The Role of Notch in Neuronal Connectivity in *C. elegans*

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Abstract

Notch receptors are conserved transmembrane proteins that regulate key developmental processes and promote stem cell proliferation and renewal. Defects in the Notch pathway are especially evident in neurons, where Notch signaling remains active in the maintenance of the nervous system from birth to adulthood. In our research, we documented the neuronal development of mechanosensory neurons at the nerve ring in *C. elegans*. Animals with an integrated transgene that expresses GFP in mechanosensory neurons (*zdIs5*) were crossed with animals with the following loss of function alleles from the Notch pathway: *sup-17*, *lin-12*, and *sel-12*. Mutant nerve rings were then compared to wild type at L4, day 1 adults, and day 2 adults. Animals of the same genotype did not exhibit different break rates across life stages, which indicates that the defect is developmental and not degeneration. Significantly higher rates of breakage were found in all of the mutant animals that have a defect in Notch signaling when compared to wild type. This suggests that the Notch pathway is involved in this neuronal connectivity defect. We are currently performing tissue specific rescue experiments and are in the process of determining the mechanism by which Notch affects neuronal connectivity in the mechanosensory neurons.

Introduction to the Notch Pathway

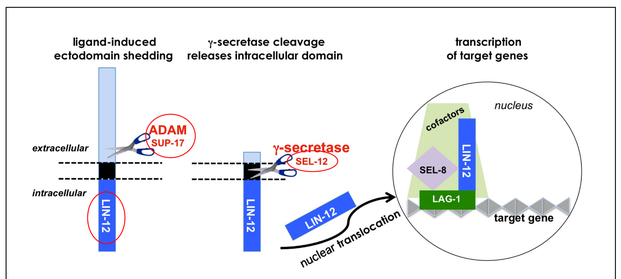

Figure 1. Diagram of the Notch pathway with labeled proteins and their roles. [1] Red circles around genes studied in this project.

Introduction to Mechanosensory Neurons

Three of a *C. elegan's* mechanosensory neurons meet in the head region of the worm and are responsible for sensing gentle touch. These specific neurons are called MT cells because they contain large diameter, 15-protofilament microtubules. The AVM (*a*nterior *v*entral *M*T cell), ALML (*a*nterior *l*ateral *M*T cell *l*eft) and ALMR (*a*nterior *l*ateral *M*T cell *r*ight) connect to form the nerve ring as indicated by the white arrows.[2]

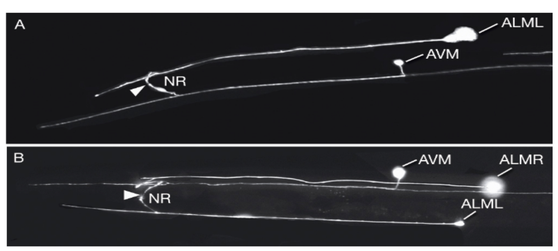

Figure 2 A. Top view of the nerve ring with labeled mechanosensory neurons.[2]
Figure 2 B. Side view of the nerve ring with labeled mechanosensory neurons.[2]

Experimental Design and Crosses

We crossed worms with a GFP marker of mechanosensory neurons (zdIs5) to our mutant animals to visualize the portion of the nerve ring we were interested in.

Experimental Steps:
1)Pick 150 mutant L4's and 150 wild type L4's
2)Place 50 of each on 6 different labeled plates
3)Blindly image 50 mutant and wild type L4's and record 1 for broken and 0 for unbroken
4)Wait 24 and 48 hours respectively then repeat step 3 for Day 1 and Day 2 animals

Normal Cross
mutant x zdIs5 male→ mixed progeny
↓
Single out zdIs5 animals and self mate
↓
Continue until progeny are mutant;zdIs5 double homozygous

Balanced Cross
Mutant/balancer(marked w/ green heads) x zdIs5 male
↓
Non green headed hermaphrodites w/ zdIs5 x green headed; zdIs5 males
↓
Select green headed; zdIs5 hermaphrodites (50/50 wild type and mutant)
↓
Verify mutant with progeny and continue selfing until balanced mutant; zdIs5 homozygous

L4 Data

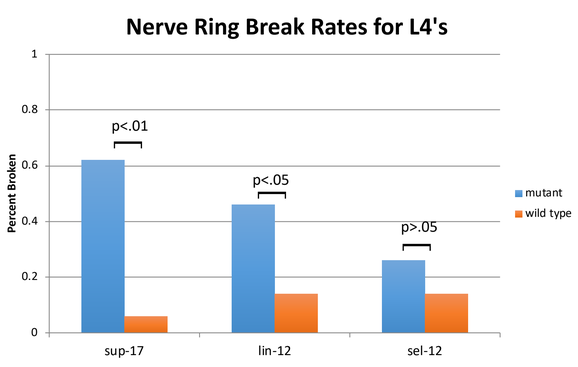

Figure 3. Break rates for L4 mutants compared to wild type. n=50. Fisher's exact was used to calculate p values

