

Physiological and Biochemical Neuroprotection in Cetaceans: Are Some Marine Mammal Species Safeguarded from Emboli Formation and Barotrauma?

Terrie M. Williams

Center for Ocean Health- Long Marine Lab, Department of Ecology and Evolutionary Biology
100 Shaffer Road, University of California-Santa Cruz, Santa Cruz, CA 95060
phone: (831) 459-5123 fax: (831) 459-3383 email: williams@biology.ucsc.edu

Award Number: N000140510808

LONG-TERM GOALS

The primary goal of this study is to evaluate the susceptibility of critical tissues in cetaceans to acoustically mediated trauma from emboli formation. By investigating tissue and whole animal mechanisms we intend to identify possible physiological/environmental factors that would allow for lipid/gas mobilization and concomitant tissue damage at depth. If successful, the results of this project will enable the development of environmentally sensitive schedules for oceanic acoustic activities by identifying those species most susceptible to tissue injury.

OBJECTIVES

To accomplish these goals we are focusing on two key questions:

1. ***Are the neural tissues of marine mammals uniquely hypoxia tolerant due to the presence of neuroglobin?*** This is being examined by measuring the concentration and function of oxygen-carrying globin proteins (hemoglobin, cytoglobin and neuroglobin) in the brain (both sensory and cognitive areas) of a wide variety of terrestrial, swimming and deep diving mammals including the beaked whales.
2. ***Is the dive response that safeguards marine mammals from decompression illness compromised by elevated environmental temperature or high levels of locomotor activity?*** In this part of the study we are measuring cardiovascular, metabolic, and gas transfer dynamics of trained bottlenose dolphins during sedentary and active periods while diving in warm and cold water.

Together these studies will enable us to determine if some marine mammal species, such as the family of beaked whales, are more susceptible to non-auditory tissue damage as may occur in conjunction with navy and oil exploration sound operations. We will take into account several recent hypotheses regarding emboli formation as well as observed behavioral responses of marine mammals to low- and mid- frequency sound production.

APPROACH

This study uses two approaches to determine the relative susceptibility of cetaceans to acoustically mediated trauma. Because stranded marine mammals often display behaviors associated with neural dysfunction (i.e. disorientation, poor localization and righting responses), and neural tissues are exceptionally vulnerable to decompression damage, we are evaluating natural variation in

Report Documentation Page

Form Approved
OMB No. 0704-0188

Public reporting burden for the collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to a penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.

1. REPORT DATE 2007		2. REPORT TYPE		3. DATES COVERED 00-00-2007 to 00-00-2007	
4. TITLE AND SUBTITLE Physiological and Biochemical Neuroprotection in Cetaceans: Are Some Marine Mammal Species Safeguarded from Emboli Formation and Barotrauma?				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S)				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of California-Santa Cruz, Center for Ocean Health- Long Marine Lab, 100 Shaffer Road, Santa Cruz, CA, 95060				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release; distribution unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT Same as Report (SAR)	18. NUMBER OF PAGES 5	19a. NAME OF RESPONSIBLE PERSON
a. REPORT unclassified	b. ABSTRACT unclassified	c. THIS PAGE unclassified			

neuroprotective mechanisms of the central nervous system. One team is conducting laboratory studies at the tissue level to assess the presence and function of oxygen binding globin proteins in the brain of marine mammals. Team members include specialists in morphology and pathology of marine mammals (M. Miller, CA Department of Fish and Game; D.A. Pabst, University of North Carolina-Wilmington), globin chemists (D. Kliger and R. Goldbeck, UCSC), molecular biologists (M. Zavanelli, UCSC) and physiologists (T.M. Williams and D. Casper, UCSC). Together we are conducting one of the first studies to examine neuroglobin and its potential role as a neuroprotectant in marine mammals. Using a comparative analysis of tissues collected from marine and terrestrial mammals we are determining the effects of dive capacity on the expression of globin proteins, and therefore the vulnerability of different species to hypoxia associated with decompression syndromes.

