Transient receptor potential ankyrin 1: exploring thermal activation of protein overexpression in *Drosophila melanogaster* behavior and physiology

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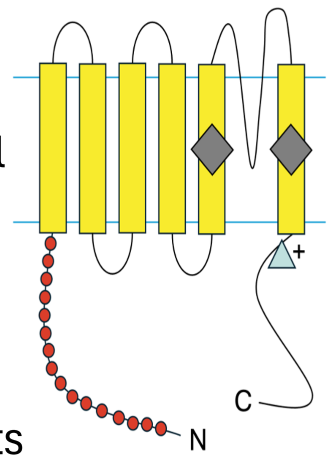
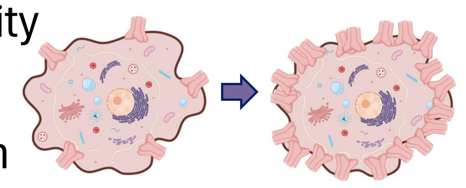
INTRODUCTION

Overview of TRPA1



- Transient receptor potential ankyrin 1 (TRPA1): a sensory nonselective ion channel mediated by dendritic Ca^{2+} influx
- TRPA1 is involved in important physiological roles such as thermosensation, chemosensation, and nociception
- TRPA1 is a promising therapeutic target for its potential ability to block pain sensitivity

Significance of TRPA1 overexpression



- In general, protein overexpression can lead to cellular malfunction, harming the organism
- TRPA1 overexpression is known to contribute to tumor growth in some cancers, while improve prognosis in others

Research Question

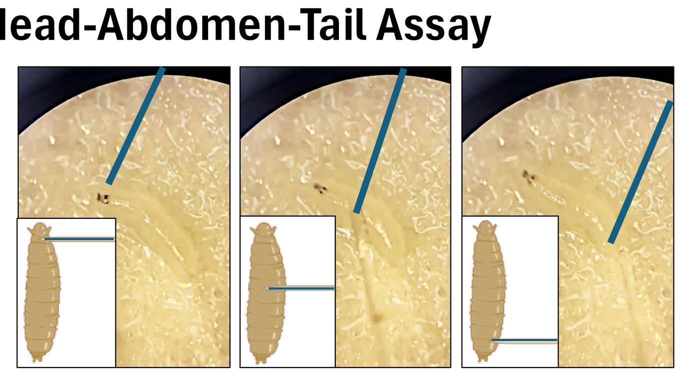
- When thermally activated, how does TRPA1 overexpression in sensory neurons, glial cells, and muscle tissues modulate (a) mechanotransduction, (b) locomotion, (c) thermotaxis, and (d) membrane potential in *D. melanogaster* larvae?

METHODOLOGY

GAL4/UAS System

1. Raise fly lines: PPK, CHA, GLIA, M6M7, and M12
2. Cross fly lines with UAS-TRPA1

(a) Mechanotransduction: Head-Abdomen-Tail Assay



1. Tap larvae on head, abdomen, and tail
2. Record responses according to an ethogram

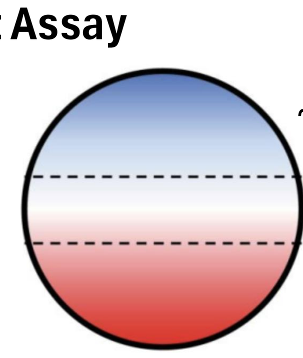
(Titlow et al., 2014)

(b) Locomotion: Body Wall & Mouth Hook Assays

1. Count body walls on Petri dish with apple juice as larva inchworms
2. Count mouth hooks in yeast solution

(Mattingly et al., 2018)

