



# THE ROLE OF SEROTONIN IN THE CONTROL OF HEART RATE AND VENTILATION IN *APLYSIA CALIFORNICA*

Sasha L. Sypher, Mary S. Shay, and M. Danielle McDonald



## Introduction

- *Aplysia californica* are marine mollusks that are exposed to low environmental oxygen levels
- The acute response to hypoxia involves changes in both heart rate and ventilation
- Cardiac contraction in *Aplysia* is controlled through pressure gradients resulting in blood flow through the gill into the cardiac atrium then to the ventricle
- The role of 5-HT in *Aplysia* muscle contraction is comparable to the role of catecholamines in vertebrate nervous systems
- Fluoxetine (FLX) blocks the reuptake of 5-HT by the 5-HT transporter, increasing the extracellular concentrations of circulating 5-HT
- Studying heart rate and ventilation response to 5-HT and FLX will provide understanding of 5-HT and the 5-HT transporter on the *Aplysia* cardio-ventilatory response to hypoxia

## Objective and Hypothesis

### Objective

To determine the acute response of heart rate and ventilation to intra-hemocoel injection of 5-HT or FLX

### Hypothesis

Heart rate and ventilation will be unchanged in *Aplysia* injected with NaCl and will increase in *Aplysia* injected with 5-HT or FLX

## Materials and Methods

- The experimental animals were obtained from the Rosenstiel School National Resource for *Aplysia californica* (n = 32; 128.6 ± 5.46 g)
- A photo was taken to evaluate physical response to injection
- A 30 sec baseline of heart rate using an ultrasound machine was taken 10 min after weighing (t = 0 min).
- Immediately after (30 sec), a dose of either 100 mL saline · g<sup>-1</sup> *Aplysia* (control), 3 μg 5-HT · 100 mL<sup>-1</sup> saline · g<sup>-1</sup> (5-HT-treated), or 0.1 μg FLX · 100 mL<sup>-1</sup> saline · g<sup>-1</sup> (FLX-treated) were injected into the hemocoel.
- 30 sec heart rate was immediately recorded after injection at (t = 2 min) (t = 7 min) (t = 32 min).
- After ultrasound recording a final picture of the *Aplysia* was taken
- Ventilation changes was measured by mantle cavity pressure using a parapodial catheter attached through surgery 24 hours prior
- Pressure transducers were connected to a Biopac System
- *Aplysia* was then connected to the pressure transducer by the parapodial catheter
- *Aplysia* were then injected with either NaCl, 5-HT, or FLX and a photo was taken to evaluate injection response
- The experimental animal was left for 30 min while the Biopac System tracked the ventilation
- At the end of the 30-min trial a final photograph was taken

