

A fluorescence microscopy image of brain tissue. The image shows a complex network of fibers and cells. A prominent feature is a large, dark, triangular-shaped region in the center, which appears to be a dense cluster of cells or a specific type of fiber. The surrounding tissue is stained with green and red dyes, creating a textured, fibrous appearance. The green staining is more widespread, while the red staining is more localized and appears as thin, branching lines.

***Dissecting the Role of MeCP2 in Brain & Implications  
for Autism Spectrum Disorders***

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# Autism

- **Twin studies examining the concordance of autism spectrum disorders for identical twins, compared to fraternal twins or siblings, indicates a strong genetic component.**
- **Many genes have been associated with autism, but each has been found in only a few individuals.**
- **No single - gene mutation linked to this disorder has been identified.**

# Autism

- **Hypothesis: Many factors can disrupt the brain pathways necessary for sociability, interactive communication, and flexible executive function.**
- **Targeting specific mutations in mice offers a rationale strategy to systemically test hypotheses about each candidate gene for autism.**
  - If the mutation results in a phenotype analogous to the symptoms of autism, then that gene may play a critical role.**
  - Because no biochemical or neuroanatomical markers for autism are known, the mouse phenotype is defined by behavioral criteria relevant to the diagnostic symptoms of autism.**

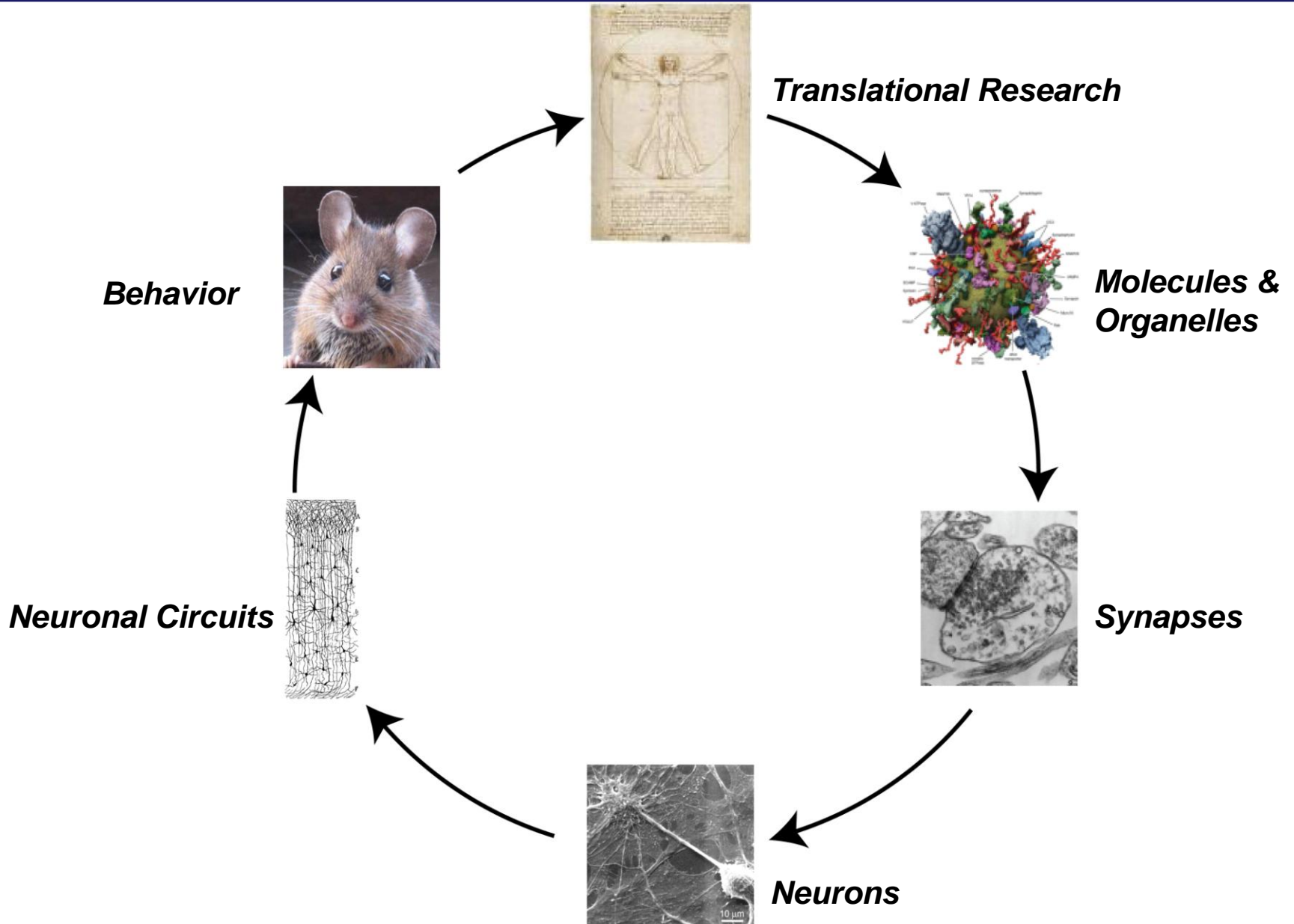
# Autism

- **Challenge:** design mouse behavioral tasks with sufficient analogies to the diagnostic symptoms.
- **Mice are social species with high levels of social interaction, as well as motor stereotypies, repetitive behaviors, and perseverative habits.**
- **Caveat:** Attempting to model symptoms without knowing causes.

# Autism

- **Approach: Perfecting animal models as mechanistic hypotheses emerge, while investigating proposed genetic, biochemical, and environmental factors, as well as any functional abnormalities resulting from the mutation that may help to better understand human disease.**

# Integrating Multiple Layers of Analysis



# MECP2

- **In 1999 the methyl-CpG binding protein 2 (MeCP2) was identified as the gene responsible for Rett Syndrome.**
- **Mutations in the MeCP2 gene have been identified in >96% of all Rett patients as well as some Autism, Asperger's and Fragile X patients.**
- **MeCP2 is believed to be a transcriptional repressor that is expressed in many cell types throughout the body.**
- **MeCP2 is the first gene that has been conclusively linked to a neuropsychiatric illness.**

***Does the loss of MeCP2 selectively in the brain contribute to behavioral abnormalities associated with Rett Syndrome and other autism spectrum disorders?***



