BUMED Animal Use Appendix for Research Involving Animals
ABBREVIATED VERSION

*****NOTE: BUMED will ONLY review protocols that have been approved by your IACUC.*****
****Animal work MAY NOT be initiated until the awardee receives BUMED approval.****
Animal work initiated without BUMED approval is noncompliant and may not be funded.

Institutions using DoD funds to support the use of animals in research, product development, testing and education projects must provide electronic copies of the following documents to the Department of the Navy (DON) Bureau of Medicine and Surgery (BUMED) Veterinary Affairs Office for review and approval prior to initiation:

a. A copy of their **IACUC-approved institutional protocol(s)** (BUMED will ONLY review **approved** protocols) and documentation of IACUC approval

b. A copy of all existing IACUC-approved protocol amendments or modifications and documentation of IACUC approval (**future modifications or amendments** must be reviewed and approved by BUMED **PRIOR** to implementation)

c. A completed Appendix for each IACUC-approved protocol.

This requirement also applies to all subcontractors using animals in support of DoD-funded projects or programs.

Specific information requested in the following animal use Appendix is derived from requirements in the Animal Welfare Regulations (AWRs), the *Guide for the Care and Use of Laboratory Animals*, and other applicable Federal and DoD regulations. The DoD policies and requirements for the use of animals in DoD-supported research, development, testing and evaluation are described in DoD Instruction 3216.01, dated September 13, 2010 and SECNAVINST 3900.38C, *The Care and Use of Laboratory Animals in DOD Programs*, dated February 16, 2005. These requirements differ from those of other funding agencies. Use of the Appendix is intended to meet the requirements of these documents.

Questions concerning animal use and review should be directed to BUMED Veterinary Affairs:

Phone: 301-619-9241 or 301-619-9224
Email: DON – VRPP, usn.ncr.bumedfchva.mbx.don---vrpp@mail.mil
Mail: Department of the Navy Bureau of Medicine and Surgery
ATTN: Director for Veterinary Affairs
Bldg 1564 Room 122
1564 Freedman Drive
Fort Detrick, MD 21702

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Each section of this Appendix must be completed. To assist you in completing this appendix, instructions and explanations are provided as *hidden, red and italicized text*. To view the instructions and/or examples for each section, select the "**Show/Hide ¶**" button on your tool bar (the button itself appears as the ¶ symbol). To print the hidden text, select "Print Hidden Text" in the print options section. It is important that you carefully **read the instructions** for each paragraph to ensure you provide a comprehensive response. Begin typing responses after the colon (":") for each section to ensure your typing is not within the hidden text. Submit electronic copies of the appendix only; please do not submit printed copies to BUMED. Any section of the Appendix that is not applicable to your proposal, e.g., no surgery or no prolonged restraint, should be marked "**No**" or "**N/A**". There are no space limitations for the responses.

It is essential that only animal studies or procedures documented in an IACUC–approved protocol or amendment be performed at your facility. BUMED will collect animal usage information for end-of-year DoD animal use reports separate from the grant annual progress reporting requirement throughout the life of your award. For this reason, Principal Investigators or other delegated research personnel should keep accurate records and be able to provide an audit trail of all animal use that correlates to their approved protocol.
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X	Yes		No	All animal studies described in the attached IACUC-approved protocol are funded by this DOD award/contract. If no, STOP ! You must use the **full version** of the Animal Use Appendix.

