Maurizio Giustetto, PhD

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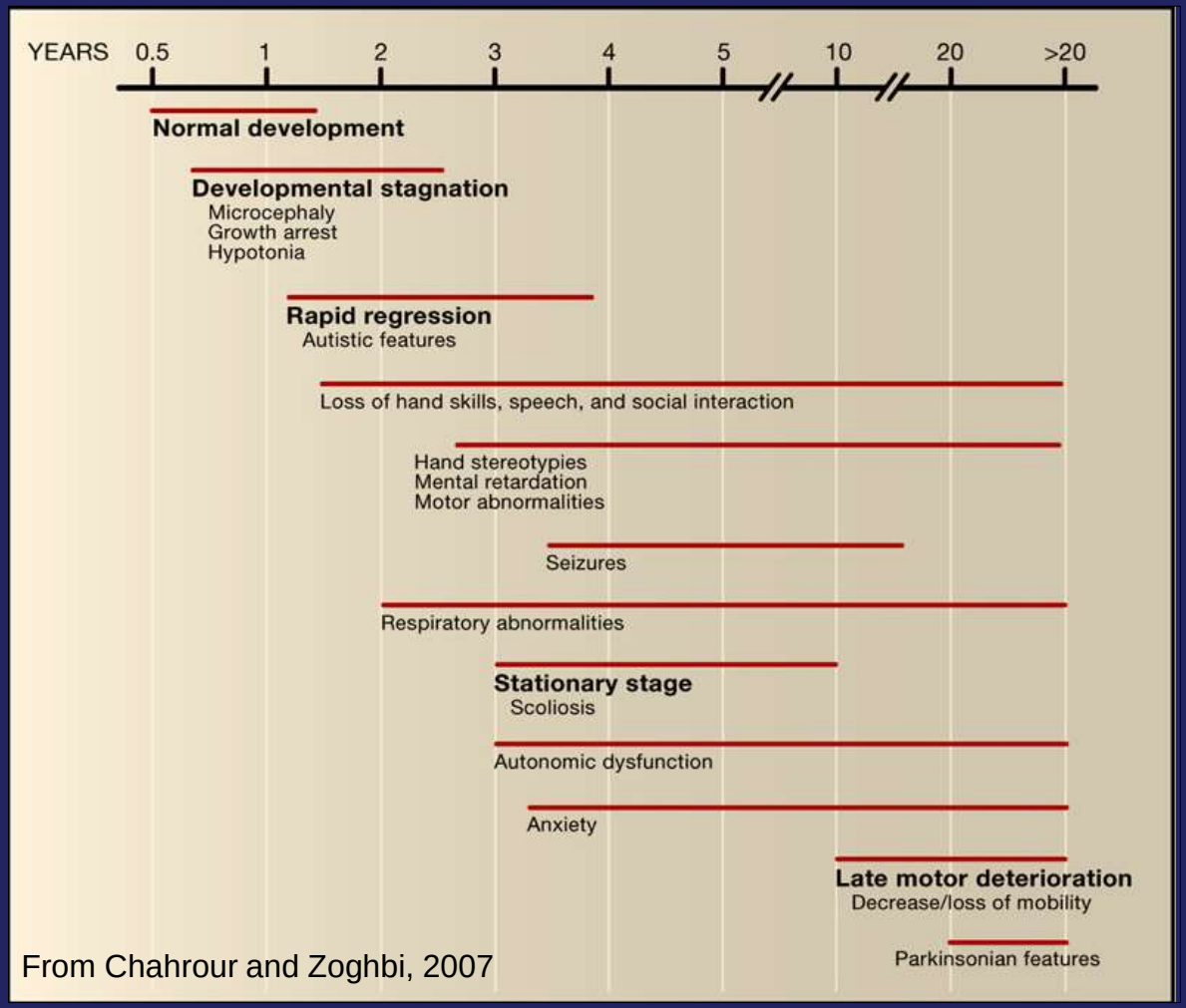
A SHORT JOURNEY INTO LIFE AS A NEUROSCIENTIST

Torino, 21.05.2018

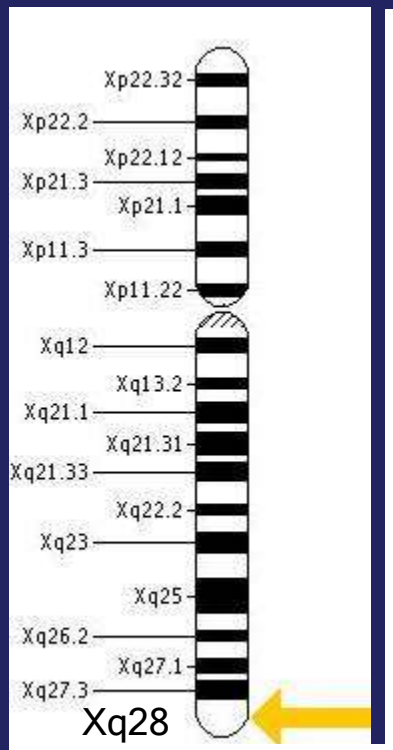
Rett syndrome: FAQs

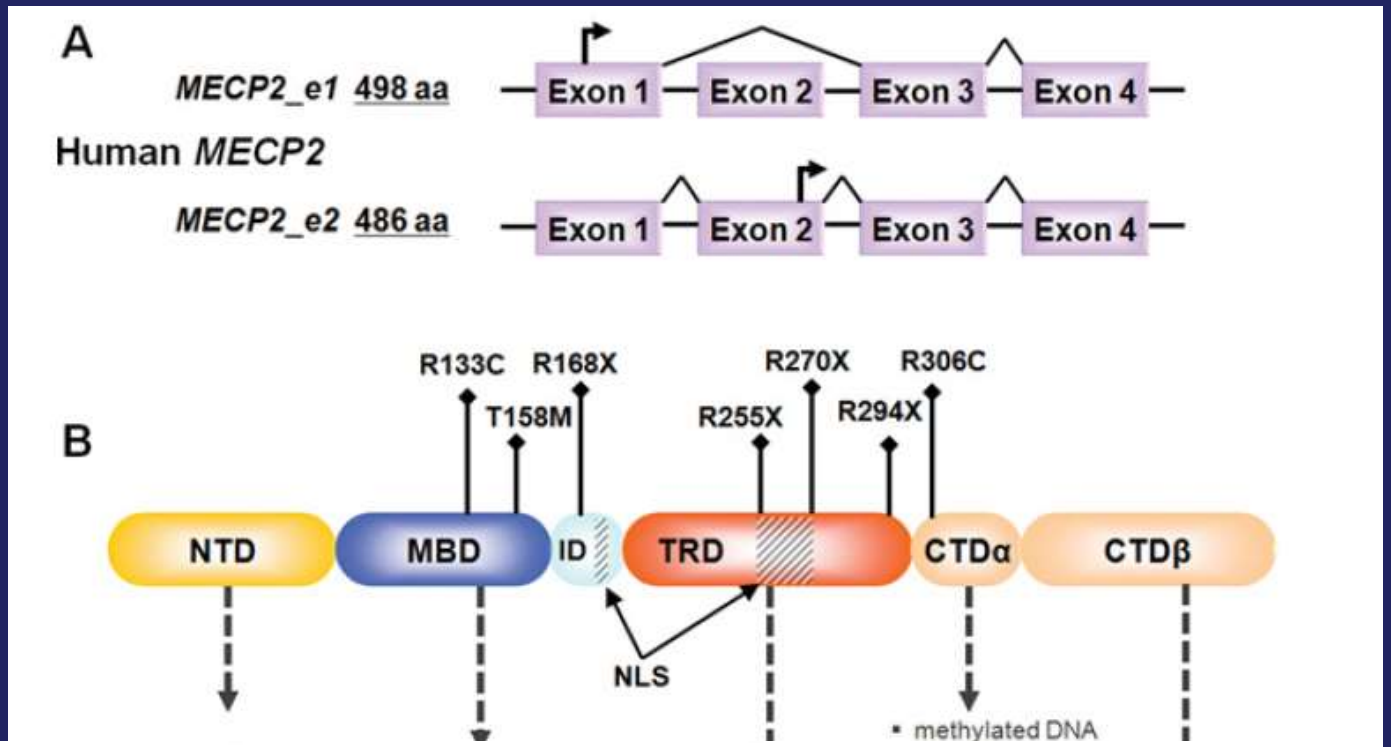
- Rett syndrome (RTT) is an X-linked childhood neurological disorder that affects 1:10,000 girls worldwide
- RTT is a progressive disease. Is characterized by loss of acquired skills, severe ID, gait ataxia, severe motor impairments and respiratory abnormalities
- Loss-of-function mutations in the X-linked gene methyl-CpG binding protein 2 (*MECP2*) cause the majority of RTT cases
- MECP2 point mutations (non-sense and missense) in exons 2-4 account for about 80% of classic Rett cases
- Other disorders (Angelman syndrome, infantile lethal encephalopathy in males, and autism) forming a group called **MeCP2-pathies** (30K/year).
- There is no cure for RTT

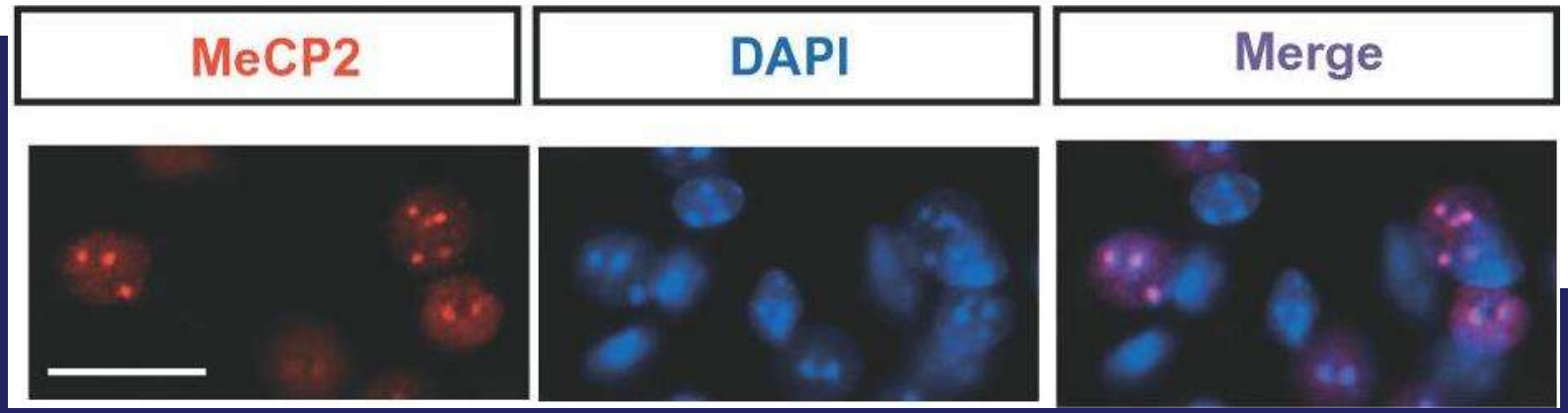


From Chahrour and Zoghbi, 2007

Composition of *MECP2*: gene structure, splicing patterns and putative functional domains





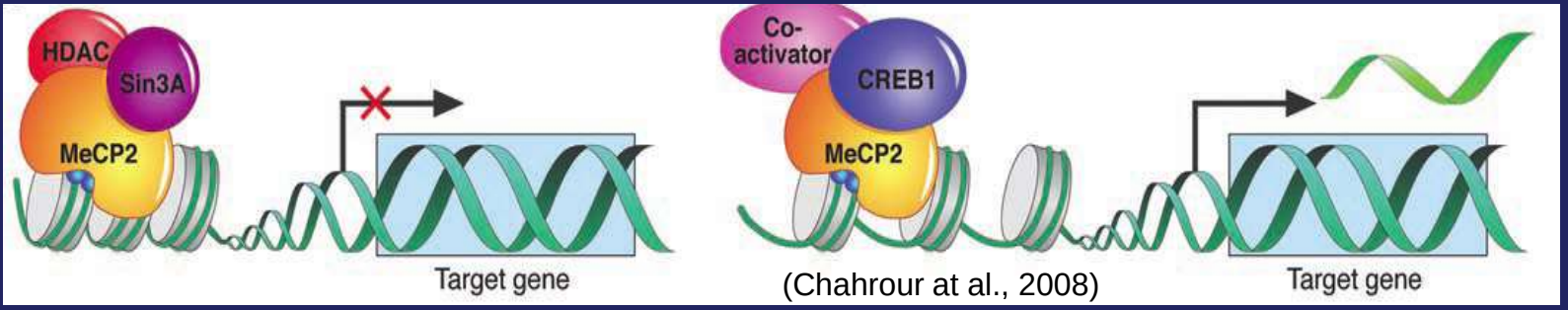


1) Why Rett Syndrome?

Rett syndrome (RTT) is the first pervasive neurodevelopmental disorder with a known genetic cause.

Because the neurobiological bases of RTT are similar in nature with other neurodevelopmental disorders, it has been postulated that the pathogenic process underlying these diseases may have common origins.

Thus, RTT has been proven a prototype for the genetic, molecular, and neurobiological analysis of neurodevelopmental disorders.