# Site Specific Cre/loxP Recombination System

Mouse 1



CaMKII- Cre recombinase

Mouse 2

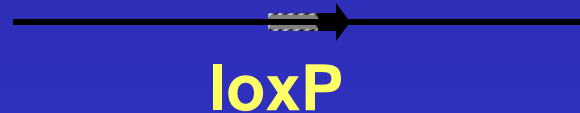


loxP

MeCP2

loxP

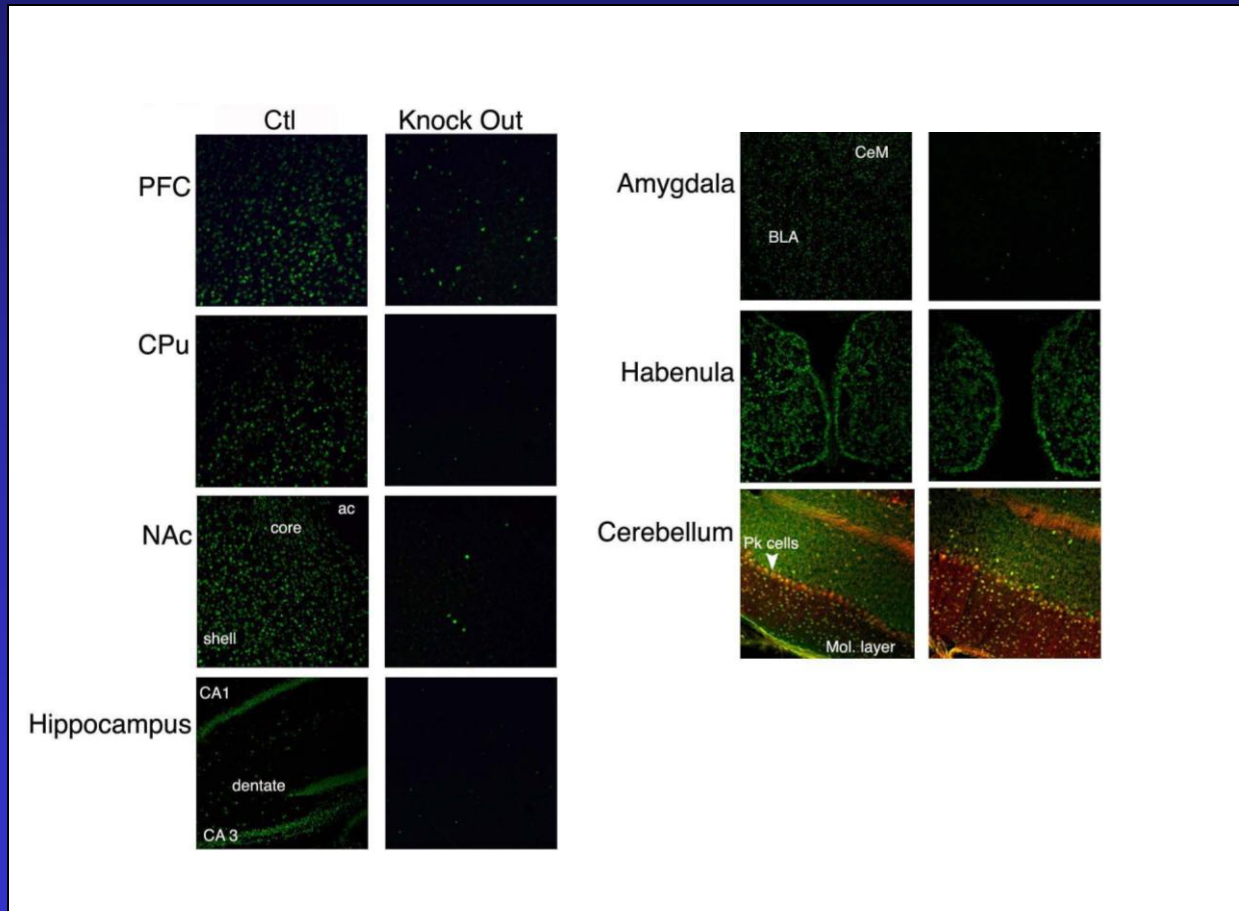
Knockout Progeny of Mouse 1 x Mouse 2



loxP

The loss of MeCP2 occurs in broad forebrain regions during early postnatal development.

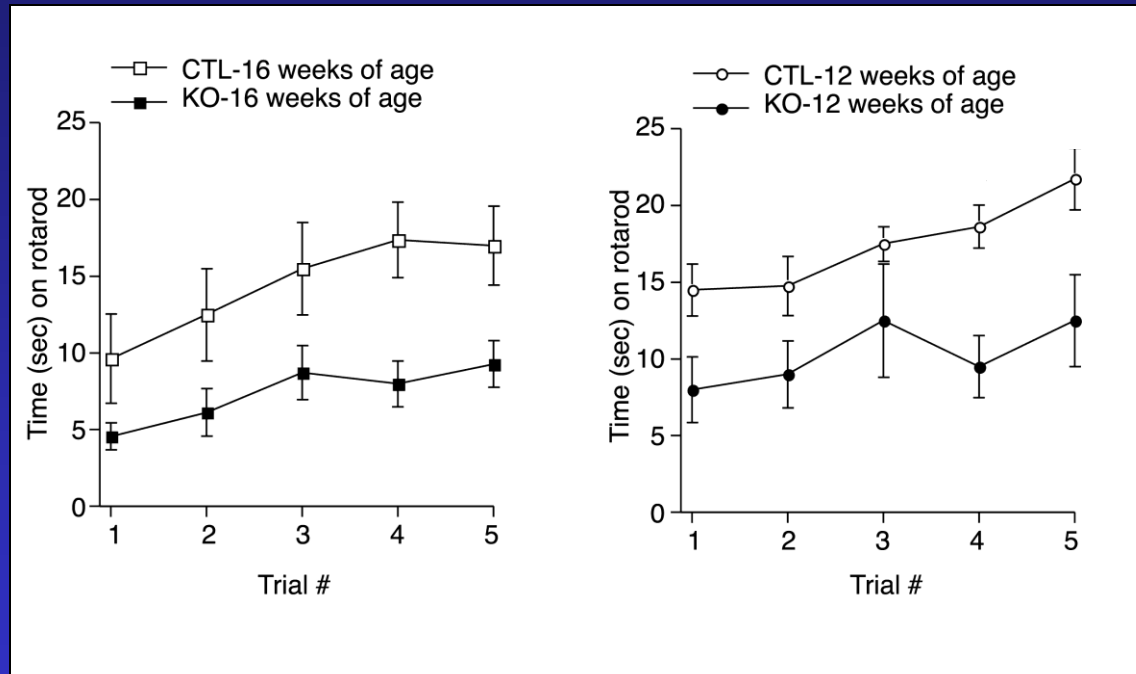
# MeCP2 is Selectively Deleted in the Forebrain of the Conditional KO's



