

Using *C. elegans* as a Model to Understand How *sax-7* Effects Canal-Associated Neurons

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Background

L1CAMs are transmembrane glycoproteins of the immunoglobulin (Ig) superfamily conserved from *C. elegans* to humans

- composed of six Ig-like domains followed by five fibronectin type III repeats, the transmembrane region, and a conserved cytoplasmic tail (Fig1)
- mediates cell-cell and cell-extracellular matrix adhesion
- functions in neuronal development, including neuronal migration and axon guidance
- Encoded by the L1 gene in humans and *sax-7* gene in *C. elegans*.
- Mutations to the L1 gene can result in L1 syndrome, which encompasses neurological disorders including hydrocephalus, a life threatening disease characterized by cerebrospinal fluid buildup within the ventricles of the brain). Currently it is a mystery how impaired L1 function causes hydrocephalus. However, interestingly, siblings with L1 syndrome carrying the identical mutant allele can differ in whether or not they have hydrocephalus (Jouet et al. 1995; Schrander-Stumpel et al. 1995; Fransen et al. 1998). Thus, we hypothesize that the L1 gene acts synergistically with other genes for enhancement of disease expression

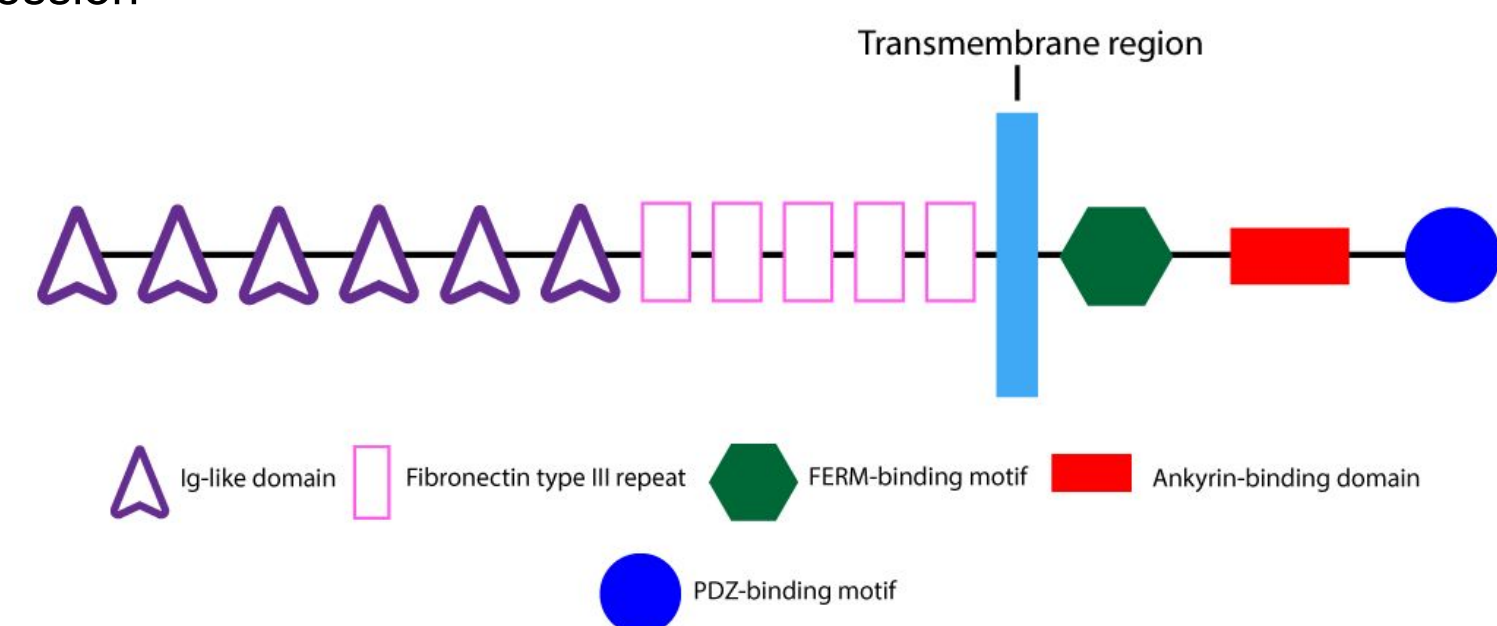


Figure 1: Cartoon of L1 Protein. L1 protein is composed (from left to right) of six Ig-like domains followed by five fibronectin type III repeats, the transmembrane region, and a cytoplasmic tail, which includes the FERM-binding motif, ankyrin-binding domain, and the PDZ-binding motif (adapted from Norris et al., 2015).