(Chahrour at al., 2008)

- (*Originally*) MeCP2 can bind to methylated DNA and it **globally** represses gene expression bound to 5-methyl-cytosines and recruiting transcriptional repressor (*A. Bird lab*).
- (*Recently*) It has been suggested that MeCP2 may act as a trascriptional activator by directly modulating transcription factors, thus enhancing the expression of **specific subset** of genes (*H. Zoghbi lab*), by binding to 5-hydroxy-methyl-cytosines in euchromatin (*N. Heintz lab*).
- A gene-specific role has been questioned in favour of a **histone-like** (core-chromatin) role suggesting that MeCP2 could be involved in fine tuning gene expression. In neurons, where the level of MeCP2 is an order of magnitude higher, MeCP2 largely substitutes for histone H1 and is distributed throughout the genome.
- Thus, it has been proposed that absence of functional MeCP2 may tamper with transcriptional regulation and cause neuronal and synaptic dysfunctions that underlie the disease appearance.

2) But, «really», why Rett syndrome?

Switching from Short- to Long-Term Memory

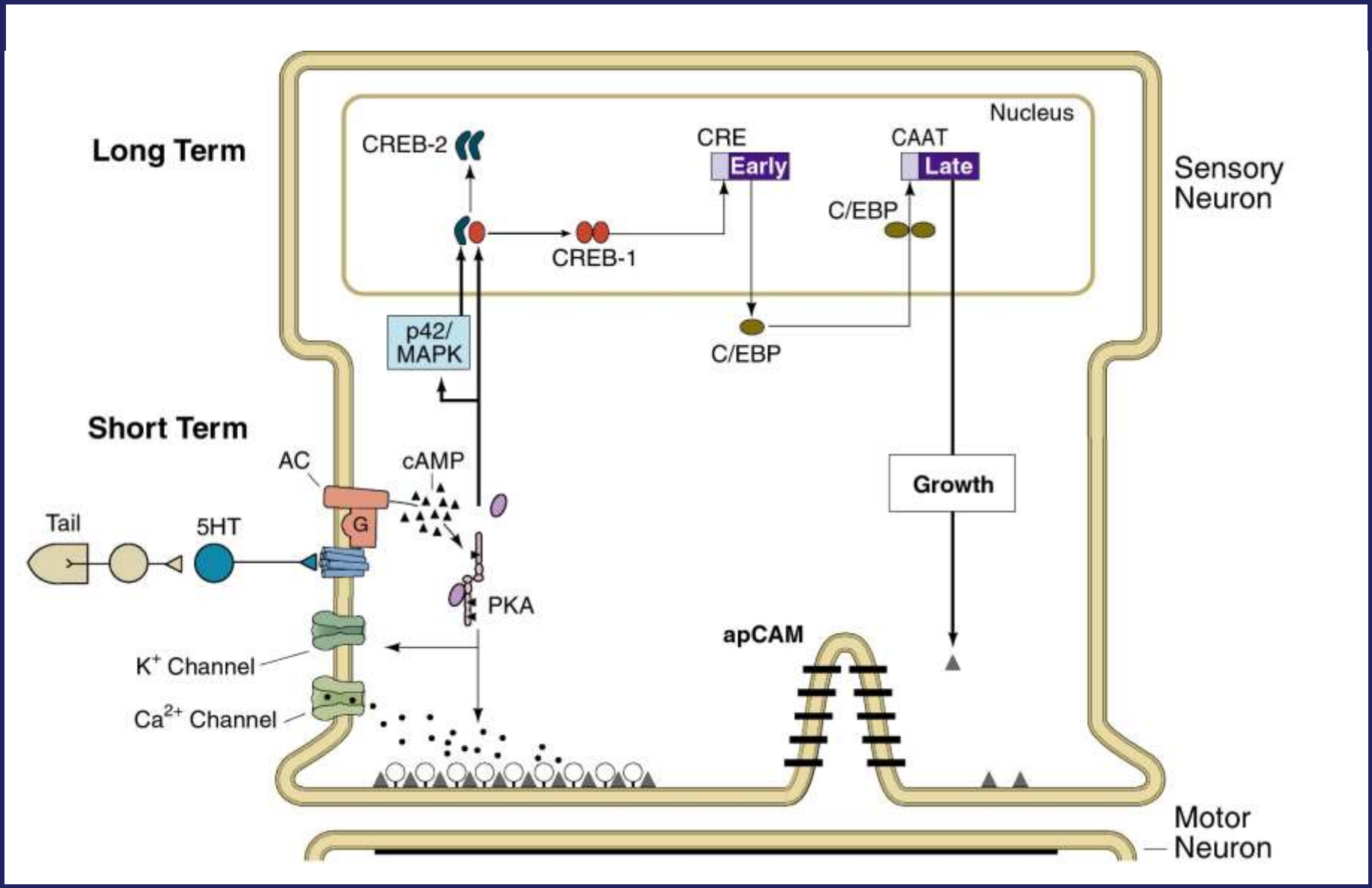
New Protein Synthesis

Short-Term Memory → **Long-Term Memory**

New Protein Synthesis

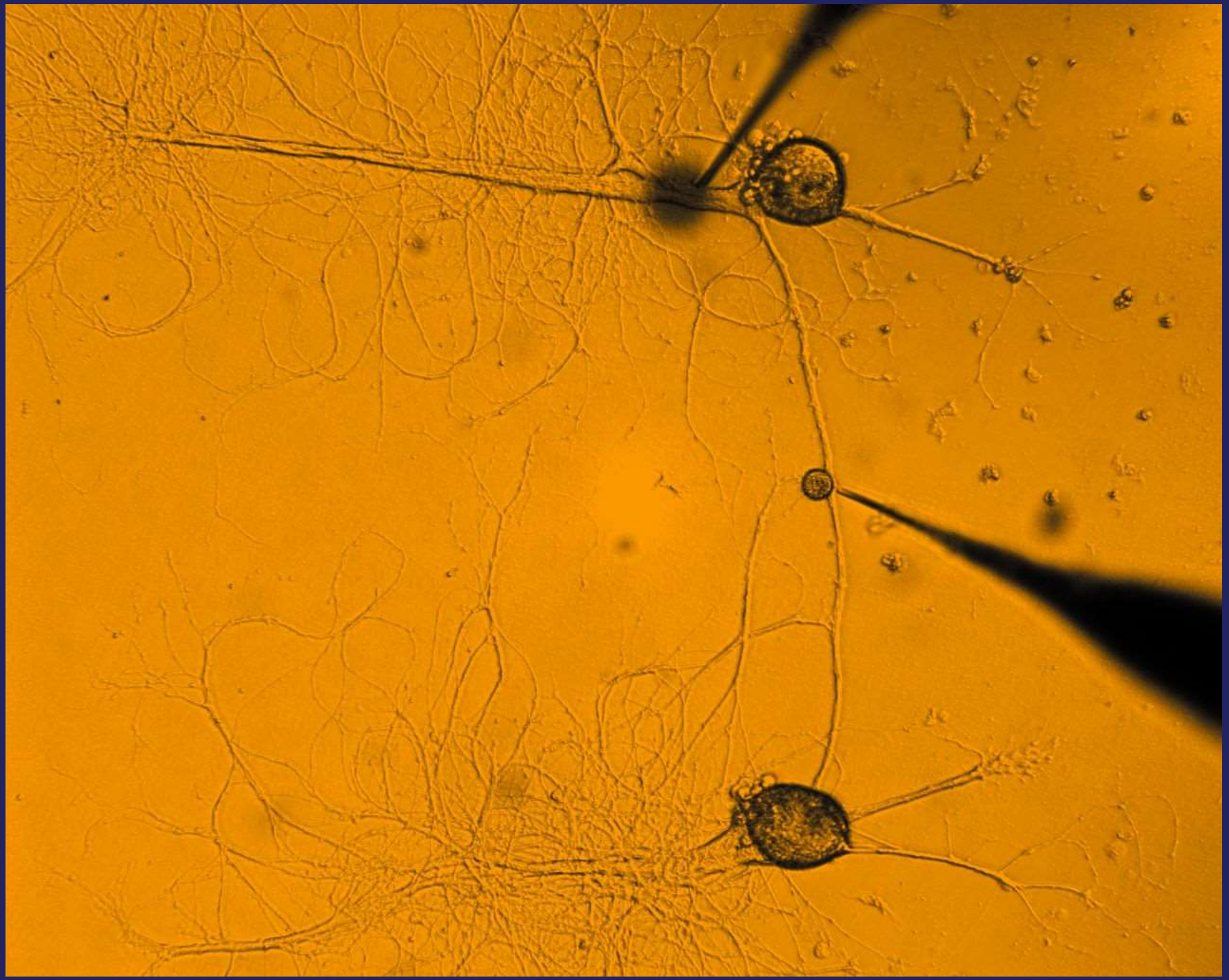
Short-Term Synaptic Plasticity → **Long-Term Synaptic Plasticity**

Long-Term Memory Requires a CREB1-Mediated Transcriptional Cascade

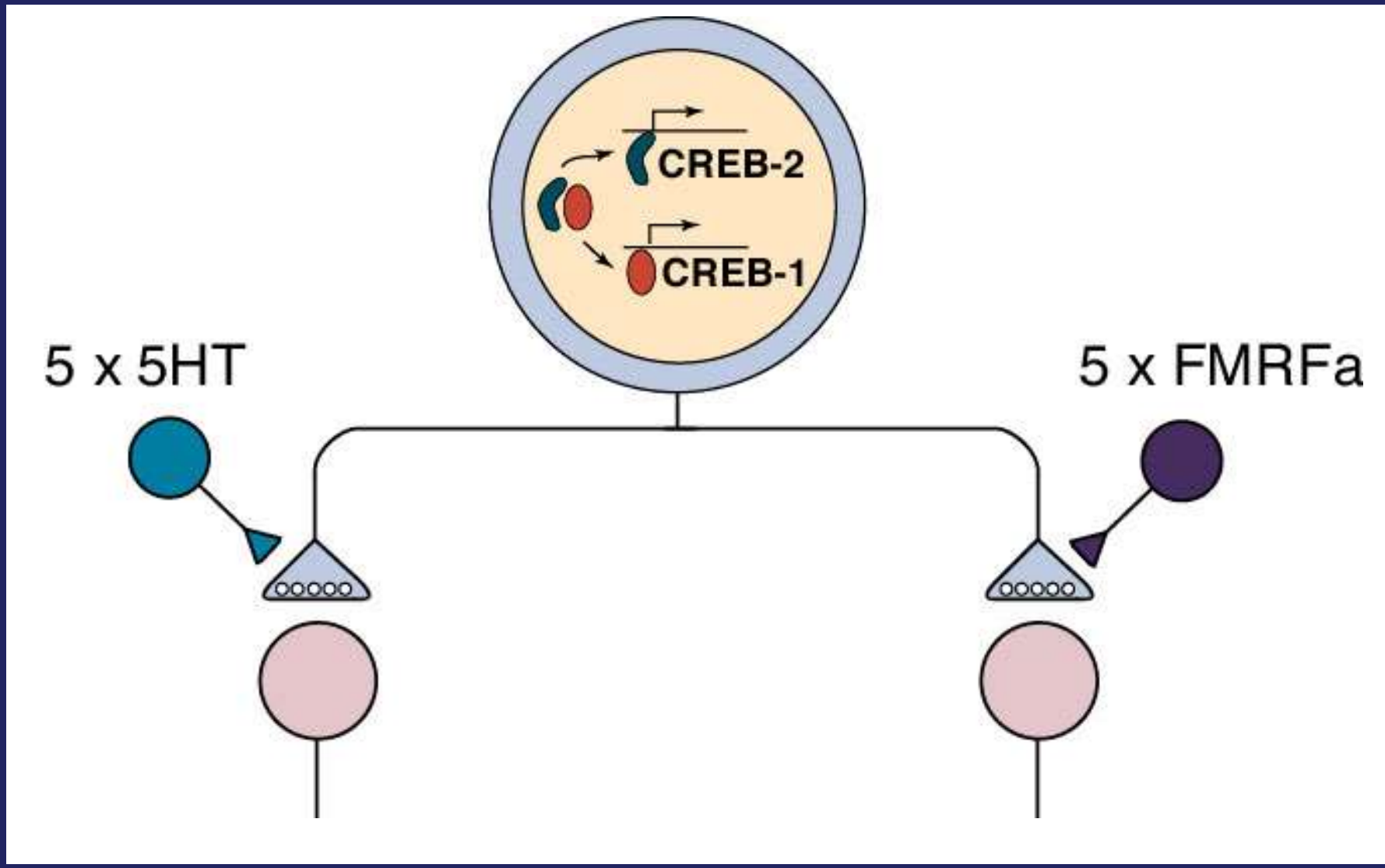


(modified from Kandel, 2000)

