rat cultured neurons (He et al., 2016). 5-HT7 receptors are coupled to Gs protein, stimulating adenylate cyclase and cAMP formation (Wirth et al., 2017), thus we might speculate that 5-HT7 receptor-induced cAMP increase might stimulate the p35/Cdk5 pathway in hippocampal neurons. This issue is particularly relevant to Fragile X Syndrome, since reduced levels of cAMP were measured in blood platelets of Fragile X patients (Berry-Kravis & Huttenlocher, 1992; Berry-Kravis & Sklena, 1993) and the cAMP signaling cascade is altered at different levels in neurons from *Fmr1* KO mice, originating a “cAMP hypothesis” of the disease (Kelley et al., 2008). In the brain of *Fmr1* KO mice, overexpression and increased activity of phosphodiesterase 2A (PDE2A), a cAMP degrading enzyme, leads to reduced cAMP formation and dysregulation of cAMP downstream signaling (Maurin et al., 2018, 2019). As above mentioned, cAMP can stimulate p35/Cdk5 expression and function in rodent neurons (He et al., 2016); thus reduced cAMP levels in mouse *Fmr1* KO hippocampal neurons might be related to the reduced Cdk5 expression recently described (Zhang et al., 2020).

Besides a possible involvement of cAMP, 5-HT7 receptors might activate Cdk5 through additional mechanisms. A just-published paper shows that 5-HT7 receptors are physically linked to Cdk5 and stimulate Cdk5 activity in a G protein-independent mode. Of note, using several in vitro and in vivo approaches, the same work shows that abnormally high constitutive activity of 5-HT7 receptors caused Tau hyperphosphorylation, formation of Tau aggregates, neuronal damage, impaired synaptic plasticity and learning deficits that were rescued by knocking down 5-HT7 receptor expression, suggesting that inhibition of 5-HT7 receptor-mediated Cdk5 activity might be used as a therapy for tauopathies (Labus et al., 2021).

Many therapeutic strategies for a potential treatment of Alzheimer's disease and Parkinson's disease aim to reduce excessive Cdk5 activity, focusing on Cdk5 inhibitors (Cheung & Ip, 2012; Gong & Iqbal, 2008). Our present results, together with the work of Zhang et al. (Zhang et al., 2020), indicate that in *Fmr1* KO neurons Cdk5 activity is instead abnormally low, suggesting that activation of Cdk5 might be beneficial in Fragile X Syndrome.

Pharmacological activators of Cdk5 are not available at present. The intracellular membrane-bound kinases p35 and p39 are physiological Cdk5 activators; only few upstream extracellular messengers are currently known to activate p35 and Cdk5, namely BDNF (Cheung et al., 2007), dopamine through D1 receptors (Lebel et al., 2009), and serotonin through 5-HT7 receptors (Speranza et al., 2013, 2015, 2017). We suggest that selective 5-HT7 receptor agonists can

be used to stimulate Cdk5 activity and might become useful pharmacological tools for Fragile X Syndrome. In addition, we suggest that the effects of 5-HT7 receptor agonists might be studied in other conditions associated with reduced Cdk5 expression and function.

## ACKNOWLEDGEMENTS

The authors thank Dr. Michael Tranfaglia (Medical Director and Chief Scientific Officer of FRAXA Research Foundation, U.S.A.) for critical reading of the manuscript. The present work was financed by Telethon Foundation (grant GGP13145) and by the University of Catania (grant Chance 2017). We wish to thank Dr. Marco Abbate for veterinary assistance, Mr Giuseppe Valastro and Mr Nicola Pulvirenti for animal care and technical assistance. Images from Motifolio drawing toolkit ([www.motifolio.com](http://www.motifolio.com)) were utilized in the graphical abstract preparation.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

## AUTHOR CONTRIBUTIONS

Lucia Ciranna designed the study, analysed data and drafted the paper; Lara Costa and Alessandra Tempio performed experiments and analysed data; Enza Lacivita and Marcello Leopoldo designed 5-HT7R agonist and analysed data.

## PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1111/ejn.15246>.

## DATA AVAILABILITY

The data that support the findings of this study are openly available in the public repository Figshare at <https://doi.org/10.6084/m9.figshare.14431205.v1>

## ORCID

Alessandra Tempio  <https://orcid.org/0000-0002-1883-0227>

Lucia Ciranna  <https://orcid.org/0000-0003-0274-6095>

## REFERENCES

- Allnutt, A. B., Waters, A. K., Kesari, S., & Yenugonda, V. M. (2020). Physiological and pathological roles of Cdk5: Potential directions for therapeutic targeting in neurodegenerative disease. *ACS Chemical Neuroscience*, *11*, 1218–1230. <https://doi.org/10.1021/acscemneuro.0c00096>
- Bear, M. F., Huber, K. M., & Warren, S. T. (2004). The mGluR theory of fragile X mental retardation. *Trends in Neurosciences*, *27*, 370–377. <https://doi.org/10.1016/j.tins.2004.04.009>
- Berry-Kravis, E., & Huttenlocher, P. R. (1992). Cyclic AMP metabolism in fragile X syndrome. *Annals of Neurology*, *31*, 22–26. <https://doi.org/10.1002/ana.410310105>
- Berry-Kravis, E. & Sklena, P. (1993). Demonstration of abnormal cyclic AMP production in platelets from patients with fragile X syndrome. *American Journal of Medical Genetics*, *45*, 81–87. <https://doi.org/10.1002/ajmg.1320450120>
- Cheung, Z. H., Chin, W. H., Chen, Y., Ng, Y. P., & Ip, N. Y. (2007). Cdk5 is involved in BDNF-stimulated dendritic growth in hippocampal neurons. *PLoS Biology*, *5*, e63. <https://doi.org/10.1371/journal.pbio.0050063>
- Cheung, Z. H. & Ip, N. Y. (2012). Cdk5: A multifaceted kinase in neurodegenerative diseases. *Trends in Cell Biology*, *22*, 169–175. <https://doi.org/10.1016/j.tcb.2011.11.003>
- Choi, C. H., Schoenfeld, B. P., Bell, A. J., Hinchey, P., Kollaros, M., Gertner, M. J., Woo, N. H., Tranfaglia, M. R., Bear, M. F., Zukin, R. S., McDonald, T. V., Jongens, T. A., & McBride, S. M. (2011). Pharmacological reversal of synaptic plasticity deficits in the mouse model of fragile X syndrome by group II mGluR antagonist or lithium treatment. *Brain Research*, *1380*, 106–119. <https://doi.org/10.1016/j.brainres.2010.11.032>
- Costa, L., Sardone, L. M., Bonaccorso, C. M., D'Antoni, S., Spatuzza, M., Gulisano, W., Tropea, M. R., Puzzo, D., Leopoldo, M., Lacivita, E., Catania, M. V., & Ciranna, L. (2018). Activation of serotonin 5-HT7 receptors modulates hippocampal synaptic plasticity by stimulation of adenylate cyclases and rescues learning and behavior in a mouse model of fragile X syndrome. *Frontiers in Molecular Neuroscience*, *11*, 353. <https://doi.org/10.3389/fnmol.2018.00353>
- Costa, L., Sardone, L. M., Lacivita, E., Leopoldo, M., & Ciranna, L. (2015). Novel agonists for serotonin 5-HT7 receptors reverse metabotropic glutamate receptor-mediated long-term depression in the hippocampus of wild-type and Fmr1 KO mice, a model of Fragile X Syndrome. *Frontiers in Behavioral Neuroscience*, *9*, 65. <https://doi.org/10.3389/fnbeh.2015.00065>
- Costa, L., Spatuzza, M., D'Antoni, S., Bonaccorso, C. M., Trovato, C., Musumeci, S. A., Leopoldo, M., Lacivita, E., Catania, M. V., & Ciranna, L. (2012). Activation of 5-HT7 serotonin receptors reverses metabotropic glutamate receptor-mediated synaptic plasticity in wild-type and Fmr1 knockout mice, a model of Fragile X syndrome. *Biological Psychiatry*, *72*, 924–933. <https://doi.org/10.1016/j.biopsych.2012.06.008>
- Drerup, J. M., Hayashi, K., Cui, H., Mettlach, G. L., Long, M. A., Marvin, M., Sun, X., Goldberg, M. S., Lutter, M., & Bibb, J. A. (2010). Attention-deficit/hyperactivity phenotype in mice lacking the cyclin-dependent kinase 5 cofactor p35. *Biological Psychiatry*, *68*, 1163–1171. <https://doi.org/10.1016/j.biopsych.2010.07.016>
- Engmann, O., Hortobagyi, T., Pidsley, R., Troakes, C., Bernstein, H. G., Kreutz, M. R., Mill, J., Nikolic, M., & Giese, K. P. (2011). Schizophrenia is associated with dysregulation of a Cdk5 activator that regulates synaptic protein expression and cognition. *Brain*, *134*, 2408–2421. <https://doi.org/10.1093/brain/awr155>
- Giese, K. P. (2014). Generation of the Cdk5 activator p25 is a memory mechanism that is affected in early Alzheimer's disease. *Frontiers in Molecular Neuroscience*, *7*, 36.
- Gomis-González, M., Busquets-García, A., Matute, C., Maldonado, R., Mato, S., & Ozaita, A. (2016). Possible therapeutic doses of cannabinoid type 1 receptor antagonist reverses key alterations in fragile X syndrome mouse model. *Genes*, *7*, 56. <https://doi.org/10.3390/genes7090056>
- Gong, C. X. & Iqbal, K. (2008). Hyperphosphorylation of microtubule-associated protein tau: A promising therapeutic target for Alzheimer disease. *Current Medicinal Chemistry*, *15*, 2321–2328.



