What guides the leaders: Identifying factors involved in pioneer axon extension.

Abigail Feresten[1], Jaffar Bhat, Alex Yu[2], Catharine Rankin[2], and Harald Hutter[1]

[1]Dept. Biological Science, Simon Fraser University, BC, Canada; [2]Dept. of Psychology, University of British Columbia, BC, Canada

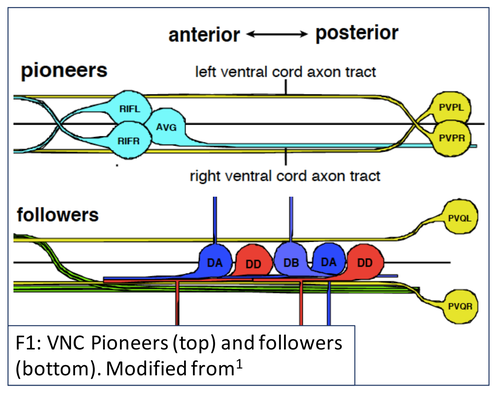
Background

Correct development of the nervous system involves precise navigation of axon growth cones as they form functional pathways. One guidance mechanism is the pioneer/follower relationship.

- Pioneer axons extend first, guided by environmental cues.
- Later "following" axons extend along the "pioneered" path.

The right ventral nerve cord (VNC) of *C. elegans* is pioneered by the AVG axon. The AVG growth cone extends posteriorly, followed by axons comprising the motor circuit (F1).

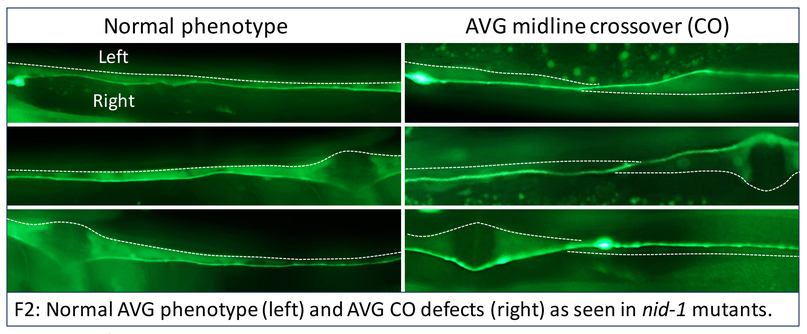
- When AVG mistakenly crosses the midline (F2: Right), command interneuron (CI) axons often cross at the same point, following AVG.



F1: VNC Pioneers (top) and followers (bottom). Modified from[1]

We conducted an enhancement screen in a *nid-1*/Nidogen null background to identify factors regulating AVG axon guidance.

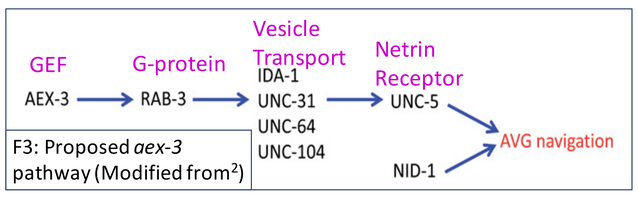
- Nidogen is a conserved basement membrane component. *nid-1* null worms exhibit 20% AVG CO defects.
- Seven mutants isolated in our screen exhibited ***nid-1* enhanced AVG CO** defects at 40% or higher.
- One of the genes identified was *aex-3*, an activator of RAB-3.



F2: Normal AVG phenotype (left) and AVG CO defects (right) as seen in *nid-1* mutants.

***aex-3* regulates AVG axon guidance via *unc-5*.[3]**

- *aex-3* affects vesicle trafficking, and interacts genetically with *unc-5*, an *unc-6*/netrin receptor.
- We hypothesized that *aex-3* regulates AVG axon navigation through transport of *unc-5* or *unc-5* associated factors (F3).