## Results

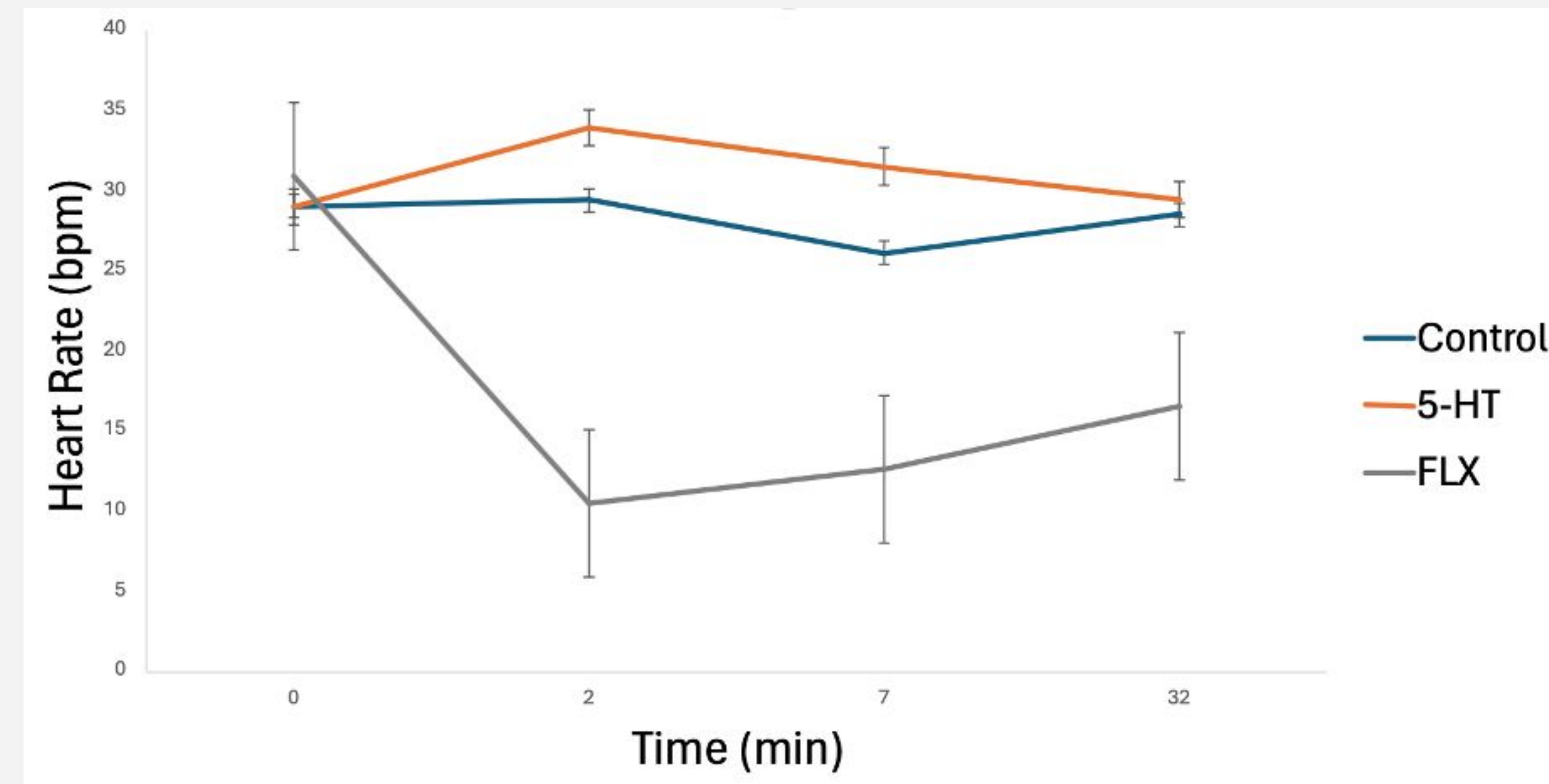


Figure 1: Mean heart beats per second over 30-min after NaCl (control), 5-HT, or FLX injection into the hemocoel. Values are means ± SEM. Different letters denote significant difference (p < 0.05).