- He, F., Qi, G., Zhang, Q., Cai, H., Li, T., Li, M., Zhang, Q., Chen, J., Ming, J., Tian, B., & Zhang, P. (2020). Quantitative phosphoproteomic analysis in alpha-synuclein transgenic mice reveals the involvement of aberrant p25/Cdk5 signaling in early-stage Parkinson's disease. *Cellular and Molecular Neurobiology*, *40*, 897–909. <https://doi.org/10.1007/s10571-019-00780-7>
- He, G., Yang, X., Wang, G., Qi, J., Mao, R., Wu, Z., & Zhou, Z. (2017). Cdk7 is required for activity-dependent neuronal gene expression, long-lasting synaptic plasticity and long-term memory. *Frontiers in Molecular Neuroscience*, *10*, 365. <https://doi.org/10.3389/fnmol.2017.00365>
- He, H., Deng, K., Siddiq, M. M., Pyie, A., Mellado, W., Hannila, S. S., & Filbin, M. T. (2016). Cyclic AMP and polyamines overcome inhibition by myelin-associated glycoprotein through eIF5A-mediated increases in p35 expression and activation of Cdk5. *Journal of Neuroscience*, *36*, 3079–3091. <https://doi.org/10.1523/JNEUROSCI.4012-15.2016>
- Huber, K. M., Gallagher, S. M., Warren, S. T., & Bear, M. F. (2002). Altered synaptic plasticity in a mouse model of fragile X mental retardation. *Proceedings of the National Academy of Sciences USA*, *99*, 7746–7750. <https://doi.org/10.1073/pnas.122205699>
- Kawauchi, T. (2014). Cdk5 regulates multiple cellular events in neural development, function and disease. *Development, Growth & Differentiation*, *56*, 335–348. <https://doi.org/10.1111/dgd.12138>
- Kelley, D. J., Bhattacharyya, A., Lahvis, G. P., Yin, J. C., Malter, J., & Davidson, R. J. (2008). The cyclic AMP phenotype of fragile X and autism. *Neuroscience and Biobehavioral Reviews*, *32*, 1533–1543. <https://doi.org/10.1016/j.neubiorev.2008.06.005>
- Ko, J., Humbert, S., Bronson, R. T., Takahashi, S., Kulkarni, A. B., Li, E., & Tsai, L. H. (2001). p35 and p39 are essential for cyclin-dependent kinase 5 function during neurodevelopment. *Journal of Neuroscience*, *21*, 6758–6771. <https://doi.org/10.1523/JNEUROSCI.21-17-06758.2001>
- Labus, J., Rohrs, K. F., Ackmann, J., Varbanov, H., Muller, F. E., Jia, S., Jahreis, K., Vollbrecht, A. L., Butzlaff, M., Schill, Y., Guseva, D., Bohm, K., Kaushik, R., Bijata, M., Marin, P., Chaumont-Dubel, S., Zeug, A., Dityatev, A., & Ponimaskin, E. (2021). Amelioration of Tau pathology and memory deficits by targeting 5-HT7 receptor. *Progress in Neurobiology*, *197*, 101900. <https://doi.org/10.1016/j.pneurobio.2020.101900>
- Lebel, M., Patenaude, C., Allyson, J., Massicotte, G., & Cyr, M. (2009). Dopamine D1 receptor activation induces tau phosphorylation via cdk5 and GSK3 signaling pathways. *Neuropharmacology*, *57*, 392–402. <https://doi.org/10.1016/j.neuropharm.2009.06.041>
- Liu, S. L., Wang, C., Jiang, T., Tan, L., Xing, A., & Yu, J. T. (2016). The role of Cdk5 in Alzheimer's disease. *Molecular Neurobiology*, *53*, 4328–4342. <https://doi.org/10.1007/s12035-015-9369-x>
- Liu, X. X., Yang, L., Shao, L. X., He, Y., Wu, G., Bao, Y. H., Lu, N. N., Gong, D. M., Lu, Y. P., Cui, T. T., Sun, N. H., Chen, D. Y., Shi, W. X., Fukunaga, K., Chen, H. S., Chen, Z., Han, F., & Lu, Y. M. (2020). Endothelial Cdk5 deficit leads to the development of spontaneous epilepsy through CXCL1/CXCR2-mediated reactive astrogliosis. *Journal of Experimental Medicine*, *217*. <https://doi.org/10.1084/jem.20180992>
- Luo, S., Vacher, C., Davies, J. E., & Rubinsztein, D. C. (2005). Cdk5 phosphorylation of huntingtin reduces its cleavage by caspases: Implications for mutant huntingtin toxicity. *Journal of Cell Biology*, *169*, 647–656. <https://doi.org/10.1083/jcb.200412071>