# BEHAVIORAL PHENOTYPE OF CONDITIONAL MeCP2 KNOCKOUT

## KO's show:

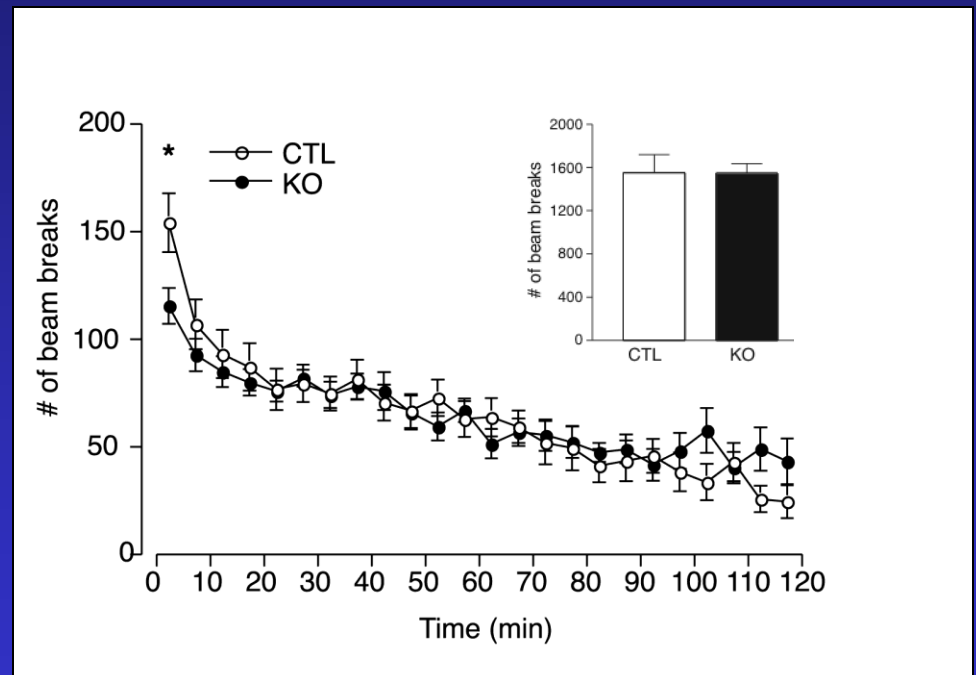
- Observable hindlimb claspings by 16 weeks of age,
- And impaired performance on the rotarod even prior to the appearance of an overt phenotype.



# BEHAVIORAL PHENOTYPE OF CONDITIONAL MeCP2 KNOCKOUT

## KO's show:

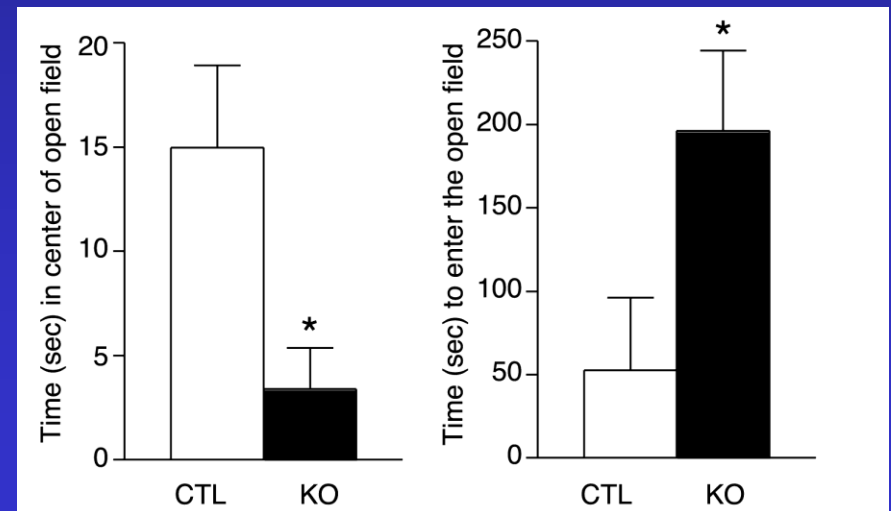
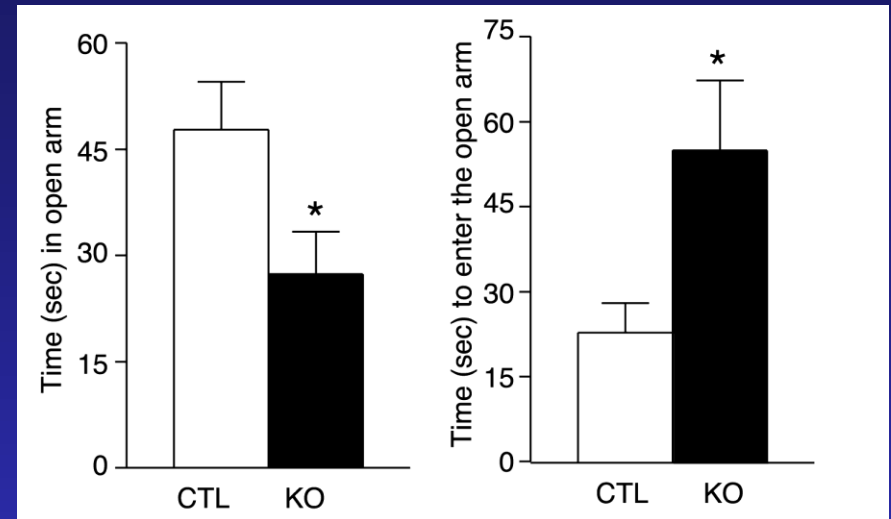
- Initial hypoactivity but then display normal locomotor activity throughout the testing period.



