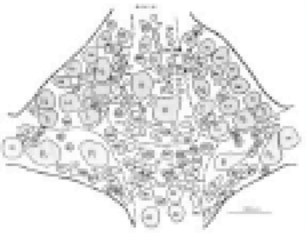
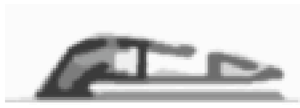
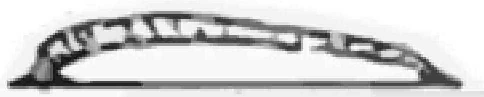


The effects of dopamine on locomotion in the medicinal leech.

Sarah E. Powers[1], Kathleen A. Klukas[2], Karen A. Mesce[2]

[1]Bryn Mawr College, [2]University of Minnesota, Departments of Entomology and Neuroscience





1. INTRODUCTION

It would be difficult to overstate how important locomotion is for most animals. The amine, dopamine (DA), is a universal modulator of locomotion, however, very little is understood about DA's specific role in locomotor rhythm generation. For example, how does DA regulate movement, and how does it interact with motor circuits?

The nervous system and the various forms of locomotion in the medicinal leech have been well studied, making it a good model system for addressing these types of questions. In addition, all of the aminergic (i.e., DA and serotonin) neurons have been identified within the leech.

The leech central nervous system (CNS) consists of a head brain, a tail brain, and 21 ganglia in between (i.e., the nerve cord). A recent study has shown that when DA is applied to the entire nerve cord, or even just one ganglion, the neural correlate of crawling can be observed (Puhl & Mesce, 2008).

2. GENERAL METHODS

Spontaneous behavior of leeches in both high and low water conditions were observed after they were placed in circular plastic containers with diameters of 30 cm. The low water condition, with barely enough water to cover the bottom of the container, promoted crawling, while high water, 5 cm deep, promoted swimming. Six leeches in 6 separate containers were simultaneously video recorded. Lighting was optimized to reduce shadows. The video recording was later analyzed for crawl cycles and time spent swimming. A single crawl cycle consists of a whole-body elongation and contraction with coordinated sucker movements. Swimming behavior is dorsal-ventral sinusoidal undulations of the flattened and hyper-elongated body.

DA was delivered to the CNS by placing the leech into a 200 mL of leech saline with a certain amount of DA. Although the rate of delivery or the final dose of DA absorbed into the CNS was unkown, this method was a success due to the leech's negligible blood brain barrier.

DA was depleted by injecting 15-25 mg of α-methyl-*p*-tyrosine (AMT), a DA-synthesis inhibitor, suspended in 100 μL of (2-hydroxypropyl)-β-cyclodextrin, 45% weight/volume. Glyoxcylic acid treatments and DA specific antibody staining was used to verify DA depletion in the head brain, anterior root ganglia (ARG) and tail brain.

General electrophysiological recordings were conducted using the methods of Puhl & Mesce (2008). A single segmental ganglion was bathed in a solution of DA while recording the extracellular activity of the dorsal posterior (DP) nerve and intracellularly from the mechanosensitive neurons.

BIBLIOGRAPHY & ACKNOWLEDGEMENTS

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•Puhl JG, Mesce KA (2008) Dopamine activates the motor pattern for crawling in the medicinal leech. *J Neurosci* 28:4192-4200.

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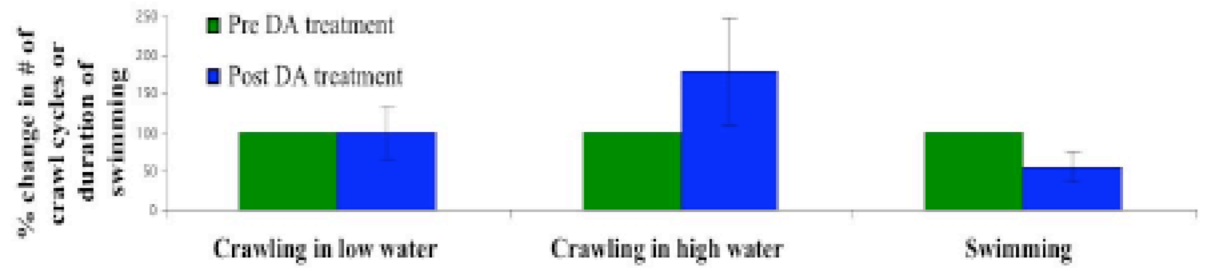
3. CENTRAL HYPOTHESIS

Fig 1. Hypothesis for how DA affects leech behavior. Crawling behavior is promoted at intermediate levels of DA, and swimming is inhibited.

