Targeting the Endocannabinoid System in the Treatment of Fragile X Syndrome

Encuentro Sectorial de Enfermedades Raras

Bilbao 19.11.2013

ehugroup
Achucarro
BASQUE CENTER FOR NEUROSCIENCE

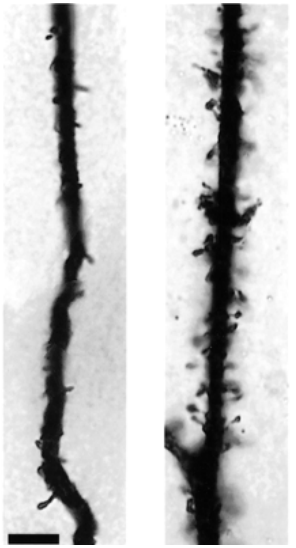
eman ta zabal zazu
Universidad del País Vasco
Euskal Herriko Unibertsitatea

ciberNed
Centro Investigación Biomédica en Red
Enfermedades Neurodegenerativas

Fragile X Syndrome (FXS)

- **FXS is the most common form of inherited mental retardation**
- **Estimated prevalence is 1 in 3600-4000 males and 1 in 6000-8000 females**
- **Features of FXS**
  - **Moderate to severe learning and intelectual disabilities/mental retardation**
  - **Autism and autistic behaviours**
  - **Atention deficit hyperactivity disorder (ADHD)**
  - **Social anxiety**
  - **Aggresive behaviours**

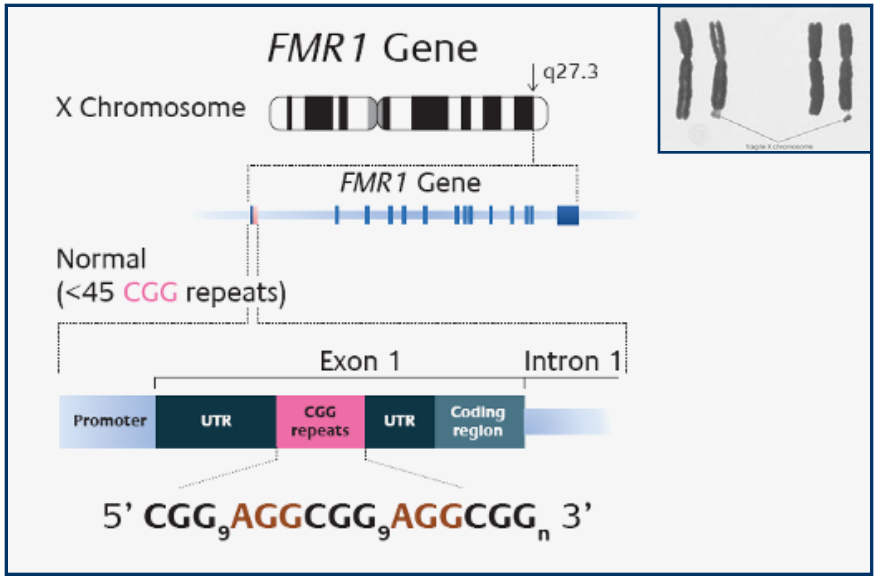
Control **FXS**

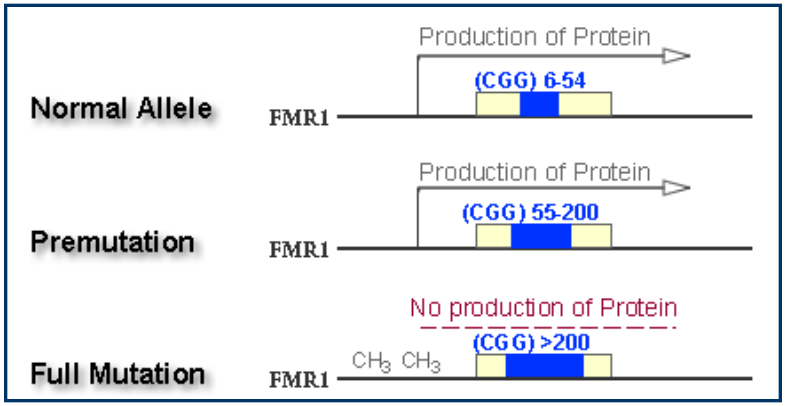


- **In the brain of FXS patients…**
  - **Abnormal dendritic spine morphology**
  - **Increased activation of the mTOR pathway**