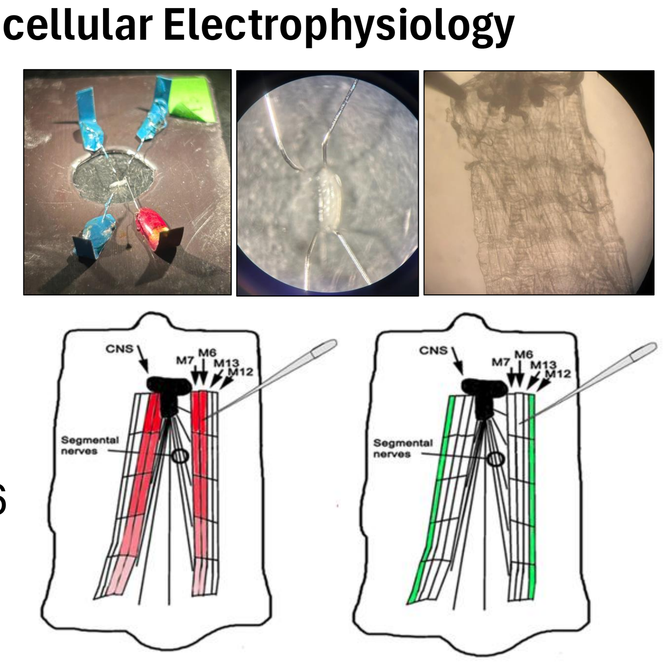
(c) Thermotaxis: Thermal Gradient Assay



1. Place larvae in the middle of gradient Petri dish
2. Record using a regular camera and with FLIR

(Bellemer, 2015)

(d) Membrane Potential: Intracellular Electrophysiology



1. Dissect larvae ventral side up, pinned at four corners in bathing saline
2. Measure resting membrane potential (RP) in M6 and M12 of M6M7>TRPA1 larvae
3. Measure RP in M12 and M6 of M12>TRPA1 larvae

(Elliott & Cooper, 2024)