4. RESULTS: EXTERNAL DA APPLICATION

Fig 2. Changes in spontaneous behavior of leeches treated with DA.



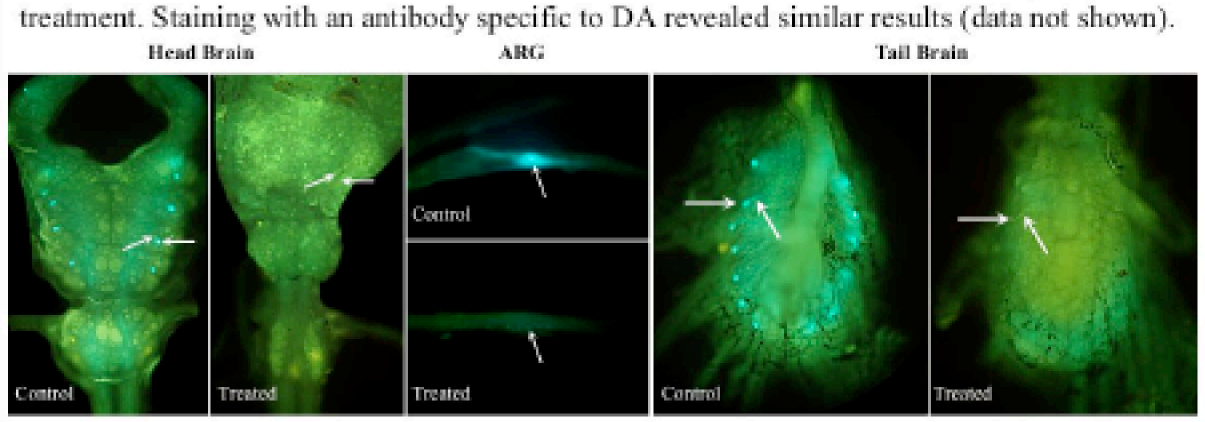
Error bars represent +/- SEM. N=10. Post-treatment data normalized to pre-treatment data. Ten minute observations pre- and post-DA treatment in high and low water. Post-DA treatment data were collected immediately after treatment. Data for high-water crawling and swimming were collected during the same observations period. Leeches were also observed once daily for 7 days post treatment (data not shown).

Table 1. Behavioral results from leeches treated with various DA bath protocols. Overall motor activity is affected more at higher concentrations of DA and with longer DA application. Green text represents leeches displaying normal behavior, while blue text represents leeches showing stronger behavioral deficits.

[DA]	Treatment duration	N=	Results
100 mM	10 min	1	Normal behavior
100 mM	40 min	1	Attempts at swimming, uncoordinated crawling
100 mM	20, 40, 180 min	1	No swimming, uncoordinated crawling
100 mM	180 min	1	No locomotion, extremely rigid
10 mM	30 min	1	Normal behavior
10 mM	20, 40 min	2	Slight decrease in overall behavior
10 mM	60 min	3	Decrease in overall behavior
1 mM	180 min	1	Normal behavior

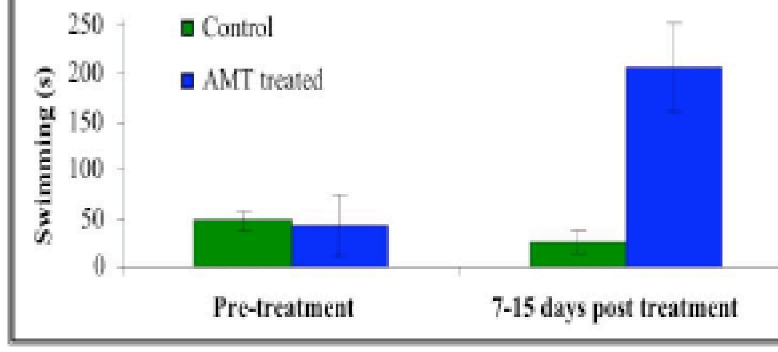
5a. RESULTS: DA DEPLETION WITH AMT

Fig 3. Reduction of DA in AMT-treated animals is indicated by loss of blue fluorescence. Shown here are the DA cells that fluoresce blue, a result of the Glyoxcylic Acid treatment. Staining with an antibody specific to DA revealed similar results (data not shown).