Fragile X Syndrome (FXS)

- **Caused by transcriptional silencing of the *FMR1* gene**
- ***FMR1* silencing occurs as a consequence of a CGG repeat in the 5´untranslated region**
- **CCGG repeats > 200 inhibits the production of the *FMR1* product FMRP**
- **FMRP is an RNA binding protein that controls translation efficacy of dendritic RNAs**

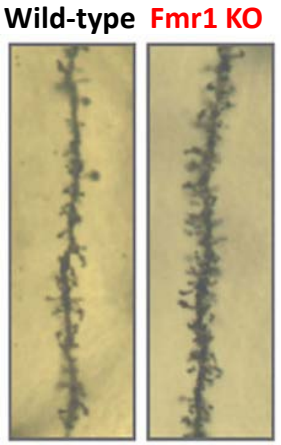




The Fmr1 Knockout Mice

- **Exhibit many features of FXS patients**
  - **Learning deficits, hiperactivity, anxiety-like behavior…**
  - **Increased number of dendritic spines and mTOR signaling**

- **Dysregulated excitatory-inhibitory balance**
- **Altered hippocampal synaptic plasticity**
- **Exagerated activity of group I metabotropic glutamate receptors (mGluR1/5)**



- **Proposed therapies normalize Fmr1 KO mouse phenotype**
  - **mGluR5 antagonists**
  - **$GABA_B$ agonist**

Brain Endocannabinoid System

- **BIOLOGICAL FUNCIONS**

  - Regulation of mood, learning and memory, movement control, pain, emesis…

- **BRAIN CANNABINOID CB1 RECEPTORS**

  - $G_{i/o}$ protein coupled receptors

  - High expression in neurons

NATURAL

Δ^9-THC
CBN

ENDOGENOUS

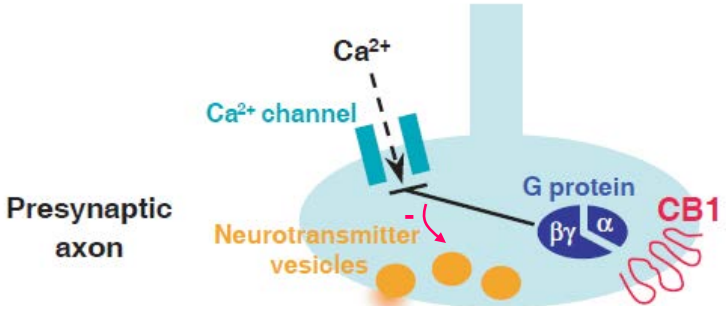
Araquidonoyletanolamine (AEA)
2-Araquidonoylglycerol (2-AG)

SYNTHETIC

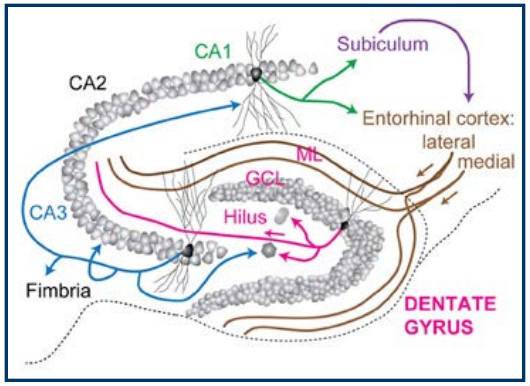
CP55,940
WIN55,212,2

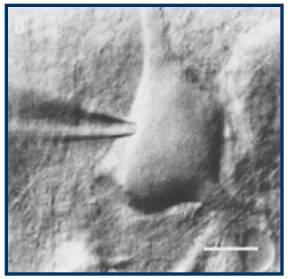
Presynaptic CB1 receptors inhibit neurotransmitter release

PRESYNAPTIC CB1Rs INHIBIT NEUROTRANSMITTER RELEASE ⟷ reduces the size of IPSCs/EPSCs

