Chapin Zerner

RESEARCH PLAN

Inner Ear Synaptic Changes in *Porichthys notatus* Supporting Seasonally Enhanced Acoustic Communication

A. RATIONALE

The Plainfin Midshipman, *Porichthys notatus,* is a species of toadfish designation, most commonly residing on the western coast of North America between 58° N and 28°N latitude and 136°W and 112° W longitude (Bass et. al., 2019). During the winter months, *Porichthys notatus* live in water of approximately 100 m in depth, migrating to intertidal or estuary zones during the spring and summer months during the reproductive periods. The Plainfin Midshipman is a species of interest due to its distinct neurological differences exhibited between winter and summer months. During summer months in shallow waters, male individuals generally fall into one of two designations as characterized by mating behavior. To better receive and process such calls, plainfin midshipman females exhibit a unique alteration in the level of dopaminergic processes and chemicals in specific areas of the nervous system. Here, afferent and efferent terminals are in constant flux, adjusting the capability of female midshipman to perceive various frequencies. The study of the CNS unique model organisms such as *Porichthys notatus* is integral to the understanding of the evolution of current auditory anatomy in a variety of species. This study contributes the role of dopamine in the fluctuation between reproductive activity in female midshipman. Dopamine was previously linked to larger catecholaminergic activities. Yet, the presence of large sites of dopamine transmission in Octavolateralis Efferent nucleus (OE) and Saccular Epithelium (SE) regions, often near afferent and efferent terminals, is poorly understood.

B. RESEARCH QUESTION

To what extent does dopaminergic activity play a role in relation to afferent and efferent terminals in *Porichthys notatus* females between summer and winter periods?

HYPOTHESIS

It was hypothesized that summer female specimen of *Porichthys notatus* would exhibit significantly increased dopaminergic activity in relation to, contacting or near, both afferent and efferent terminals. It was also hypothesized that the frequency and size of such terminals would increase in summer females. Moreover, the quantity and contact area of synaptic surfaces was expected to be increased in summer females.

C. PROCEDURE

(Note that items marked with an asterisk (Pre-experimentation or steps 1-7) were previously completed by qualified research personnel including but not limited to associate professor and/or graduate students at CUNY Brooklyn College Department of Biology; steps 8-13 will be completed by the student researcher)

Pre-experimentation

1. *Ample supply of specimen (n = 2-3) of *Porichthys notatus* were obtained per situational anatomical structure studied (i.e. 2 specimen of winter females).
2. *Entire supply of specimen (n = 28-32) of *Porichthys notatus* was subjected to identical temperatures (~5°C) to stabilize neuroanatomy immediately following capture and transit.
3. *Each specimen was injected with enzyme-specific antibodies, such as 1,1-dioctadecyl-3 3 3 3-tetramethylindocarbocyanine (dil).
4. *An allotted 2.5 hours passed, at which point each treated specimen was be subjected to euthanasia under IACUC protocol.

5. *Specimen underwent dissection, taking careful measure to ensure both the chemical preservation of brain structure as well as proper laboratory safety when handling potentially disease-bearing fish.
6. *Desired sections of Plainfin midbrain (OE and Saccule) were continuously sectioned according to use, ensuring lack of potentially disruptive contamination, until desired thickness of 60 nm was reached.
7. *Electron microscopy was performed according to necessity to image and were processed at a later date. Microscopy performed such that each given frame contained four (4) equal quadrants within field of view, following clockwise movement pattern. Process repeated first for each specimen and hereafter for any necessary duplicate slides.

<u>To be completed by Student Researcher</u>

8. Electron microscopic images will be digitally processed using Fiji Image Analysis Software and TrakEM2 ImageJ plugin.
9. Quadrants will be digitally stitched together to create cohesive image, with 18-28 images per set, and 5 sets per individual specimen will be observed.
10. Such a process will be completed for each of 12 individual organisms such that 60 image sets of 18-28 images are extracted.
11. Digitally processed images will then be annotated to expose areas of interest including dopamine terminals.
12. Jython code will be written to extract measures of and between areas of interest including the size and quantity of dopaminergic terminals in each organism. Key areas of interest

will be afferent and efferent terminals and their proximity to hair cells and dopamine terminals, as well as their size and count.

13. Measurements recorded and analyzed by use of R statistical computing language. These will include Tukey Post-hoc ANOVA, T-testing, and creation of graphic elements to aid understanding as deemed necessary.

Health Risk & Safety: None. Student researcher will not interact or handle live or dead specimens. Images only will be obtained.

D. BIBLIOGRAPHY

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NO ADDENDUMS EXIST