5b. RESULTS: EFFECTS OF DA DEPLETION

Fig 4. Average swim duration pre- and post-AMT treatment.



Error bars represent +/- SEM. N=4 (N=2, 15 days post treatment; N=2, 7 days post treatment.) $p = 0.03$, paired Student's *t*-test.

Note: There was no significant change in crawling (data not shown). Pilot data indicate AMT-treated animals can recover motor function when DA is re-introduced (data not shown).

6. CELLULAR-LEVEL ASPECTS OF DA-INDUCED CRAWLING

Fig 5. Schematic of a leech ganglion, highlighting the mechanosensory cells. Figure adapted from Muller et al., 1981 by J. Puhl.

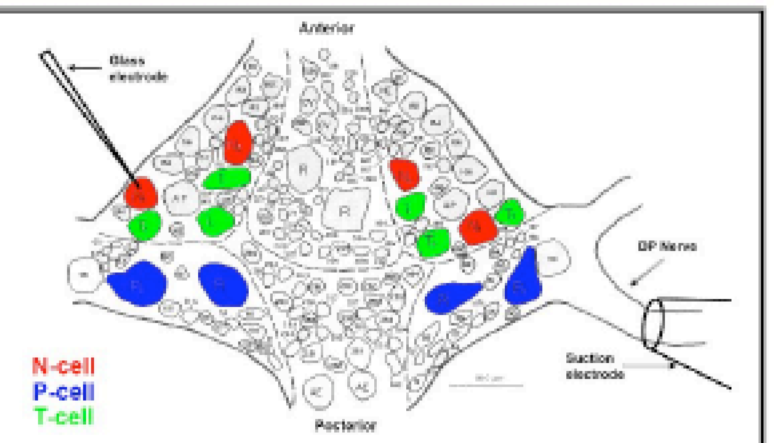
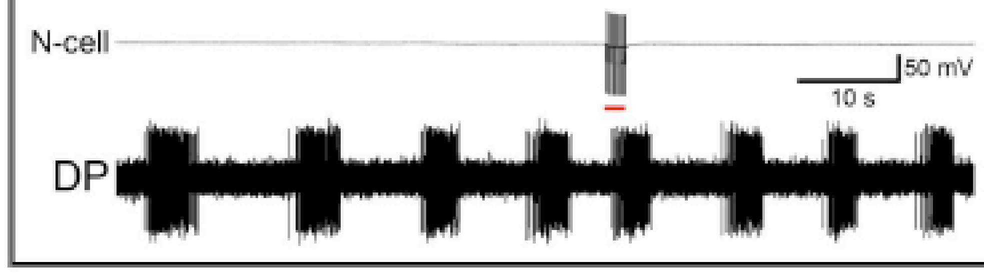


Fig 6. In the presence of DA, electrical stimulation of a mechanosensory neuron, called the "N" cell, will reset the fictive crawling rhythm. Other preliminary data (not shown) support the idea that additional mechanosensory neurons, such as the "P" cell can both reset and activate the crawling rhythm (S. Powers & J. Puhl, personal observations).



N-cell was stimulated with +1.5 nA, delivered for 2 s via a train of 50 ms long pulses at 5 Hz.

7. SUMMARY & CONCLUSIONS

- **The behavior of leeches treated with DA supports our central hypothesis that DA biases behavior towards crawling and away from swimming. After treatment with DA, leeches will crawl more and swim less in high water.**
- **Spontaneous locomotor activity decreases when leeches are exposed to higher doses of DA, which could factor into the leech's decision to stop locomotion in preparation to bite.**
- **AMT appears to be a promising protocol for the depletion of DA. AMT-treated leeches will swim significantly more than controls.**
- **Mechanosensory neurons can influence the crawl rhythm, helping us to understand the circuitry regulating crawling behavior.**