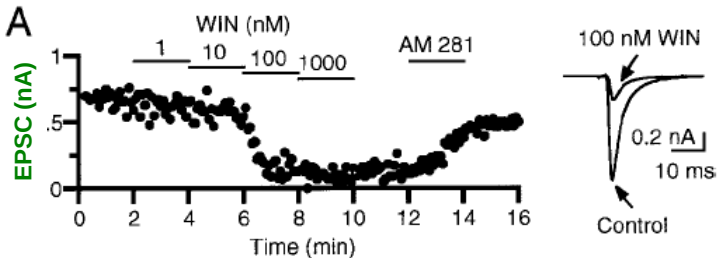


Inhibition of pharmacologically isolated eEPSC and IPSCs in CA1 pyramidal cells by CB1R

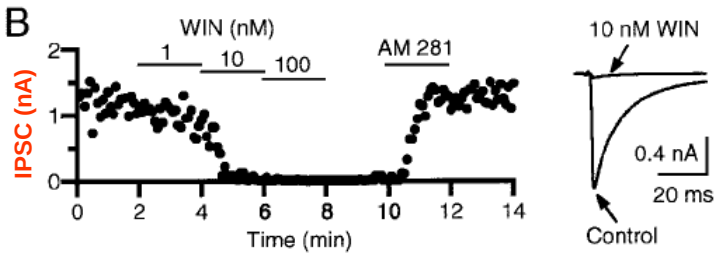




Glutamatergic transmission

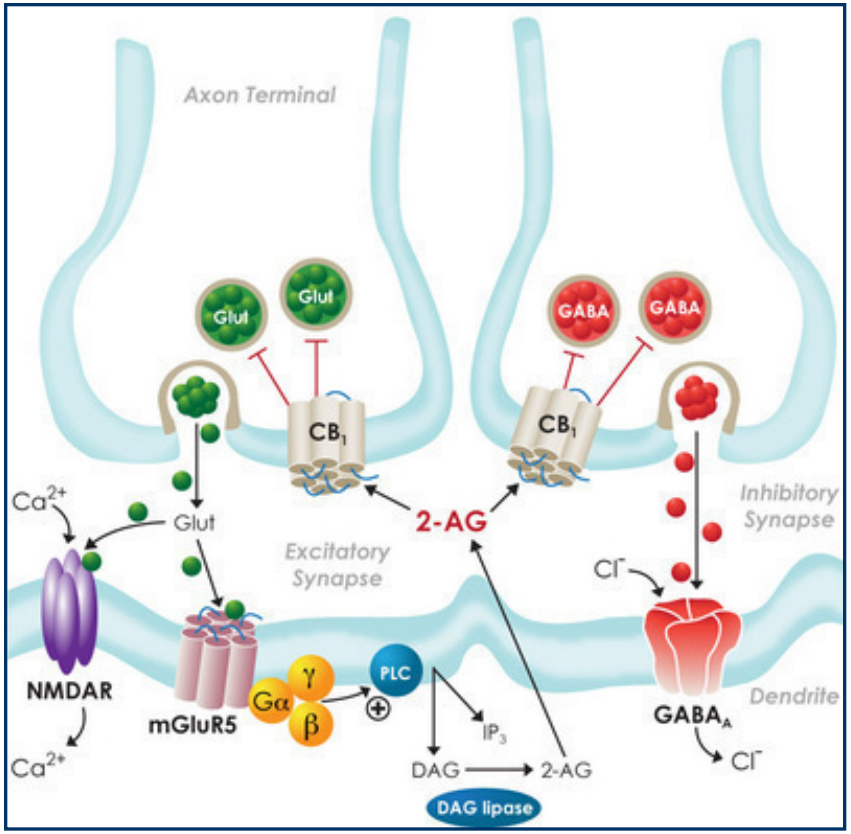


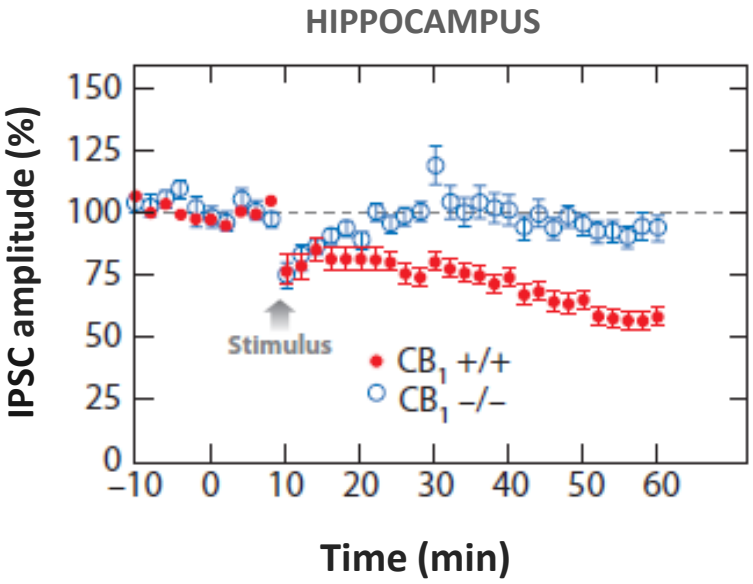
GABAergic transmission