# BEHAVIORAL PHENOTYPE OF CONDITIONAL MeCP2 KNOCKOUT

## KO's show:

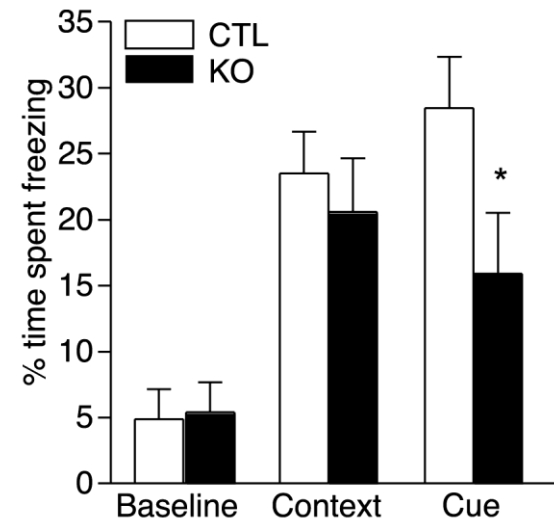
- Increased anxiety-like behavior in three independent tests measuring anxiety behavior.
- The majority of RTT patients have increased incidence of heightened anxiety.
- This data suggests that MeCP2 may be an important mediator of anxiety-like behavior.



# BEHAVIORAL PHENOTYPE OF CONDITIONAL MeCP2 KNOCKOUT

## KO's show:

- Normal context-dependent fear-conditioning but deficits in cue-dependent learning.

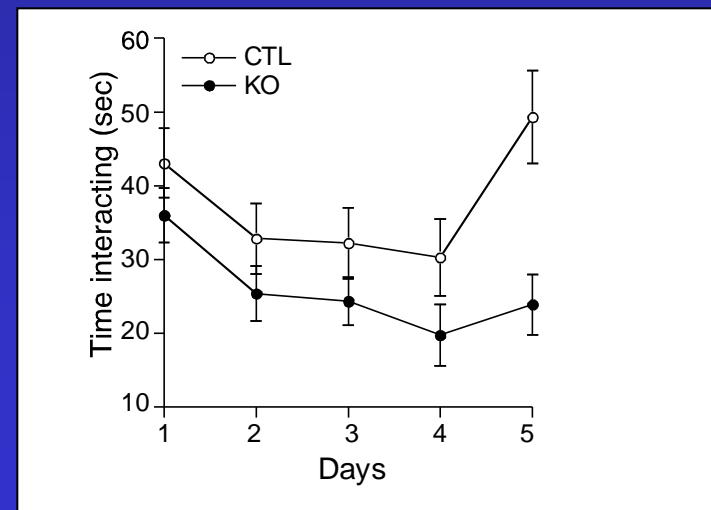
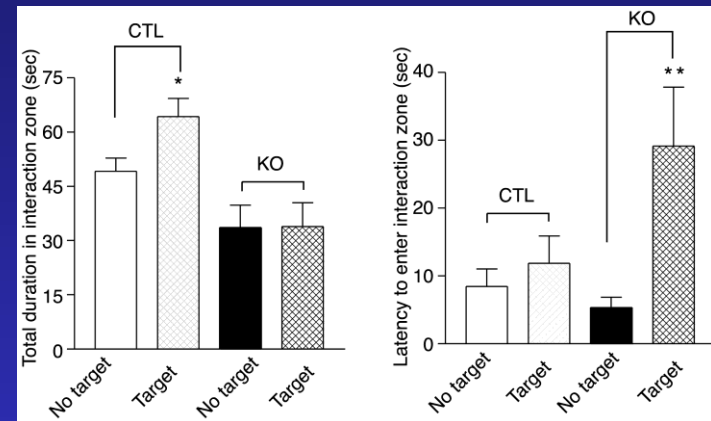


# BEHAVIORAL PHENOTYPE OF CONDITIONAL MeCP2 KNOCKOUT

KO's also show:

- Impaired social interactions with other mice,
- But can distinguish between a novel animal and one they've seen before.
- These data suggest that forebrain MeCP2 is important in mediating aspects of social interaction and that this conditional KO's may serve as a useful animal model for studying deficits in social interaction.

