Project Description

1) Title: Neuron counts in the unusually large brains of whales

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2) **Abstract**: Within mammals, dolphins and beluga whales are known to have a large brain size relative to body size. However, little is known of the composition of these brains. To increase our understanding of the different cells that make up the brains of terrestrial vs. marine mammals, this study aims to establish a fundamental understanding of the number of neurons in the brains of an echolocating and agile beluga whale compared to a slow move and non-echolocating bowhead whale. This study will use fluorescent labels to stain the neurons in the brains of both animals. Numbers of neurons will be counted using a confocal microscope. We hypothesize that the cerebellum of both animals will be roughly equal in their neuron density, but the cortex of the beluga brain will display a greater neuron density. Results will be compared with published accounts of neuron densities within terrestrial mammals (i.e., bats, elephants, carnivores, and ungulates). We expect our results will add a critical understanding of the architecture of big brains in cetaceans as well as elucidate the evolution of brains within aquatic and terrestrial mammals.

3) **Significance of the Research:** The brains of cetaceans (whales, dolphins, and porpoises) are unusual among mammals in that they process different locomotor and sensory information. We expect the life in an aquatic habitat has altered brain function. Comparative studies of the brains of cetaceans have so far shown that massive increases in brain size are found in dolphins and their close relatives, but baleen whales retain relative brain sizes similar to that of terrestrial mammals. Critical to our understanding of the architecture of these brains is understanding the cellular architecture of the brains. This study will increase our understanding of the evolutionary origins by quantifying cell type within the brains of a large-brained beluga compared to that of a small-brained bowhead whale. By comparing neuron counts, our data will elucidate whether the large brain of some cetaceans is the result of an expansion in the number of neurons, or supportive glial cells, or both. Fresh tissues of these arctic species are exceptionally rare. One of us, J.G.M. Thewissen, conducts field work in the Arctic and has collected brains of each species that are currently fixes and ready for analysis.

Our contribution is expected to further the goal of understanding the unique architecture of the brains of echolocating and non-echolocating whales compared to terrestrial mammals. Our contribution will be significant because the data will assist in developing a nuanced understanding of the cellular evolution leading to the expanded brain size in modern whales. It is also likely that our results will vertically advance our understanding of neural plasticity within the mammalian brain associated with life in novel habitats.

4) Goals and Objectives of the Research: By comparing neuron counts, our data will elucidate whether the large brain of some cetaceans is the result of an expansion in the number of neurons, or supportive glial cells, or both.

5) Research Methods Learned by the Summer Fellow: First, the researcher will be trained to participate in every phase of project research, including specimen preparation and analyses. Opportunities for students to gain experience with unusual model organisms are rare, and the skills gained through involvement with this project should substantially broaden the researcher's skill sets. The student will learn to homogenize and stain brain tissue, count neurons, and analyze data.

6) Research Methods and Data Analysis – The brains of one beluga and bowhead are fixed. The brains will be cut into 5-gram sections, and these sections will then be homogenized, stained, and counted for neurons. DAPI stain will be used to stain all nuclei, while an anti-NeuN antibody stain will stain neurons. This protocol is already established the lab of the PIs. Using a confocal microscope, we will count number of positively DAPI and anti-NeuN nuclei throughout homologous regions in both taxa. We will compare neuron densities of these parts to those of terrestrial mammals through use of ANOVA.

7) Expected Outcomes – Three possible outcomes are anticipated for this study. Our null hypothesis is that neuron densities will be similar between the two whales. If the beluga cerebellum has greater neuron density, this is likely associated with its agile swimming style relative to the slow-moving bowhead. Our findings, regardless of outcome, will lay the foundation for future work quantifying total brain neuron counts between the two taxa. We expect the cerebellum work to be the first of several publications.

Student Fellow Training/Mentoring Plan: Funding is requested to support one summer research fellow. PI's Cooper, Smith and Thewissen are committed to fostering the researcher's development for the summer. This goal will be achieved through a structured mentoring program, as described below.

Research will be conducted in RG200 using fixed brains that are ready for analysis. Protocols are already established, and all necessary laboratory equipment and disposables are already in use as this is an ongoing project.

Besides benefiting from working alongside the PI's, the student will be required to attend and present once at weekly laboratory meetings. The Musculoskeletal Research Focus Area - a joint effort of the Department of Anatomy and Neurobiology and the Department of Integrated Medical Sciences at NEOMED – also sponsors a weekly journal club on the general topic of "Evolutionary Morphology", where the fellow would have the opportunity to share and discuss ongoing research findings and pertinent scientific publications. Finally, the student will design and present a poster for the end-of-program poster symposium at NEOMED.