1. Administrative Data: (Provide Attending Vet, IACUC, and Research Office info for the work site.)

DoD/Contract PI Name:	Jay B. Dean, PhD		
Grant/Contract PI Email:	jaydean@usf.edu	**Phone:**	813-974-1547
Protocol PI Name:	Jay B. Dean, PhD		
Protocol PI Email:	jaydean@usf.edu	**Phone:**	813-974-1547
Animal Research Site (RS):	University of South Florida, Tampa, FL		
RS Attending Veterinarian:	Dr. Norman Wiltshire, DVM, DACLAM, Assoc. Dir. Comp. Med.		
Attending Vet Email:	wiltshire@usf.edu	**Phone:**	(813) 974-3673
RS IACUC Point of Contact (POC):	Dr. Shari Pilon-Thomas (Vice-Chair USF IACUC); [Note: Dr. Jay B. Dean (P.I.) is the Chair of the USF IACUC]		
IACUC POC Email:	Shari.Pilon-Thomas@moffitt.org	**Phone:**	(813) 745-4247
RS Grants Office Point of Contact (POC):	Ms. Bethany Kincaid		
Grants Office POC Email:	bkincaid@usf.edu	**Phone:**	(813) 974-5044
Animal Protocol Title:	Mitigation of CNS oxygen toxicity: mitochondria-targeted antioxidant therapy and aquaporin gas channel		

2. Total Number of Animals Used (by Species) and USDA Pain/Distress Category:

SPECIES	HIGHEST USDA PAIN/DISTRESS CATEGORY (B, C, D, E*) See table below for definitions	TOTAL NUMBER
Sprague-Dawley rat	B	25
Sprague-Dawley rat	C	122
Sprague-Dawley rat	E	153
Mouse: Aquaporin-4 KO	E	20
Mouse: C57BL/6 WT	E	50

USDA Pain/Distress Category Definitions:

- ***Column B:*** *Animals being bred, and animals being held for use in research, testing, teaching, experiments or surgery but not yet used for those purposes.*

- ***Column C:*** *List the number of animals that will experience no more than slight or momentary pain or distress as a result of experimental manipulations or procedures on this protocol.*

- ***Column D:*** *List the number of animals that will potentially experience more than momentary or slight pain or distress that* ***WILL*** *be alleviated through the use of anesthetics and/or analgesics.*

- ***Column E:*** *List the number of animals that will experience more than momentary or slight pain or distress that* ***WILL NOT*** *be alleviated or relieved with anesthetics or analgesics.* **If any animals are listed in USDA Column E (Unalleviated Pain or Distress), the PI must provide a scientifically valid justification for withholding pain relieving medication:**. .

  - USDA Pain Category E (= pain category C in USF IACUC animal use protocol): Animals listed in USDA Pain Category E will be exposed to hyperbaric oxygen until onset of two bouts of seizures, which may cause discomfort. The potential for discomfort is why these animals are placed in USDA pain category E. The animals are unanesthetized in these experiments so that their visible behaviors can be used as one of the criteria to score onset of a seizure; we use a six-point scale of behavior to evaluate the type of seizures. Sensitivity to hyperbaric oxygen is blunted in an anesthetized animal and thus the latency time to seizures is severely lengthened plus a higher oxygen pressure is required. After the second bout of seizures occurs the breathing atmosphere is changed from 100% O_2 to air and the animal chamber is decompressed at a rate of ~1 atmosphere/minute. The 1st bout of seizures is short lasting (seconds) and during the interictal period before the 2nd bout of seizures the animal, remarkably, appears fine for all intents and purposes based on its behavior. After recovery at room pressure, the animal appears normal based on its behavior.

3. Literature Search for Unnecessary Duplication: This search is required for all animal use proposals. Note the DoD-specific database requirements in subparagraph a.

a. Literature Source(s) Searched:

http://www.ntis.gov/products/fedrip.aspxhttp://projectreporter.nih.gov/reporter.cfm

☐ Federal Research in Progress (FEDRIP) http://www.ntis.gov/products/fedrip.aspx

or

☒ Research Portfolio Online Reporting Tool Expenditures and Results (RePORTER) http://projectreporter.nih.gov/reporter.cfm

b. Date of Search: 16-17 December 2019

c. Years Covered By Search: 1994-2019

d. Key Words and/or Search Strategy:

Search 1: ((mitochondria targeted antioxidant therapy) or MitoQ or Mitoquinone), hyperbaric oxygen, oxygen toxicity, rat, ((brain slice) or (radio telemetry))

Search 2: hyperbaric oxygen, oxygen toxicity, seizure, aquaporin, gas channel, mouse

e. Results of Search:

Search 1: Searched in Projects,Publications and publication year limited between 1994 and 2019, Limit to:Project Abstracts, Admin IC: All, Fiscal Year: All Fiscal Years. Your search resulted in 0 projects. There were no relevant projects and publications found, even with altering the combination of words used in a search.