## Social Interaction Tests



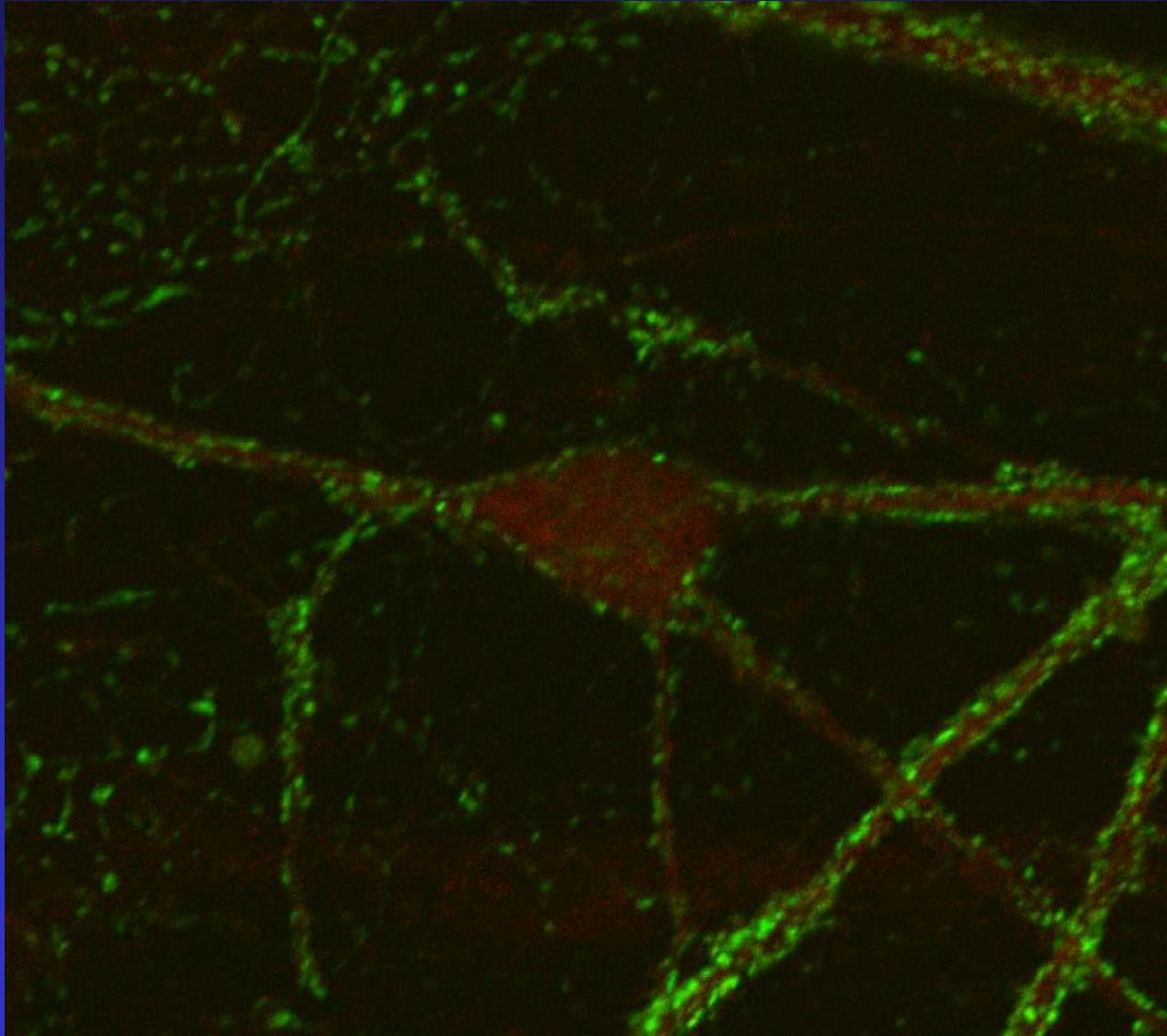
# BEHAVIORAL PHENOTYPE OF CONDITIONAL MECP2 KNOCKOUT

- The loss of MeCP2 occurs in broad forebrain regions during early postnatal development.
- Suggests that the behavioral deficits observed are the result of loss of MeCP2 function in postnatal brain and not generalized global deficits.
- Partial reduction in the forebrain is sufficient to recapitulate symptoms of RTT suggesting that slight alterations in MeCP2 levels can produce profound behavioral effects.
- Starts to provide a framework of the neural circuitry that is involved in mediating aspects of the disorder.