***Caenorhabditis elegans* can be a good model organism to study L1 function and mechanisms of action**

Preliminary studies in the Chen lab uncovered *Ras* as a gene that interacts with L1 in *C. elegans*. RAS encodes a GTPase that activates MAPK/ERK signaling pathway (Fig 2). In this identified gene interaction, *Ras(gf)* in a *sax-7(0)* mutant results in synthetic lethality caused by fluid buildup in the animal. Interestingly, *Ras(gf)* in humans is known to cause a set of congenital diseases collectively termed Rasopathies which most interestingly includes variable hydrocephalus (Rauren 2013; Kang 2019). Additionally, it has been found that SAX-7 plays a role within fluid regulation that is also dependent on SAX-7 function within neurons. Conditional knockout (KO) of *sax-7* in the nervous system also results in progressive fluid accumulation in *Ras(gf)* animals. This conditional knockout suggests that SAX-7 functions in the neurons in a fluid regulation capacity.

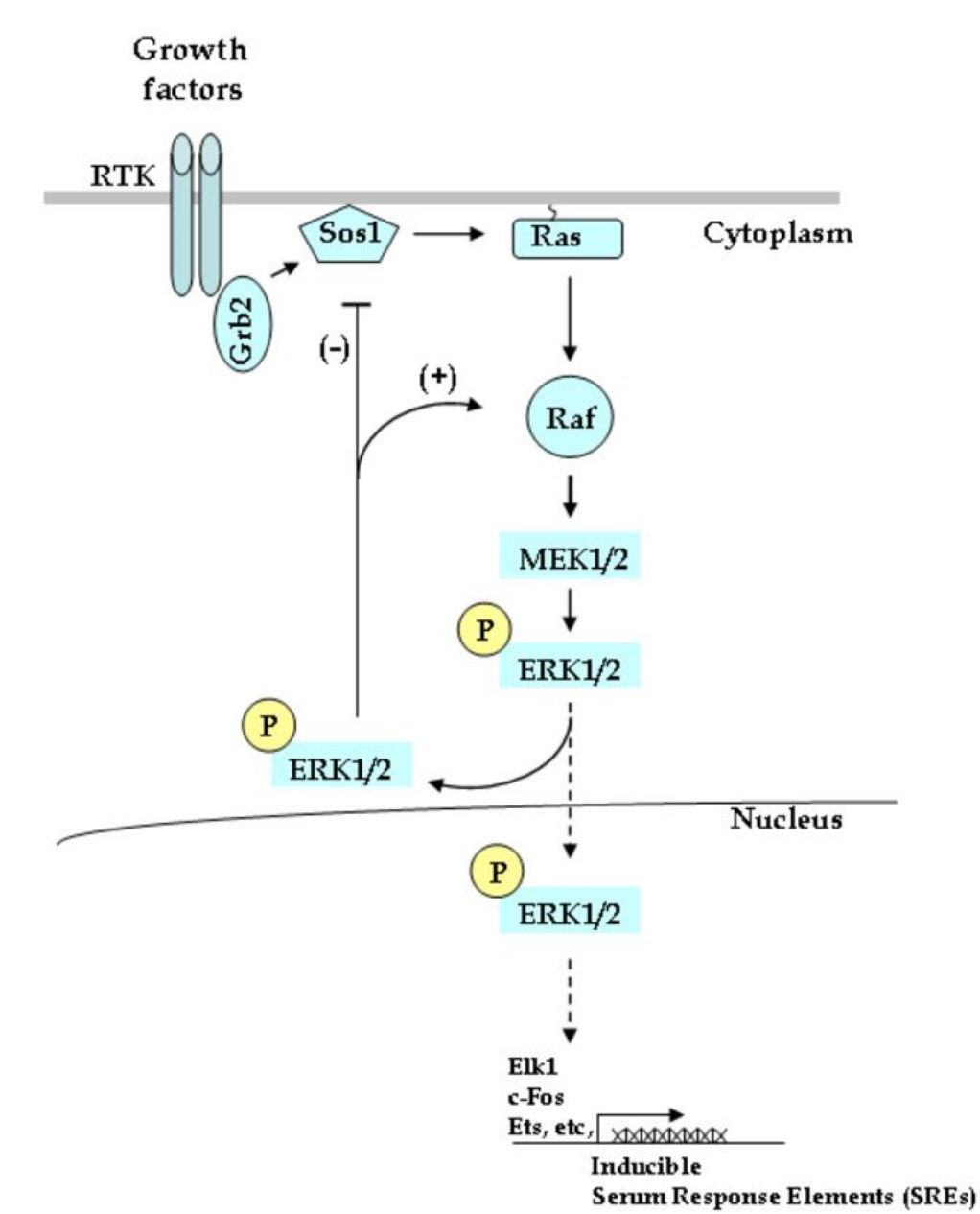


Figure 2: Simple Cartoon Depiction of MAPK/ERK Pathway. Ras tyrosine Kinase or (RTK) is activated after the binding of growth factors. This then leads to autophosphorylation of the RTK and activation of the Ras MAPK/ERK cascade. This ultimately leads to regulation of cell growth and differentiation (Taken from Wigerius et al. 2010).

Excretory System and Canal Associated Neurons

The function of the excretory system in the worm is analogous to the kidney in humans. Both are important for fluid regulation in that they excrete soluble waste, excess salts, and fluid to help maintain body fluid homeostasis. The excretory system of the animal is composed of epithelial cells and associated neurons. Similarly, in humans, this is analogous to the choroid plexus (a plexus of cells lining the ventricles of the brain) which is composed of neuroepithelium and is responsible for regulation of cerebrospinal fluid production and absorption.

Within the excretory system are two neurons known as the canal associated neurons (CANs). The CANs are a pair of neurons that are documented to function within fluid regulation. Killing the CANs results in fluid accumulation within the pseudocoelom of the animal. Since the conditional KO of *sax-7* within the nervous system results in progressive fluid accumulation in *Ras(gf)* animals, it can be hypothesized that *sax-7(0)* and *Ras(gf)* affect specifically the CAN neurons in some way which would cause fluid dysregulation.