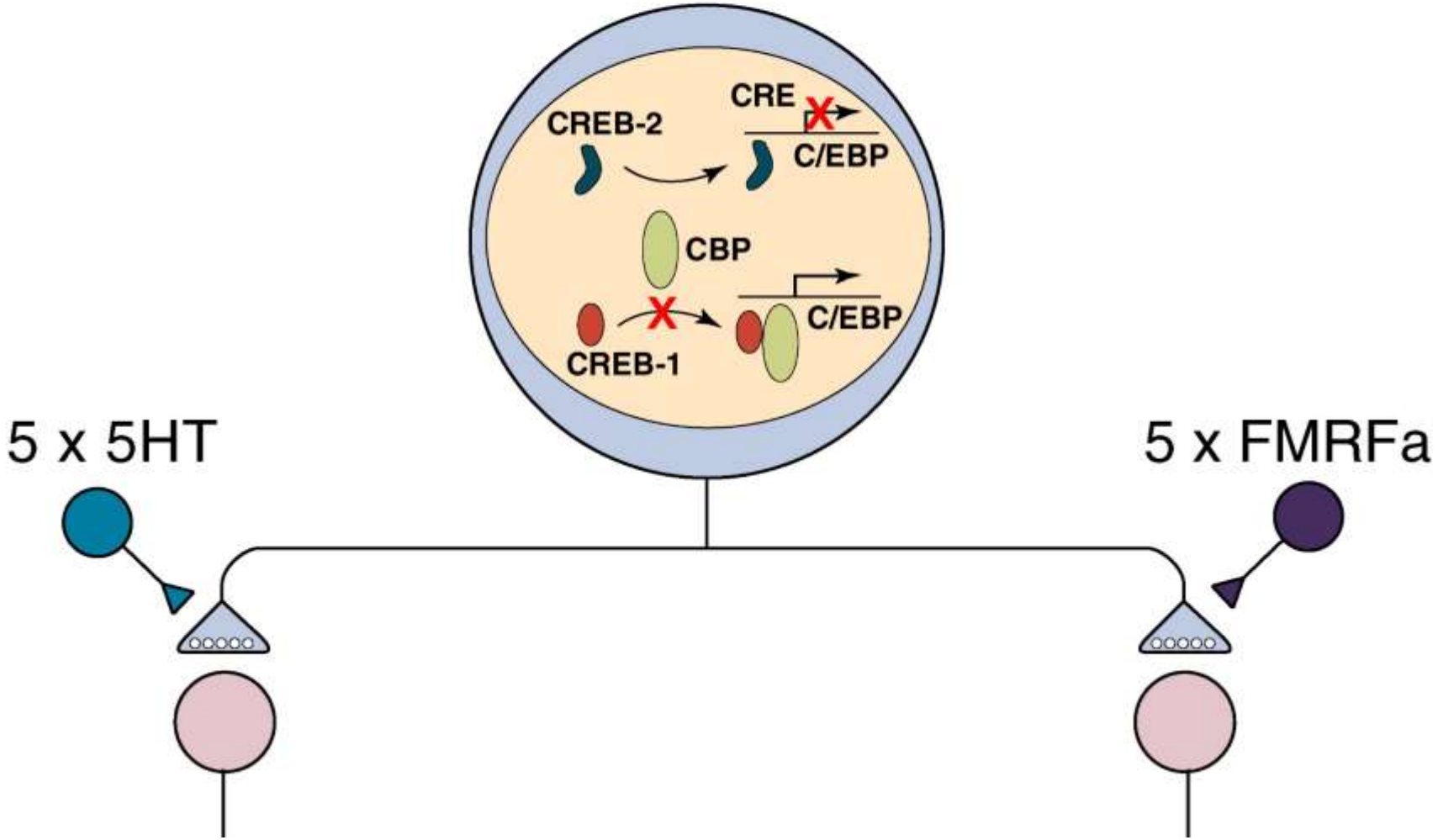

….and after



Nuclear Activation by Inhibitory and Facilitatory Signals in the Long-Term Control of Synaptic Action



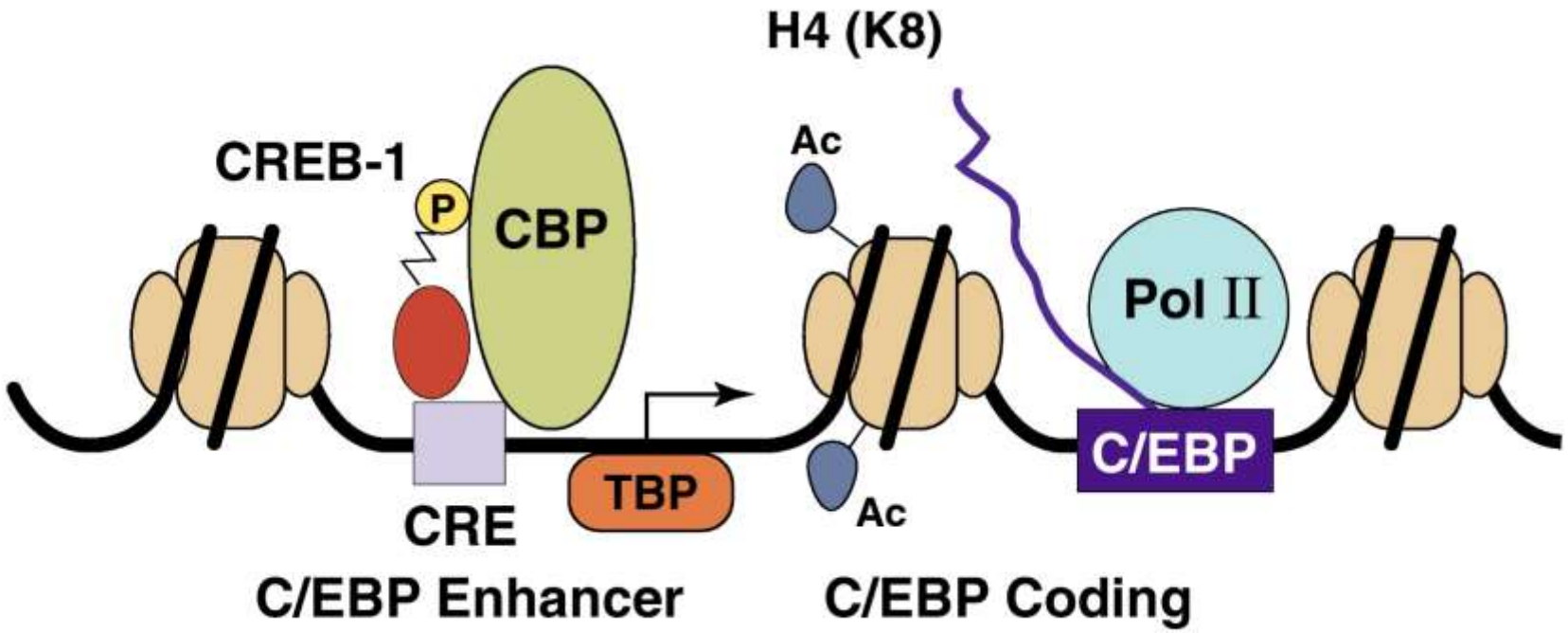
Long-Term Synaptic Integration is Mediated Through a Loop of Molecular Competition in the Nucleus



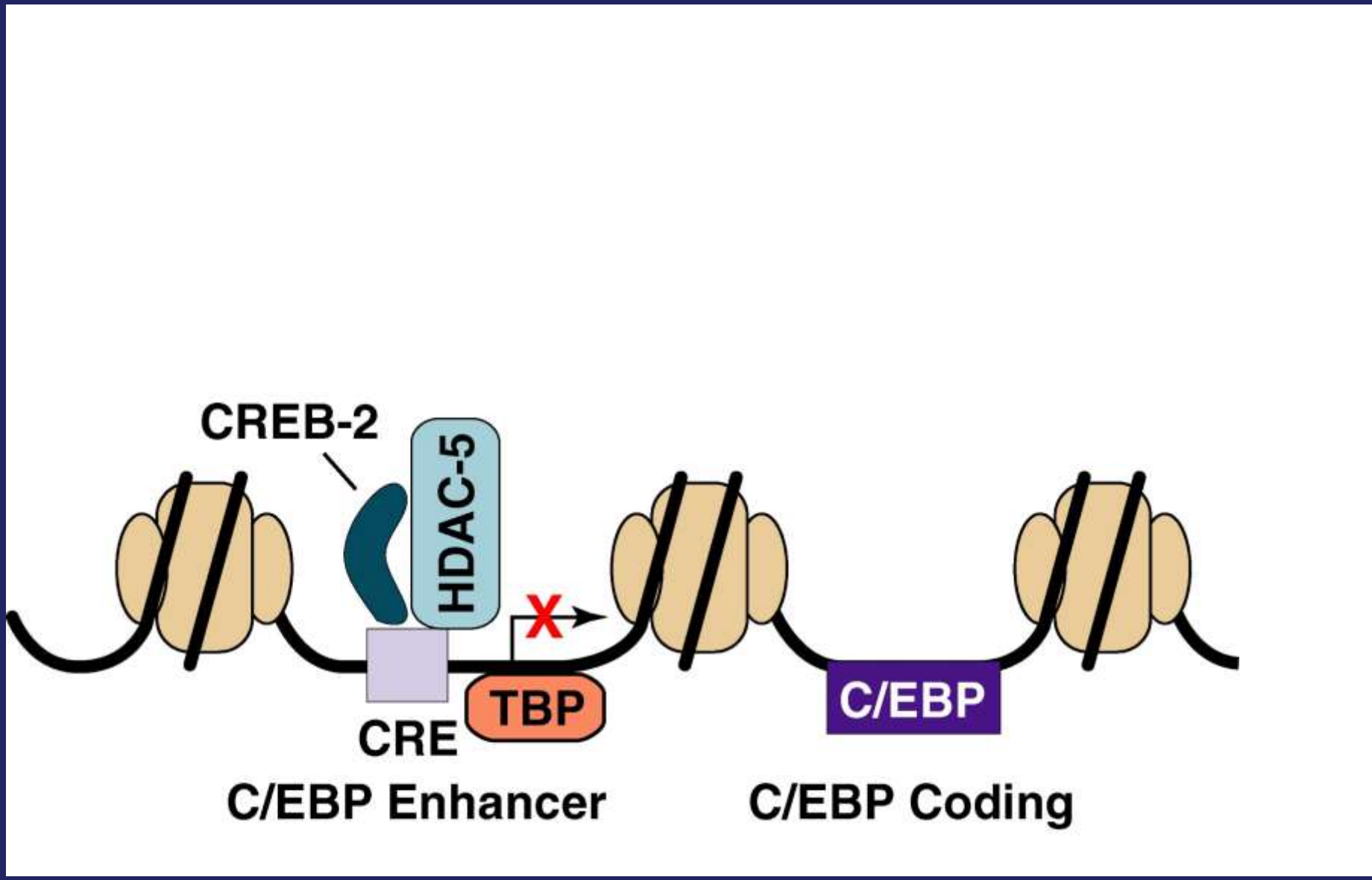
- CREB-1 recruits Creb Binding Protein (CBP)
- CBP is a histone acetylase
- CBP alters chromatin structure facilitating new gene expression

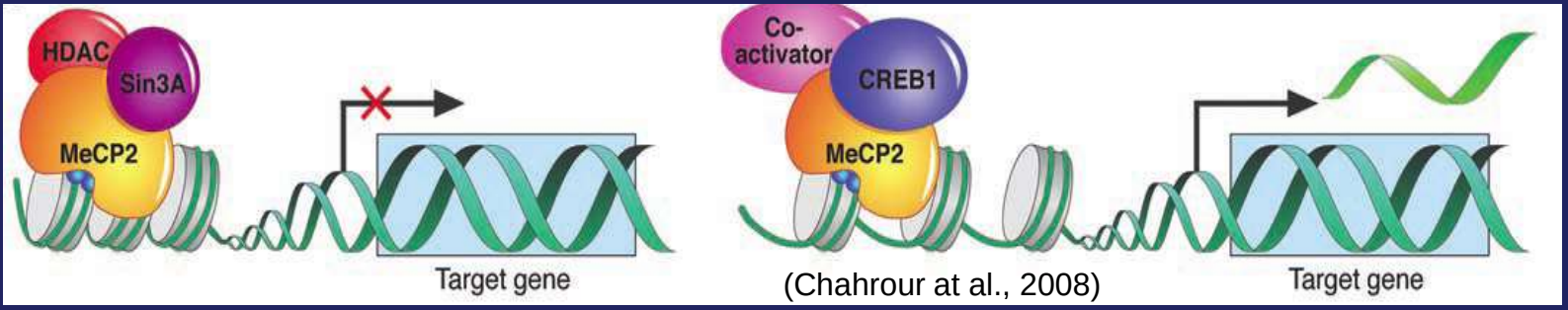
Is Synaptic Integration Regulated By Chromatin Modification at the C/EBP Promoter Region ?

5HT Produces Modifications in Chromatin Structure



C/EBP Transcription is Shut Off by CREB-2 and HDAC-5





(Chahrour at al., 2008)

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- Thus, it has been proposed that absence of functional MeCP2 may tamper with transcriptional regulation and cause neuronal and synaptic dysfunctions that underlie the disease appearance.