Search 2: Text Search: Search in Projects, Publications and publication year limited between 1994 and 2019, Limit to: Project Abstracts, Admin IC: All, Fiscal Year: Active Projects. Your search resulted in 0 projects. There were no relevant projects and publications found, even with altering the combination of words used in a search.

****Questions 4-10 refer to the Research Site and Protocol Principal Investigator.****

Information and/or documents required in questions 4-8 should be obtained from the research site's IACUC or veterinary staff. Documents may be provided directly to BUMED by the Protocol Principal Investigator (PI) or institution staffs. If the Protocol PI prefers that BUMED staff contact the institution to obtain this information and/or documents, the PI must specifically request this action in writing. The Protocol PI should contact BUMED (see contact information on cover page) to arrange for submission of this written request.

4. Institutional Animal Care and Use Committee(s) (IACUC) Approval(s):

Institutional Animal Use Protocol Number:	IS00007438		
IACUC (initial) approval date:	December 13, 2019	Protocol expiration (rewrite) date:	December 10, 2022

5. Veterinary Care: Provide a brief description of the veterinary care plan at the research site. Describe routine care; weekend, holiday, and emergency care; and identify whether the attending veterinarian is on staff full-time or by contract.

USF MCOM Comparative Medicine is fully accredited by AAALAC International as program #000434, is managed in accordance with the Guide for the Care and Use of Laboratory Animals, the Animal Welfare Regulations, the PHS Policy, the FDA Good Laboratory Practices, and the IACUC Principles and Procedures of Animal Care and Use, has an assurance #D-16-00589 (A4100-01) on file with OLAW/PHS and maintains registration #58-R-0015 with USDA/APHIS/AC.

Comparative Medicine veterinarians are full-time staff, Florida-licensed, members of the AVMA, and are specialty board certified by ACLAM, and/or have research doctorates. Comparative Medicine maintains institutional membership with AALAS. All program staff are AALAS-certified, and many are either registered with the Society of Quality Assurance, and/or certified by the Academy of Surgical Research or are State of Florida-certified veterinary technicians.

Daily routine, weekend, holiday, and emergency animal care is provided by animal care program staff, all of whom are certified by the American Association for Laboratory Animal Science (AALAS), and more staff are certified at the highest, technologist level {LATG) than any other level. Cage changes occur twice weekly. Health surveillance occurs twice daily and assesses: food and water intake, fecal output, assurance of water supply and patency, and general appearance of animals (e.g. presence of lesions/injuries, coat condition, disposition). If at any time there is a medical emergency, the clinical veterinarian is notified immediately.

6. Institutional Accreditation / Assurances:

a. Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) Accreditation (do **NOT** provide AAALAC correspondence)**:**

[X] Yes [ ] No Animal work is being performed at an AAALAC International-accredited facility.

b. Public Health Service Animal Welfare Assurance Statement:

[X] Yes [ ] No Animal work is being performed at a PHS-assured facility.

c. Non-accredited, Unassured Facilities: If neither 6.a. nor 6.b. above apply to the facility where animal work is being performed, submit a statement signed by the Institutional Official that states the care and use of animals will be conducted in accordance with the National Research Council's *Guide for the Care and Use of Laboratory Animals* and applicable Federal and DoD regulations.

7. Animal Procurement:

[ ] Yes [ ] No If the protocol involves Animal Welfare Act-regulated species, are the animals obtained legally from suppliers licensed by the USDA? If the supplier claims exemption from USDA licensing, provide confirmation from the research site's IACUC that the exemption criteria have been met. If work is conducted outside the US, have animals been obtained legally in accordance with national policy? If wildlife are used, provide IACUC assurance that animals have been obtained legally and provide copies of applicable state, federal and/or international capture and use permits.

8. Overseas / Foreign Country Animal Work:

[ ] Yes [X] No Animal work will be performed outside the United States.