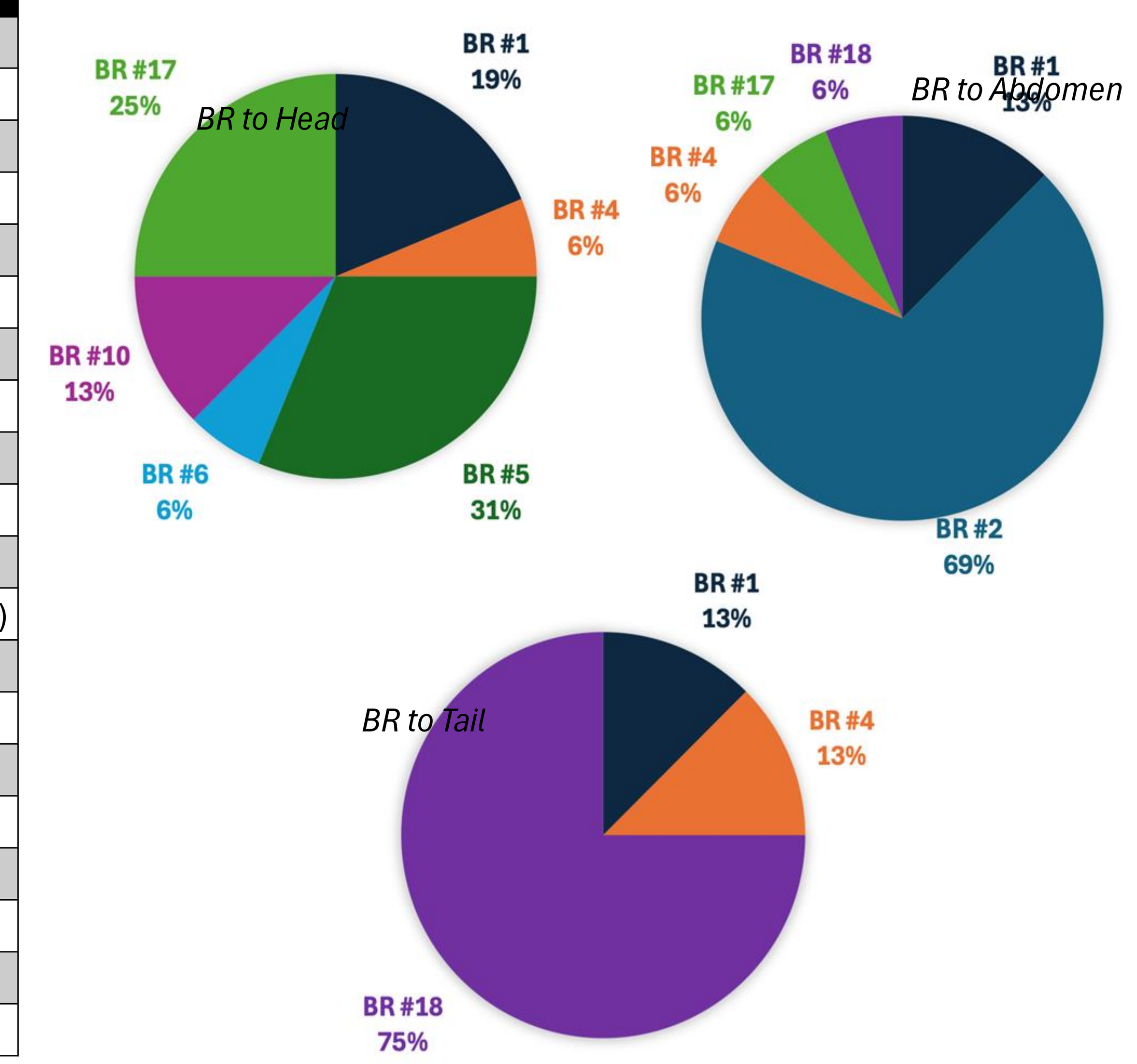
RESULTS

(a) Mechanotransduction: Head-Abdomen-Tail Assay

Summary Table of Behavioral Responses (% Frequency)

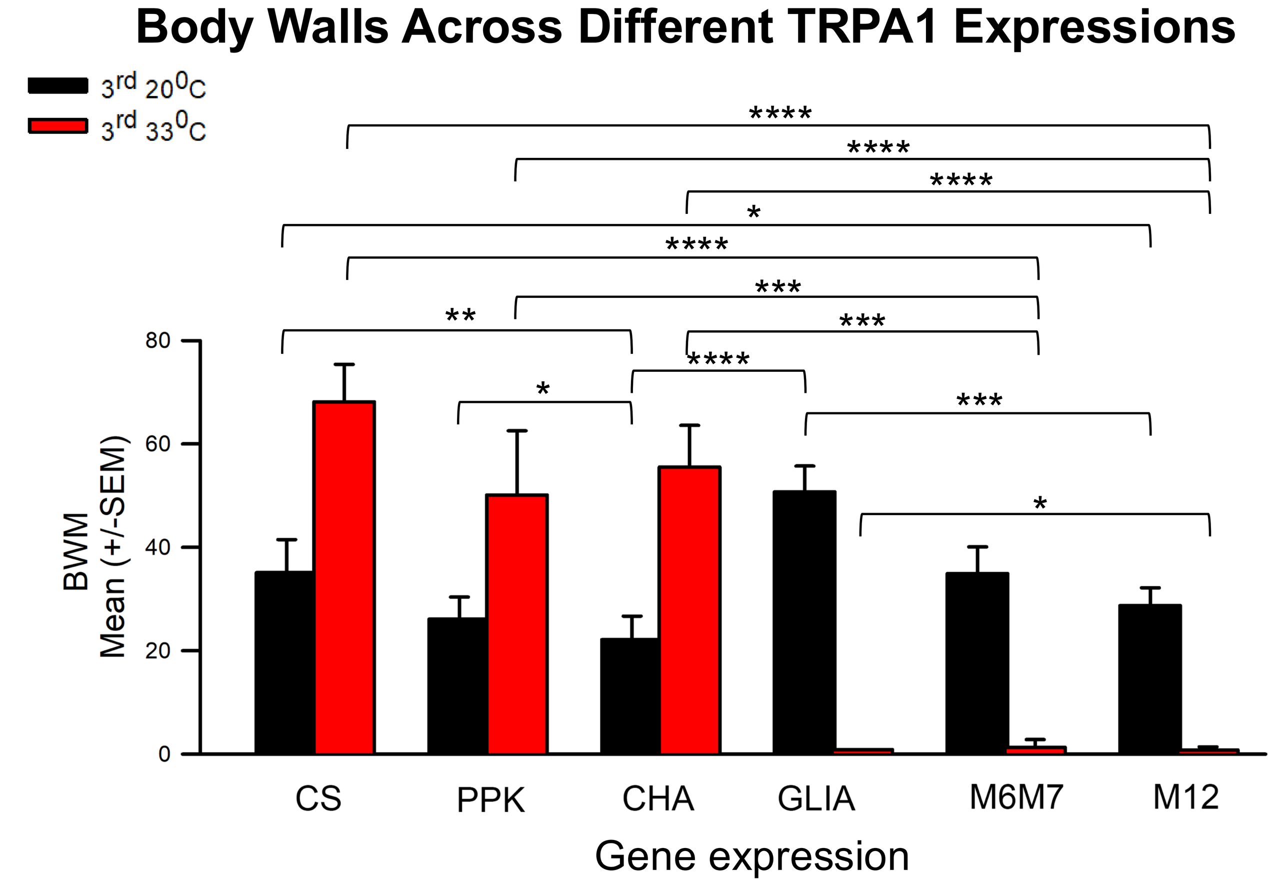
Genotype	Stage	Temp	BR to Head (%)	BR to Abd. (%)	BR to Tail (%)
Canton S	2nd	20°C	BR #10 (20%)	BR #2 (50%)	BR #2 (40%)
		33°C	BR #5 (50%)	BR #2 (50%)	BR #18 (50%)
	3rd	20°C	BR #5 (40%)	BR #2 (60%)	BR #18 (70%)
		33°C	BR #10 (20%)	BR #2 (50%)	BR #18 (60%)
PPK>TRPA1	2nd	20°C	BR #5 (70%)	BR #2 (90%)	BR #2 (60%)
		33°C	BR #6 (30%)	BR #17 (60%)	BR #2 (70%)
	3rd	20°C	BR #17 (40%)	BR #2 (40%)	BR #18 (60%)