Endocannabinoids and CB1 receptors mediate synaptic plasticity

SYNAPTIC mGluR ACTIVATION TRIGGERS eCB PRODUCTION and LONG-TERM DEPRESSION

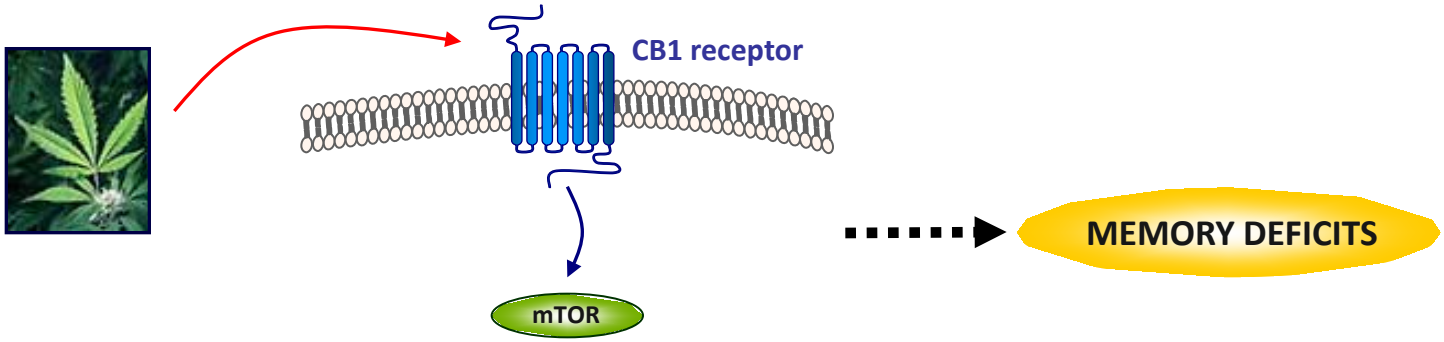




FXS and the Endocannabinoid System

- **Exagerated mGluR5 activity detected in Fmr1 mice is expected to mobilize eCBs**