If "Yes," answer the following questions.

a. What is the law or regulation governing the use of animals in research in the research site's host country? Please provide a copy or link to this law or regulation in English.

b. Does the research site's host country adhere to European Union (EU) Directive 86/609 or EU Directive 2010/63 standards of animal housing and care?

c. If the research site is in Canada, does the institution hold a Canadian Council on Animal Care (CCAC) certificate?

d. Does the research site adhere to any national or international standards of animal housing and care that are more stringent than the host country's laws or regulations (such as AAALAC or CCAC)? If so, please describe below or provide a document, in English, that describes these standards.

e. Does the research site's host country or local institute require a local ethical committee review or Animal Care and Use Committee review? If so, please describe below or provide a document, in English, describing the committee's membership, purpose, authority and function.

9. Site Visits

	Yes	**X**	No	Does animal work involve at least one of the following species: dogs, cats, nonhuman primates, marine mammals? If yes, provide a planned start date for work in these species and point of contact for site visit coordination. Based on accreditation status, species used, and type of research conducted, a site visit to the performance site may be required.

10. Protocol Principal Investigator Assurances:

The law specifically requires several written assurances from the P.I. Please read and sign the assurances as indicated (this page may be photocopied and signed).

As the Principal Investigator on this protocol, I acknowledge my responsibilities and provide assurances for the following:

A. Painful Procedures: I assure that discomfort and injury to animals will be limited to that which is unavoidable in the conduct of scientifically valuable research and that analgesic, anesthetic, and/or tranquilizing drugs will be used where indicated and appropriate to minimize pain and/or distress to animals.

B. Animal Use: The animals authorized for use in this protocol will be used only in the activities and in the manner described herein, unless a modification is specifically approved by the IACUC and the DoD Department of the Navy **Bureau of Medicine and Surgery** (BUMED) Veterinary Affairs Office prior to its implementation.

C. Duplication of Effort: I have made a reasonable, good faith effort to ensure that this protocol is not an unnecessary duplication of previous experiments.

D. Statistical Assurance: I assure that I have consulted with a qualified individual who evaluated the experimental design with respect to the statistical analysis, and that the minimum number of animals needed for scientific validity will be used.

E. Training: I verify that the personnel performing the animal procedures/manipulations/ observations described in this protocol are technically competent and have been properly trained to ensure that no unnecessary pain or distress will be caused to the animals as a result of the procedures/manipulations.

F. Responsibility: I acknowledge the inherent moral, ethical and administrative obligations associated with the performance of this animal use protocol, and I assure that all individuals associated with this project will demonstrate a concern for the health, comfort, welfare, and well-being of the research animals. Additionally, I pledge to implement animal use alternatives where feasible, and conduct humane and lawful research.

G. Scientific Review: This proposed animal use protocol has received appropriate peer scientific review, and is consistent with good scientific research practice.

Jay B. Dean (b) (6)

(Protocol Principal Investigator Printed Name) (Protocol Principal Investigator Signature and Date)

NOTE: In accordance with SECNAVINST 3900.38C, the DON BUMED Veterinary Affairs Officer (or designee thereof) will conduct a site visit to all sites using nonhuman primates, dogs, cats or marine mammals in the proposal, or where a site visit is deemed warranted.

United States Department of Agriculture
Animal and Plant Health Inspection Service

EGONZALEZ
2016090000486416 Insp_id

Inspection Report

UNIVERSITY OF SOUTH FLORIDA
12901 BRUCE B DOWNS BLVD MDC 20
TAMPA, FL 33612

Customer ID: **930**
Certificate: **58-R-0015**
Site: 001
UNIVERSITY OF SOUTH FLORIDA

Type: FOCUSED INSPECTION
Date: 03-DEC-2020

No non-compliant items documented on this inspection report.

This inspection and exit interview were conducted with IACUC representatives.