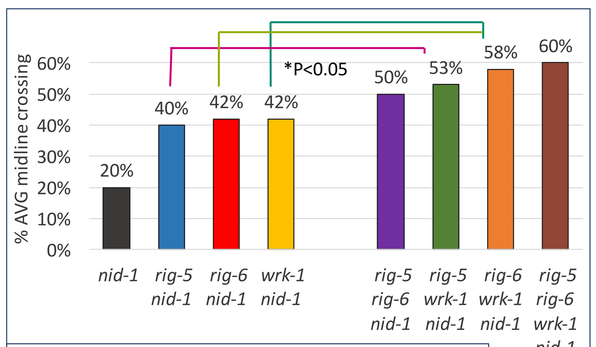
F3: Proposed *aex-3* pathway (Modified from[2])

rig-5, *rig-6*, and *wrk-1* contribute to AVG guidance

I Identified three GPI anchored IgCAMs that also affect AVG axon guidance: *rig-5*, *rig-6*, and *wrk-1*.

- Genetic interaction results (F4) demonstrate that *rig-5* and *rig-6* genetically interact, while *wrk-1* does not interact with *rig-6*.

rig-5 and *rig-6* may be binding partners, or they may interact indirectly. They may be anchored to a shared cell surface, or on different cell membranes. **We are in the process of generating GFP constructs to determine cell type expression and colocalization.**



F4: IgCAMs redundantly Support AVG axon guidance

Effect of *rig-5* on motor function

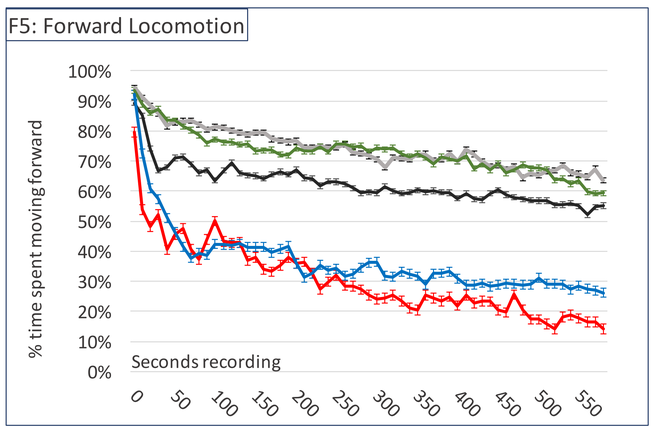
We assessed spontaneous and elicited movements in *rig-5 nid-1 otIs182[inx-18::GFP]* mutants and controls, using a **multi worm tracker**.[4]

- AVG does not differentiate in *lin-11* mutants, so this line serves as a positive control.
- *rig-5* was chosen for locomotive assessment as it has the fewest known pleiotropic functions that may influence mobility independent of AVG axon guidance.

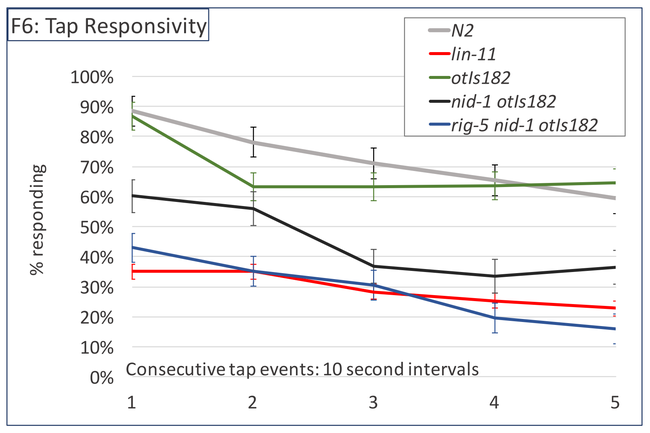
Forward locomotion (F5) and tap responsivity (F6) impaired in *rig-5 nid-1* mutants and *lin-11* mutants.

