Discovering Potential Novel Anthelmintics using C. elegans

Rida Naqvi, John Regan, & Bruce Bamber

Department of Biological Sciences, University of Toledo, Ohio

Introduction

Anthelmintic drugs inhibit pharyngeal pumping and typically attack several neuronal pathways in the pharynx, among other organs. Pharyngeal locomotion is controlled by neuronal pathways both known and unknown. C. elegans are a particularly ideal test subject for these drugs due to their simple nervous system, short lifespan, and ease to grow. Breaking down the anthelmintic PAPP into smaller compounds allows a controlled method of testing to identify affected pathways. The use of mutants may allow for the identification of novel neuronal pathways for further testing. One component of PAPP, 3009, decreased pharyngeal pumping in wild-type C. elegans but was ineffective in mutant strains lacking Acetylcholine (Ach) receptors. Conclusions drawn from other compounds were increasingly difficult to draw, which indicates that relationships between neuronal pathways is more intricate than previously thought.

Background

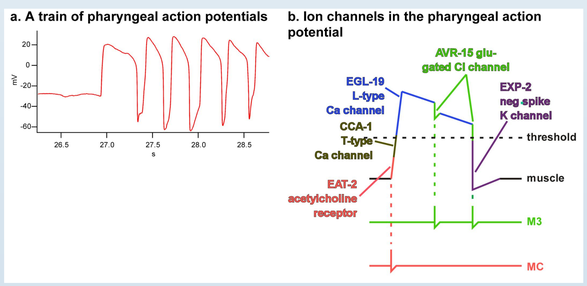

Figure 1: The mechanism of pharyngeal pumping: ACh binds to the eat-2/eat-18 receptor complex, beginning depolarization. Voltage gated Ca++ channels open, furthering depolarization; the muscle contracts, then Glu is secreted onto the avr-14/15/glc-1 receptors to start to relax.

Figure 2: 5-HT stimulates pharyngeal pumping, egg laying and paralysis in C. elegans.

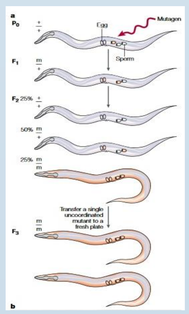

Figure 3: Gametes in the P_0 are randomly mutated via ethyl methyl sulfide. The F_2 generation is exposed to test drugs and resistant worms are transferred to seeded, drug-free plates. Tests are repeated on recombinant offspring (F_3). Surviving F_3's undergo genome sequencing.

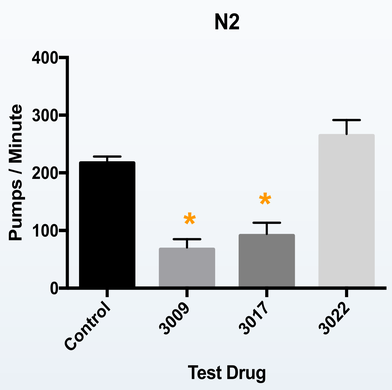

Figure 4: Drugs 3017 and 3009 significantly decrease pharyngeal locomotion in wild-type worms.

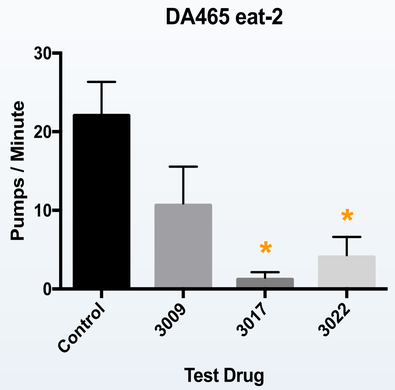

Figure 5: Both 3017 and 3022 cause a substantial decrease in pharyngeal pumping in worms containing *eat-2* receptor mutations.

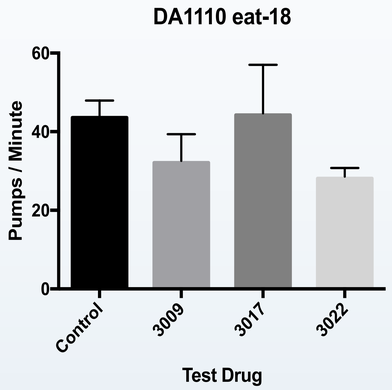

Figure 6: *Eat-18* receptor mutants failed to provide remarkable data points regarding pharyngeal locomotion

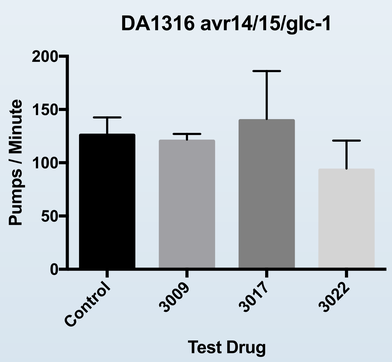

Figure 7: *Avr-14/15/glc-1* receptor mutants did not display any significant changes in pharyngeal locomotion.