		33°C	BR #6 (40%)	BR #2 (45%)	BR #18 (50%)
CHA>TRPA1	2nd	20°C	BR #5 (30%)	BR #2 (70%)	BR #18 (80%)
		33°C	BR #17 (50%)	BR #2 (40%)	BR #18 (50%)
	3rd	20°C	BR #17 (60%)	BR #2 (70%)	BR #2 (70%)
		33°C	BR #5 (50%)	BR #18 (50%)	BR #18 (100%)
GLIA>TRPA1	2nd	20°C	BR #5 (50%)	BR #2 (70%)	BR #18 (60%)
		33°C	BR #1 (30%)	BR #2 (50%)	BR #1 (30%)
	3rd	20°C	BR #17 (50%)	BR #2 (70%)	BR #18 (70%)
		33°C	BR #1 (40%)	BR #1 (40%)	BR #1 (40%)
M6M7>TRPA1	3rd	20°C	BR #17 (30%)	BR #17 (50%)	BR #18 (50%)
		33°C	BR #1 (60%)	BR #1 (60%)	BR #1 (40%)
M12>TRPA1	3rd	20°C	BR #5 (25%)	BR #2 (30%)	BR #2 (80%)
		33°C	BR #4 (50%)	BR #4 (40%)	BR #4 (40%)

