The Genetic Basis Of The First Connections In The Brain

Anisha Adke & Adam Miller

Abstract

There are an estimated 100 billion neurons in the human brain, equal to the number of stars in our galaxy. These neurons are connected at specialized junctions called synapses, one type of which is electrical. **Gap junction channels connect neurons at electrical synapses**, allowing ions and small molecules to pass between cells. Initial synapse formation is determined by the genetic code, which instructs where, when, and how neurons wire together to create circuits. Genetic defects believed to alter normal circuit wiring have been linked to neurodevelopmental disorders such as autism and schizophrenia, but the exact circuits and molecular mechanisms affected remain unclear.[1,2] Critical to normal circuit wiring is the formation of the first synapses between neurons, as these lay the foundation upon which mature circuits are built; research has shown that these first synapses are electrical but it is unknown which genes are responsible and drive the continued development of the networks.[3,4] **This project aims to identify the genes required for the first electrical synapses and investigate their roles from a genetic, neural circuit, and behavioral standpoint.**

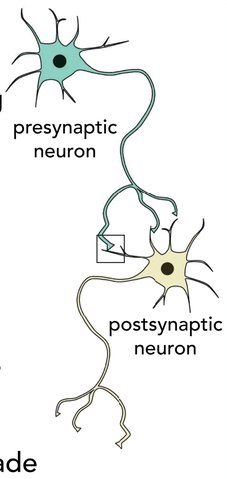
To investigate the first synapses, we examined the spinal cord circuits in zebrafish. These neural networks provide advantages to studying synapse formation because they wire together within 24 hours post fertilization (hpf) using electrical synapses, are visualizable at the levels of neurons, circuit function, and behavior, and are genetically accessible. With this approach, we can identify the genes responsible for the first synapses formed in the brain and examine how these synapses impact early circuit wiring.

Background

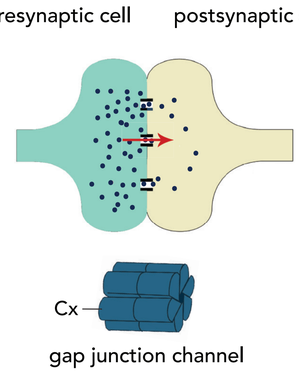
neurons are the building blocks of the brain

neurons are specialized cells that communicate with one another by receiving and transmitting electrical signals

electrical signals are passed from the presynaptic cell across junctions called **synapses** to the postsynaptic cell



electrical synapses form the first connections during development



electrical synapses directly couple two neurons and pass electrical impulses from cell to cell

they are formed by **gap junction channels** - made from **gap junction proteins [connexins (Cxs)]**

the first neural networks that form during development use electrical synapses

genes in the DNA encode 20 Cxs in humans - a subset of these are expressed in neurons

What Cxs are responsible for the first synapses?

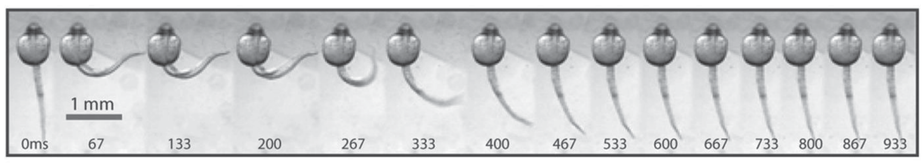
What are their roles in neural network formation throughout development?

zebrafish as a model organism

externally developing embryos - accessible for genetic manipulations

spinal cord circuits develop within 24 hours post fertilization (hpf) via electrical synapses

exhibits rhythmic behavior at 18 hpf that requires electrical synapse function



Spontaneous coiling behavior in a 26 hpf embryo (modified from Knogler et al., 2014).

Methods

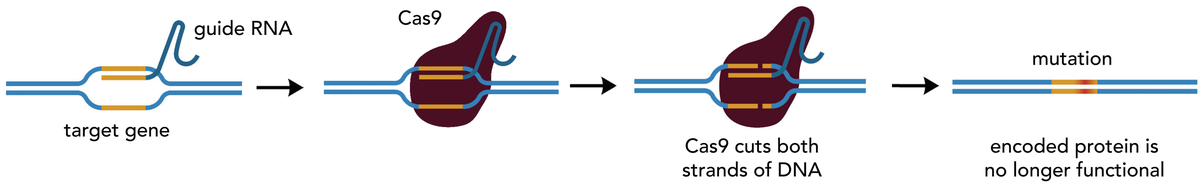
Which Cxs are expressed early in development?

Analysis of RNAseq data from ~24 hpf zebrafish spinal cord neurons identified 7 Cxs.[5]

Cx46.8 *Cx35.1* *Cx34.7* *Cx35.5* *Cx35.5* *Cx30.3* *Cx43.3*

To determine whether these Cxs are involved in early wiring, knock out the genes.