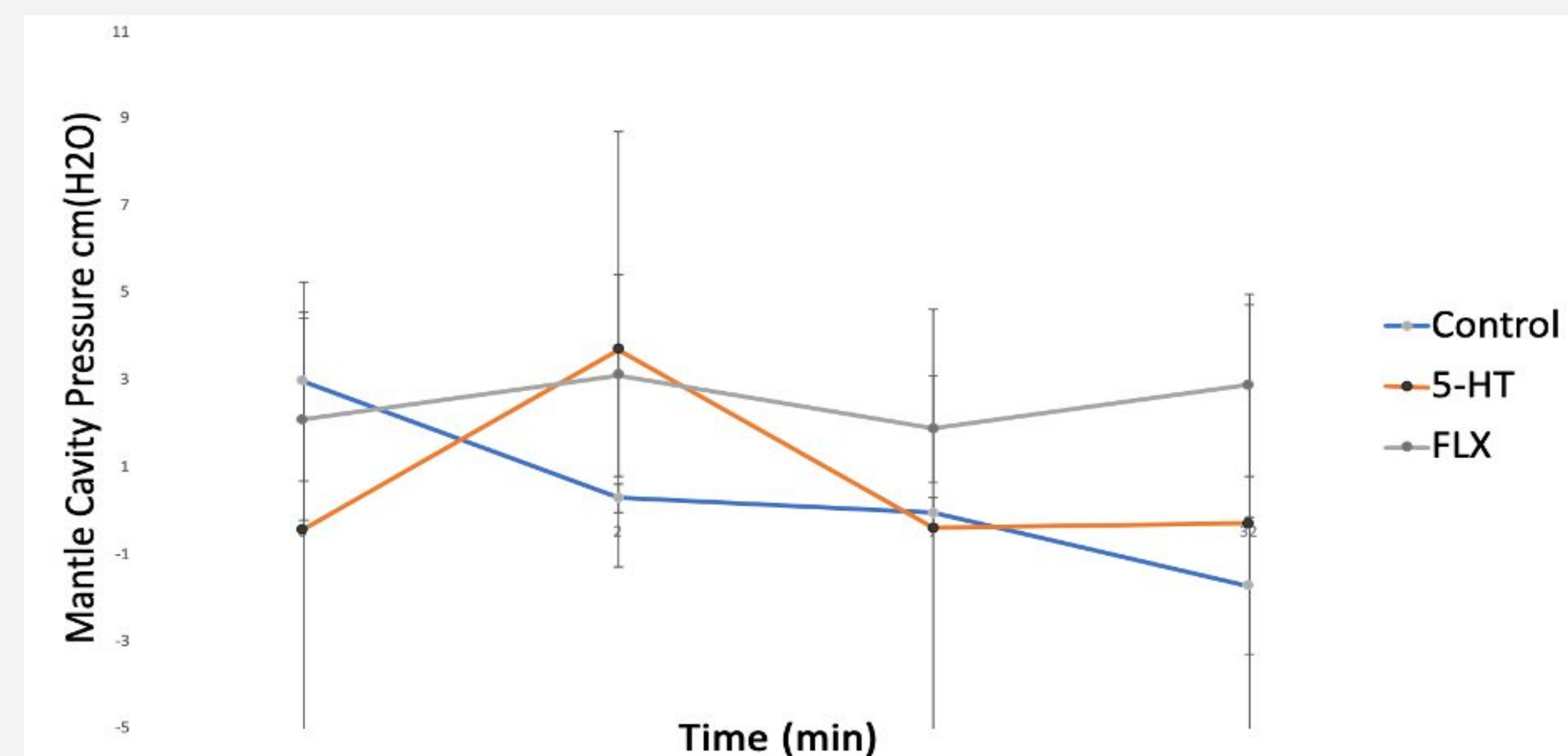


Figure 2: Mantle cavity pressure over 30-min after NaCl (control), 5-HT, or FLX injection into the hemocoel. Values are means ± SEM.

