Apr 22nd, 3:00 PM - 4:00 PM

Ion Channel Screen Reveals a Role for SERCA in Brain Tumor Growth

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Ion Channel Screen Reveals a Role for *SERCA* in Brain Tumor Growth

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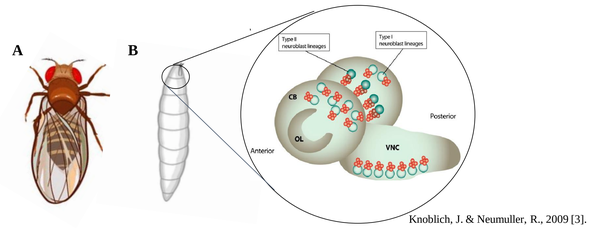
1) Introduction

Ion channels, playing a wide variety of roles within cells including excitability, maintaining gradients, and volume control, are essential for neural function. Recently, it has emerged that neural precursors may be affected by channelopathies indicating that ion channels may play critical roles in neural development and pathology [1]. Prior work in the Piggott lab used the model system *Drosophila melanogaster* to test the effect of ion channel mis-expression on larval brains, finding evidence that changes to key ion channels affect cell proliferation-- a subset of development-- within the organism [2]. We are currently examining the effect of several channel types in a highly proliferating *D. melanogaster* model and screening for changes in both larval brain volume and number of cells expressing proliferation markers that would indicate increased or decreased proliferation.

Ion Channel Types:	
Exchangers	Calcium
TRP	Gap Junction

Figure 1. Ion channel types used in these experiments.

Experiments in this project are testing the decreased expression of various ion channels to determine whether these channels affect proliferation and thus development of the organism.

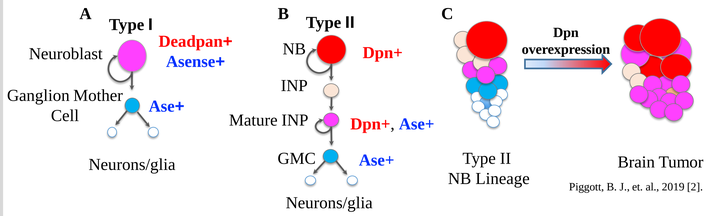


Knoblich, J. & Neumuller, R., 2009 [3].

Figure 2. ***Drosophila Melanogaster* Schematic. (A)** Adult *Drosophila*. **(B)** Third Instar Larvae. Inset shows a diagram of the larval brain, including the brain lobes and the ventral nerve cord (VNC).

2) Generation of a Brain Tumor Model

For our experiments, we used a tumor model caused by the overexpression of *deadpan* (*dpn*)-- a gene that maintains self-renewal-- in all neural stem cells called neuroblasts (NB) in the fly. This overexpression (OE) leads to ectopic Dpn proteins that cause normally differentiating cells to gain "stem cell characteristics" like self-renewal; thus, causing uncontrolled proliferation at the expense of neural development, a process that results in tumor formation.



Piggott, B. J., et. al., 2019 [2].

Figure 3. Neuroblast differentiation. **(A)** Type I NB lineage. **(B)** Type II NB lineage. **(C)** Overexpression of Dpn protein causes cells to abnormally self-renew and proliferate uncontrollably.

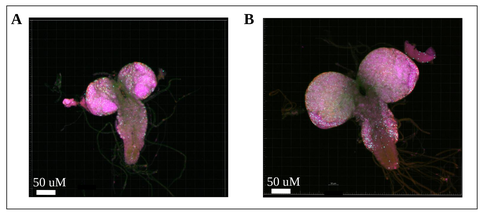


Figure 4. Day 7 larval brains, stained with GFP-488, Dpn-568, pH3-640, and DAPI, showing visible volume difference between (**A**) the non-tumor control brain and (**B**) the *dpn*-tumor control brain.

3) Experimental Setup

RNAi knockdown:

The under-expression of ion channels in the larval brain is accomplished utilizing a RNAi knockdown driven by the GAL4/UAS system, a *Drosophila* system used to direct expression of genes in specific tissues. This results in the binding of the siRNA to the mRNA, signaling for the destruction of the mRNA.

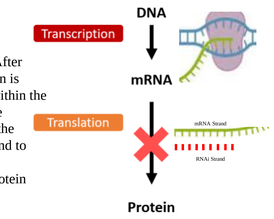


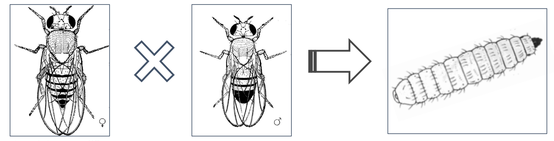
Figure 5. After transcription is complete within the nucleus, the binding of the siRNA strand to the mRNA prevents protein synthesis.

Experimental Model:

Drosophila Dpn^{OE} stocks were crossed to the stock containing the desired knockdown channel, the produced progeny then had highly proliferating neuroblasts as well as a reduction of the specified ion channel. Brains were then imaged using confocal microscopy, the volumes were measured using Imaris and were compared to controls.

Cross genotypes:
w-,UAS-mCD8GFP; insc-GAL4/cyo,tub-GAL80; UAS-Dpn,UAS-Dcr2/Tm6B,tb X *y¹v¹;; UAS-RNAi*

Figure 6. Genotypes of the Dpn-tumor stock (left) and the general RNAi knockdown stock (right).