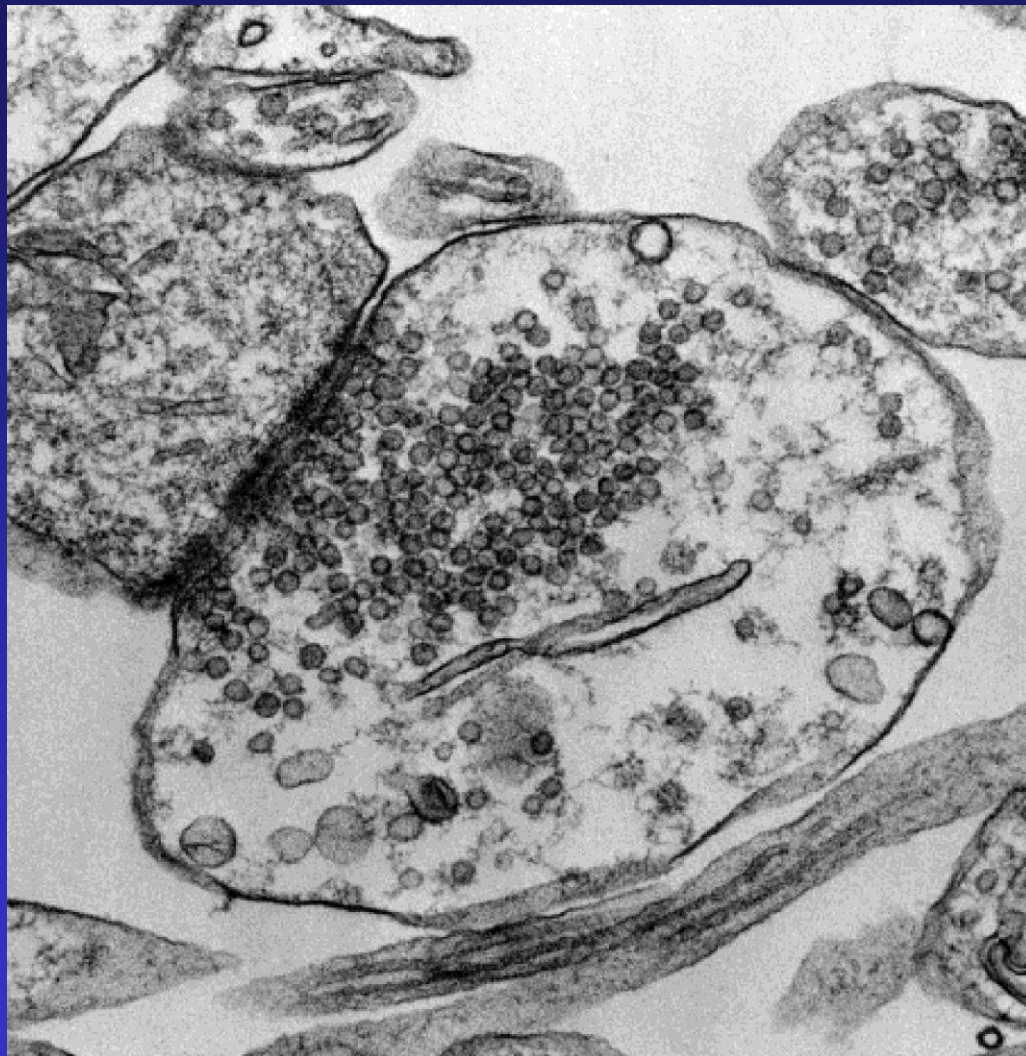


***How does the loss of MeCP2 affect neuronal function?***

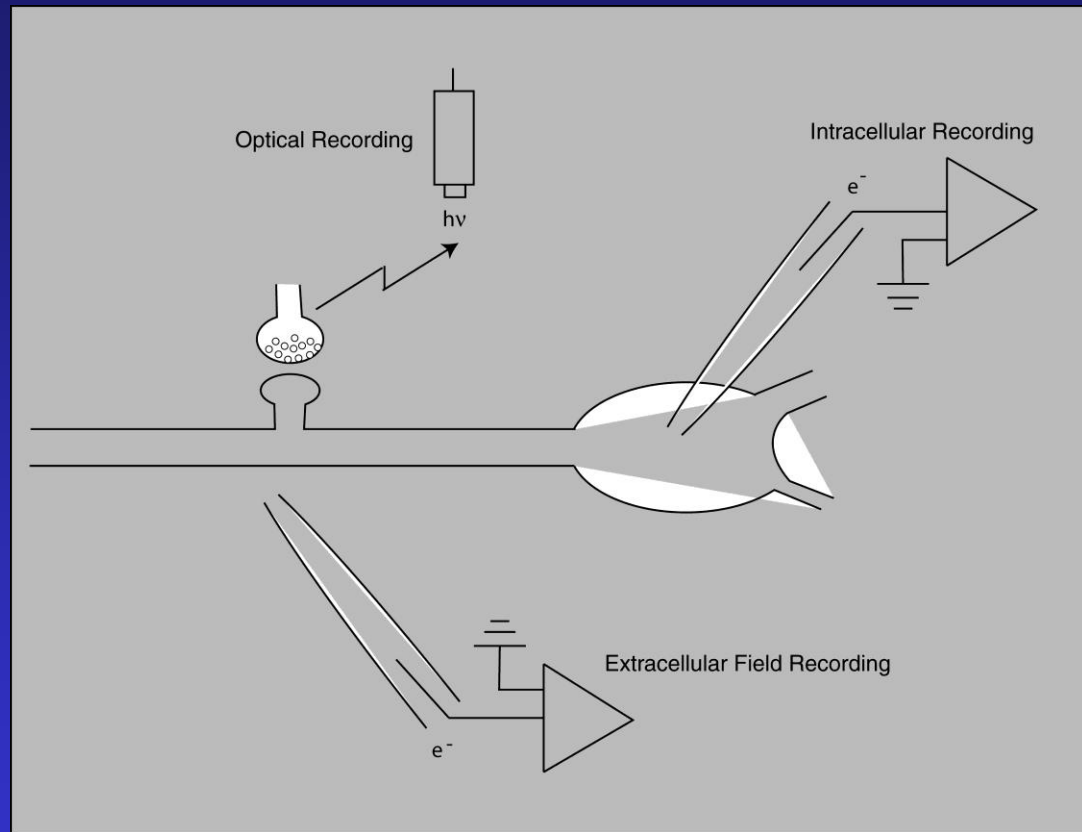
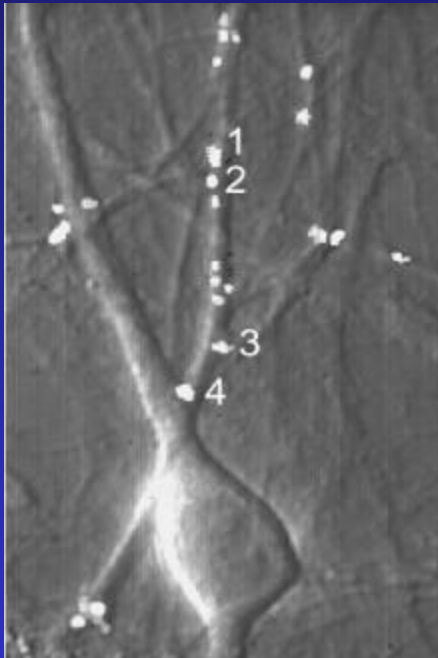
# CNS Neurons