Cell

A Model for Neural Development and Treatment of Rett Syndrome Using Human Induced Pluripotent Stem Cells

Maria C.N. Marchetto,[1,5] Cassiano Carromeu,[2,5] Allan Acab,[2] Diana Yu,[1] Gene W. Yeo,[3] Yangling Mu,[1] Gong Chen,[4] Fred H. Gage,[1] and Alysson R. Muotri[2,*]

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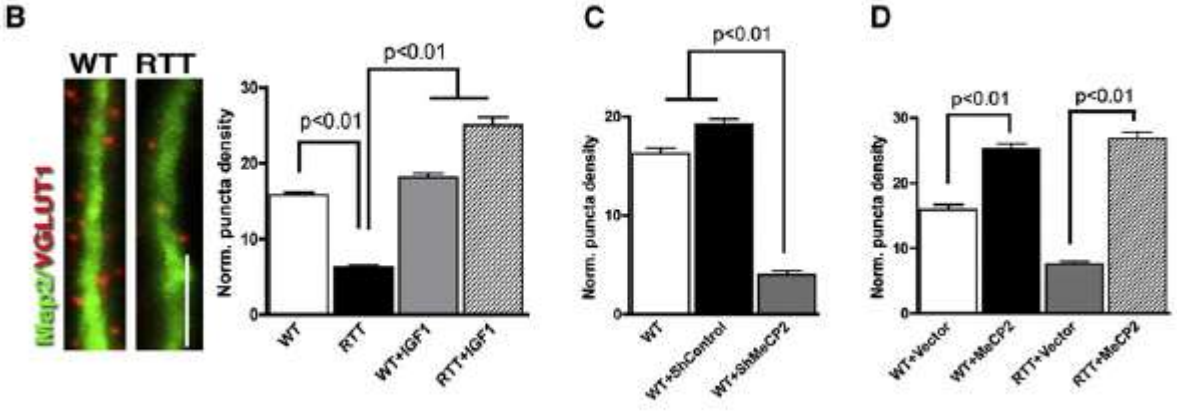
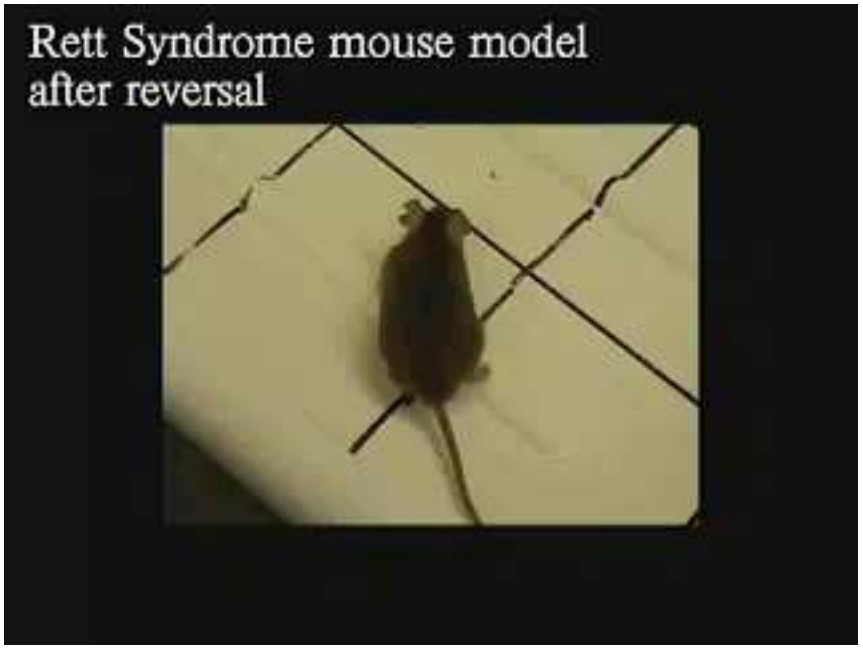
DOI 10.1016/j.cell.2010.10.016



3) Why Rett Syndrome?

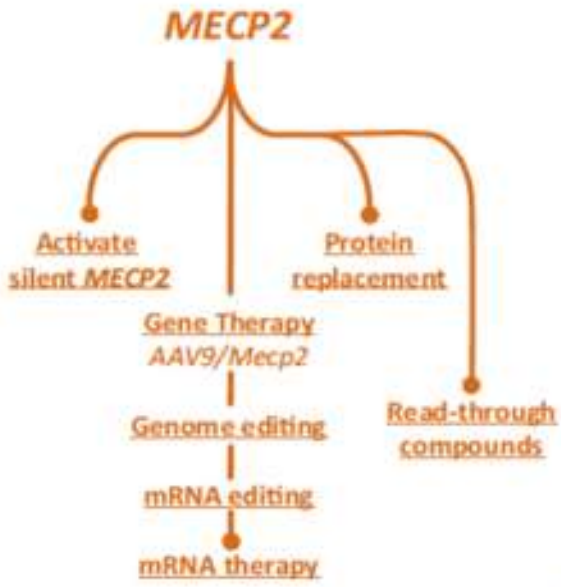
Is Rett syndrome reversible ?

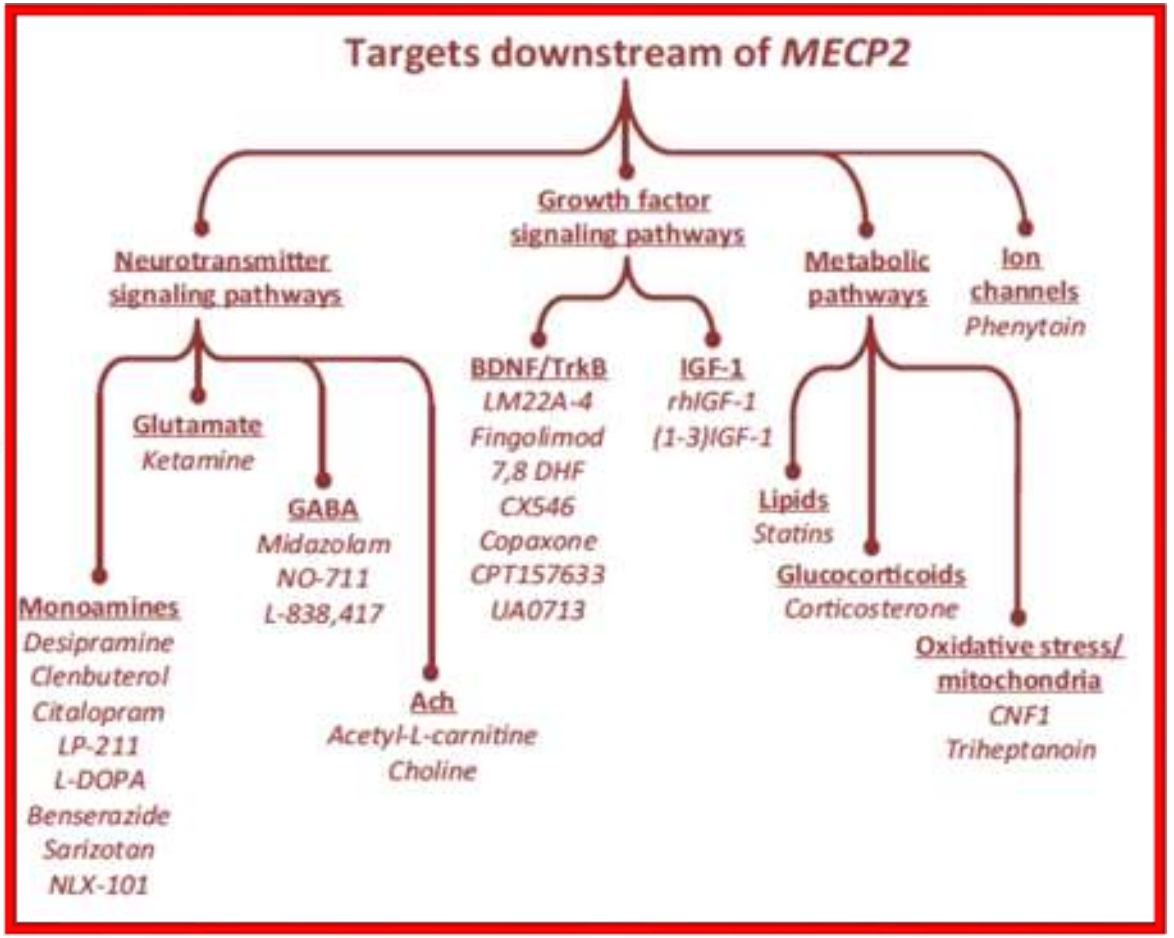
Mice with lox-STOP-Lox cassette before exon 3 (*Mecp2*^{Stop}) that can be removed by Cre-mediated recombination using tamoxifen



(Guy et al., Science, 2007, 315:1143)

Rett Syndrome therapies





Katz DM et al. Trends in Neuroscience, 2016

Rett Syndrome therapies

- **Treating Rett syndrome's symptoms**

  Pharmacology (short term)

- **Treating Rett syndrome's causes – MECP2 mutations**
  - **Stop codon read-through therapy**
  - **X chromosome reactivation**
  - **Replacement therapy**
    - **Viral and non-viral gene therapy (long term)**

Rett Syndrome therapies

- Pharmacology
  - Identification of new therapeutical compounds for Rett syndrome through research
    - BDNF (Brain-Derived Neurotrophic Factor)
    - IGF1 (Insulin-like Growth Factor 1)
    - mTOR reactivation
    - Glutamate receptor modulators and neurostransmitters
    - Dietary supplements (curcuma, PUFA oils)
  - Clinical trials
    - BDNF – Cysteamine, Fingolimod
    - IGF1 – Mecasermin
    - Desipramine

mTOR defects in Rett syndrome

Human Molecular Genetics, 2011, Vol. 20, No. 6 **1182–1196**
doi:10.1093/hmg/ddq563
Advance Access published on January 6, 2011

Reduced AKT/mTOR signaling and protein synthesis dysregulation in a Rett syndrome animal model

Sara Ricciardi[1,2,†], Elena M. Boggio[4,5,†], Stefano Grosso[3,6,†], Giuseppina Lonetti[7,†], Greta Forlani[8], Gilda Stefanelli[1,2], Eleonora Calcagno[4], Noemi Morello[4], Nicoletta Landsberger[2,8], Stefano Biffo[3,6], Tommaso Pizzorusso[5,7], Maurizio Giustetto[4,*] and Vania Broccoli[1,2,*]

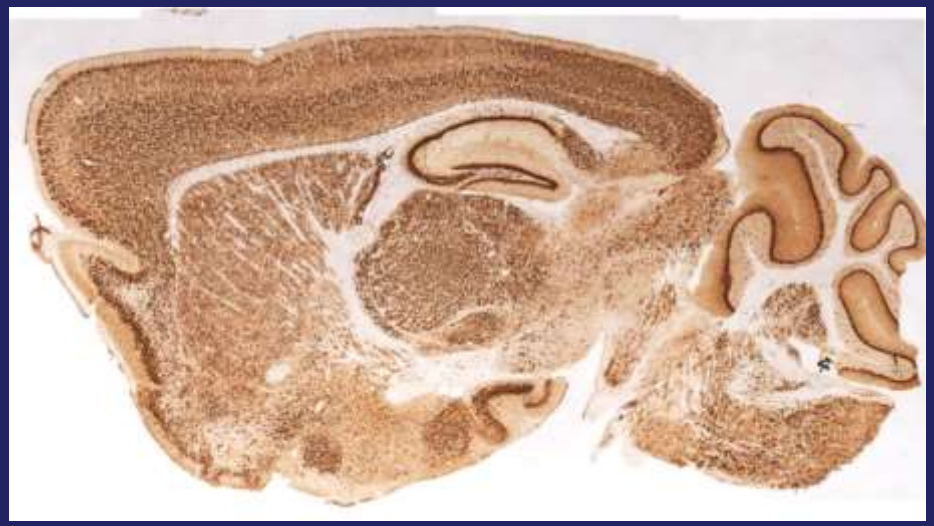
[1]Stem Cells and Neurogenesis Unit, Division of Neuroscience, [2]San Raffaele Rett Research Center, Division of Neuroscience and [3]Laboratory of Molecular Histology and Cell Growth, Division of Oncology, San Raffaele Scientific Institute, 20132 Milano, Italy, [4]Department of Anatomia, Farmacologia e Medicina Legale, Università di Torino and National Institute of Neuroscience-Italy, Turin, Italy, [5]CNR, Neuroscience Institute Pisa, Pisa, Italy, [6]DISAV, University of Eastern Piedmont, 15100 Alessandria, Italy, [7]Department of Psicologia, Università di Firenze, Firenze, Italy and [8]Laboratory of Genetic and Epigenetic Control of Gene Expression, Department of Structural and Functional Biology, University of Insubria, 21052 Busto Arsizio, VA, Italy