CRISPR/Cas9 system to mutate genes-of-interest



Preliminary Results

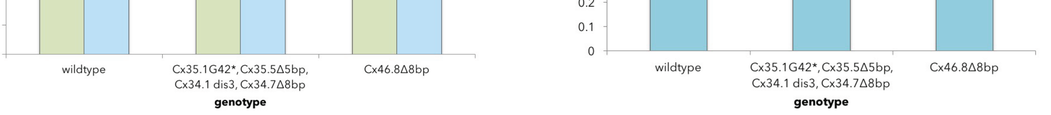
progress in generating mutant animals

gene/Cx	status
Cx46.8	CRISPR/Cas9 injected, 8 base pair deletion identified, growing up homozygous mutant line, initial behavioral analysis complete
Cx35.1	generating line with other genes (Cx35.5, Cx34.7, and Cx34.1) to account for genetic redundancy
Cx35.5	generating line with other genes (Cx35.1, Cx34.7, and Cx34.1) to account for genetic redundancy
Cx34.7	generating line with other genes (Cx35.1, Cx35.5, and Cx34.1) to account for genetic redundancy
Cx34.1	generating line with other genes (Cx35.1, Cx35.5, and Cx34.7) to account for genetic redundancy
Cx43.3	CRISPR/Cas9 injected, 4 base pair deletion identified, generating homozygous mutant line
Cx30.3	CRISPR/Cas9 injected, 4 base pair deletion identified, generating homozygous mutant line

highly related Cxs may have redundant functions

Which Cxs are involved in early circuit formation?

when a fish is mutant for a gene required for electrical synapses, coiling behavior will be affected, highlighting which genes are important for network formation and further narrowing genes-of-interest

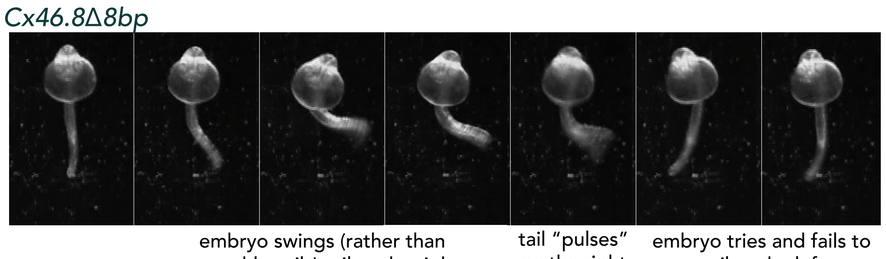


Python program tracks coiling behavior to identify changes in mutant animals

mutations in *Cx46.8* cause defects in coiling

An 8 base pair frame-shifting deletion (*Cx46.8Δ8bp*) causes coiling defects

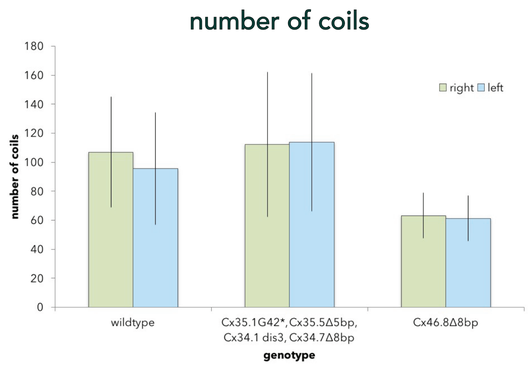
Cx46.8Δ8bp

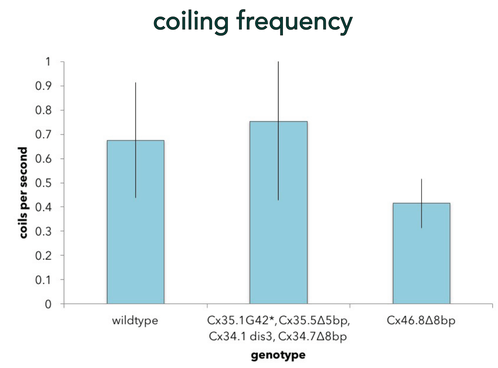


embryo swings (rather than smoothly coils) tail to the right

tail "pulses" on the right

embryo tries and fails to coil to the left





Next Steps

generate stable mutant fish lines for *Cx43.4* and *Cx30.3* and analyze behavior in detail

conduct further behavioral analysis on *Cx35.1*, *Cx34.7*, *Cx34.1*, & *Cx35.5* mutants to conclusively eliminate their role in the first synaptic connections

perform RNA *in situ* hybridization to find where genes-of-interest are expressed in neurons - this will suggest where the Cxs are required for neural circuit function

use a neuronal activity marker (GCaMP) expressed in all neurons to visualize circuit dynamics, patterns of synapse formation, and roles of Cxs in this process

Acknowledgements & References

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