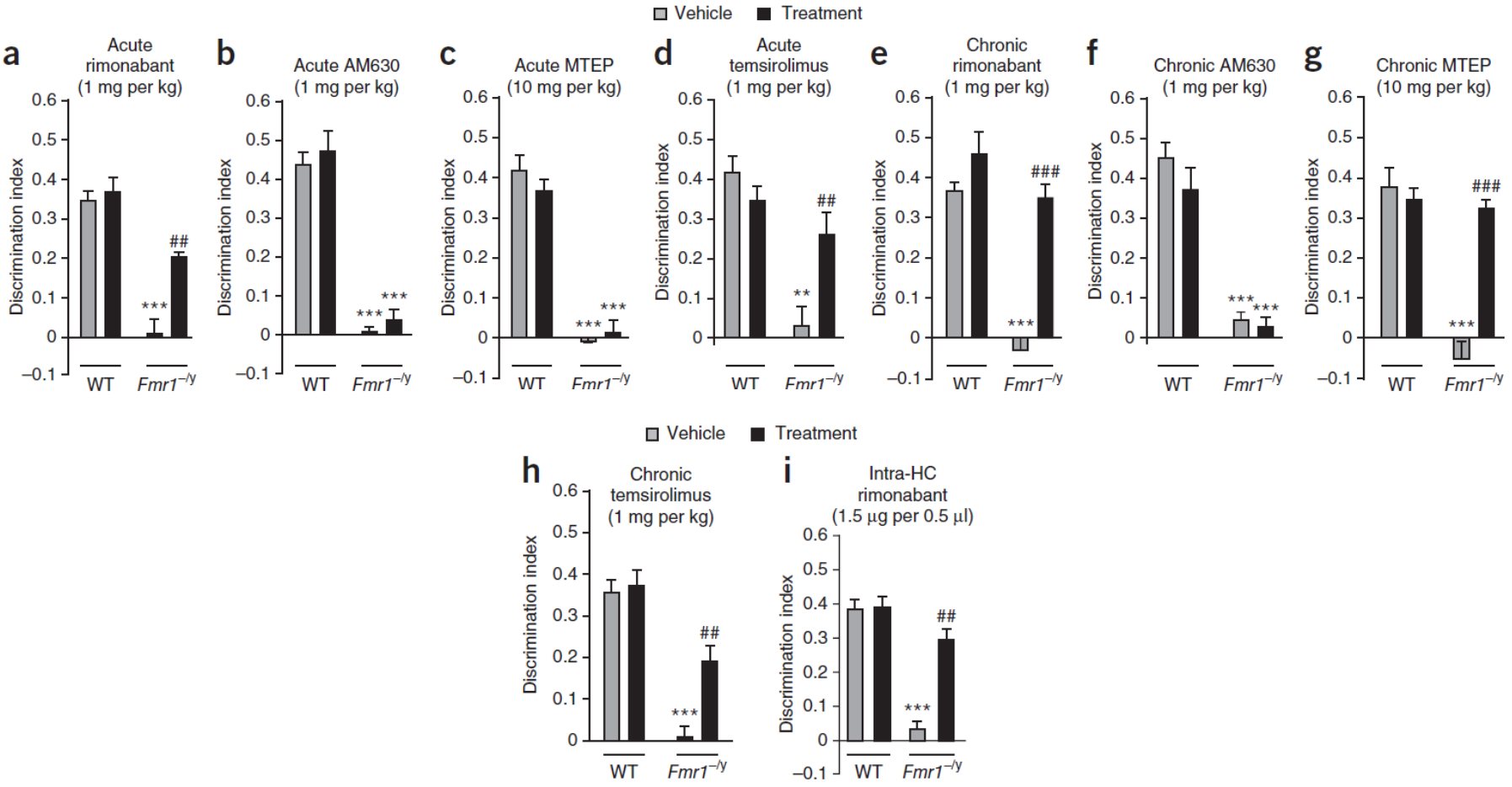
- ***In vivo* administration of Δ^9-THC induces CB1 receptor-dependent cognitive deficits in wild-type mice through the activation of mTOR pathway in the hippocampus**



Does enhanced activity of brain eCB system contribute to the etiopatogeny of FSX?