- *rig-5 nid-1* null worms have movement defects that are significantly more severe than all negative controls and behaviorally approach *lin-11* null worms in which AVG is absent.

F5: Forward Locomotion



F6: Tap Responsivity



ccd-1 affects CI guidance through AVG defects

Elevated CI defects may result from CI growth cones following misguided AVG axons, or defects of both cell types may be due to the loss of a common guidance cue.

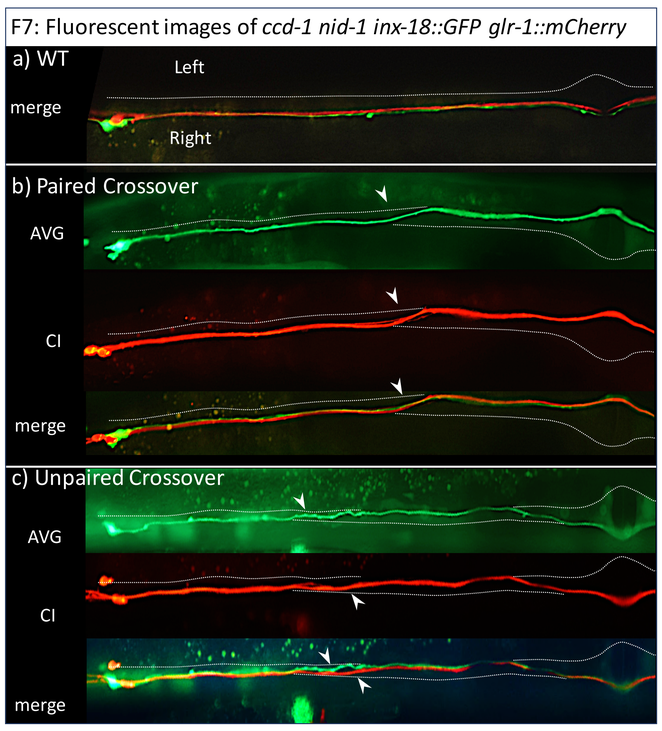
To determine if CI follower axon CO are correlated with AVG pioneer axon CO, **we labeled CI and AVG cells with fluorescent markers** (F7).

- If CI CO result from CI growth cones **following** the AVG pioneer axon, then **both CO events should coincide spatially.**
- CI CO occurring **without physical contact** with an AVG CO suggest that these CI **axon navigation defects are independent** of observed AVG pioneer axon defects.

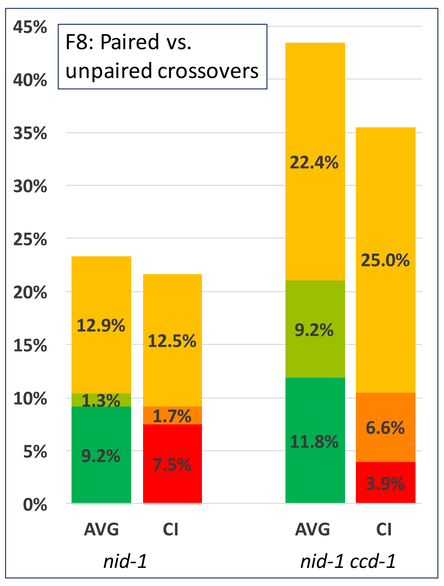
The chart below (F8) shows **unpaired CI CO in red** and **unpaired AVG CO in green**. **Paired CO are shown in orange**. Overlapping regions (**dark orange** and **light green**) represent animals that exhibit **multiple** COs, some paired and some unpaired.

- **Loss of *ccd-1* leads to an increase in paired CO and in unpaired AVG CO, but no change in unpaired CI CO rates were observed.**
- These results indicate that the *ccd-1* associated changes in CI CO are due exclusively to paired CO events.

F7: Fluorescent images of *ccd-1 nid-1 inx-18::GFP glr-1::mCherry*



F8: Paired vs. unpaired crossovers



We conclude that *ccd-1* contributes to CI axon guidance through its contributions to AVG axon guidance, and not by influencing CI guidance directly.