The second team involved in this study is examining the susceptibility of cetaceans to decompression illness at the whole animal/physiological level by monitoring disruption of bradycardia during diving tests. Tests with bottlenose dolphins trained to dive at varying depths and water temperature are assessing the variability in bradycardia and peripheral vasoconstriction as occurs with the dive response. The effects of two physiological mechanisms known to alter blood flow are being investigated, exercise and heat. Both have the capacity to alter the dive response, and therefore, nitrogen transfer dynamics between tissues. In this study we are examining this by measuring heart rate, and post-dive blood parameters in trained dolphins under three conditions, 1) shallow diving in cold and warm water, 2) moderate depth, warm water dives varying in exercise intensity, and 3) deep dives. Team members for this part of the program include physiologists (T.M. Williams, UCSC) and animal behaviorists (T. Fink and B. Richter, UCSC)

WORK COMPLETED

Tissue Globin Analyses. Our team has successfully developed two assays for brain globins, a spectrophotometric test that provides globin concentration and an mRNA expression test for relative cytoglobin and neuroglobin levels. Currently, we have used these assays to detect the presence and concentration of globins in the cerebral cortex of 16 species of mammals. This includes five species of terrestrial mammal ranging in body mass from 0.1 kg to 100 kg, and 11 species of marine mammal ranging in mass from 30 – 300 kg. Among the marine species, we have examined both coastal and pelagic divers among the small cetaceans, pinnipeds and sea otters. All have demonstrated the presence of globins, although the concentration varies among the various species. Both the cerebral cortex and cerebellum have yielded similar results for the species in which we were able to sample both areas.

We are continuing to refine our isolation techniques in order to quantify the level of globins as well as characterize the exact molecular structure of the globins. One of the major challenges of this project has been obtaining fresh brain samples for the analysis. Although the globins appear robust we find that the morphological structure of neural tissue quickly degrades during postmortem events. As a result, identification of specific areas of globin concentration becomes difficult.

Variation in Diving Bradycardia. The second component of this study examines variability in the dive response of cetaceans. Two major challenges were immediately encountered on this project, 1) obtaining marine mammal permits, and 2) developing heart signal instrumentation that could withstand the rapid swimming movements of dolphins. During this year we were able to meet both of these challenges. We succeeded in obtaining the live animal permits and we have tested novel

instrumentation for monitoring variability in the dive response of dolphins. Two instrumentation systems are currently being developed. The first uses a medical Holter monitor designed for ambulatory human patients. Initial tests successfully recorded the ECG of two bottlenose dolphins in our facility. This system and the associated software enables detailed analysis of each heart beat for the animals. Consequently, we will be able to obtain a detailed profile of the electrocardiogram of the dolphins as well as identify subtle and gross changes in the signals as occurs from rest to high levels of activity. The second instrumentation system incorporates an accelerometer that will be used to correlate the level of activity with changes in heart rate in diving dolphins. Both instruments and underwater housings have been delivered and are currently being tested on diving dolphins.

RESULTS

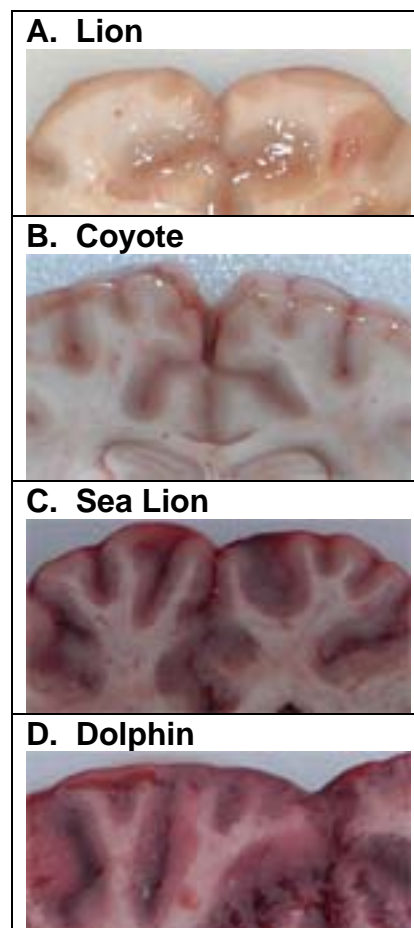


Figure 1. Comparison of pigmentation in the cerebral cortex of terrestrial (mountain lion, coyote) and marine (sea lion, dolphin) mammals. Note the relatively light pigmentation of the terrestrial species compared to the dark coloration of both marine species.

Our primary accomplishment was in the completion of the first series of assays for neuroprotective globins in the mammalian brain. Based on these assays we found marked variation in the ability of the mammalian brain to protect itself from hypoxic-ischemic injury as indicated by the levels of circulating (hemoglobin) and resident (neuroglobin, cytoglobin) globin proteins. Differences in

pigmentation of the cerebral cortex were apparent for terrestrial and marine mammals (Fig. 1) and attributed to the relative presence of hemoglobin in the tissues. Resident globin protein concentration also differed between terrestrial, swimming and diving mammals (Fig. 2).