Pharmacological modulation of memory impairment in Fmr1$^{-/y}$ mice

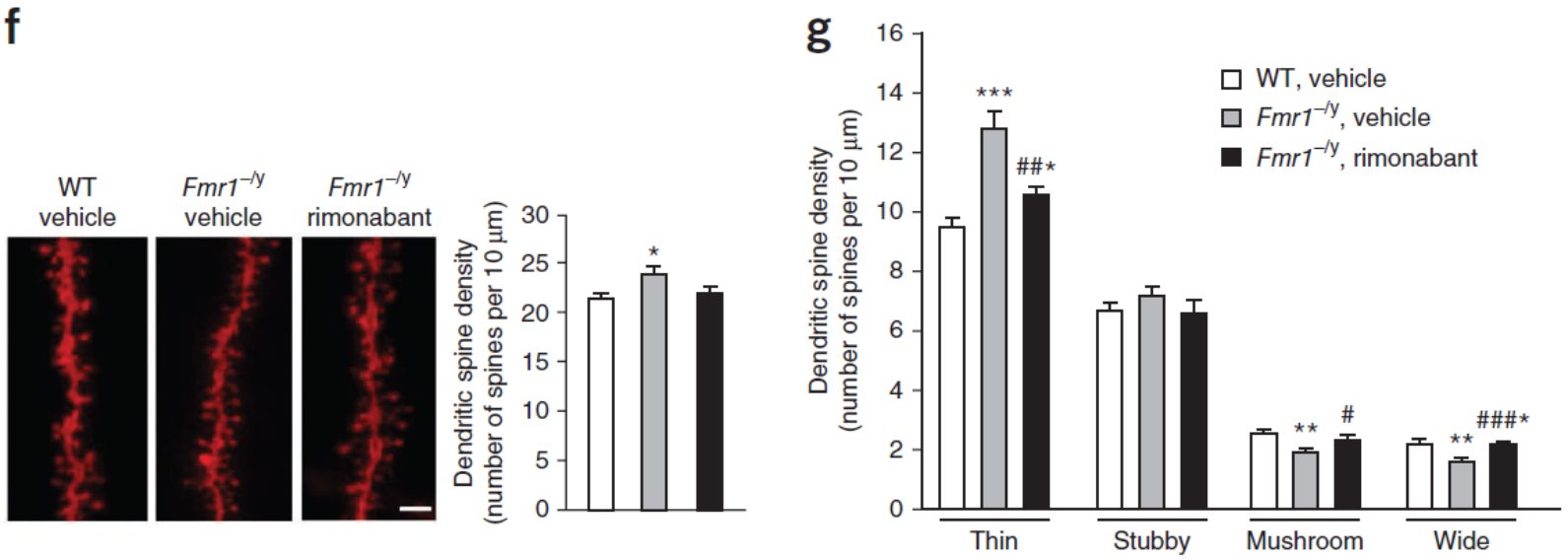
OBJECT RECOGNITION MEMORY TEST



Acute and chronic CB1R, mGluR5 and mTOR blockade nomalizes cognitive deficits in Fmr1$^{-/y}$ mice

Pharmacological modulation of memory impairment in Fmr1$^{-/y}$ mice

HIPPOCAMPAL CA1 DENDRITIC SPINE MORPHOLOGY

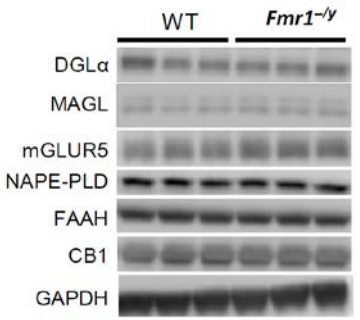


Chronic CB1R blockade corrects increased spine density in Fmr1$^{-/y}$ mice

The endocannabinoid system in the hippocampus of Fmr1$^{-/y}$ mice

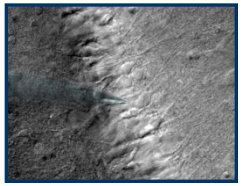
BIOCHEMICAL EXPERIMENTS IN HIPPOCAMPAL HOMOGENATES

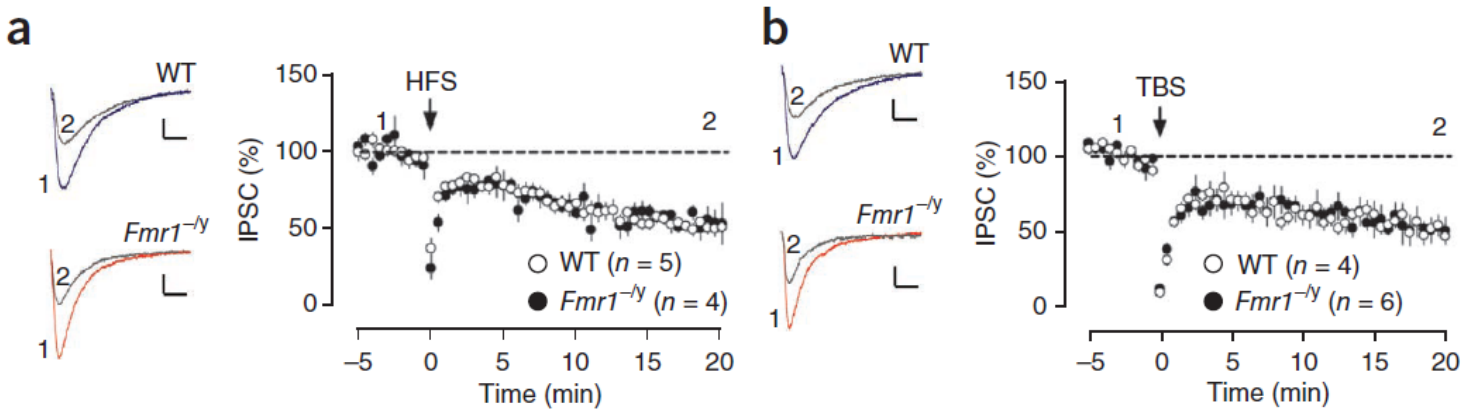
- Unaltered expression of CB1Rs and mGluR5s
- No changes in the expression of eCB synthetic/degrading enzymes
- Unaltered levels of 2-AG and AEA in the hippocampus