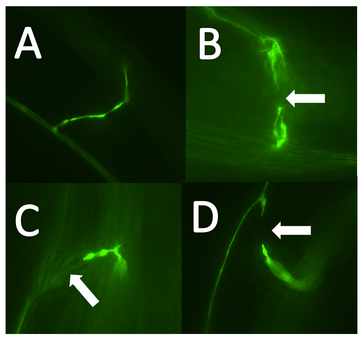

Figure 1. L4 animals. A-wild type; not broken. B-sup-17; broken. C-lin-12; broken. D- sel-12; broken. Arrows indicate breaks.

Day 1 Adult Data

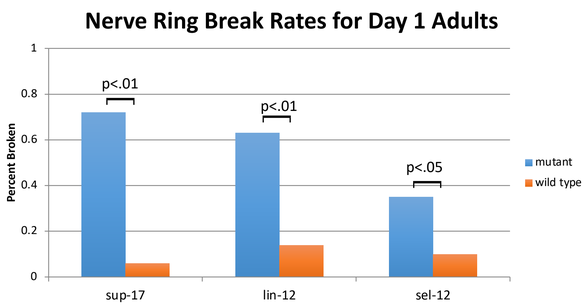

Figure 5. Break rates for Day 1 adult mutants compared to wild type. n=50. Fisher's exact was used to calculate p values.

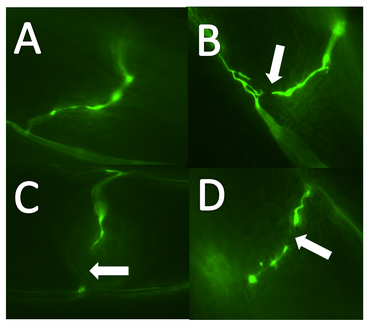

Figure 6. Day 1 adult animals. A-wild type; not broken. B-sup-17; broken. C-lin-12; broken. D- sel-12; broken. Arrows indicate breaks.

Day 2 Adult Data

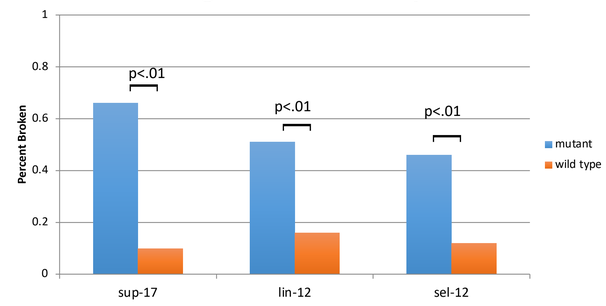

Figure 7. Break rates for Day 2 adult mutants compared to wild type. n=50. Fisher's exact was used to calculate p values.

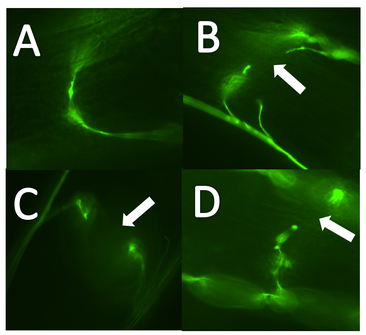

Figure 8.Day 2 adult animals. A-wild type; not broken. B-sup-17; broken. C-lin-12; broken. D- sel-12; broken.

Data Analysis

- All mutant nerve ring break rates were significantly different from wild type except for *sel-12* L4's
- None of the mutants differed between life stages on breakage rates
- L3's were considered, but not compared because wild type nerve ring's were not fully formed at this stage even in the wild type

Discussion

- The defect in the nerve ring is the result of a developmental defect and not degeneration
- The nerve ring is normally developed at the stage of L4, however it seems to never completely form in the mutants

Further Directions

- Quantify this defect in other Notch signaling mutants such as *pen-2*, *aph-1*, and *glp-1*
- Determine if Notch functions cell autonomously in neurons or glia
- Perform a rescue experiment on *sup-17* to confirm its involvement
- Image other mutants that are known to be involved with neuronal development such as *sax*, *wnt*, *ror* and *slt*.

Acknowledgments & References

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References:

1) Greenwald, I. LIN-12/Notch signaling, *Iva GreenWald Lab*, web, accessed August 15, 2016, http://www.greenwaldlab.org/research.html#lin-12.
2) Altun, Z.F. and Hall, D.H. 2011. Nervous system, general description. In *WormAtlas*. doi:10.3908/wormatlas.1.18
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