UNIVERSITY OF MIAMI
ROSENSTIEL SCHOOL of MARINE & ATMOSPHERIC SCIENCE

The Effects of Cold Stress During Hypoxia on *Aplysia californica*

Seré E. Politano, Dr. Lynne A. Fieber
University of Miami, sxp867@miami.edu

Introduction

- The California sea hare, *Aplysia californica*, is a commonly used animal model in neurophysiological studies due to the simplicity of its nervous system and the relatively large size of its neurons
- In the wild, *Aplysia* species inhabiting intertidal regions are regularly exposed to air for at least 4 hours at a time[1] and may experience a variety of temperatures throughout a 24 hour period[2]. This has led to adaptations that allow them to withstand and recover quickly from prolonged hypoxia exposure.
- This study served to see whether adding an additional stressor of colder temperature during hypoxia would alter reflex behaviors and recovery of *A. californica*.

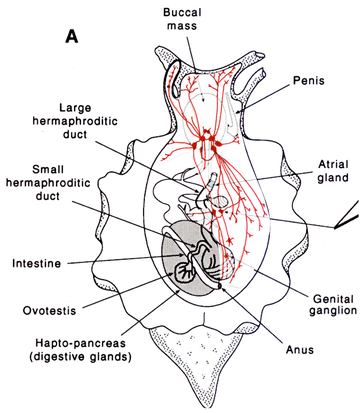


Figure 1. Anatomy of *A. californica* nervous system.

Methods

- Three experimental groups:
  - No-hypoxia controls
  - Ambient hypoxia
  - Cold hypoxia
- Ambient and cold hypoxia animals were exposed to air for 6 hours to induce a hypoxic state.
- The tail withdrawal reflex (TWR) and the righting reflex (TTR) were performed on animals before hypoxia, directly after 6 hr. hypoxia, and 48 hours following hypoxia.
- Other behavioral observations were made directly after hypoxia and 48 hours following hypoxia.

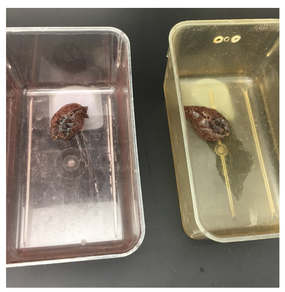


Figure 2. Setup for ambient hypoxia experiments.

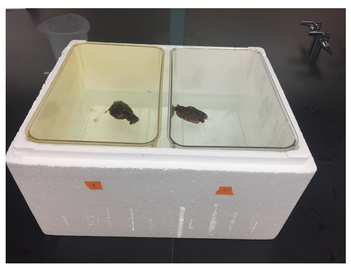


Figure 3. Setup for cold hypoxia experiments.

Results

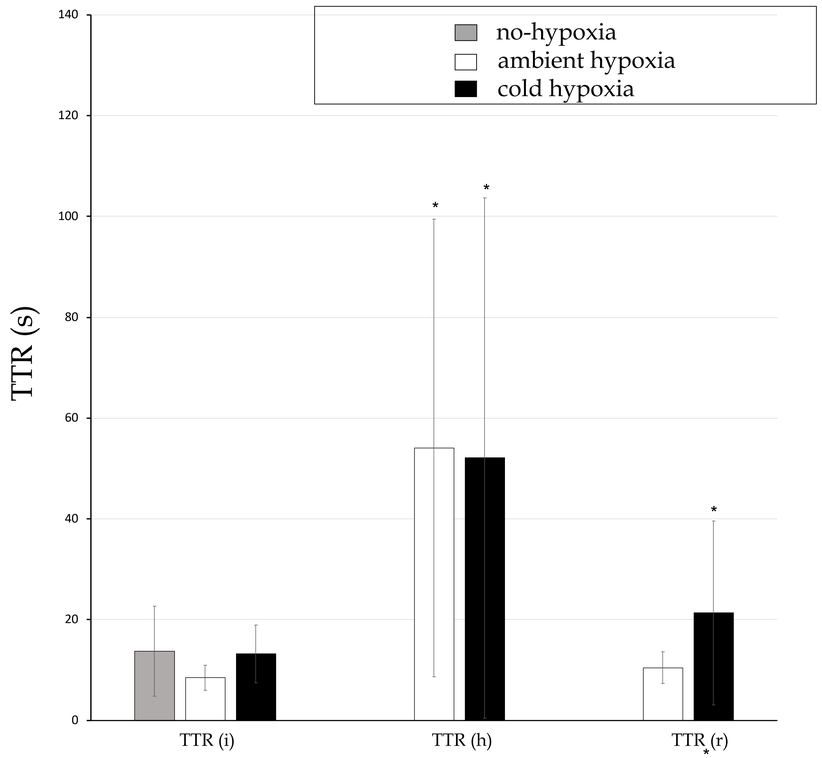


Figure 4. TTR means±SD, before 6 hr. hypoxia exposure [TTR (i)], directly after 6 hr. hypoxia exposure [TTR (h)], and after the 48 hour recovery period following hypoxia exposure [TTR (r)]. *above bars denotes the values that were significantly different from corresponding TTR (i) values (Wilcoxon signed-rank statistical test, $p \leq 0.05$). *below bars denotes two values that were significantly different from one another ($p \leq 0.05$).