Female of the Dpn^{OE} stock. Male of the RNAI stock. Resulting progeny of the cross.

Figure 7. Females of the Dpn^{OE} genotype are crossed with males of the RNAi knockdown genotypes (in a 2:1 ratio), producing progeny with tumors and decreased ion channel function for dissection. Memorial University, n.d. [4].

4) RNAi Knockdown of Several Ion Channels Reduces Brain Volume

Volume Data From:

- Control:
  Dpn^{OE} tumor X Luciferase RNAi
- Experiments:
  Dpn^{OE} tumor X Ion Channel RNAi
  - Day 6:
    - *Ir76A*
    - *Wtrw*
    - *SERCA*
    - *TrpA1*
  - Day 7:
    - *Inx3*
  - Day 6/7:
    - *Itp-r83A*

Ca²⁺ ATPase gene SERCA displays a consistent statistically significant reduction in (A) whole brain, (B) brain lobe, and (C) VNC volumes.

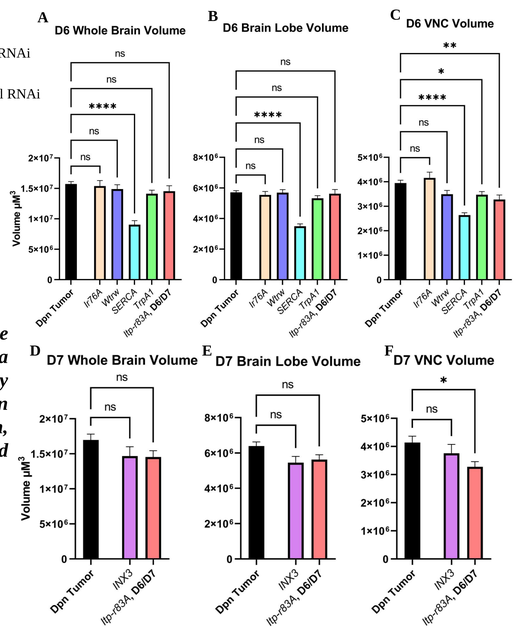


Figure 8. (A-C) Day 6 brain volume comparisons between the control and the RNAi experiment larvae. **(D-F)** Day 7 brain volume comparisons between the control and the RNAi experiment larvae. One-way ANOVA tests with Dunnett Multiple Comparisons tests were conducted in **(A-F)** to determine statistical significance. Error bars denote S.E.M. All groups have n≥13. * equals p≤0.05, ** equals p≤0.01, *** equals p≤0.001, **** equals p≤0.0001.

5) Tumor Reduction in *SERCA* Knockdown

What is *SERCA*?

The *SERCA* (*Sarco/endoplasmic reticulum Ca²⁺ATPase)* gene encodes an endoplasmic reticulum (ER) calcium pump with roles in ER calcium homeostasis and lipid storage. These ATPases drive transmembrane transport of Ca^{2+} from the cytoplasm back into organelle lumens.

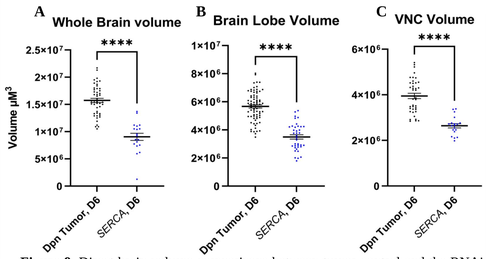


Figure 9. Direct brain volume comparisons between tumor control and the RNAi SERCA knockdown tumor, analyzed with parametric t-tests. A statistically significant reduction is displayed in the **(A)** whole brain volume, **(B)** brain lobe volume, and **(C)** VNC volume comparisons. Error bars denote S.E.M. Control n≥49, SERCA n≥19. **** equals p≤0.0001.

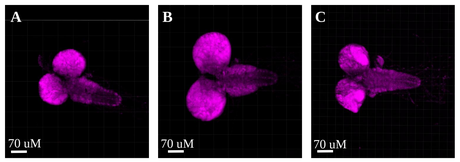


Figure 10. Representative images of DAPI stained larval brain of **(A)** *SERCA* RNAi knockdown, **(B)** Dpn^{OE} Tumor Control, **(C)** Non-Tumor Control. *SERCA* RNAi knockdown suppresses brain tumor size to wild-type levels.

Hypothesis:

If the SERCA gene is knocked down in fly tumor brains, then there will be an increase in cytosolic calcium ion concentrations. This will also cause ER stress, leading to cellular apoptosis (cell death) and a decrease in overall brain volume. The current hypothesis is supported in literature and preliminary analysis of SERCA knockdown cells showing a decrease in cells with mitotic marker phosphohistone-3 (pH3), thus indicating reduced proliferation.

6) Conclusions, Future Directions and Acknowledgements

Conclusions:

- Our results show that a reduction of the *SERCA* ion channel gene causes decreased brain volume in tumor model larval brains.
- There is also relevant volume decrease shown in the VNC following a reduction of the gene *Itp-r83A.*

Future Directions:

- We are currently working with the *SERCA* gene in further experiments, quantifying Deadpan positive, proliferating, and apoptotic cells to better understand the role of *SERCA* in development.
- Functional calcium live-cell imaging is also underway.
- Other ion channels are being studied for their role in development, including other calcium pumps and sodium exchangers.

References
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Acknowledgements:
BJP startup funding was provided by CBSD and the UM Vice President of Research Funds & BJP and HMC by a SURF grant.