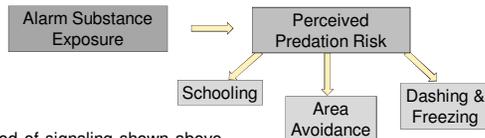


Variability in Ostariophysan Fish Brain Anatomy in Relation to the Neural Pathway of Innate Fear Response



Cynthia Koenigsberg, Claudia Seravalli & Philip Schadegg Department of Biology University of Wisconsin-Eau Claire
Faculty Mentors: Dr. David Lonzarich & Dr. Winnifred Bryant

Behavioral Response



The method of signaling shown above is rapidly propagated within schools. While ostariophysans heavily rely on chemical communication as alarm cues, visual cues compensate for a lack of perceived predation risk under sub-threshold Schreckstoff concentrations. Overt anti-predator behaviors can also serve as secondary visual cues that elicit increased anti-predator behavior in nearby fish. Recent evidence suggest that this response is more intensely displayed in juvenile fish than in adults. [1]



Figure 1. Zebrafish tank grid including graph showing fish velocity over time.

Selective Pressures

Inquiry of the alarm response continues to surround the evolutionary function of this phenomenon. Proposed hypotheses for the evolution of alarm response include:

Kin selection hypothesis

Many of these fish live in groups, and if these groups consist of relatives, this would suggest that the purpose of the alarm chemical is to increase the chances of survival of the kin of the preyed upon fish, thus passing on the individual's genes. [2]

Predator attractant hypothesis

The substance could also function as a secondary predatory attractant; supposedly, when the substance is released in the presence of a predator, this attracts a secondary predator that interferes or deters the predator that originally induced the substance release. [3]

Immune function hypothesis

Schreckstoff may serve as protection against pathogens, parasites and UV radiation. Recent evidence has shown that the presence of pathogens and parasites stimulates alarm substance production in Ostariophysans [4]

Neuro-Chemical Activity

Characteristics of Alarm Substance

Alarm substance is found in homogenized fish skin tissue, or Schreckstoff's Substance (Fig. 2). Characterization of the active alarm chemical remains elusive, however chondroitin has been hypothesized as one compound involved [5]

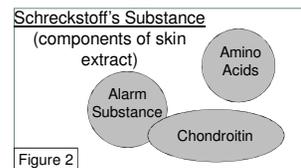


Figure 2

Neural Pathway: Processing of Odorant Molecules

Upon binding of the odorant molecules to the receptors located in the olfactory epithelium, the neural response progresses as shown below in Figure 3.

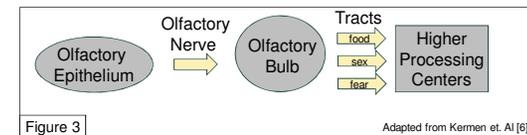


Figure 3

Adapted from Kermen et. Al [6]

C-FOS Staining

Upon prolonged exposure to stimuli, C-FOS, a proto oncogene, is expressed in the nuclei of neural cells. This makes the examination of C-FOS activity an effective way to observe the neural pathways of alarm response.

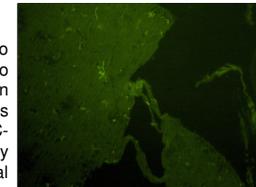


Figure 4. Portion of sagittal cut of creek chub telencephalon with C-FOS fluorescent antibody staining.

Introduction

Upon exposure to odorant molecules, such as alarm substance, fish exhibit a distinct behavioral response as a result of the neural pathway that is activated by the chemical ligand. Various hypotheses have been developed for the evolutionary purpose of this phenomenon. Brain morphology variation within the Ostariophysan superorder in relation to the processing of alarm is of interest as well. We seek to use this interdisciplinary collection of information in order to further characterize the ostariophysan alarm response.

Brain Morphology



Figure 7. Dorsal view of creek chub brain, displaying the peduncle olfactory bulb arrangement.

Olfactory Tract
Olfactory Bulb
Nasal Epithelium

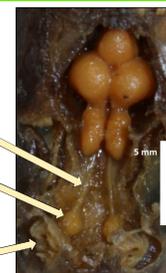


Figure 8. Dorsal view of zebrafish brain, displaying the sessile olfactory bulb arrangement.

Our research has focused on Zebrafish (*Danio rerio*) and Creek Chub (*Semotilus atromaculatus*), two species which have distinguished applications in the inquiry of the alarm response. While Zebrafish are easily bred have a widely established genome, the Creek Chub is larger (thus more easily manipulated) and shows variation in size and alarm response behavior between juveniles and adults. Additionally, we see divergent olfactory morphology in the olfactory bulb of the zebrafish (peduncle vs. sessile) as shown in figures 5-8.



Figure 5. Sagittal cut of zebrafish brain from Neuroanatomy of the zebrafish brain: a topological atlas.



Figure 6. Sagittal cut of creek chub brain.

Implications

Biomedical

The characterization of innate fear in the zebrafish has implications in medical research on the pathways of anxiety in humans. The zebrafish is an effective vertebrate model with neural pathways that correspond to those found in humans.

Ecological

The behavioral response of Creek Chub to skin extracts containing the alarm substance varies dramatically with age. Young individuals exhibit an alarm response while adult behavior develops from that of prey to predation, which may have implications within fish communities. The characterization of alarm response has the potential to provide mechanistic understanding about this transition.

This interdisciplinary approach gives us the opportunity to further explore the implications of the alarm response in fish of the Ostariophysan superorder.

References

- [1] Brown, E. G., Poirier, J., Adrian, J. C. (2004). *Behavioral Ecology*, 15.
- [2] Mathis, A., and R. J. F. Smith. 1993. *Behavioral Ecology* 4:260-265.
- [3] Chivers, D. P., G. E. Brown, and R. J. F. Smith. 1996. *The American Naturalist* 148:649-659.
- [4] Chivers, D. P., B. D. Wisenden, C. J. Hindman, T. A. Michalak, R. C. Kusch, S. G. W. Kaminsky, K. L. Lack, M. C. O. Ferrari, R. J. Pollock, C. F. Halbgewachs, M. S. Pollock, S. Alemadi, C. T. James, R. K. Savelloja, C. P. Goaler, A. Corwin, R. S. Mirza, J. M. Kiesecker, G. E. Brown, J. C. J. Adrian, P. H. Krone, A. R. Blaustein, and A. Mathis. 2007. *Proceedings of the Royal Society* 274:2611-2619.
- [5] Kermen, F., Franco, L. M., Wyatt, C., Yaksi, E. (2013). *Frontiers in Neural Circuits*, 7.
- [6] Mathuru, A. S., Kibat, C., Cheong, W. F., Shui, G., Wenk, M. R., Friedrich, R. W., Jesuthasan, S. (2012) *Current Biology*, 22, R183-R186.

Acknowledgements

We thank the Office of Research and Sponsored Programs for supporting this research, Student Differential Tuition for funding CERCA, and Learning & Technology Services for printing this poster.