Frequency of Behavioral Responses to Stimuli

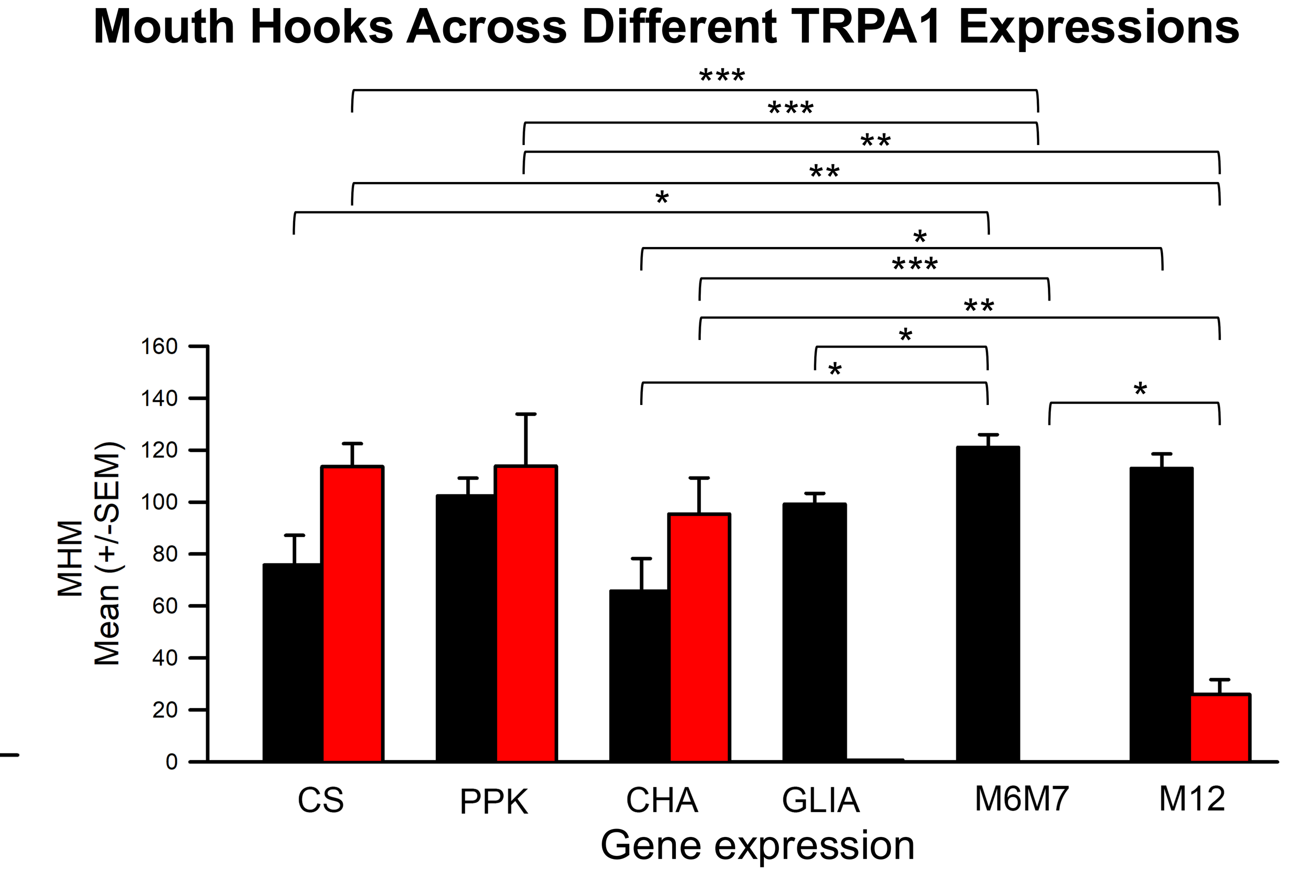


(b) Locomotion: Body Wall & Mouth Hook Assays

Body Walls Across Different TRPA1 Expressions

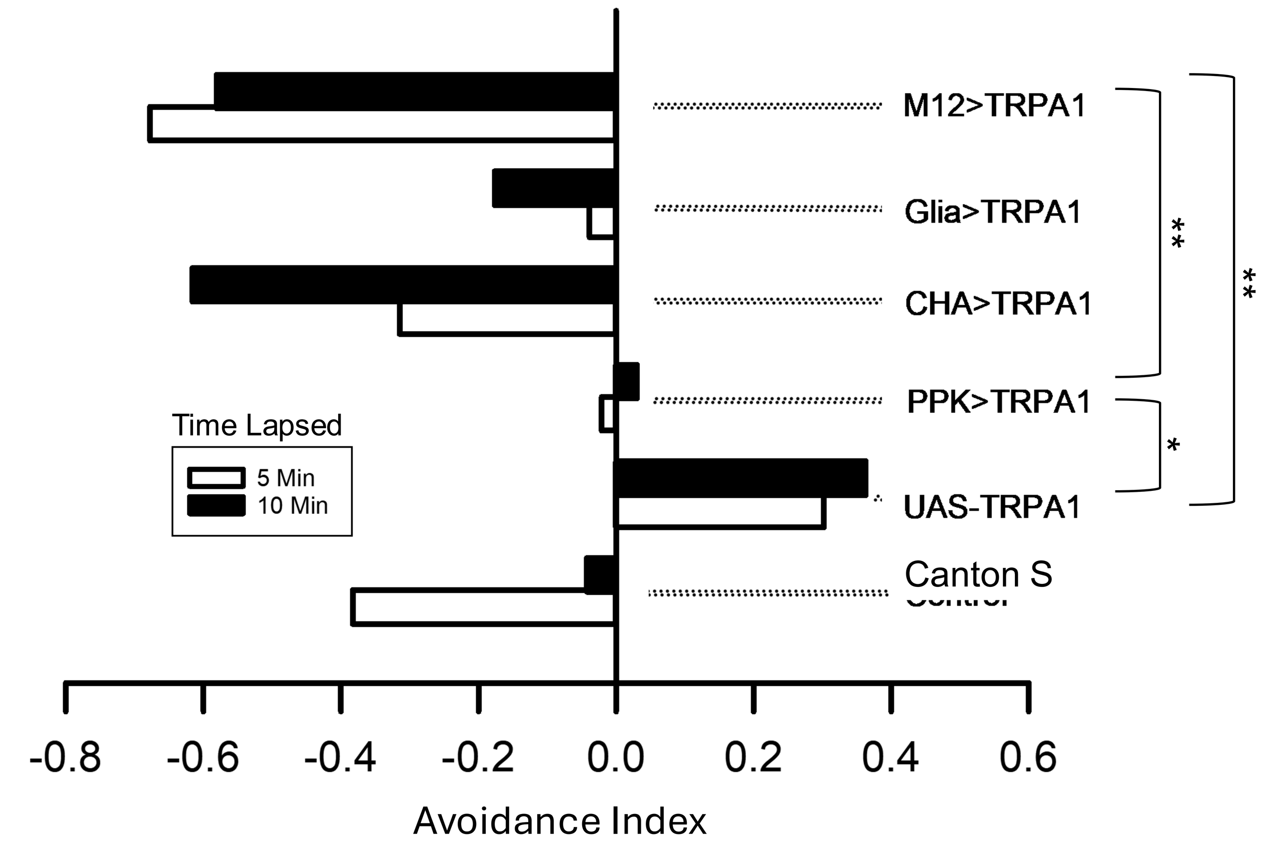


Mouth Hooks Across Different TRPA1 Expressions



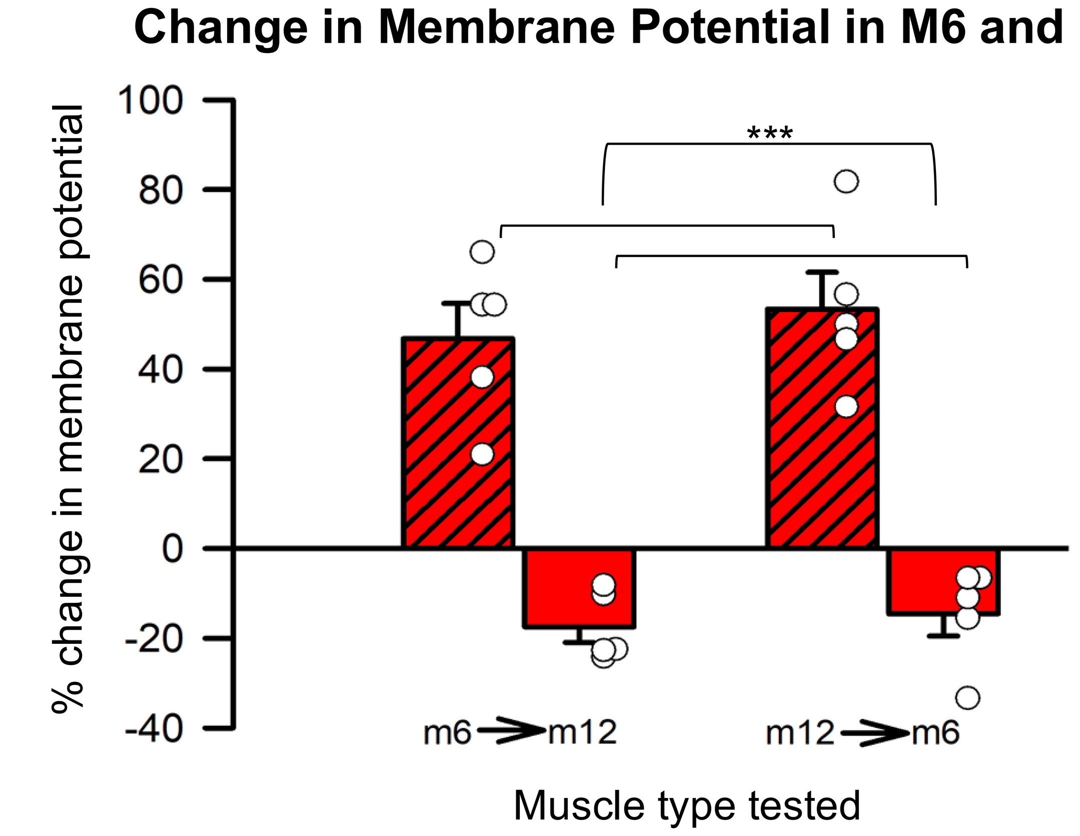
(c) Thermotaxis: Thermal Gradient Assay

Avoidance Indices Across TRPA1 Expressions



(d) Membrane Potential: Intra. Electrophysiology

Change in Membrane Potential in M6 and M12



*$p<0.05$
**$p<0.01$
***$p<0.001$
****$p<0.0001$

DISCUSSION

Concluding Ideas

- Altered expressions of TRPA1 thermal receptors in sensory neurons, glial cells, and muscle tissues cause significant differences in the behavior and physiology of *Drosophila melanogaster* larvae.
- The effects of TRPA1 overexpression depended on whether TRPA1 was thermally activated and where TRPA1 was overexpressed (i.e., the specific cell or muscle type)
  - Spike-timing dependent plasticity at the neural circuit level (i.e., the behavioral responses to the stimuli depended on the activation of the motor nerve) may have accounted for the variety in responses to stimuli
  - TRPA1 overexpression may have heightened sensory responsiveness in PPK>TRPA1 and CHA>TRPA1 but induced muscle contraction in GLIA>TRPA1, M6M7>TRPA1, and M12>TRPA1