Prepared By: ELBA GONZALEZ
USDA, APHIS, Animal Care
Title: VETERINARY MEDICAL OFFICER
Date: 04-DEC-2020

Received by Title: Attending Veterinarian
Date: 04-DEC-2020

United States Department of Agriculture
Animal and Plant Health Inspection Service

Customer: 930
Inspection Date: 03-Dec-2020

Species Inspected

Cust No	Cert No	Site	Site Name	Inspection
930	58-R-0015	001	UNIVERSITY OF SOUTH FLORIDA	03-DEC-2020

Count	Scientific Name	Common Name
000000	NONE	NONE
000000	Total	

New Animal Use Protocol (AUP) Submission Checklist

This checklist has been prepared to assist PIs with a new AUP submission. Please check off each relevant item applicable to this protocol. The protocol will not be accepted without this form. Additionally, incomplete protocols will be returned.

☒ Animal Use Protocol (AUP) Form (*Version Date: 02/2021*)

☒ All Species (x NHP) Enrichment / Socialization Form (*Version Date: 02/2021*)

☐ Non-Human Primate (NHP) Enrichment / Socialization Addendum (*Version Date: 02/2021*)

☒ Electronic copy of funded grant(s) supporting this work (*Emailed to IACUC on:* Click here to enter a date.)

☒ Rodent Breeding Addendum (*Version Date: 10/2021)*

☐ Hazardous Agent Addendums – Pathogenic Organisms, Radioactive Materials & Other (*Version Date: 09/2020*)

☐ Hazardous Agent Addendums – Chemical Level 1 or Chemical Level 2 (*Version Date: 09/2020*)

☐ PI-Managed Satellite Housing Facility Addendum (*Version Date: 02/2021*)

☐ IBC Approval Letter or IBC application status: _____

☐ RSC Approval Letter or RSC application status: _____

☐ ESCRO Approval Letter or ESCRO application status: _____

☐ UMMC Administration Approval Letter or UMMC review status: _____

☒ Survival Surgery Consult **ALL SPECIES**
Date of Consult (*within last 3 months*): Feb 2, 2022
Veterinarian Consulted: Dr. Jessie Duggan

☐ Non-survival Surgery Consult **USDA regulated species only**
Date of Consult (*within last 3 months*): _____
Veterinarian Consulted: _____

☐ BVAMC Attending Veterinarian Consult
Date of Consult (*within last 3 months*): _____
Veterinarian Consulted: _____

☐ BVAMC SRS Approval or RPSS application status: _____
****NOTE: A new RPSS must be submitted with each new IACUC protocol submission.****

I certify that all required components of a new AUP submission (*as applicable*) are attached and complete.

SRT
PI or PI Designee Name (printed)

OAWA Internal Use:

____ All Relevant Items Attached
____ Current versions utilized
____ Adherence to AUP format
AUP Date Rec'd: _____

IACUC # _____

OAWA Staff Initials: _____
Date: _____

UNIVERSITY OF MARYLAND BALTIMORE
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE
-- ANIMAL USE PROTOCOL --

Principal Investigator: Stephen R. Thom Degree: MD, PhD Employee ID#: _____
Campus Mail Address: (b) (6) Dept: Emergency Medicine
E-mail: (b) (6) Office Phone: (b) (6) Emergency Contact # *(24 hr access)*: (b) (6)
Secondary Lab Contact: (b) (6) Email: (b) (6) Phone: (b) (6)

AUP Title: Neuroinflammation and glymphatic dysfunction in decompression sickness

I. RESEARCH CATEGORIES
Major Categories of Research: Please check all that apply.

☐ Antibody Production
☒ Behavioral Studies
☒ Breeding *(*attach breeding protocol as an addendum to the experimental protocol. Breeding template on OAWA Website)*
☐ Chronic or Prolonged Restraint
☒ Exogenous Substances *(e.g., any substance administered to an animal except anesthetics or analgesics)*
☒ Euthanize and Harvest Tissue
☐ Food / Fluid Regulation
☐ Hazardous Agents
☒ Imaging
☐ Investigational Device
☐ Irradiation
☒ Non-Survival Surgery
☐ Oncology / Tumor Production
☐ Physiologic Measurements *(e.g., blood pressure, telemetry, electrophysiology, etc.)*
☐ Sampling (*e.g., blood, urine, bone marrow, biopsies, etc. – collected antemortem*)
☐ Satellite Housing *[e.g. animals maintained outside animal facility >12 hours (USDA) or >24 hours (rodents)]*
☐ Specialized Diet
☐ Specialized Husbandry (*e.g., Singly housed animals, BSL3 housing, metabolic cages, reverse light cycle, etc.*)
☒ Survival Surgery
  ☐ Multiple Survival Surgery (*e.g., major + minor surgeries or multiple minor surgeries in one animal*)
  ☐ Multiple *Major* Survival Surgery (*e.g., opening body cavity more than once in one animal)*
☐ Teaching / Training
☐ Use of Hospital Equipment [(*e.g., imaging or radiation producing equipment) *refer to Use of Hospital Equipment Guidelines on OAWA Website*]