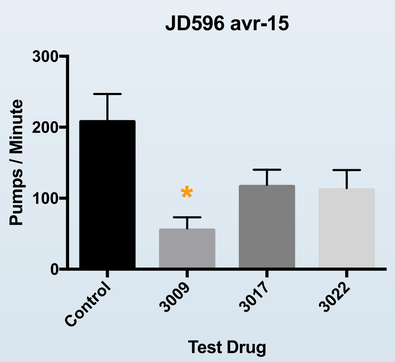

Figure 8: 3009 remarkably decreased pharyngeal pumping in nematodes with *avr-15* receptor mutations.

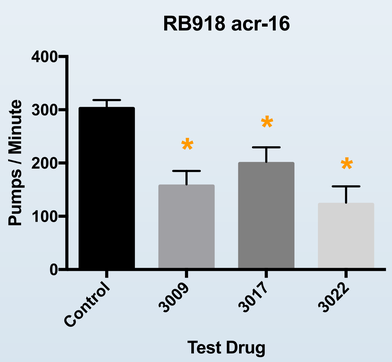

Figure 9: Each of the drugs significantly inhibited pharyngeal locomotion in *acr-16* receptor mutants.

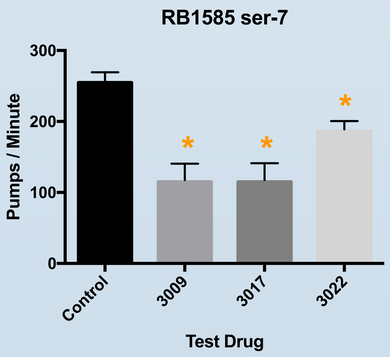

Figure 10: In *ser-7* mutants, all drugs remarkably decreased pharyngeal pumping.

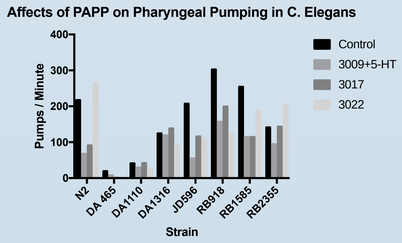

Figure 11: A compiled representation of all the data side-by-side is presented to visualize trends.

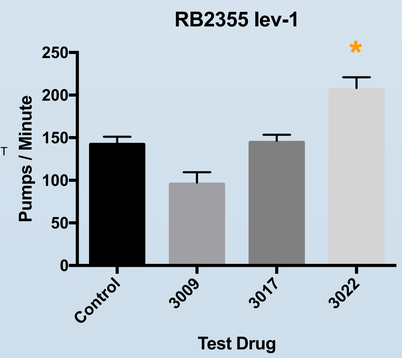

Figure 12: *Lev-1* mutants displayed a substantial increase in pharyngeal pumping when 3022 was present.

Methods

Candidate/Biased Approach

- Test worms with known mutant neuronal pathways
- Goal:
  - Determine mechanism of action
  - Identify pathways that regulate pumping
- Control
  - 13mM 5-HT: paralyze worm, stimulate pharyngeal pumping
  - 1mM DMSO: drug vehicle
- 5-HT & 1mM of each compound in DMSO (3009, 3017, 3022)
- USB camera
- Slowed down over 250 videos and counted pumps
- ANOVA- multiple comparisons

Mutagenesis/Unbiased Approach

- Create new mutants and identify resistant worms
- Goal:
  - Working backwards so all mutations are unknown
  - Random mutant genomes may show different neuronal pathways necessary for pharyngeal pumping
- Mutagen: ethyl methyl sulfide

Summary

Evaluation		
Drug	Mutations Affecting Drug Sensitivity	Conclusions
3009	• Eat-2, Eat-18	• 3009 acts through ACh pathway
	• Avr-14/15/glc-1	• 3009 may act through Avr-14/15/glc-1 pathway (similar to Ivermectin)
	• Avr-15	• Loss of Avr-15 subunit may alter properties of remaining subunits
3017	• Eat-18	• 3017 acts through ACh pathway
	• Avr-14/15/glc-1, Avr-15	• 3017 may act through Avr-14/15/glc-1 pathway
3022	• Eat-2	• Loss of ACh pathway may unmask 3022-sensitive pathway

Future Directions

- Can electrophysiology be used on worm tissues to identify specific pathways involved in pharyngeal pumping?
- Can studies of compound interactions allow us to target ion channels?