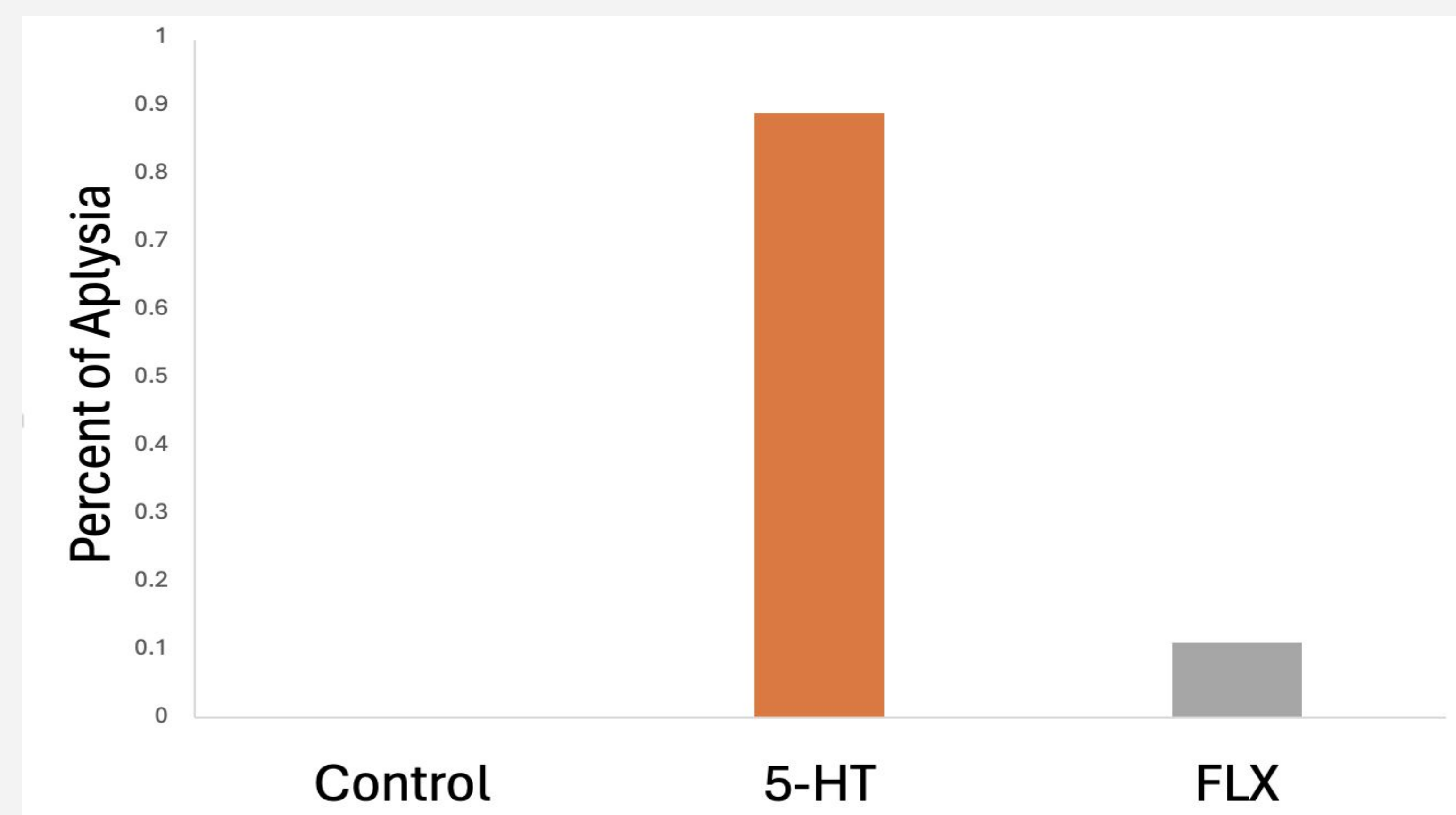


Figure 3: Number of 5-HT injected *Aplysia* and fluoxetine- injected *Aplysia* that exhibited scrunching after injection.

## Discussion

Our data supported the hypothesis in 5-HT- injected *Aplysia* heart rate experiments as the heart rate increased

Our FLX injection experiments did not support the original hypothesis, as heart rate decreased in response to FLX

5-HT and FLX had no effect on ventilation

The NaCl injected animals acted as a control, so their heart rates and ventilation remained the same after the baseline reading

89% of the animals that exhibited scrunching were the FLX-injected *Aplysia* compared to only 11% that were 5-HT injected *Aplysia*

- Ventilatory amplitude and respiratory pumping are major components in *Aplysia* and could be analyzed in response to 5-HT and FLX injection
- The physical reaction could indicate that the heart rate and ventilation response was because of the injection
- More studies understanding what the scrunching behavior means would need to be conducted

## Broader Impacts

There is an increase in hypoxic dead zones occurring with climate change

The 5-HT ability to regulate heart rate could implicate functions of *Aplysia* hypoxia resistance as shifting heart rate is a strategy for hypoxic resistant organisms

Fluoxetine is metabolized and excreted into many wastewater systems resulting in increased levels in fish in the areas nearby

## Acknowledgements

I would like to express my sincere gratitude to the *Aplysia* Resource staff. I would like to thank Mary Shay for guiding and instructing me through this project. I would also like to thank Danielle McDonald for providing me with multiple valuable opportunities and experiences.