Received October 19, 2010; Revised and Accepted December 27, 2010

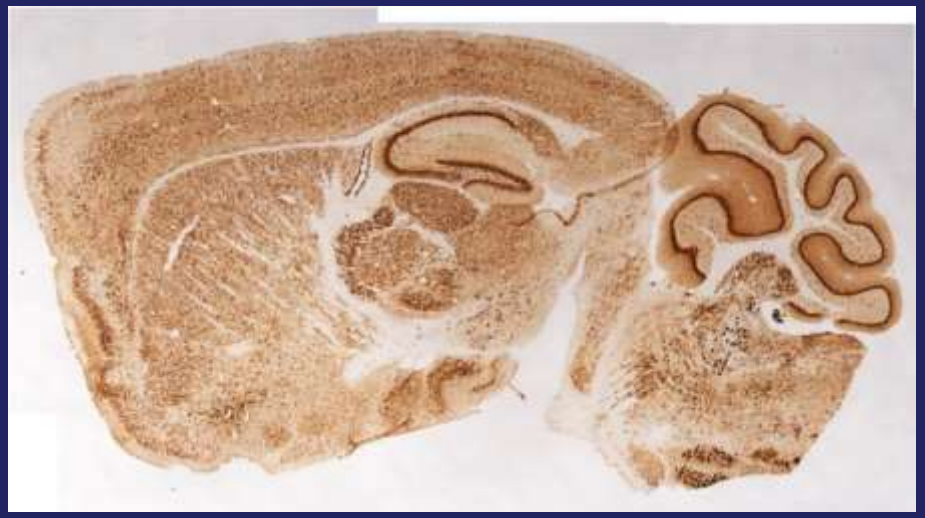
Phosphorylation of rp-S6 is severely reduced in asymptomatic *Mecp2*-KO mice

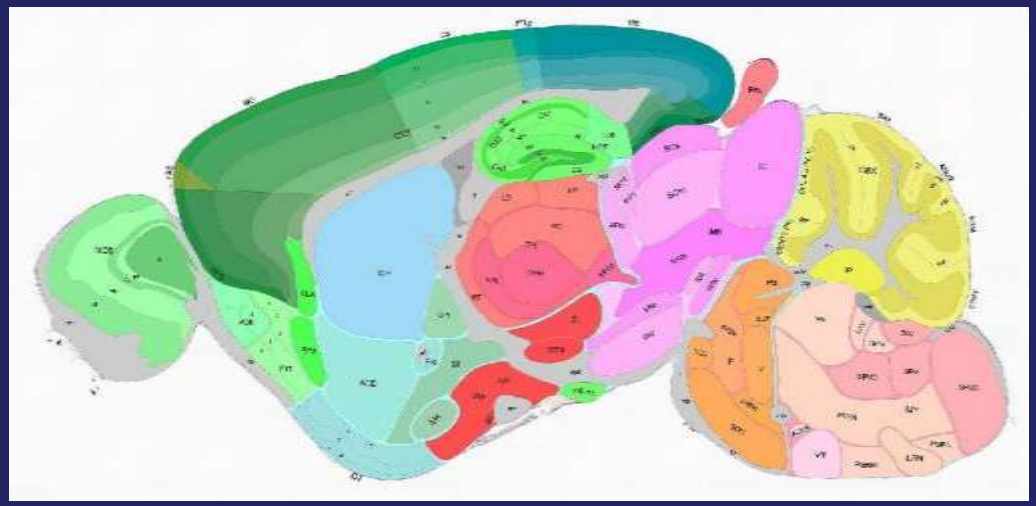
IHC P-rpS6 (Ser240/244)

WT



KO

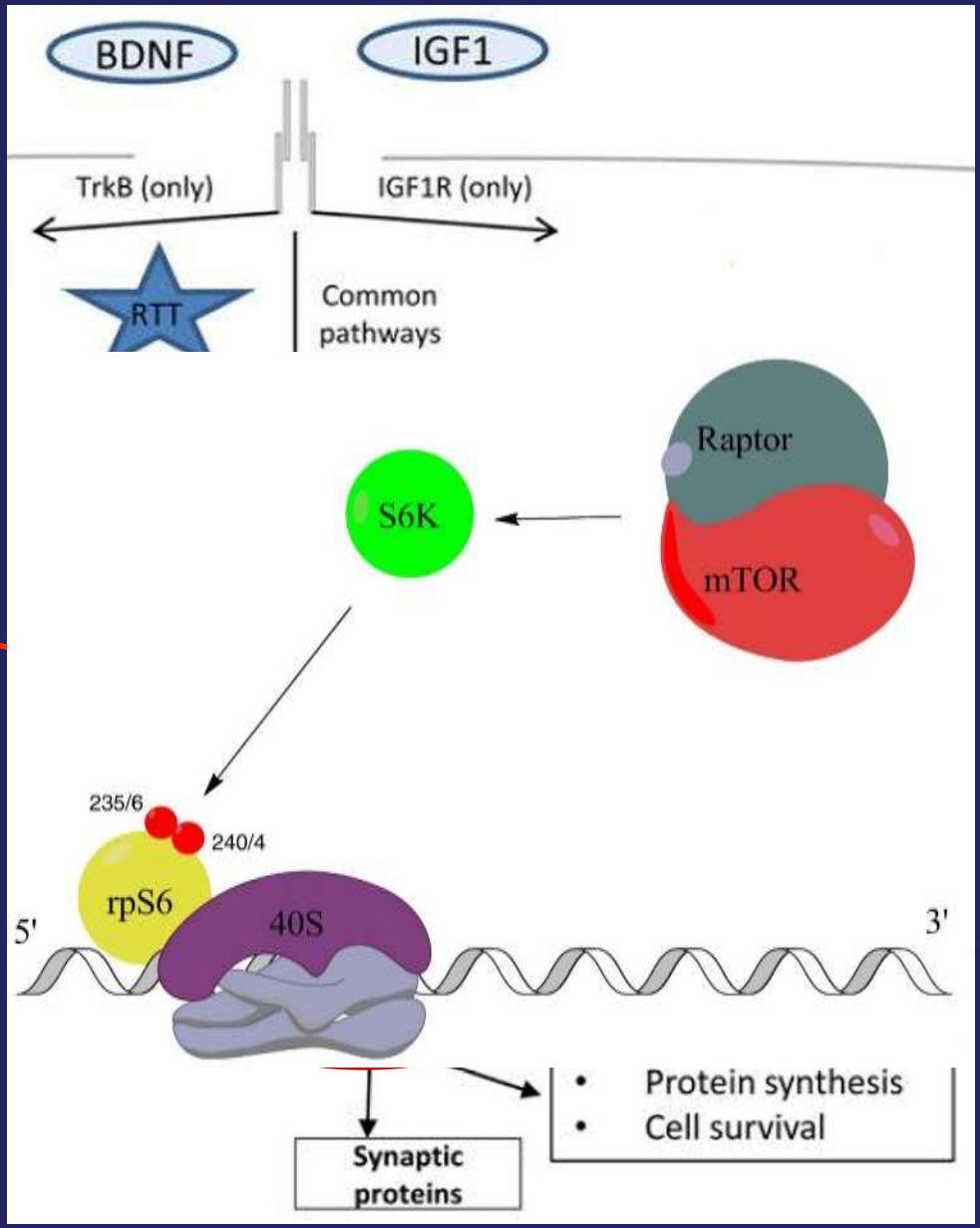




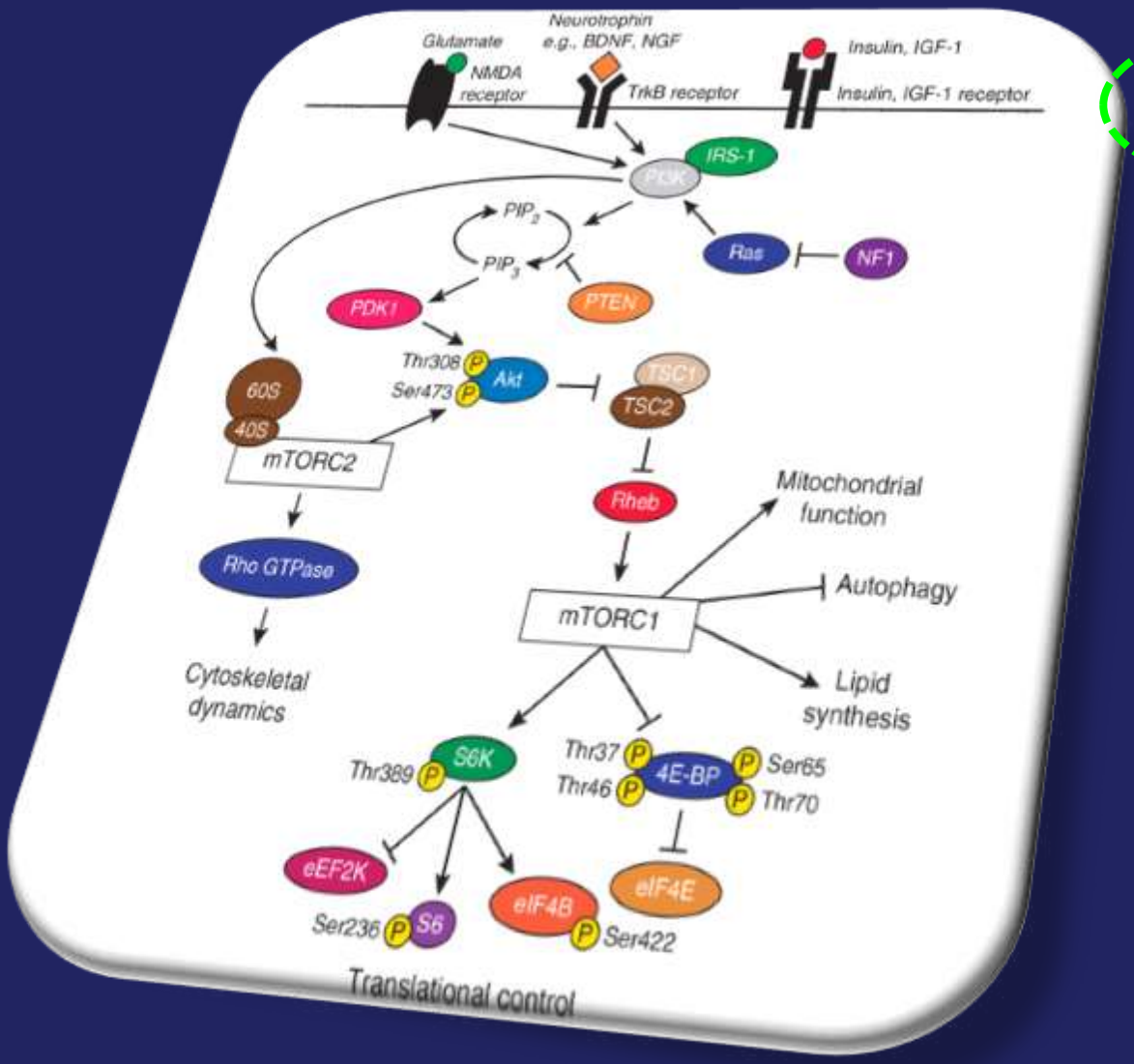
E. Castroflorio and N. Morello

Analysis of m-TOR signalling in the brain of *Mecp2*-KO mice

- AKT/mTOR pathway and protein synthesis are hypofunctional in MeCP2-KO brains
- mTOR activity controls cell soma size, axon pathfinding, dendritic arborization, dendritic spine morphology and synaptic plasticity
- mTOR phosphorylates rpS6 through p70S6k and this correlates with the translational rates



Impaired pathways and putative therapies



IGF treatment to re-activate the intracellular pathway

Glutamate NMDA-receptor antagonist

Pharmacological inhibition of Phosphatase and tensin homolog (PTEN)

Genetic and pharmacological activation of AKT kinase

TrkB agonists pharmacological compounds

Activation of mGluR1/5 metabotropic glutamate receptors

SCIENTIFIC REPORTS

OPEN

The short-time structural plasticity of dendritic spines is altered in a model of Rett syndrome

SUBJECT AREAS:
IMAGING
NEUROSCIENCE
NEUROANATOMY
NEURODEVELOPMENTAL DISORDERS

Silvia Landi[1,2*], Elena Putignano[1,3*], Elena Maria Boggio[3], Maurizio Giustetto[4], Tommaso Pizzorusso[1,2] & Gian Michele Ratto[1,2,3]

[1]NEST, Scuola Normale Superiore, Pisa, Italy, [2]NEST, Institute Nanoscience CNR, Pisa, Italy, [3]Institute of Neuroscience CNR, Pisa, [illegible] Department of Anatomy, Pharmacology and Forensic Medicine, University of

Archival Report

Biological Psychiatry

Dendritic Spine Instability in a Mouse Model of CDKL5 Disorder Is Rescued by Insulin-like Growth Factor 1

Grazia Della Sala, Elena Putignano, Gabriele Chelini, Riccardo Melani, Eleonora Calcagno, Gian Michele Ratto, Elena Amendola, Cornelius T. Gross, Maurizio Giustetto, and Tommaso Pizzorusso