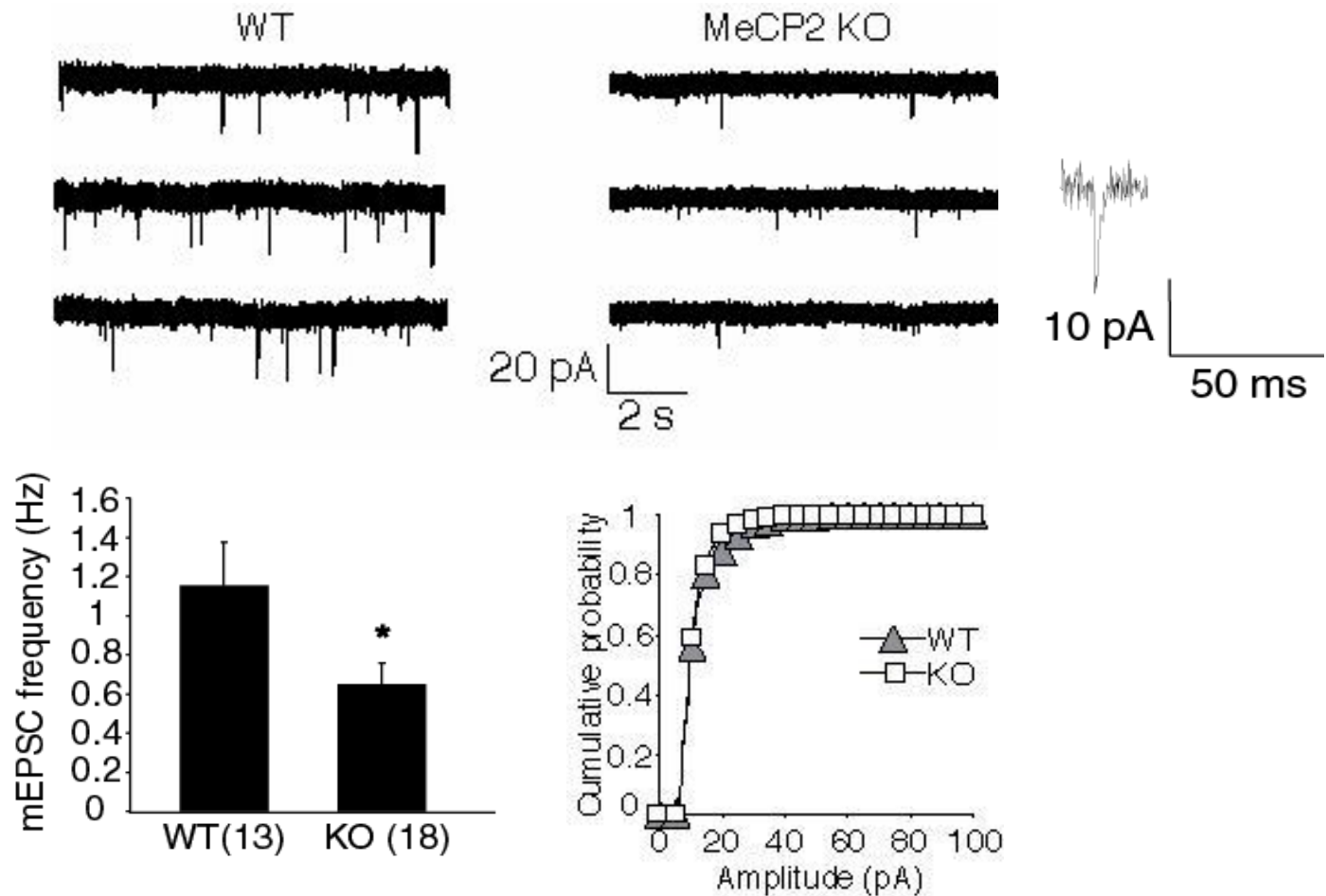
# The Synapse



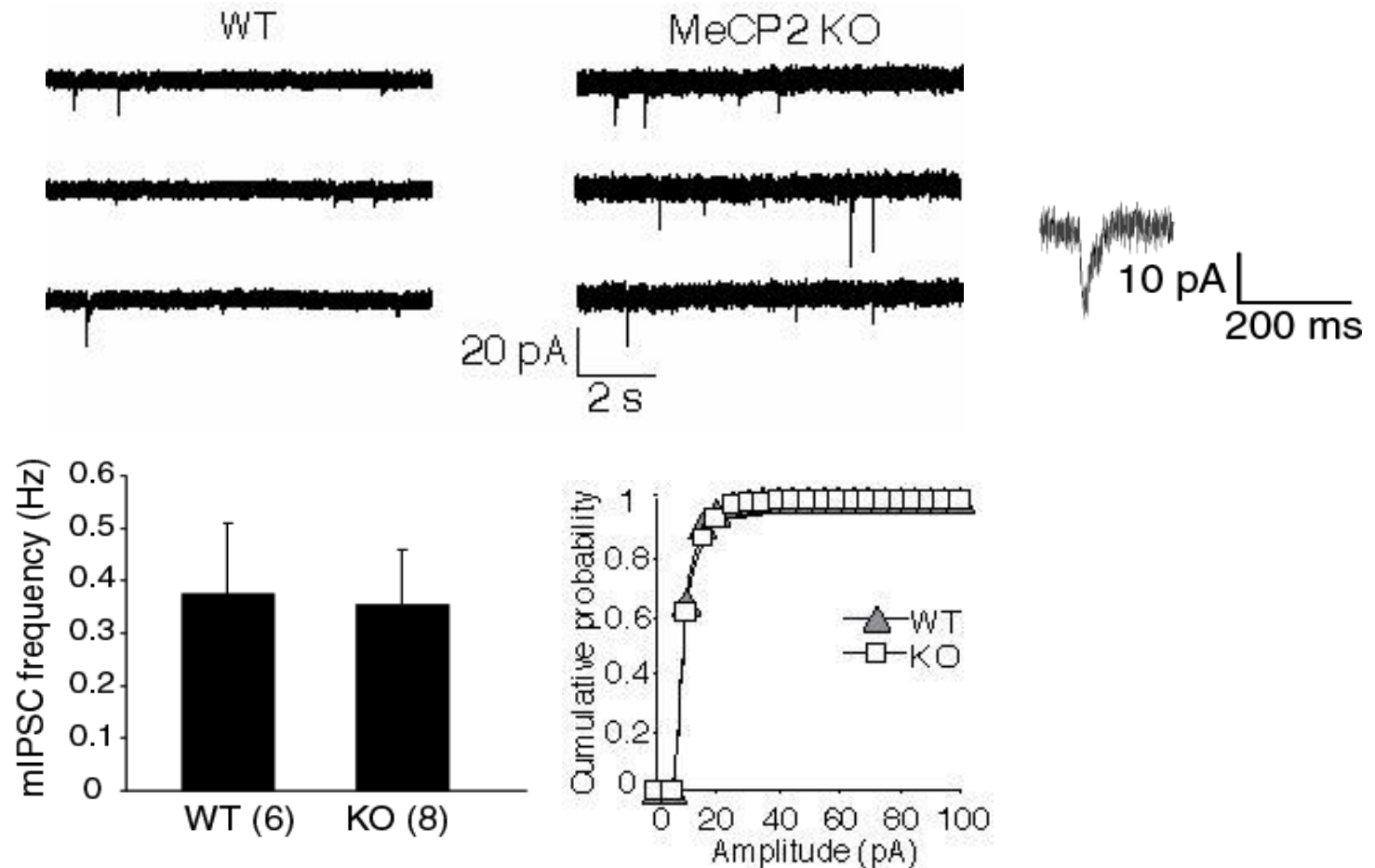
# Elucidating synaptic transmission in neurons using electrophysiological & optical imaging



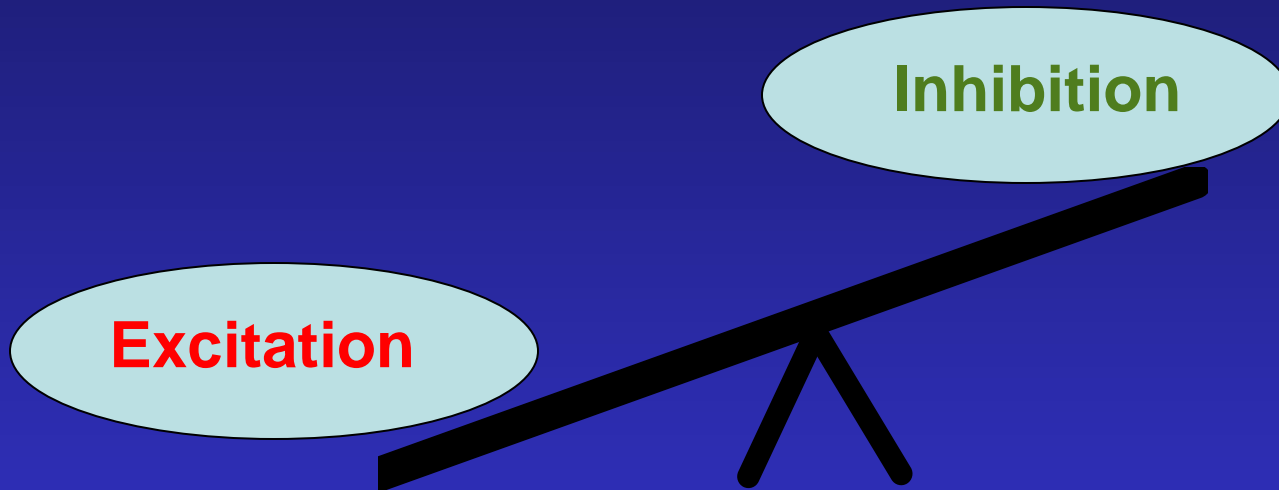
# mEPSC FREQUENCY IS DECREASED IN MECP2 KO NEURONS