- Luscher, C. & Huber, K. M. (2010). Group 1 mGluR-dependent synaptic long-term depression: Mechanisms and implications for circuitry and disease. *Neuron*, *65*, 445–459. <https://doi.org/10.1016/j.neuron.2010.01.016>
- Maurin, T., Lebrigand, K., Castagnola, S., Paquet, A., Jarjat, M., Popa, A., Grossi, M., Rage, F., & Bardoni, B. (2018). HITS-CLIP in various brain areas reveals new targets and new modalities of RNA binding by fragile X mental retardation protein. *Nucleic Acids Research*, *46*, 6344–6355. <https://doi.org/10.1093/nar/gky267>
- Maurin, T., Melancia, F., Jarjat, M., Castro, L., Costa, L., Delhay, S., Khayachi, A., Castagnola, S., Mota, E., Di Giorgio, A., Servadio, M., Drozd, M., Poupon, G., Schiavi, S., Sardone, L., Azoulay, S., Ciranna, L., Martin, S., Vincent, P., ... Bardoni, B. (2019). Involvement of phosphodiesterase 2A activity in the pathophysiology of fragile X syndrome. *Cerebral Cortex*, *29*, 3241–3252. <https://doi.org/10.1093/cercor/bhy192>
- Meijer, L., Borgne, A., Mulner, O., Chong, J. P., Blow, J. J., Inagaki, N., Inagaki, M., Delcros, J. G., & Moulinoux, J. P. (1997). Biochemical and cellular effects of roscovitine, a potent and selective inhibitor of the cyclin-dependent kinases cdc2, cdk2 and cdk5. *European Journal of Biochemistry*, *243*, 527–536. <https://doi.org/10.1111/j.1432-1033.1997.t01-2-00527.x>
- Paoletti, P., Vila, I., Rife, M., Lizcano, J. M., Alberch, J., & Gines, S. (2008). Dopaminergic and glutamatergic signaling crosstalk in Huntington's disease neurodegeneration: The role of p25/cyclin-dependent kinase 5. *Journal of Neuroscience*, *28*, 10090–10101. <https://doi.org/10.1523/JNEUROSCI.3237-08.2008>
- Salcedo-Arellano, M. J., Dufour, B., McLennan, Y., Martinez-Cerdeno, V., & Hagerman, R. (2020). Fragile X syndrome and associated disorders: Clinical aspects and pathology. *Neurobiology of Diseases*, *136*, 104740. <https://doi.org/10.1016/j.nbd.2020.104740>
- Sanderson, T. M., Hogg, E. L., Collingridge, G. L., & Correa, S. A. (2016). Hippocampal mGluR-LTD in health and disease: Focus on the p38 MAPK and ERK1/2 pathways. *Journal of Neurochemistry*, *139*, 200–214.
- Schang, L. M., Bantly, A., Knockaert, M., Shaheen, F., Meijer, L., Malim, M. H., Gray, N. S., & Schaffer, P. A. (2002). Pharmacological cyclin-dependent kinase inhibitors inhibit replication of wild-type and drug-resistant strains of herpes simplex virus and human immunodeficiency virus type 1 by targeting cellular, not viral, proteins. *Journal of Virology*, *76*, 7874–7882. <https://doi.org/10.1128/JVI.76.15.7874-7882.2002>
- Schmetsdorf, S., Gartner, U., & Arendt, T. (2005). Expression of cell cycle-related proteins in developing and adult mouse hippocampus. *International Journal of Developmental Neuroscience*, *23*, 101–112. <https://doi.org/10.1016/j.ijdevneu.2004.07.019>
- Shah, K. & Rossie, S. (2018). Tale of the good and the bad Cdk5: Remodeling of the actin cytoskeleton in the brain. *Molecular Neurobiology*, *55*, 3426–3438. <https://doi.org/10.1007/s12035-017-0525-3>
- Speranza, L., Chambery, A., Di Domenico, M., Crispino, M., Severino, V., Volpicelli, F., Leopoldo, M., Bellenchi, G. C., di Porzio, U., & Perrone-Capano, C. (2013). The serotonin receptor 7 promotes neurite outgrowth via ERK and Cdk5 signaling pathways. *Neuropharmacology*, *67*, 155–167. <https://doi.org/10.1016/j.neuropharm.2012.10.026>
- Speranza, L., Giuliano, T., Volpicelli, F., De Stefano, M. E., Lombardi, L., Chambery, A., Lacivita, E., Leopoldo, M., Bellenchi, G. C., di Porzio, U., Crispino, M., & Perrone-Capano, C. (2015). Activation of 5-HT7 receptor stimulates neurite elongation through mTOR, Cdc42 and actin filaments dynamics. *Frontiers in Behavioural Neurosciences*, *9*, 62. <https://doi.org/10.3389/fnbeh.2015.00062>