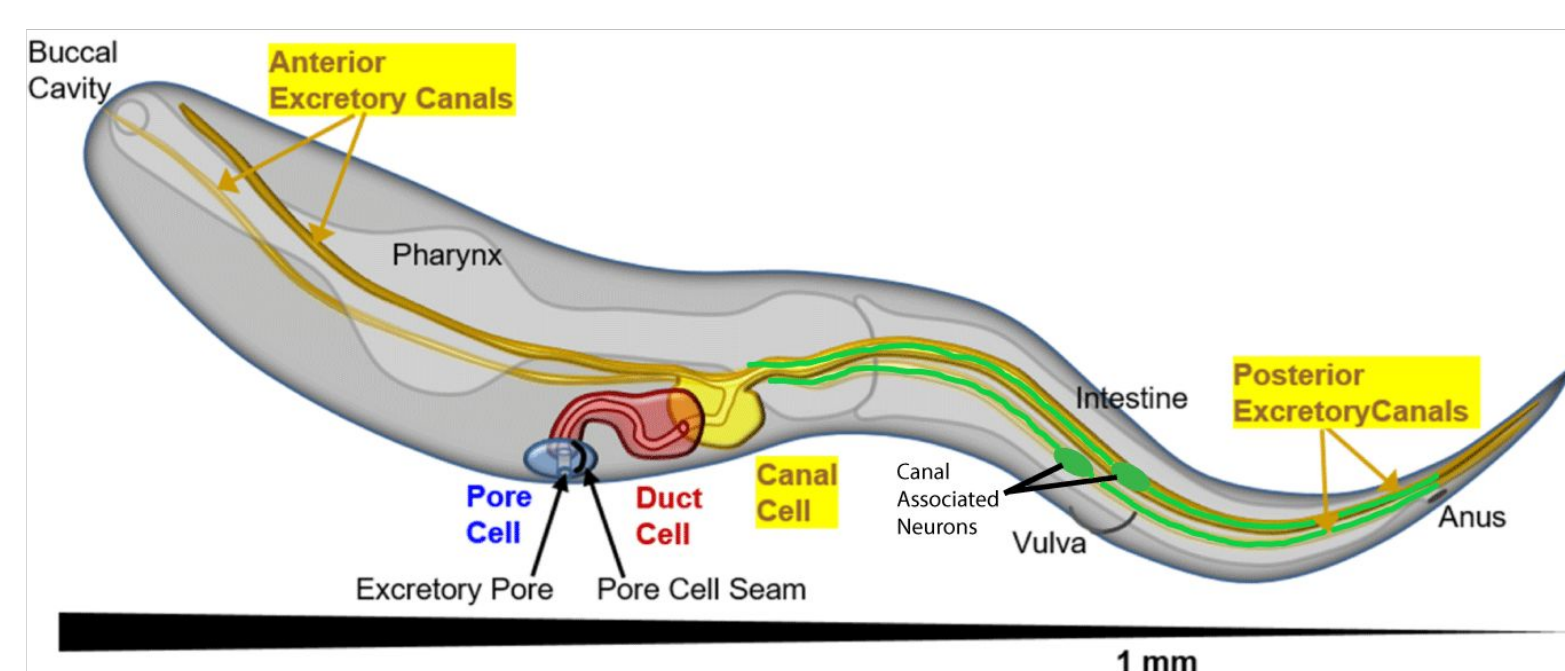


Figure 3: Cartoon Depicting Excretory System of *C. Elegans*. This diagram showcasing the different cells within the excretory system of *C. elegans*. Green lines along the CANs represent the posterior and anterior axon processes (adapted from Buechner et al., 2020).

Hypothesis: We hypothesize that the CAN neurons are defective in *sax-7(0) Ras(gf)* mutant animals to cause fluid dysregulation.

Materials and Methods

To test this hypothesis...

- *sax-7(0) Ras(gf)* animals were examined for structural abnormalities within the CANs and its axonal processes. To examine the CAN neurons, *kyIs4* a transgene that drives green fluorescent protein (GFP) behind the *ceh-23* promoter in a handful of neurons, including the CANs was used (the reporter allows for visualization of the CAN soma and axonal processes).
- As a control, WT animals were examined (WT animals were also crossed with the *kyIs4* transgene).

Procedure:

Microscopy: L4-staged larvae were picked onto an agar pad made on a microscope slide. A drop of 10 mM levamisole (in M9 buffer) was added to the animals to anesthetize them (Meneely, P. M., Dahlberg, C. L., & Rose, J. K., 2019). The Axioplan 2 Imaging scope was used along with the AxioCam Mrm to image the animals. The position of the cell body/soma of the CAN neuron and the lengths of its axon process were examined using fluorescence microscopy. The position of the vulva and the anus were examined using DIC microscopy.

Image Analysis:

Images of the CANs and the axons taken using fluorescence microscopy were superimposed onto DIC images of the vulva and anus respectively (with the vulva and anus acting as reference points for the position of the CAN and distance from the end of the the posterior axon respectively).