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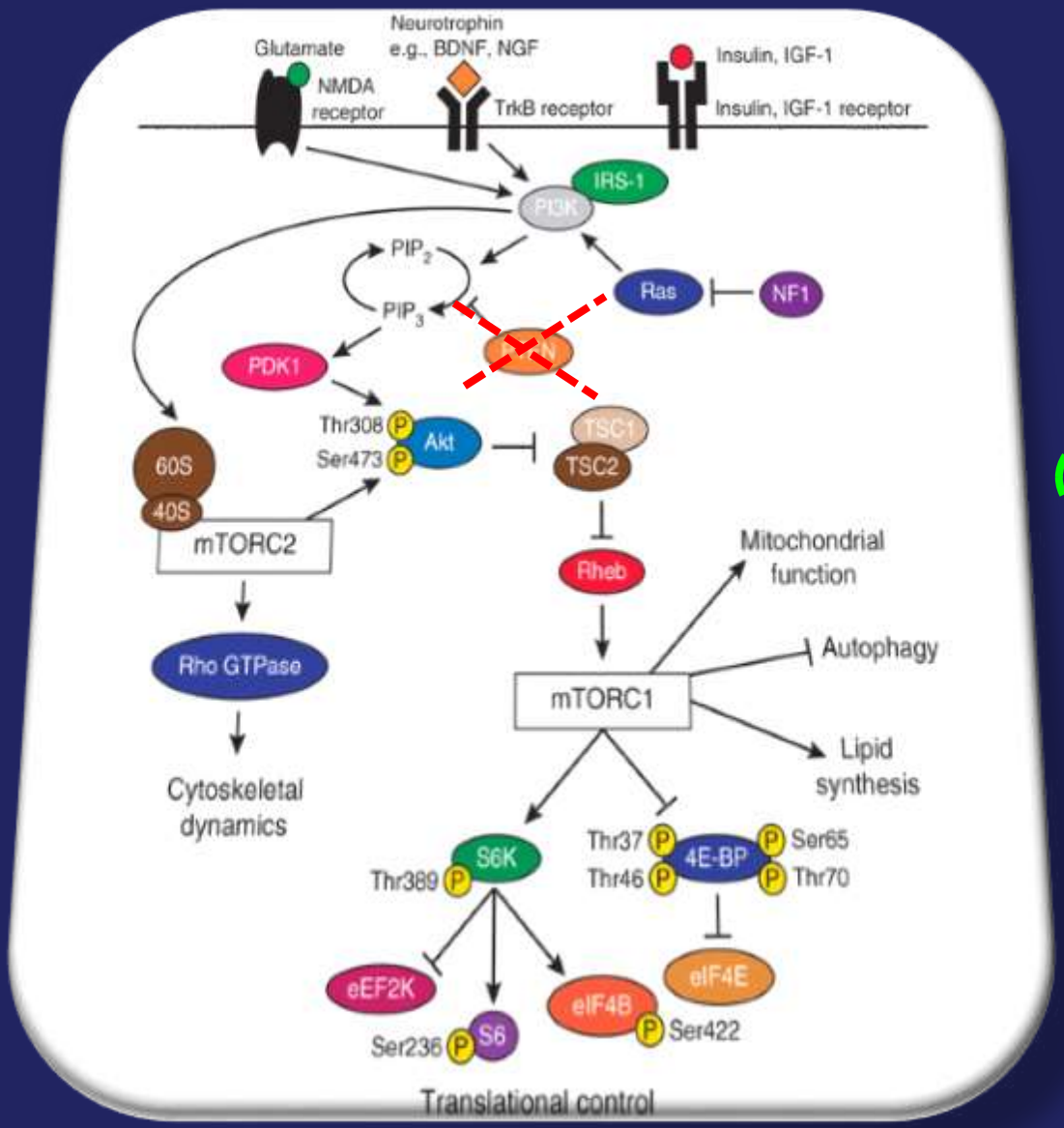


IGF treatment to re-activate the intracellular pathway

Glutamate NMDA-receptor antagonist

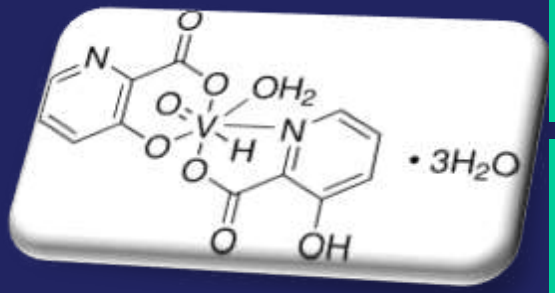
Pharmacological inhibition of Phosphatase and tensin homolog (PTEN)

Genetic and pharmacological activation of AKT kinase

TrkB agonists pharmacological compounds

Activation of mGluR1/5 metabotropic glutamate receptors

Phosphatase and tensin homolog (PTEN) small molecule inhibitor



VO-OHpic (10 ug/kg i.p.)

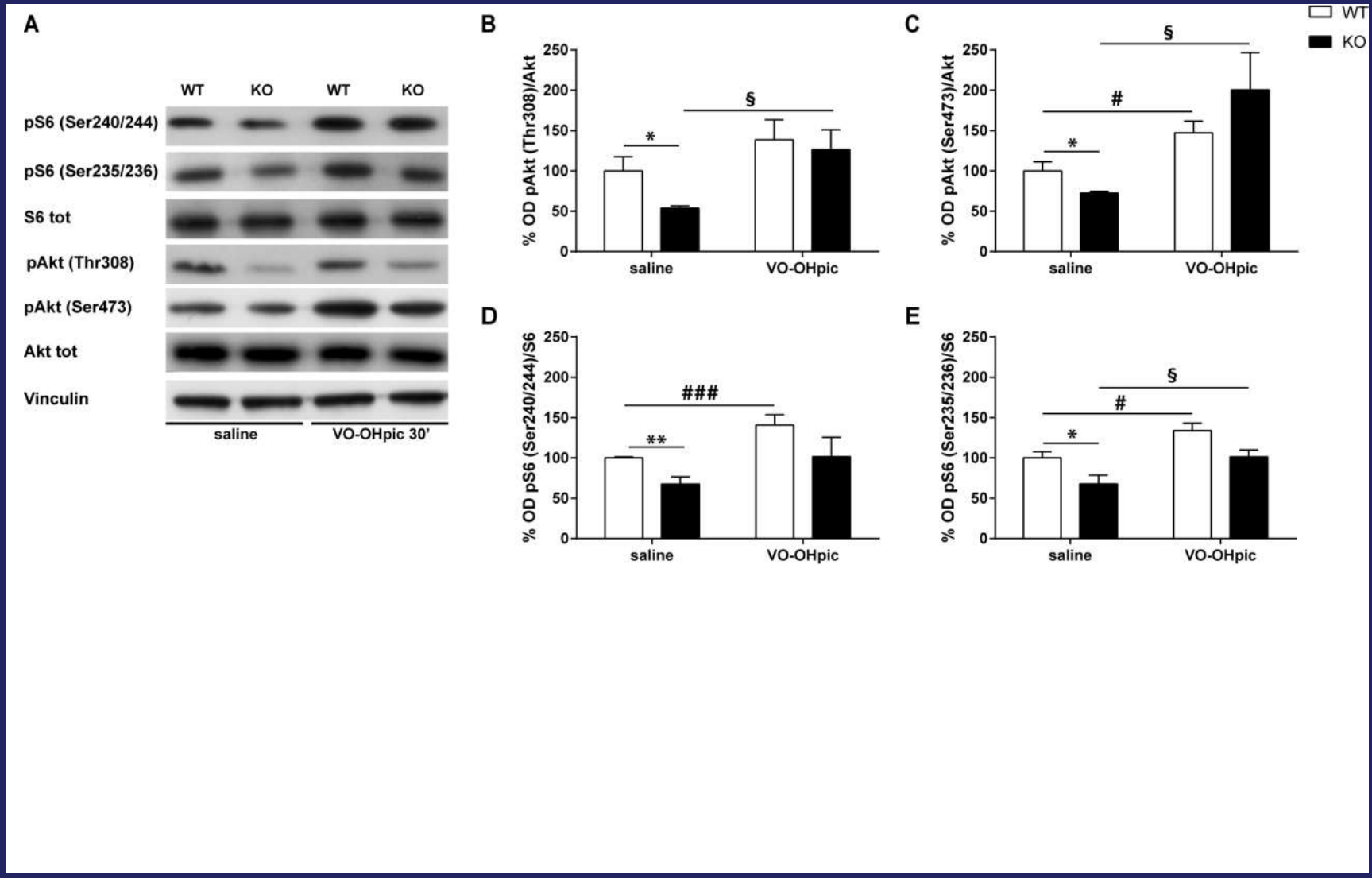
(OC-6-45) Aqua (3-hydroxy-2-pyridinecarboxylato-kapaN1,kapaO2)[3-(hydroxy-kapaO)-2-pyridinecarboxylato(2-)-kapaO2]oxo-vanadate(1-), hydrogen, trihydrate

A small molecule inhibitor for phosphatase and tensin homologue deleted on chromosome 10 (**PTEN**). Rosivatz E, Matthews JG, McDonald NQ, Mulet X, Ho KK, Lossi N, Schmid AC, Mirabelli M, Pomeranz KM, Erneux C, Lam EW, Vilar R, Woscholski R. ACS Chem Biol. 2006 Dec 15;1(12):780-90.

PTEN inhibitors cause a negative inotropic and chronotropic effect in mice. Zu L, Shen Z, Wesley J, Cai ZP. Eur J Pharmacol. 2011 Jan 10;650(1):298-302.

Calcagno E., in preparation

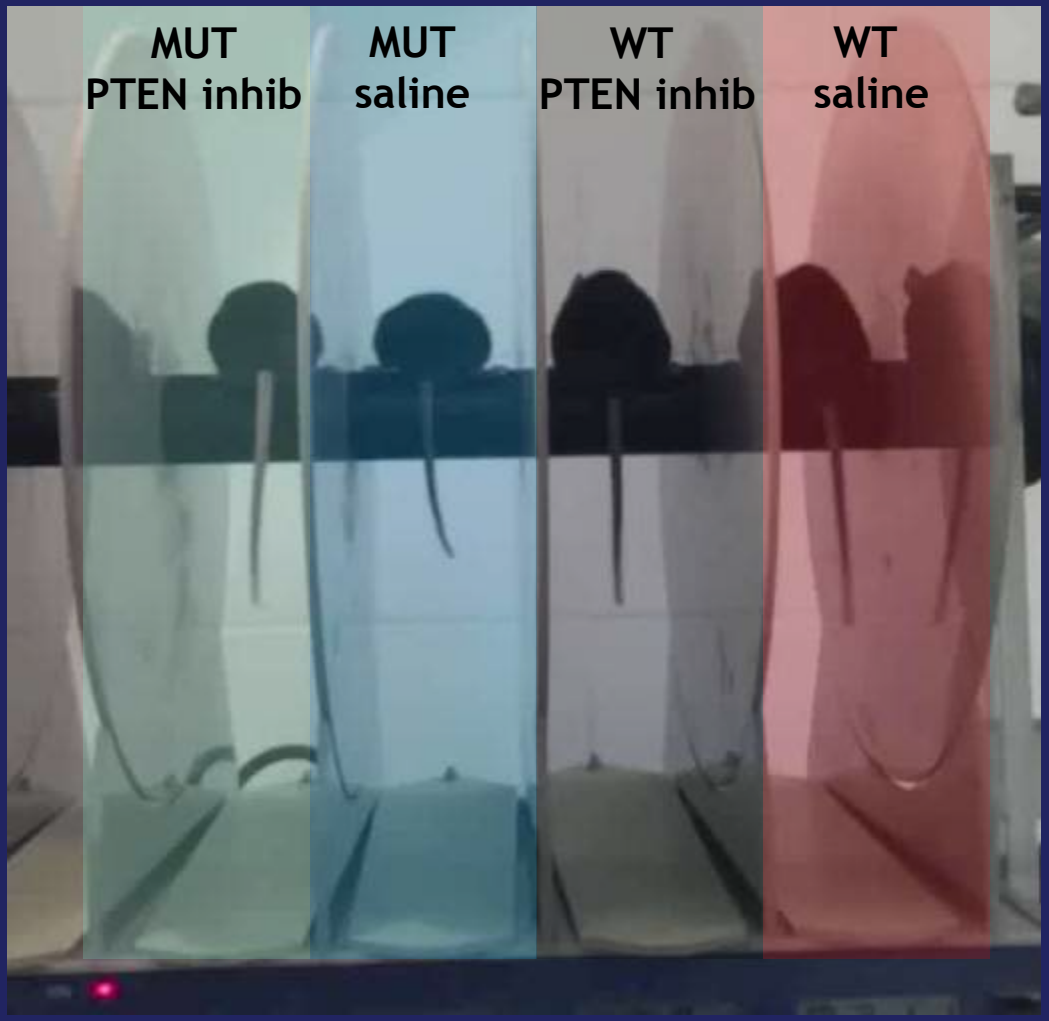
Acute PTEN inhibition normalizes both Akt/mTOR and BDNF signaling deficits in *Mecp2*-KO mice



*p<0.05, **p<0.01 WT saline vs KO saline
# p<0.05; ### p<0.001 WT saline vs WT VO-Ohpic
§ p<0.05KO saline vs KO VO-OHpic
n=3