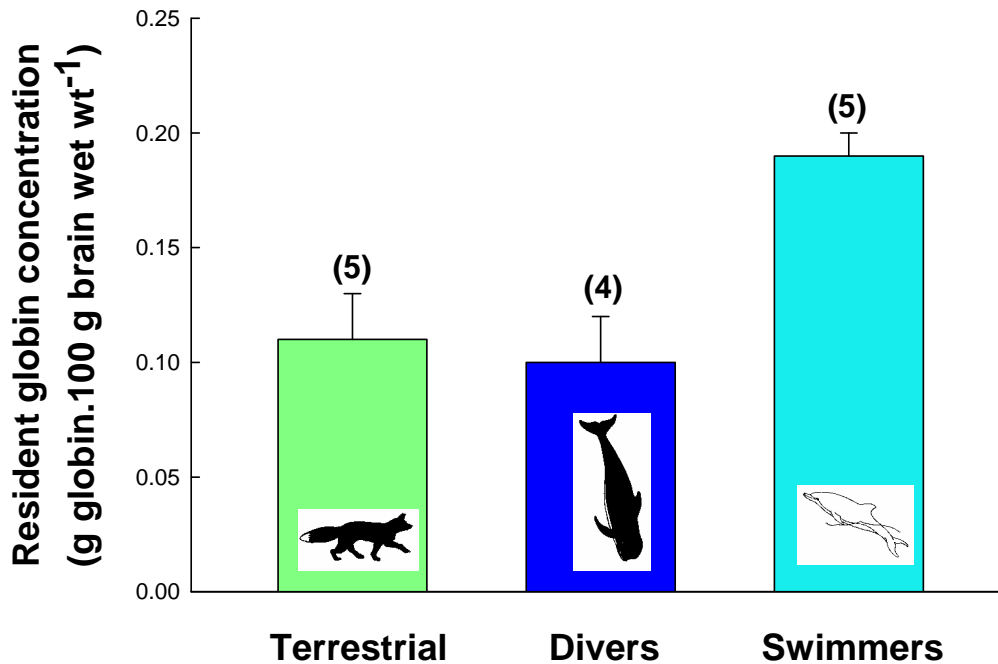


Figure 2. Relationship between routine activity pattern and globin protein concentrations in the mammalian cerebral cortex. Resident neural globin concentration was higher for marine mammals specialized for swimming (light blue bar) than for diving specialists (dark blue bar) or terrestrial mammals (green bar). Bar height and lines denote mean + 1 S.E.M. Numbers in parentheses are total numbers of species in each category.

Together these globins provide complimentary mechanisms for facilitating oxygen transfer into neural tissues as well as the potential for protection against reactive oxygen and nitrogen groups when marine mammals are submerged. By safeguarding neural tissues challenged by hypoxia, this array of concentrated globin proteins represents an important adaptation for maintaining activity while diving by marine mammals, and may lead to novel approaches for averting oxygen-mediated neural injury in comparatively poor divers including humans.

IMPACT/APPLICATIONS

Our recent findings on tissue globins in the cerebral cortex provide:

1. A new perspective on neuroprotection. By examining sixteen different mammalian species, we demonstrate how malleable the mammalian brain can be when placed under extreme chronic hypoxia, which occurs not only in air-breathing vertebrates who dive but also in response to various common medical conditions in humans and other species.

2. An assessment of the importance of globin proteins. Since neuroglobin and cytoglobin have been associated with neuronal survival following stroke and other ischemic insults with cardiovascular accidents, the results are relevant to many of the leading causes of mortality in the United States. Furthermore, although further research is needed, differences in resident neuroglobins may help to explain the relative susceptibility of deeper diving species to barotrauma following exposure to anthropogenic noise.

3. New techniques for clinical, ecological, behavioral and physiological studies. Our study is the first to measure the concentration of resident neural globins by developing new biochemical methods and animal models. To our knowledge this is the first time that these globins have been investigated for any non-laboratory mammalian species, and provides new techniques for use by a wide variety of comparative and medical neurophysiologists.

RELATED PROJECTS

None.

PUBLICATIONS

Williams, T.M. and Zavanelli, M. (2007) Natural Neuroprotection in the Brains of Marine Mammals: Why swimming dolphins don't stroke. *Integrative and Comparative Biology* [in press, refereed].

HONORS/AWARDS/PRIZES

T.M. Williams, UCSC, 2007 Women of Discovery Sea Award, WingsWorldquest