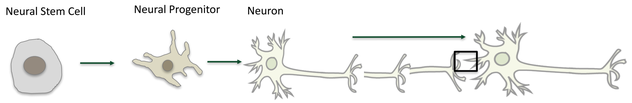
Role of the gene *Ncad* in neuronal circuit formation

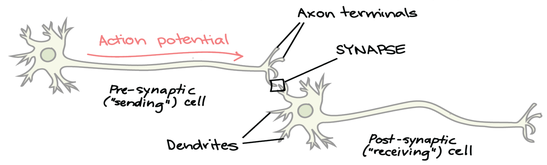
Hatun Mine Şahin[1], Adam Miller[2]
[1]Dokuz Eylul University Faculty of Medicine, Turkey; [2] Institute of Neuroscience, University of Oregon, USA

Introduction

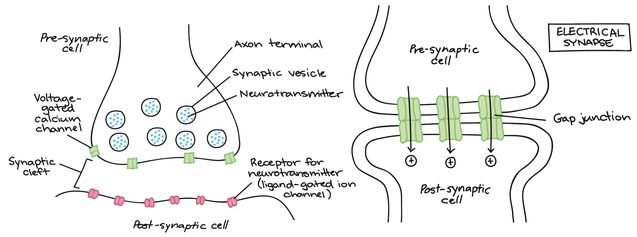
In order to form neural circuits in the brain, neural stems cells must give rise to the correct neuronal types and then the neurons must reach out long processes to find one another.



Once neurons find one another, they form connections called synapses to communicate.



There are two main types of synapses: chemical and electrical.

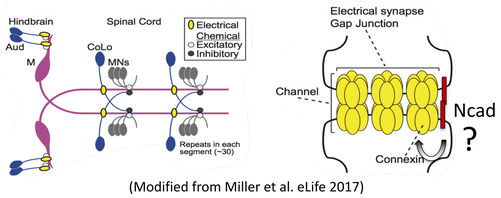


The genes required for electrical synapses are not well understood.

***Danio rerio* (zebrafish) as a model:**

- Accessible neural circuits
- Easily visualizable electrical synapses
- Gene and synapse function conserved from fish to humans

In *Danio rerio* (zebrafish), **the Mauthner (M) circuit** coordinates a fast escape response which is driven by both chemical and electrical synapses.



(Modified from Miller et al. eLife 2017)

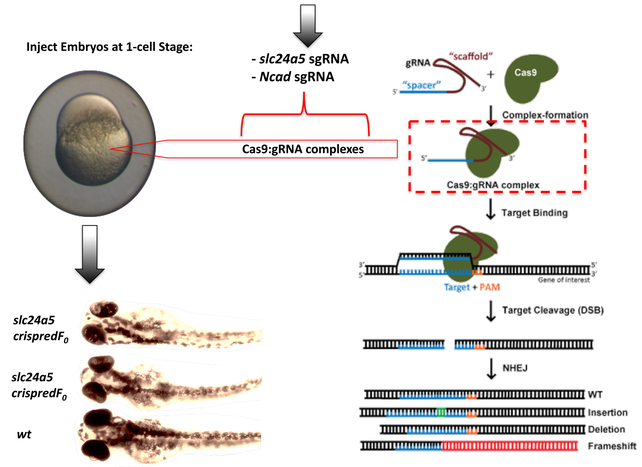
Synaptic transmission is a complex cellular function that requires the interaction of multiple proteins. A cell adhesion protein called **N-cadherin (Ncad)** is found in neurons and might be important for electrical synapse function.

Main Question

➢ **Does the gene *Ncad* play a role in Mauthner neuronal circuit and electrical synapse formation in zebrafish?**

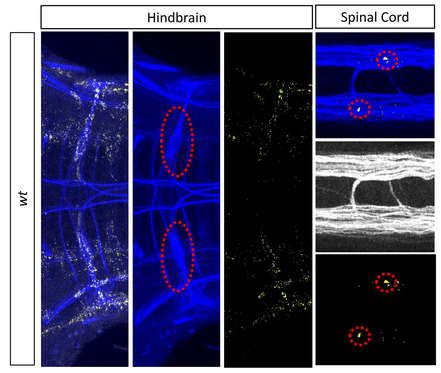
Methods

To examine the gene encoding Ncad protein, we generated mutations in the *slc24a5* locus (pigmentation gene) as a positive control and in *Ncad* to test for an effect on electrical synapse formation. To knock-out genes, we used **CRISPR/Cas9** gene-editing technique with pooled sgRNAs.