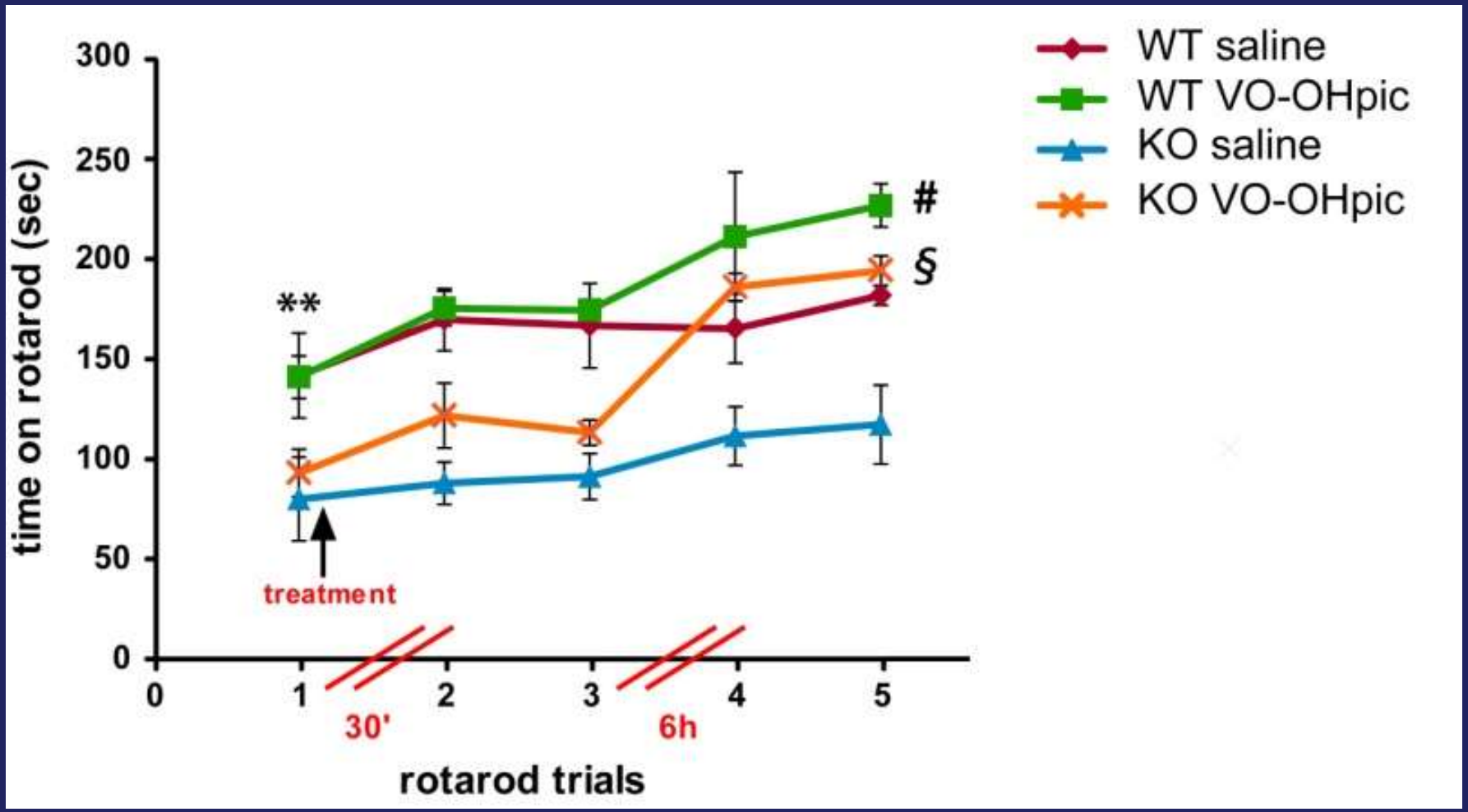
Calcagno E., in preparation

Motor domain: rotorod test in Mecp2 mutant mice with or without PTEN inhibition



E. Calcagno, in preparation

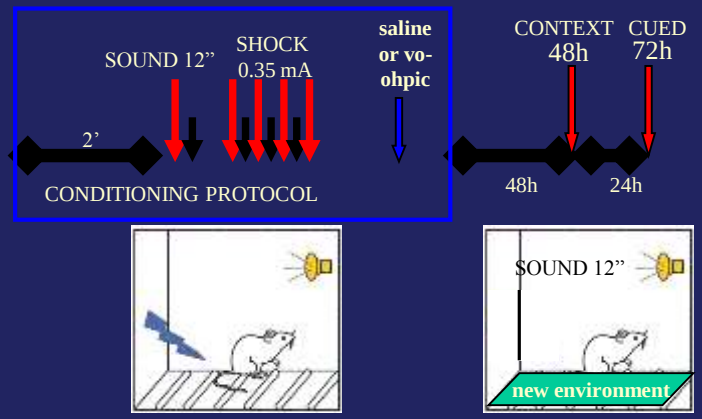
Acute VO-OHpic treatment corrects motor learning defects in *Mecp2*-KO mice

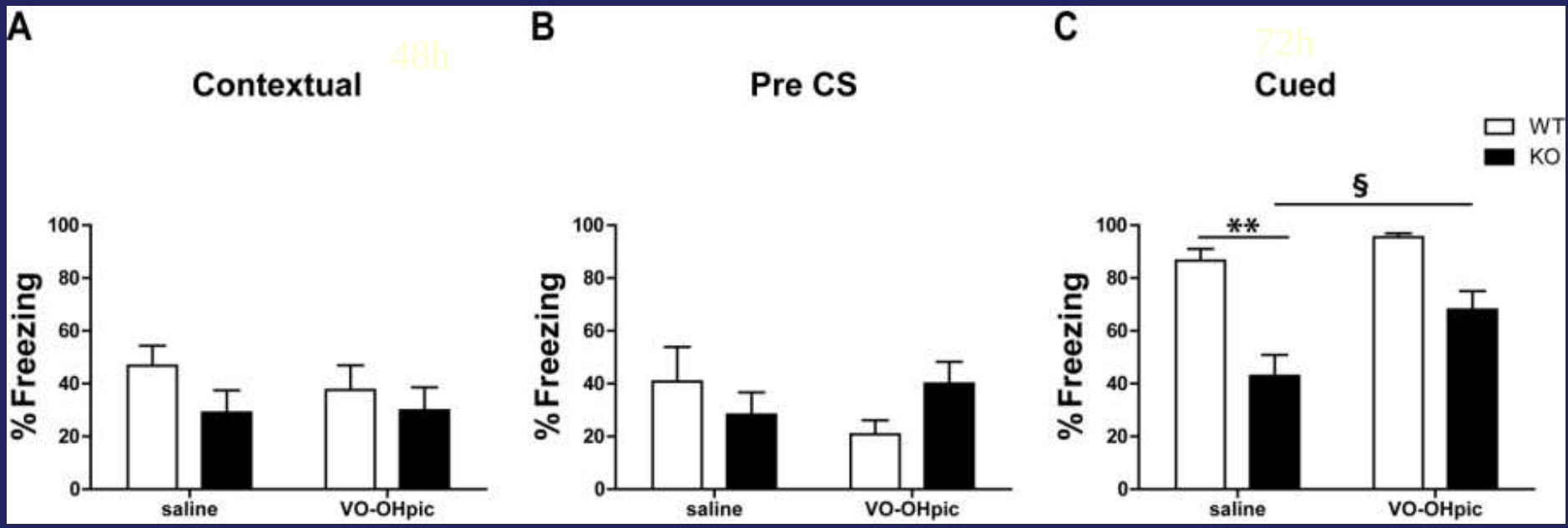


**p<0.01 WT vs KO
# p<0.05 WT saline vs WT VO-Ohpic
§ p<0.05KO saline vs KO VO-OHpic
n=4/5

Calcagno E., in preparation

Cognitive domain: acute VO-OHpic reverses loss of cued-fear conditioning memory in *Mecp2*-KO mice





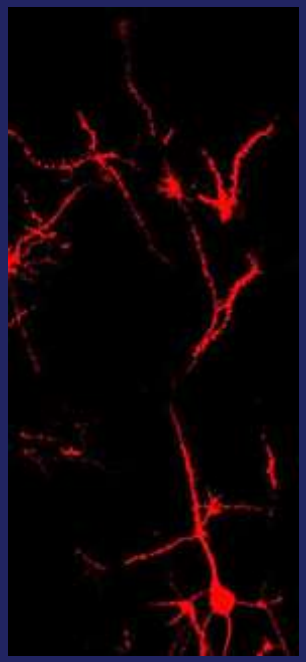
**p<0.01 WT saline vs KO saline
§ p<0.05KO saline vs KO VO-OHpic
n=5/6

Calcagno E., in preparation

Dendritic spine dysgenesis in the cerebral cortex of *Mecp2*-KO mice is reversed by chronic PTEN inhibition

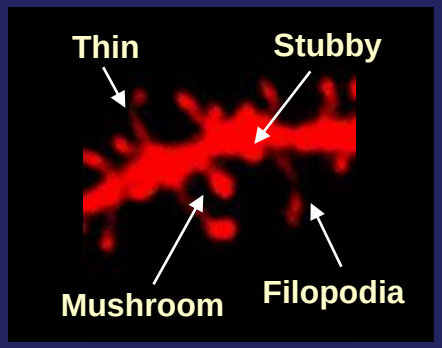
S1 cortex

L2-3



L5

DiOlistic labeling



Calcagno E., in preparation

GENE THERAPY FOR RETT SYNDROME

Rett Syndrome is a candidate for gene therapy because it is caused by a single gene, it is not degenerative, and symptoms may be reversible at any age.

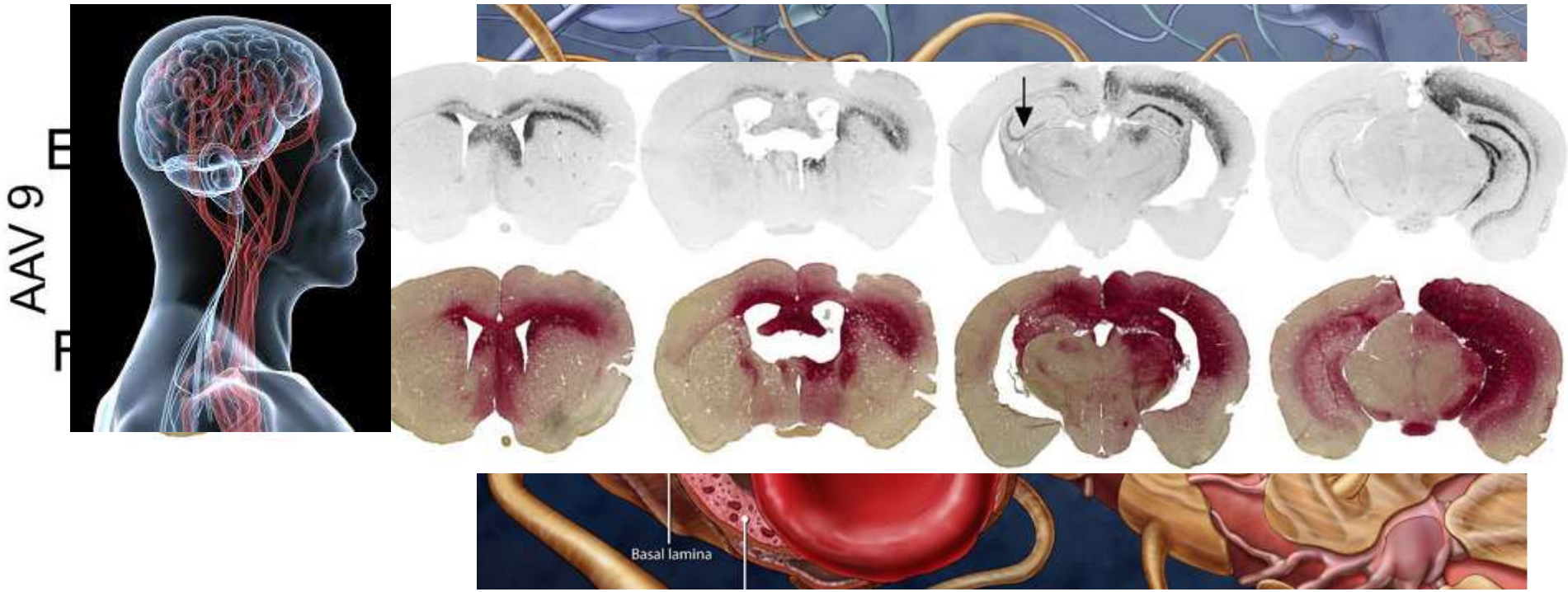
Studies in mouse models of Rett Syndrome demonstrate that a single dose of gene therapy can significantly improve symptoms even after the syndrome has progressed.

But…..