  - TRPA1 overexpression likely caused M12>TRPA1 and PPK>TRPA1 larvae to prefer or become unable to detect and respond to nociceptive temperatures
  - TRPA1 induced significant depolarization in membrane potential, like the results from Raisinghai et al. (2011), likely due to significant Ca^{2+} influx

Limitations & Future Research

- TRPA1 channel studied as a whole → Separate studies with isoforms (types) of dTRPA1
- Four behavioral and physiological responses investigated → Additional responses to further the overall understanding of the effects of TRPA1 overexpression
- One species, *D. melanogaster* → Other species, such as *Lucilia sericata* to compare inter-species responses
- Behavioral and physiological responses quantification → Quantification of cellular mechanisms, such as through Goldman-Hodgkin-Katz simulations
- Glial optogenetics project idea

Implications

- Potential applications to human TRPA1
- Insights into cancer pathophysiology
  - Night sweats/hot flashes
  - Fevers
  - Pain therapies
- Insights into mechanisms of nociception

REFERENCES

- Images are created using Biorender.com
- Bellemer A. (2015). Thermotaxis, circadian rhythms, and TRP channels in *Drosophila*. *Temperature, 2*(2), 227–243. https://doi.org/10.1080/23328940.2015.1004972
- Dillon et al. (2009). Review: Thermal preference in *Drosophila*. *Journal of Thermal Biology, 34*(3), 109–119. https://doi.org/10.1016/j.jtherbio.2008.11.007
- ElGindi et al. (2021) May the Force Be with You (Or Not): The Immune System under Microgravity. *Cells, 10*(8), 1941. https://doi.org/10.3390/cells10081941
- Elliott, E. R., & Cooper, R. L. (2024). The effect of calcium ions on resting membrane potential. *Biology, 13*(9), 750. https://doi.org/10.3390/biology13090750
- Mattingly et al. (2018). Hyperpolarization by activation of halorhodopsin results in enhanced synaptic transmission: Neuromuscular junction and CNS circuit. *PIOS One, 13*(7), e0200107. https://doi.org/10.1371/journal.pone.0200107
- Raisinghani et al. (2011). Activation characteristics of transient receptor potential ankyrin 1 and its role in nociception. *American Journal of Physiology. Cell Physiology, 301*(3), C587–C600. https://doi.org/10.1152/ajpcell.00465.201
- Titlow et al. (2014). Anatomical and genotype-specific mechanosensory responses in *Drosophila melanogaster* larvae. *Neuroscience Research, 83*, 54–63. https://doi.org/10.1016/j.neures.2014.04.003