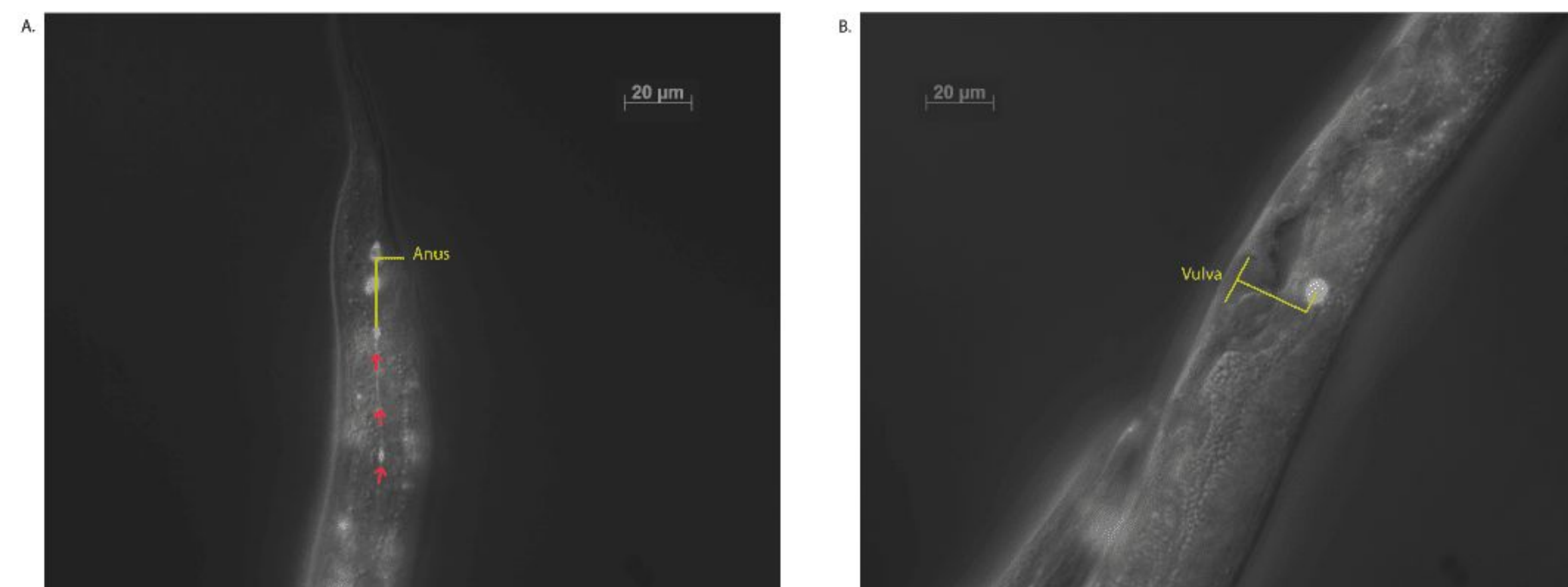


Figure 4: Diagram of Measurement Techniques Used. Demonstration of the measurement technique used to characterize the location of the CAN cell body and axon process length. (A) represents the method used for measuring the distance from the end of the posterior axon to the anus. Red arrows represent the path of the axon process. (B) represents the method used for measuring the location of the CAN cell body relative to the vulva.