- Speranza, L., Labus, J., Volpicelli, F., Guseva, D., Lacivita, E., Leopoldo, M., Bellenchi, G. C., di Porzio, U., Bijata, M., Perrone-Capano, C., & Ponimaskin, E. (2017). Serotonin 5-HT7 receptor increases the density of dendritic spines and facilitates synaptogenesis in forebrain neurons. *Journal of Neurochemistry*, *141*, 647–661. <https://doi.org/10.1111/jnc.13962>
- Tsai, L. H., Takahashi, T., Caviness, V. S. Jr, & Harlow, E. (1993). Activity and expression pattern of cyclin-dependent kinase 5 in the embryonic mouse nervous system. *Development*, *119*, 1029–1040.
- Wirth, A., Holst, K., & Ponimaskin, E. (2017). How serotonin receptors regulate morphogenic signalling in neurons. *Progress in Neurobiology*, *151*, 35–56. <https://doi.org/10.1016/j.pneurobio.2016.03.007>
- Yousuf, M. A., Tan, C., Torres-Altora, M. I., Lu, F. M., Plautz, E., Zhang, S., Takahashi, M., Hernandez, A., Kernie, S. G., Plattner, F., & Bibb, J. A. (2016). Involvement of aberrant cyclin-dependent kinase 5/p25 activity in experimental traumatic brain injury. *Journal of Neurochemistry*, *138*, 317–327. <https://doi.org/10.1111/jnc.13620>
- Zhang, J., Hou, L., Klann, E., & Nelson, D. L. (2009). Altered hippocampal synaptic plasticity in the FMR1 gene family knockout mouse models. *Journal of Neurophysiology*, *101*, 2572–2580.
- Zhang, M., Li, X., Xiao, D., Lu, T., Qin, B., Zheng, Z., Zhang, Y., Liu, Y., Yan, T., & Han, X. (2020). Identification of differentially expressed microRNAs and their target genes in the hippocampal tissues of Fmr1 knockout mice. *American Journal of Translational Research*, *12*, 813–824.

**How to cite this article:** Costa L, Tempio A, Lacivita E, Leopoldo M, Ciranna L. Serotonin 5-HT7 receptors require cyclin-dependent kinase 5 to rescue hippocampal synaptic plasticity in a mouse model of Fragile X Syndrome. *Eur J Neurosci*. 2021;00:1–9. <https://doi.org/10.1111/ejn.15246>