PATCH-CLAMP EXPERIMENTS IN CA1 PYRAMIDAL CELLS

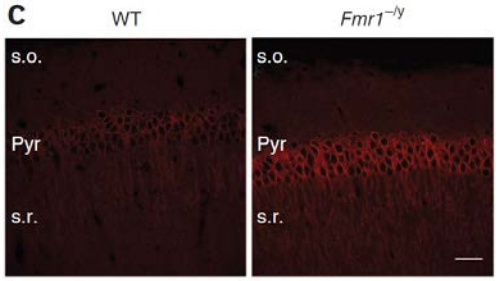
- Unaltered CB1R and mGluR5 inhibition of GABAergic currents
- Unaltered eCB-LTD of GABA tranmission

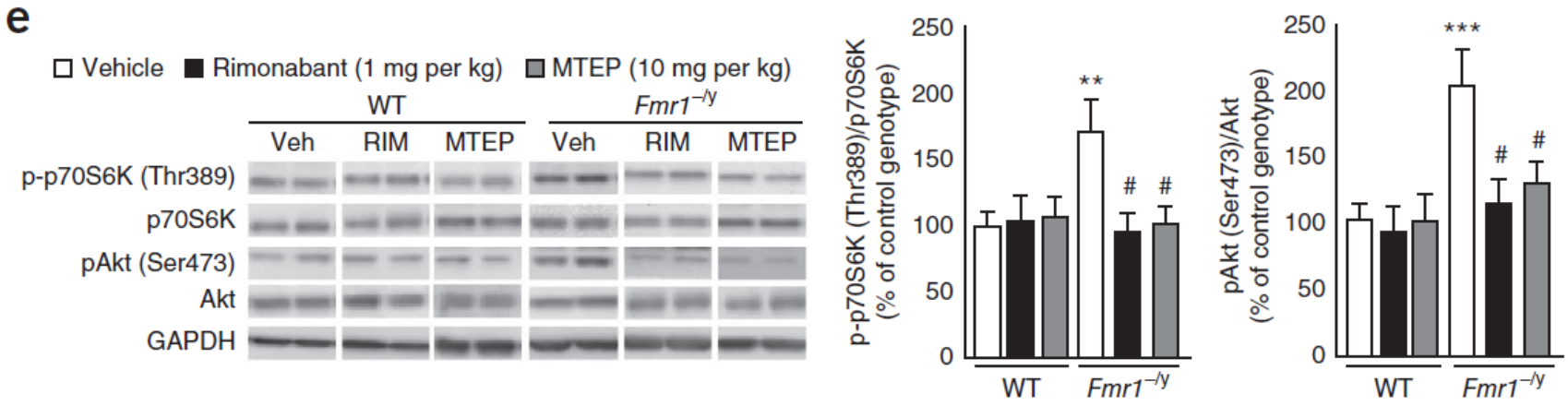




Enhanced activation of mTOR pathway in the hippocampus of Fmr1$^{-/y}$ mice

IMMUNOHISTOCHEMICAL AND BIOCHEMICAL EXPERIMENTS IN HIPPOCAMPAL HOMOGENATES





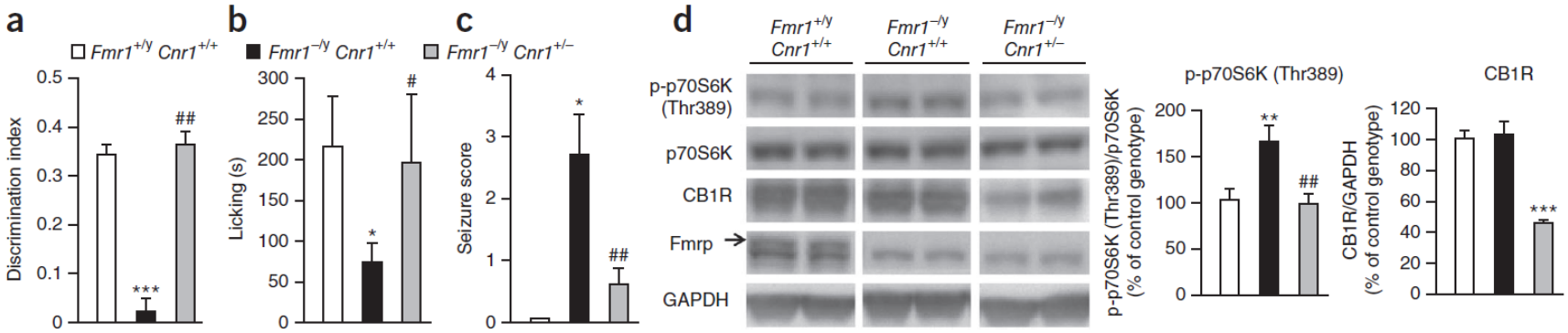
Increased p70S6K phosphorylation in CA1 pyramidal cells of Fmr1$^{-/y}$ mice is normalized by chronic treatment with rimonabant and MPEP

Genetic rescue of *Fmr1*$^{-/y}$ mice phenotype by CB1R attenuation

Fmr1$^{+/-}$ female x *Cnr1*$^{+/-}$ male

Fmr1$^{+/y}$: *Cnr1*$^{+/+}$	*Fmr1*$^{-/y}$: *Cnr1*$^{+/+}$	*Fmr1*$^{+-/y}$: *Cnr1*$^{+/-}$
Wild-type	FXS	Rescued

Conductual and biochemical experiments in genetically rescued Fmr1$^{-/y}$: Cnr1$^{+/-}$ mice



Genetic atenuation of CB1R corrects behavioral deficits and mTOR overactivation in Fmr1$^{-/y}$ mice

nature medicine

LETTERS

Targeting the endocannabinoid system in the treatment of fragile X syndrome

Arnau Busquets-Garcia[1], Maria Gomis-González[1], Thomas Guegan[1], Carmen Agustín-Pavón[2,3,11], Antoni Pastor[4,5], Susana Mato[6–8], Alberto Pérez-Samartín[6–8], Carlos Matute[6–8], Rafael de la Torre[1,4,9], Mara Dierssen[2,3,10], Rafael Maldonado[1] & Andrés Ozaita[1]