Results

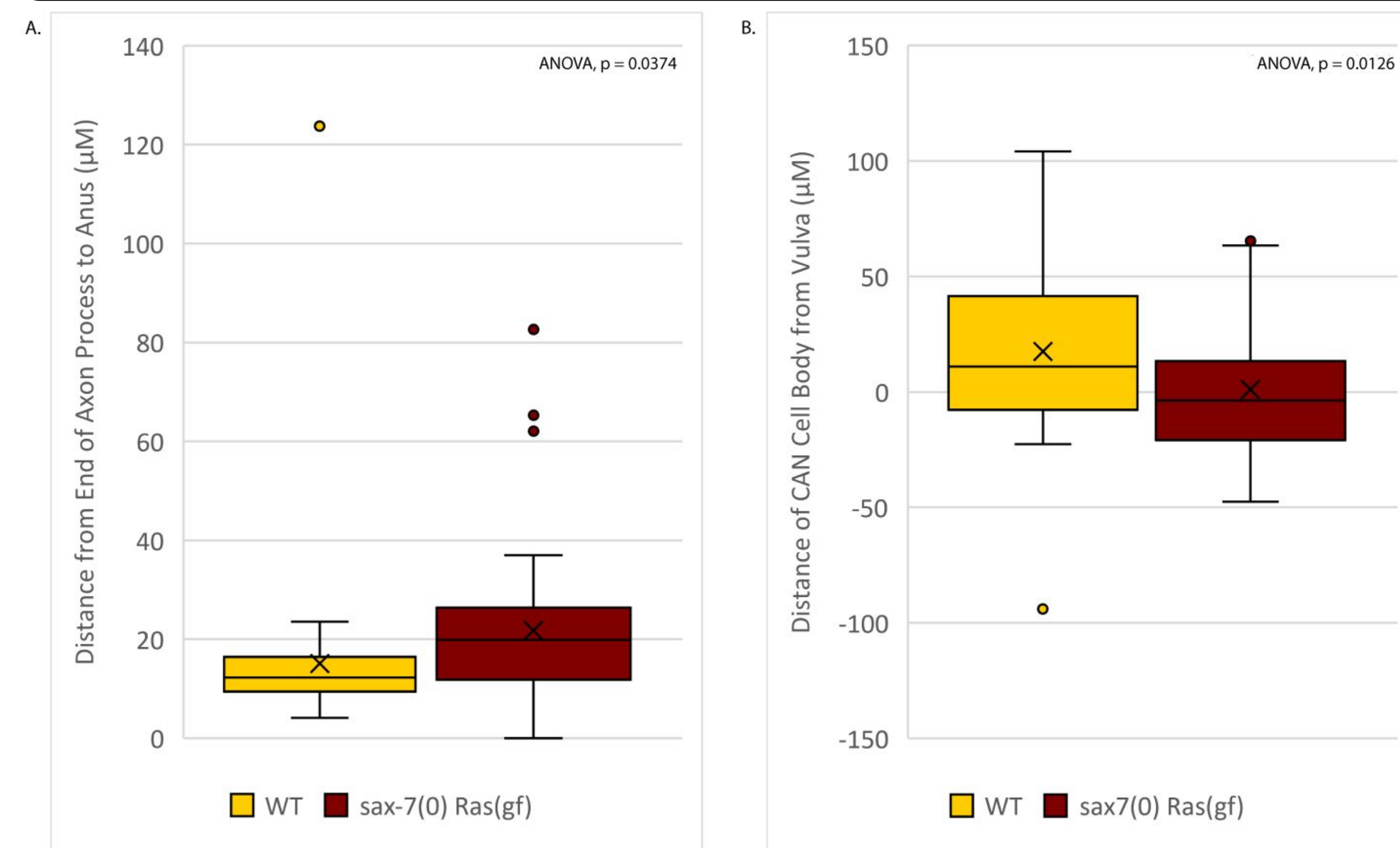


Figure 5: *sax-7(0) Ras(gf)* leads to truncation of CAN axon process and a shift in CAN cell body positioning. (A) *sax-7(0) Ras(gf)* double mutants showed a significantly greater distance from the end of the posterior axon process to the anus compared to WT animals. (B) *sax-7(0) Ras(gf)* double mutants showed a significantly greater distance from the vulva in the posterior direction compared to WT animals (negative values represent distances in the posterior direction while positive values represent distances in the anterior direction). The upward error bars on each graph represent the difference from the maximum to the third quartile while the downward error bars on each graph represent the differences from the first quartile to the minimum. The X represents the average of the data while the middle bar in each box represents the median.

Discussion and Conclusions

***sax-7(0) Ras(gf)* animals exhibit an abnormal positioning of CAN axon cell body**

Our results reveal that are morphological abnormalities in the CANs of *sax-7(0) Ras(gf)* animals. These defects may underlie the fluid buildup observed in *sax-7(0) Ras(gf)* animals. To test this possibility, we will examine for similar CAN defects in each single mutant, which do not exhibit fluid buildup. If each single mutant does not display the CAN defects, it suggests that the combination of both mutations leads to observed CAN defects, which contributes to the fluid buildup in *sax-7(0) Ras(gf)* animals. On the other hand, if at least one single mutants also shows similar CAN defects, then the fluid buildup observed in *sax-7(0) Ras(gf)* animals may be caused by another as-yet-undefined defect.