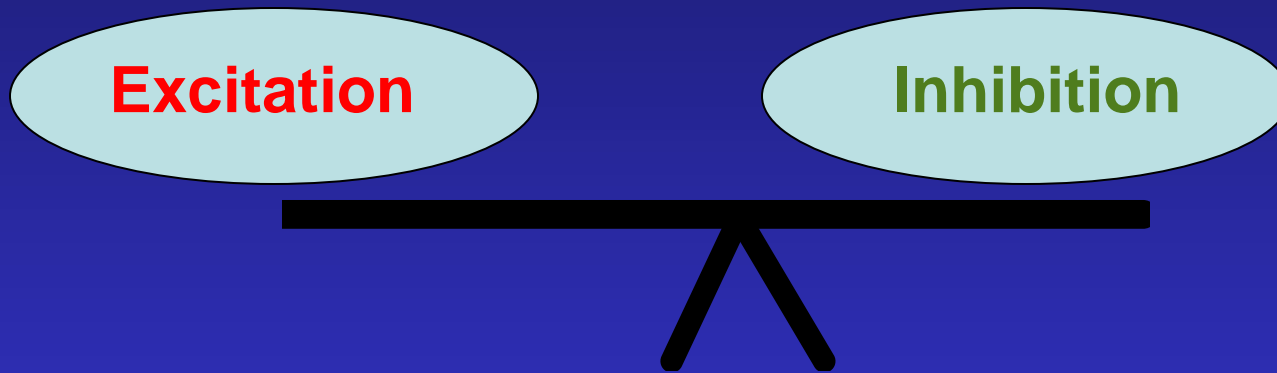
# mIPSC PROPERTIES ARE UNALTERED IN MECP2 KO



# DECREASED EXCITATORY TRANSMISSION IN THE BRAIN



# RESTORE THE BALANCE OF EXCITATORY:INHIBITORY TRANSMISSION IN THE BRAIN

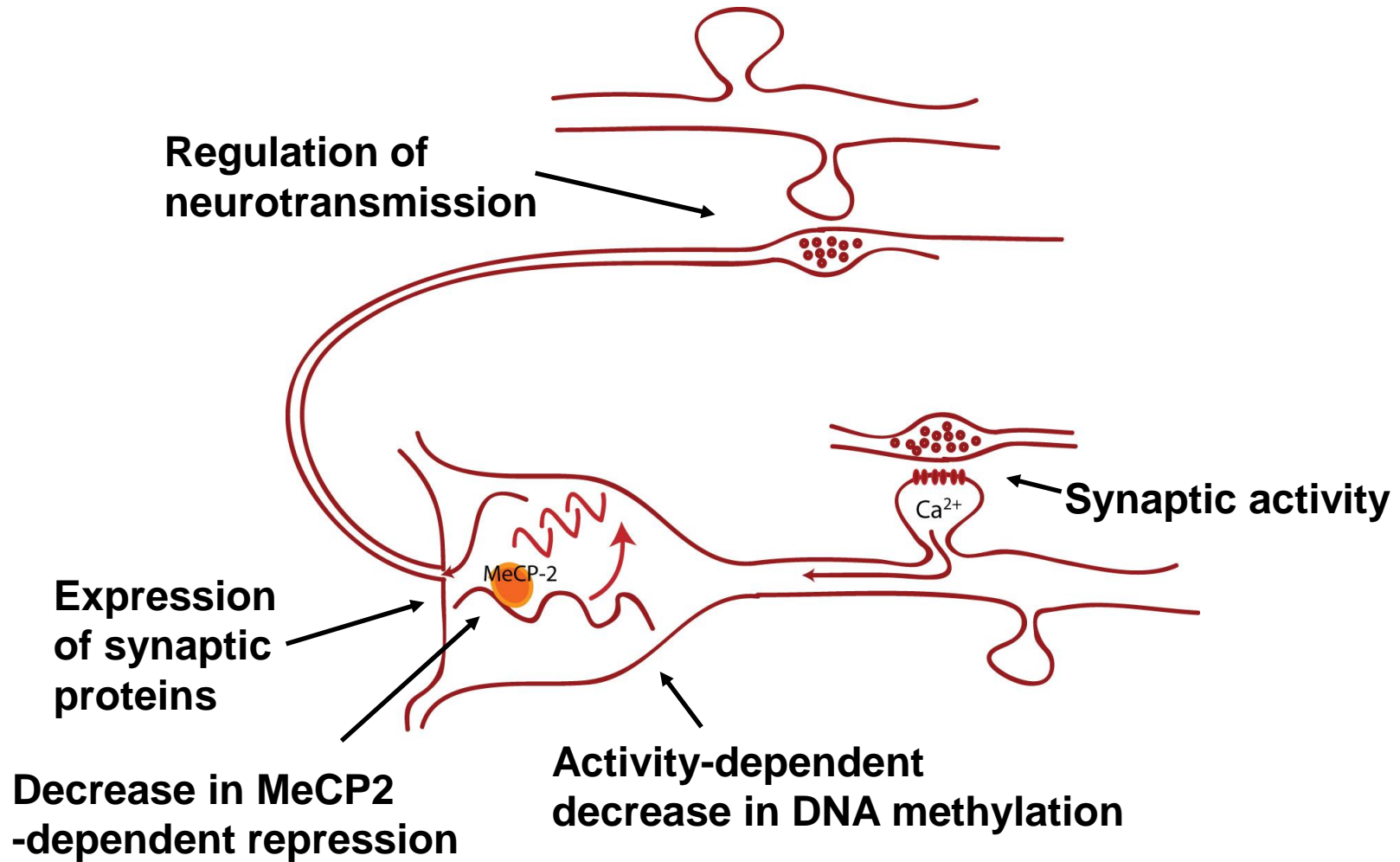




# **Functional Findings in Neurons Lacking MeCP2**

- **MeCP2 plays a specific role in the regulation of excitatory presynaptic function.**
- **Our findings are consistent that a change in excitation and inhibition contributes to the pathogenesis of autism spectrum disorders.**
- **Future work is currently underway to more fully examine how this imbalance may underlie changes in network activity in the brain.**

# TRANSCRIPTIONAL REGULATION OF NEUROTRANSMISSION



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