## References

- [1] "Producing *Aplysia*." University of Miami. *Aplysia*. earth.miami.edu/biology-and-production-of-aplysia/20/producing-aplysia/index.html. Accessed 8 Apr. 2024.
- [2] Byrne JH, Koester J. Respiratory pumping: neuronal control of a centrally commanded behavior in *Aplysia*. *Brain Res*. 1978 Mar 17;143(1):87-105. doi: 10.1016/0006-8993(78)90754-0. PMID: 630406.
- [3] Koester J, Mayeri E, Liebeswar G, Kandel ER. Neural control of circulation in *Aplysia*. II. Interneurons. *J Neurophysiol*. 1974 May;37(3):476-96. doi: 10.1152/jn.1974.37.3.476. PMID: 4363778.
- [4] Feinstein R, Pinsker H, Schmale M, et al. Bradyarrhythmia response in *Aplysia* exposed to air. *J Comp Physiol B* 122, 311–324 (1977). <https://doi.org/10.1007/BF00692518>
- [5] Sawada M, Ichimose M, Ito I, Maeno T, McAdoo DJ. Effects of 5-hydroxytryptamine on membrane potential, contractility, accumulation of cyclic AMP, and Ca<sup>2+</sup> movements in anterior aorta and ventricle of *Aplysia*. *J Neurophysiol*. 1984 Feb;51(2):361-74. doi: 10.1152/jn.1984.51.2.361. PMID: 6323644.
- [6] Hiller, Martha J. A model of the combined effects of chemical and activity-dependent mechanisms in topographic map formation. *Computation and Neural Systems*, 1993, pp. 415-422. [https://doi.org/10.1007/978-1-4615-3254-5\\_63](https://doi.org/10.1007/978-1-4615-3254-5_63)
- [7] Carrigan, I.D., Croll, R.P. and Wyeth, R.C. (2015). Morphology, innervation, and peripheral sensory cells of the siphon of *Aplysia californica*. *J. Comp. Neurol.*, 523: 2409-2425. <https://doi.org/10.1002/cne.23795>
- [8] Barbas, D., Zappulla, J.P., Angers, S., Bouvier, M., Castellucci, V.F. and DesGrossillers, L. (2002). Functional characterization of a novel serotonin receptor (5-HT<sub>7</sub>) expressed in the CNS of *Aplysia californica*. *Journal of Neurochemistry*, 80: 335-345. <https://doi.org/10.1046/j.0022-3042.2001.00703.x>
- [9] Bymaster FP, Zhang W, Carter PA, Shaw J, Chernet E, Phebus L, Wong DT, Perry KW. Fluoxetine, but not other selective serotonin uptake inhibitors, increases norepinephrine and dopamine extracellular levels in prefrontal cortex. *Psychopharmacology (Berl)*. 2002 Apr;160(4):353-61. doi: 10.1007/s002130010986x
- [10] xandrine Robert, Tiphaine Monssinjon, Romain Pédén, Virginie Rasoamampianina, Jean-Claude Le Mével, Thomas Knigge. In vivo effects of serotonin and fluoxetine on cardio-ventilatory functions in the shore crab *Carcinus maenas* (L. 1758). *Aquatic Toxicology*, Volume 207, 2019. Pages 132-141. ISSN 0166-445X. <https://doi.org/10.1016/j.aquatox.2018.12.004>
- [11] Panilio, J., Marin, S., Lobl, M. et al. Treatment with the selective serotonin reuptake inhibitor, fluoxetine, attenuates the fish hypoxia response. *Sci Rep* 6, 31148 (2016). <https://doi.org/10.1038/srep31148>
- [12] JE Goldman, K S Kim, J H Schwartz. Axonal transport of [3H] serotonin in an identified neuron of *Aplysia californica*. *J Cell Biol* 1 August 1976, 70 (2): 304–318. doi: <https://doi.org/10.1083/jcb.70.2.304>
- [13] Goldman, James E., Schwartz, James H., (1974). Cellular specificity of serotonin storage and axonal transport in identified neurones of *Aplysia californica*. *The Journal of Physiology*, 242 doi: 10.1113/jphysiol.1974.sp010694.
- [14] Marc Kermorgant, Frédéric Lancien, Nagi Mimassi, Charles R. Tyler, Jean-Claude Le Mével. Effects of intracerebroventricular administered fluoxetine on cardio-ventilatory functions in rainbow trout (*Oncorhynchus mykiss*). *General and Comparative Endocrinology*, Volume 205, 2014. Pages 176-184. ISSN 0016-6480. <https://doi.org/10.1016/j.ygcen.2014.03.012>