Gene therapy for Rett syndrome: future challenges

The therapeutic vector will need to reach the whole CNS

- Spread widely through the brain if administered directly in the brain
- To cross the BBB if administered intravenously

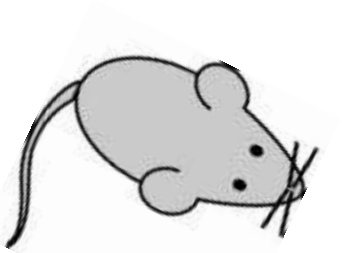


Gene therapy for Rett syndrome: preclinical studies

Gadalla et al, 2013

Rescue of RTT mice when the virus was injected **directly in the brain** of **neonatal** Mecp2-deficient **male** mice

Weak rescue when the virus was injected intravenously in **young adult male mice**

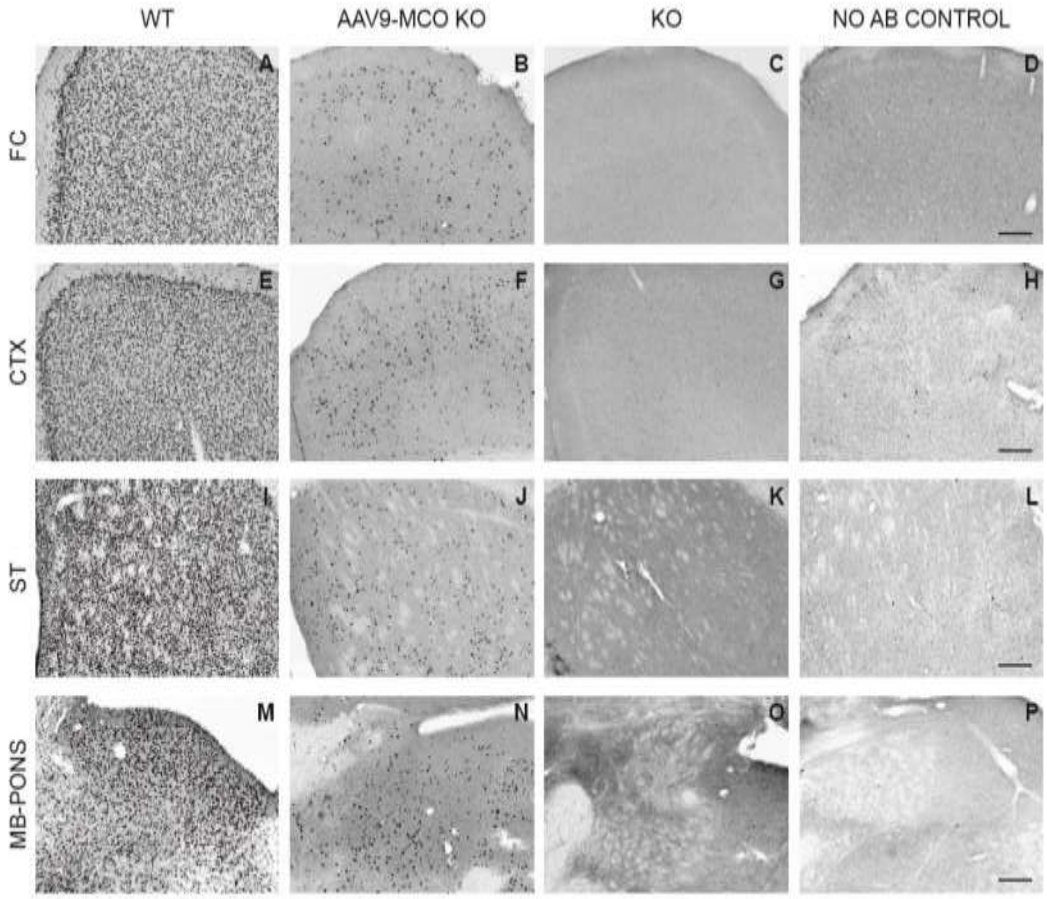


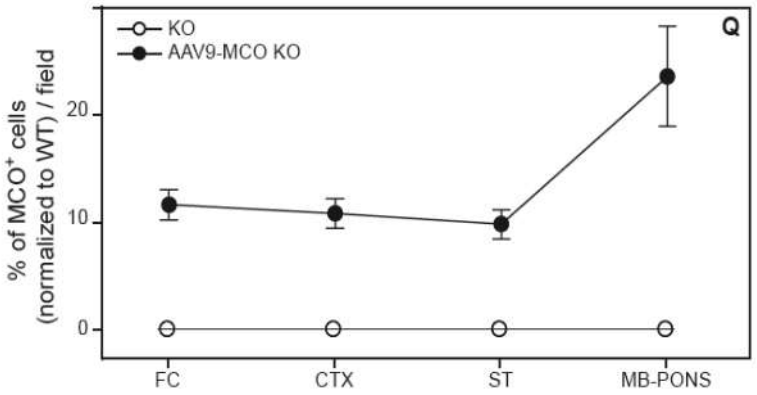
Garg et al, 2013

Partial rescue of Mecp2-deficient **adult male** mice when the virus was injected intravenously

Partial rescue of Mecp2 – deficient **adult female** mice.

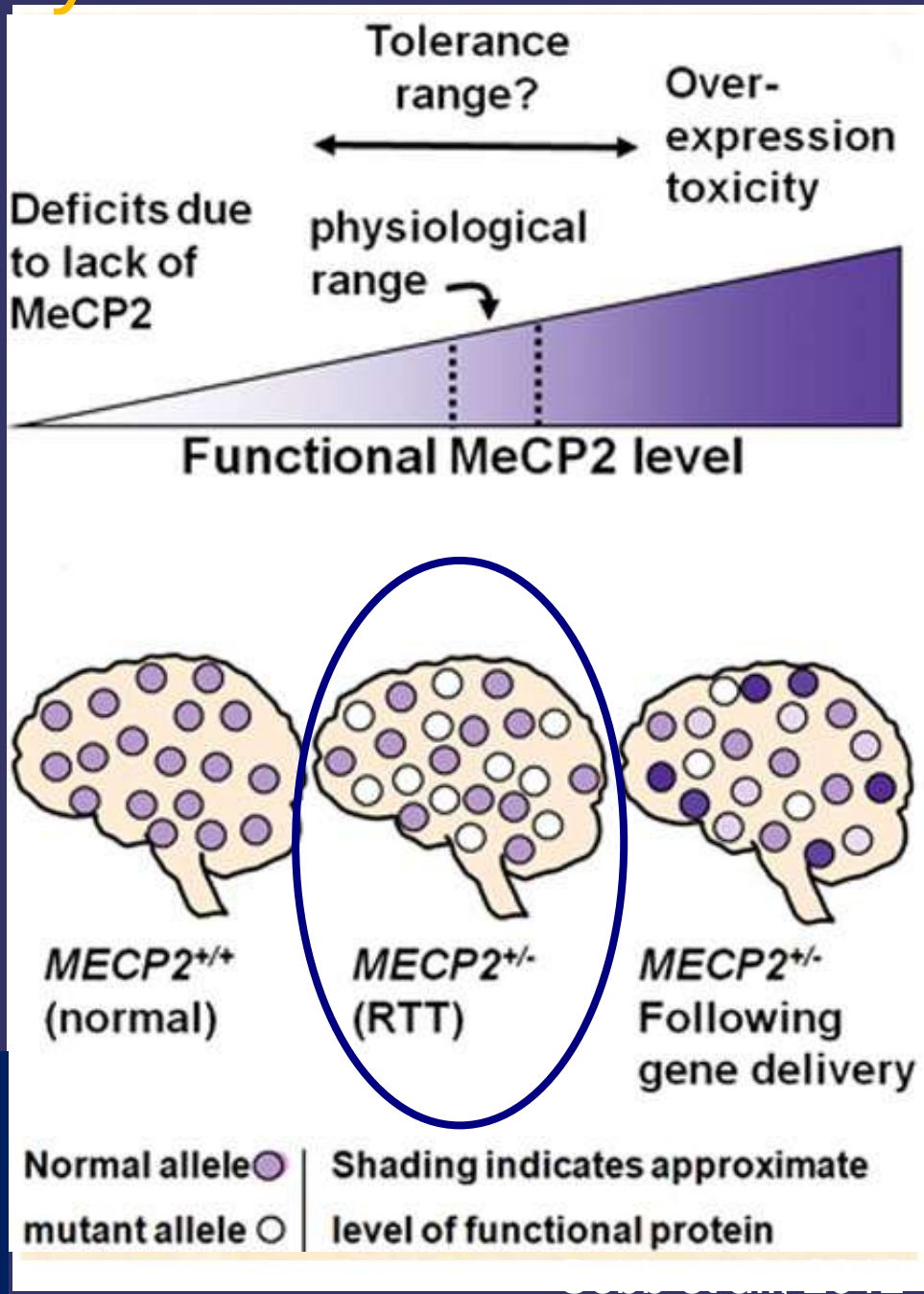
MeCP2 expression in the brain of Mecp2 KO mice





Gene therapy for Rett syndrome: future challenges

Girls with Rett have two *MECP2* genes, one that is mutated and one that is normal.



Gene therapy for Rett syndrome: from preclinical studies to the clinics

\+

Rett Syndrome is a candidate for gene therapy because it is caused by a **single gene**, it is **not degenerative**, and **symptoms may be reversible** at any age. Studies in mouse models of Rett Syndrome demonstrate that a **single dose of gene therapy** can significantly improve symptoms even after the syndrome has progressed.

\–

However, translating this to humans is challenging.
MeCP2 is needed in all regions of the brain so the gene must be delivered broadly. At the same time, some **cells already have healthy copies so overexpression is a risk.** Choosing the correct dose and vector is vital. In addition, MECP2 is elaborately regulated so ideally the introduced copy would incorporate these checks and balances. **Toxicity in peripheral organs** (liver) has to be controled

rett syndrome research trust

PRESS RELEASE:

AveXis to Advance Gene Therapy Program Initiated by the Rett Syndrome Research Trust

June 7, 2017

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NOVARTIS

Novartis enters agreement to acquire AveXis Inc. for USD 8.7 bn to transform care in SMA and expand position as a gene therapy and Neuroscience leader

Apr 09, 2018

INNOVATIVE STRATEGY TO ENHANCE THE EFFICIENCY OF BRAIN GENE THERAPY

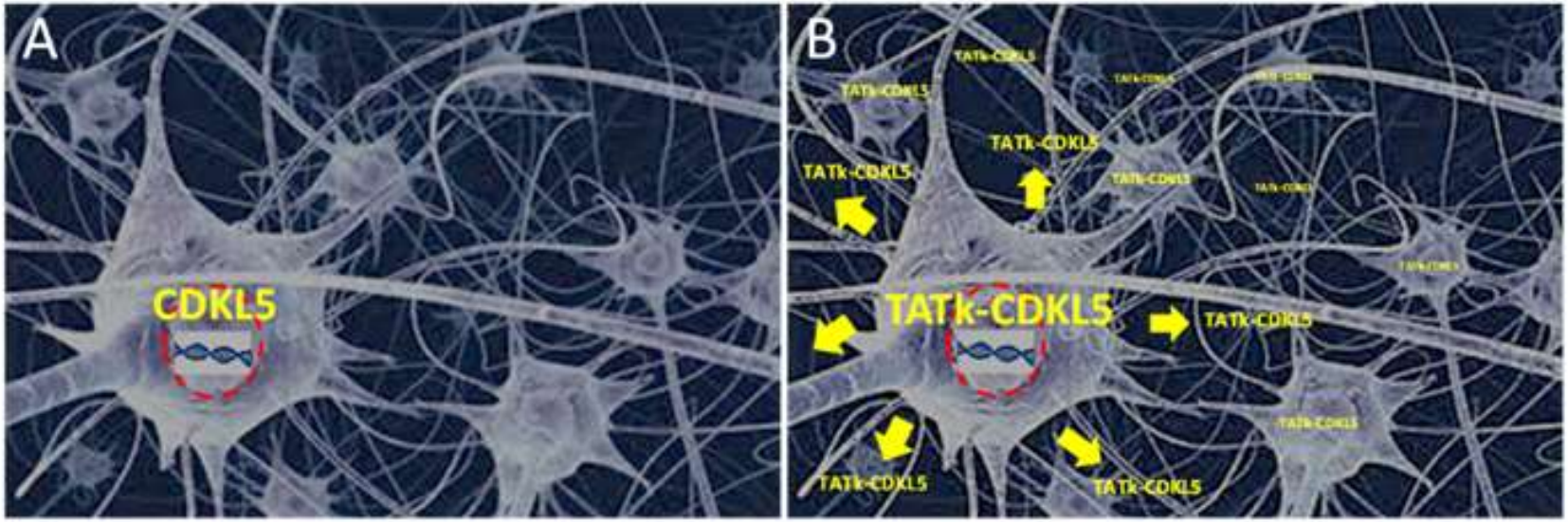


Figure 1: Comparison of the potential protein delivery between classical gene therapy (A) and innovative gene therapy (B) based on the insertion of a protein transduction domain in the target gene.

Key dates in Rett-related research

1966 — Rett Syndrome description

1981 — Description

1992 — Identification of MECP2

1999 — Link between MECP2 mutation and RTT

2001 — First RTT mouse model

2005 — CDKL5 mutations and atypical RTT variants

2008 — FOXG1mutations and atypical RTT variants

2007 — Reversibility of RTT

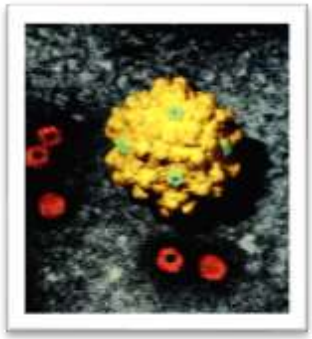
2010-2013 — IGF1 clinical trail

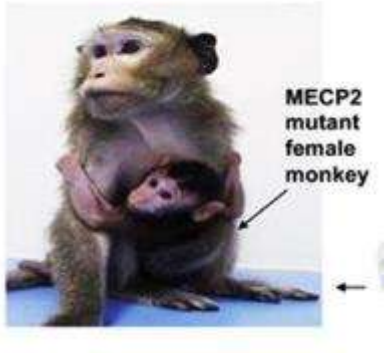
2013 — Proof-of-principle for gene therapy in RTT

2013 — First RTT primate model

2014 — 8 active clinical trials

??? — Replacement therapy





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