Mosaicism observed in loss of pigmentation

After CRISPRing for *slc24a5* and *Ncad*, larval zebrafish were fixed and stained using polyclonal antibody against the human Cx36 protein to visualize the Connexins at electrical synapses.

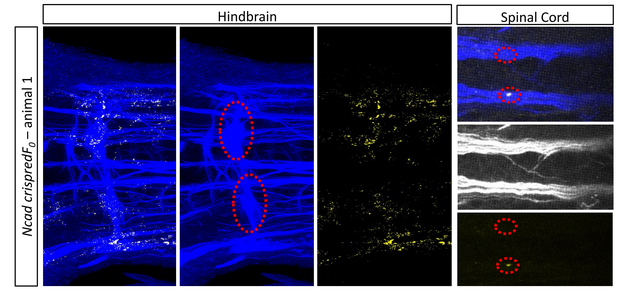


(blue: neurons in the circuit, yellow: electrical synapses)

Images were taken using a confocal microscope.

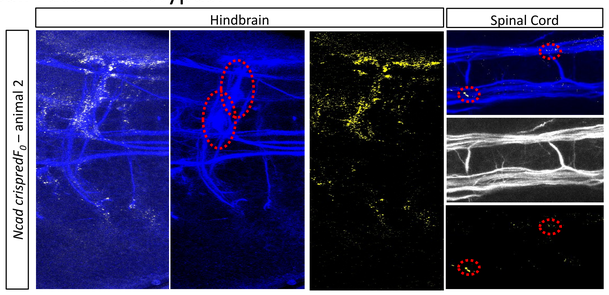
Results

Synapse Formation Phenotype:

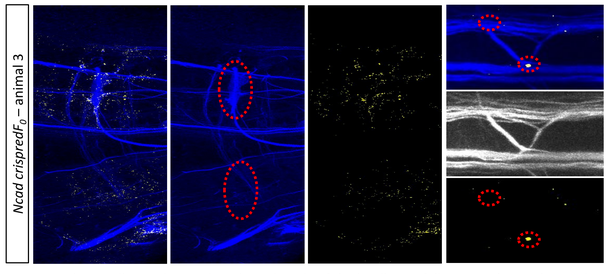


Mauthner cells and CoLo interneurons were observed, however there was a mosaic loss of M/CoLo electrical synapses. Since we didn't observe this phenotype in convincing number of the fish, we concluded that *Ncad* might be important for electrical synapse formation.

Cell Fate Phenotypes:



Two Mauthner cells were observed on the right side of the hindbrain. Correspondingly, we observed electrical synapse formation only on the continuing axons on the left side in spinal cord.



One Mauthner cell was observed on the right side of the hindbrain, following M/CoLo electrical synapses were only present on continuing axon on the left.

We conclude that *Ncad* is a candidate gene for neuronal circuit formation, both in specifying the correct types of neurons and forming the correct synapses.

We propose that *Ncad* is most likely playing a role at different phases of neurodevelopment, first at the state of specification from the neural stem cell, then during synapse formation.

Future Directions

- How does *Ncad* function to form M/CoLo electrical synapses?
- What are the molecular mechanisms underlying Ncad's effect on Mauthner specification?
- In which stages of neurodevelopment Ncad is important for neuronal circuit formation?

Acknowledgement & References

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- Korn, H., and Faber, D.S. (2005). The Mauthner cell half a century later: a neurobiological model for decision-making? Neuron 47, 13–28.
- Elife. 2017 May 22;6.pii: e25364. doi:10.7554/eLife.25364. A genetic basis of molecular asymmetry at vertebrate electrical synapses. Miller AC[1], Whitebirch AC[1], Shah AN[1], Marsden KC[2], Granato M[2], O'Brien J[3],Moens CB[1].
- Methods Cell Biol. 2016;135:89-106. doi: 10.1016/bs.mcb.2016.01.008. epub 2016 Feb26. Targeted candidate gene screens using CRISPR/Cas9 technology. Shah AN[1], Moens CB[1], Miller AC[1].
- Khan Academy