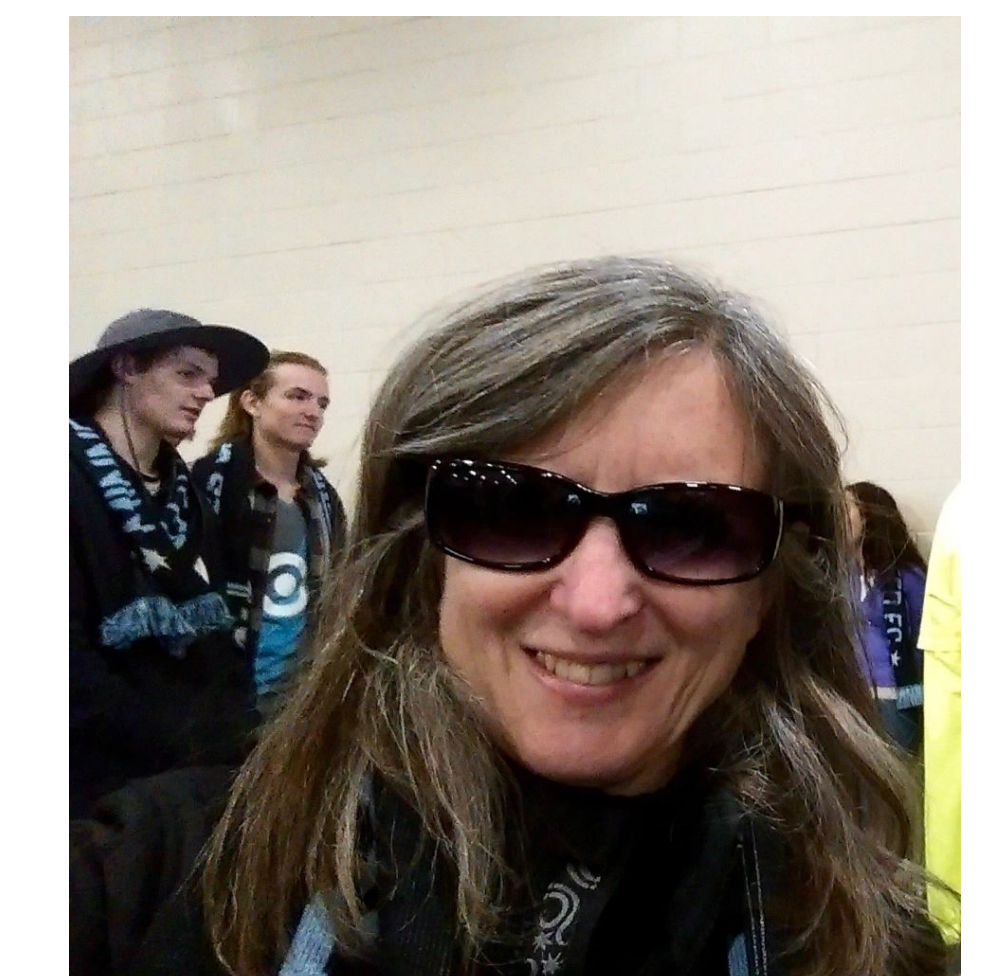
Future Directions

In this study we only looked at WT and *sax-7(0) Ras(gf)* animals (crossed with the *kyIs4* transgene). As stated in the discussion we would additionally like to explore how *sax-7(0)* and *Ras(gf)* single mutants affect the CANs. It is possible that single mutants do not display the same positional defects as double mutants and through this analysis we can assess the strength and extent of the synergistic effect between *sax-7* and *Ras*. To begin, the following strains would be needed:

- *Ras(gf)* animals (crossed with the *kyIs4* transgene)
- *sax-7(0)* animals (crossed with the *kyIs4* transgene)

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