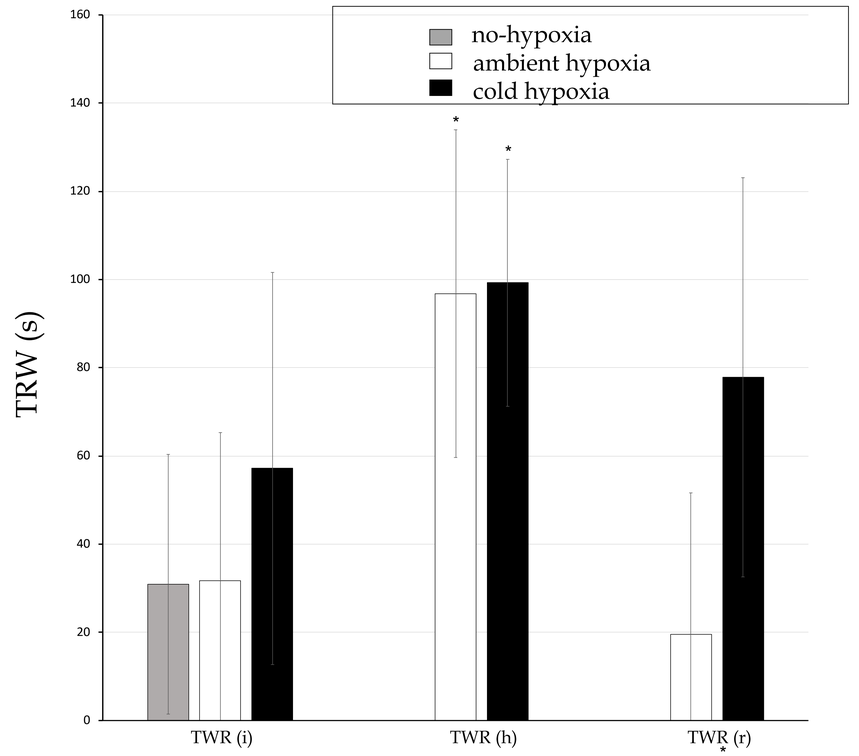
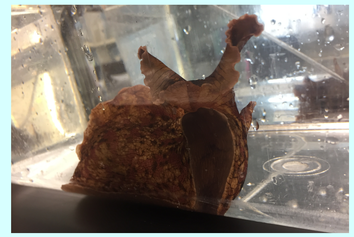


Figure 5. TWR means±SD, before 6 hr. hypoxia exposure [TWR (i)], directly after 6 hr. hypoxia exposure [TWR (h)], and after the 48 hour recovery period following hypoxia exposure [TWR (r)]. *above bars denotes the values that were significantly different from corresponding TWR (i) values (Wilcoxon signed-rank statistical test, $p \leq 0.05$). *below bars denotes two values that were significantly different from one another ($p \leq 0.05$).

Conclusions

- Hypoxia exposure significantly increased time to complete TTR and TWR in both ambient hypoxia and cold hypoxia animals directly after hypoxia.
- TTR and TWR returned to normal 48 hours after hypoxia in ambient hypoxia animals but not in cold hypoxia animals.
- Cold hypoxia animals did not perform typical hypoxia-recovery behaviors directly after hypoxia, compared to ambient hypoxia animals.
- Animals exposed to cold hypoxia continued to exhibit abnormal behaviors 48 hours following hypoxia, with some appearing to have physical damage.
- Addition of the cold stressor impairs the ability of the animals to fully recover from hypoxia and thus extends the recovery time needed for the animals to exhibit normal reflexes and normal behaviors.
- Cold hypoxia may have negative impacts on neurons that control simple behaviors in *Aplysia californica*.



Acknowledgements

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References

1. Kupfermann, I., & Carew, T. J. (1974). Behavior patterns of Aplysia californica in its natural environment. *Behavioral biology*, *12*(3), 317-337.
2. Kanz, J. E., & Quast, W. D. (1992). Respiratory pumping behavior in the marine snail Aplysia californica as a function of ambient hypoxia. *Physiological zoology*, *65*(1), 35-54.