[1]Departament de Ciències Experimentals i de la Salut (DCEXS), Universitat Pompeu Fabra (UPF), Barcelona, Spain. [2]Centre for Genomic Regulation (CRG), Barcelona, Spain. [3]Universitat Pompeu Fabra (UPF), Barcelona, Spain. [4]Grup de Recerca Clínica en Farmacologia Humana i Neurociències, Institut Hospital del Mar d'Investigacions Mèdiques (IMIM), Barcelona, Spain. [5]Facultat de Medicina, Universitat Autònoma de Barcelona (UAB), Barcelona, Spain. [6]Laboratorio de Neurobiología, Departamento de Neurociencias, Universidad del País Vasco/Euskal Herriko Unibertsitatea (UPV/EHU), Leioa, Spain. [7]Achucarro Basque Center for Neuroscience, Zamudio, Spain. [8]Instituto de Salud Carlos III (ISCIII), Centro de Investigación Biomédica en Red de Enfermedades Neurodegenerativas (CIBERNED), Leioa, Spain. [9]CIBER de Fisiopatología de la Obesidad y Nutrición (CB06/03) (CIBEROBN), Hospital Clínico Universitario Santiago de Compostela, Santiago de Compostela, Spain. [10]CIBER de Enfermedades Raras (CIBERER), Barcelona, Spain. [11]Present address: EMBL-CRG Systems Biology Research Unit, Centre for Genomic Regulation (CRG), Barcelona, Spain. Correspondence should be addressed to A.O. (andres.ozaita@upf.edu).

Received 14 May 2012; accepted 12 February 2013; published online 31 March 2013; doi:10.1038/nm.3127

Conclusion

Pharmacological blockade of CB1R may be a useful therapeutic strategy to treat FXS

Future and ongoing work…

- **Study the ability of lower Rimonabant doses to correct the conductual, electrophysiological and biochemical phenotype of Fmr1$^{-/y}$ mice**

- **Analyze the effects of other CB1R antagonists/inverse agonists in Fmr1$^{-/y}$ mice**

Thanks for your attention!