Understanding the Role of Interneurons in CDD

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Abstract

CDKL5 Deficiency Disorder (CDD) is a neurodevelopmental condition affecting predominantly females with an incidence of 1:40,000/60,000. Clinical features of CDD include developmental delays, hypotonia, deficits in learning, memory, and social behaviors, and most notably seizure onset before the age of 3 months.[1]

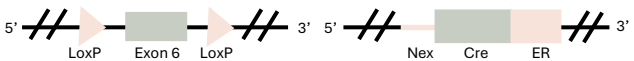
CDD is caused by mutations in the X-Linked gene Cyclin-Dependent Kinase-Like 5 (CDKL5). It encodes a Serine/Threonine kinase that implicates neuronal migration, axon outgrowth, dendritic morphogenesis, and synapse development.[2]

How interneurons are affected remains unknown. In this project, our goal is to investigate how CDKL5 dysfunction impairs interneuron development. We used a conditional KO mouse model to address this question. We found that early knockout of CDKL5 in excitatory neurons can increase interneuron density in the hippocampus and medial prefrontal cortex.

Methods

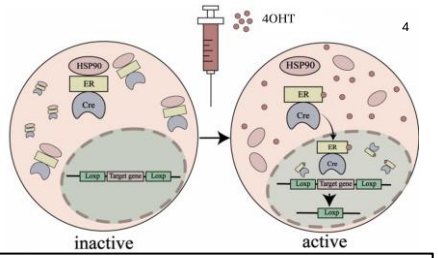
Nex CreLoxP Conditional Gene Knockout (cKO)

We use the Nex promotor to drive recombinase fused with an Estrogen Receptor.[3]



Hydro tamoxifen (4OHT) Injections at P0

Inject 0.3mg/g of body weight of 4OHT for 3 consecutive days. 4OHT activates the Estrogen Receptor (ER) which translocates Cre into the nucleus.



Tissue Collection P80

After perfusion and freezing of mice brains, we cut 40um sections of the hippocampus and medial prefrontal cortex (mPFC) where Nex is active.

Immunohistochemistry (IHC) Staining

Day 1: Wash, Permeabilize, Block, Incubate with Primary Antibody
Day 3: Permeabilize, Incubate with Secondary Antibody
Day 4: Permeabilize, Incubate with DAPI, Permeabilize, Wash, Mount

Imaging & Counting

mPFC & hippocampus are imaged at 10X & 5X respectively on the Leica Microscope. Images were then analyzed on ImageJ. Density was calculated by manually counting the cells, dividing by area, and using a linear mixed model on R.

Results

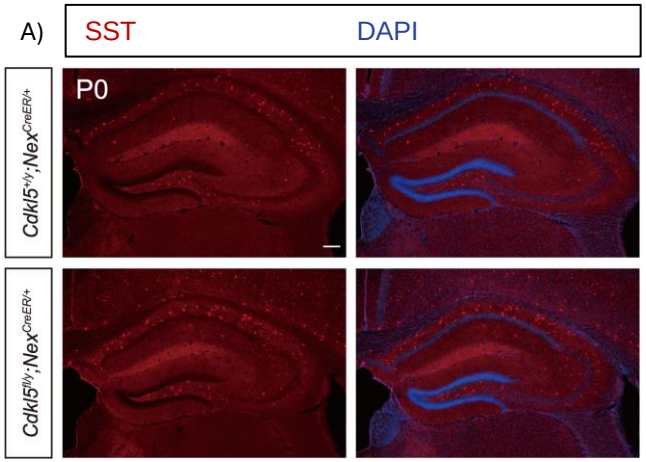


Figure A. Somatostatin (SST)+ interneuron staining of hippocampus in WT (top) and CDKL5 KO (bottom) mouse. Left column: SST antibody only; Right column: SST stained with 4-dimethyl (DAPI).

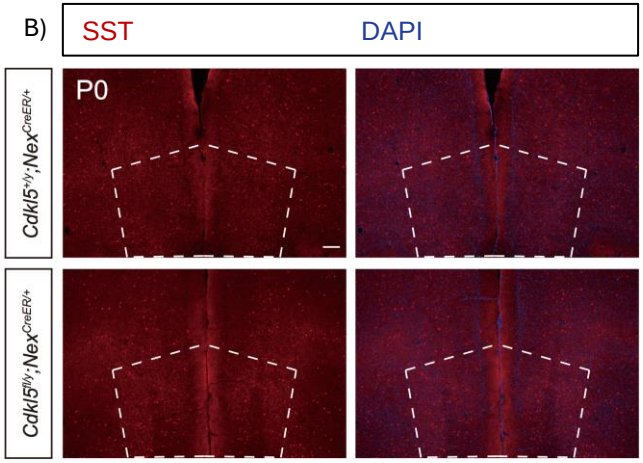


Figure B. Somatostatin (SST)+ interneuron staining of medial Prefrontal Cortex (mPFC) in WT (top) and CDKL5 KO (bottom) mouse. Left column: SST antibody only; Right column: SST stained with 4-dimethyl (DAPI).

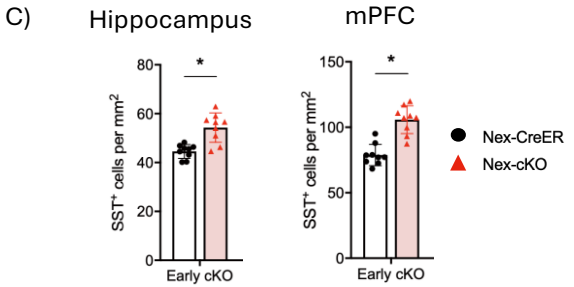


Figure C. Density of SST+ interneurons located in the hippocampus and mPFC of WT (Nex-Cre ER) and mutant (Nex-cKO) mice.

Conclusion

From the bar graphs, we see that CDKL5 expression leads to an increase in interneuron density only when knocked out early in excitatory neurons in the hippocampus and mPFC. When interneurons migrate to cortex, their survival is dependent on excitatory neuron interactions. If they do not receive input, they undergo programmed cell death, which can explain the trends in the graph. SST+ interneurons are involved in feedback inhibition, which is important in maintaining the balance between excitatory and inhibitory neurons. An imbalance could lead to seizures like in CDD.

Future Directions

In addition to early KO, we will inject Tamoxifen between P21-P24 to study the late cKO of CDKL5 in excitatory neurons. We will use the flex mouse model in which CDKL5 Exon 4 is initially inverted and can be reactivated upon Cre expression.

References

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