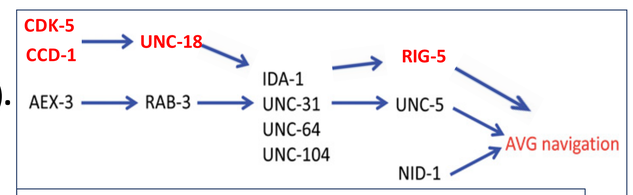
ccd-1 and *cdk-5* interact with *unc-5* and *rig-5*

Another allele isolated in our enhancement screen mapped to the gene F52H2.7, **a homologue of human C2CD5. This protein interacts with the CDK5 kinase[3]**.

- We have temporarily dubbed this gene *ccd-1*.

Mutant analysis demonstrates that *ccd-1, cdk-5, rab-3*, unc-18, and *unc-5* interact genetically (F5).

- This places *ccd-1* and *cdk-5* into the previously identified *aex-3* pathway (F6).

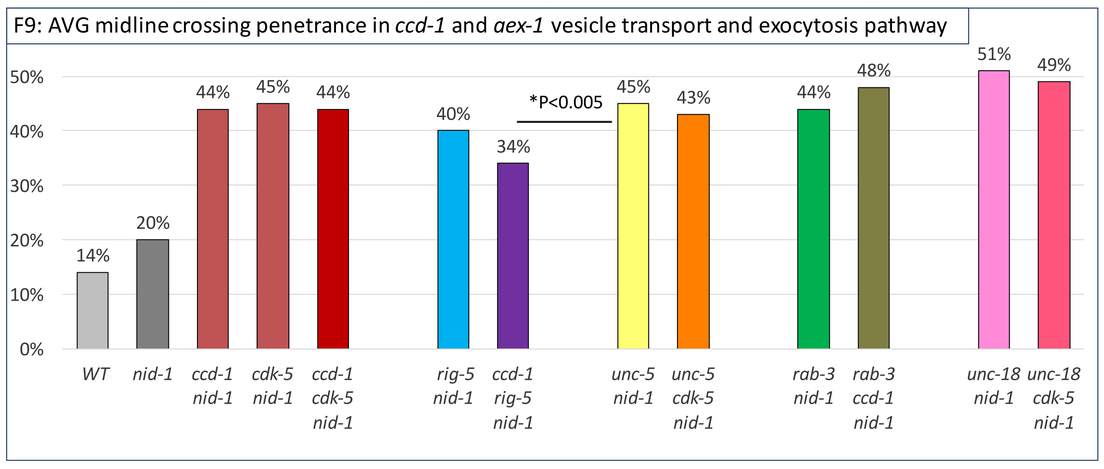


F10: Proposed *ccd-1* and *aex-3* gene interaction pathway

***rig-5* suppresses *ccd-1* defects.**

- Results suggest that *ccd-1* regulates *rig-5* mediated AVG axon guidance.

F9: AVG midline crossing penetrance in *ccd-1* and *aex-1* vesicle transport and exocytosis pathway



Questions?

Contact me at:
afereste@sfu.ca
https://www.sfu.ca/biology/faculty/hutter/lab

Special thanks to the Moerman lab at UBC for their generous sequencing support, the Hutter lab, my committee members, and everyone who let me rehearse presentations at them. You are the reason I show up.

Works Cited:

1. Hutter, H., (2003). **Development,** 130, 5307-5318
2. Bhat, J. M., & Hutter, H. (2016). **Genetics**, 115.
3. Xu, S., … & Chen, J. (2014). **Mol. & Cel. Proteomics**, 13(11), 2986-3000
4. McDiarmid, T. A., … & Rankin, C. H. (2018). **Genes, Brain and Behavior**, 17(3), e12437.

CIHR IRSC

NSERC CRSNG

SFU