Will the animal work proposed in this protocol be carried out under Good Laboratory Practices (GLP) in support of, or intended to support, applications for research or marketing permits for products regulated by the Food and Drug Administration (FDA) or the Environmental Protection Agency (EPA), e.g., human and animal drugs, medical devices for human use, etc.? ☐ YES or ☒ NO
If YES, please contact the SOM GLP Office (b) (6) *for more information.*

II. CATEGORY OF PAIN OR DISTRESS
Only the highest category of pain or distress should be indicated.

☐ CATEGORY B – Animals bred, conditioned or held for use in teaching, testing, research, but not yet used for such purposes. (*NOTE: If tail snips are necessary for genotyping, this category is not appropriate.*)

☐ CATEGORY C – Procedures that involve no or only very brief pain or distress, with no need for or use of pain relieving drugs.

☒ CATEGORY D – Procedures involving potential pain or distress for which appropriate anesthetics, analgesics, or tranquilizers are given. *(NOTE: Contact a Veterinary Resources (VR) veterinarian for "appropriate" agents as needed)*

☐ CATEGORY E – Painful or distressful procedures for which drugs to relieve the pain or distress would adversely affect the research study. *Under 'Detailed Description of Animal Procedures' in the written protocol, please include subheading Category E Justification and provide strong scientific justification for conducting procedures that involve unrelieved pain or distress. Refer to the last page for guidance.*

III. FUNDING SOURCE (*CURRENT*)

Provide one copy of each funded grant for IACUC file. At a minimum, the Research Methods and Vertebrate Animal Section should be included with the submission.

- ☐ US Public Health Service (e.g., NIH)
- ☐ Department of Veterans Affairs
  - ☐ This research is funded by the Department of Veterans Affairs, or
  - ☐ This research is administered through the Baltimore BREF
- ☐ Private or Charitable Foundation
- ☐ Private Company
- ☐ Departmental Funds
- ☐ Other

Sponsor / Agency	Grant Identification Number*	Grant Title
Office of Naval Research	**N00014-21-S-B001**	Neuroinflammation and glymphatic dysfunction in decompression sickness

Please add rows to these tables by placing cursor at end of last row and clicking return.

Does the PI or any of the research staff have a financial interest related to the proposed research or the sponsor that must be disclosed accordingly to UMB's Conflict of Interest in Research and Development and UMB's Policy and Procedures on Financial Conflict of Interest to Promote Objectivity in Public Health Service-Funded Research?

☐ YES or ☒ NO

If YES, please contact the Research Integrity Office and speak with (b) (6) [redacted]

IV. EXPERIMENTAL ANIMAL USAGE

Each species (*by age*) should be listed separately. This table is for adult and/or juvenile (*post-weaning*) experimental animals only. ***Strain designations and the number of breeders are no longer required in this table. # of breeders should be provided in the Breeding Addendum only.*

Species	Age	Weight range *(USDA Covered Species Only)*	Sex	Total # *(for 3 yrs)*	Max. Daily Census*
Mouse	☒ Juvenile (*weaned*) or Adult (n=2144) ☐ Timed pregnant ☐ Dams w/ pups ☒ Other - *Specify*: 36 breeders		☐ Male ☐ Female ☒ Both	**2180**	6
	☐ Juvenile (*weaned*) or Adult		☐ Male		

	☐ Timed pregnant ☐ Dams w/ pups ☐ Other - *Specify*:		☐ Female ☐ Both		
	☐ Juvenile (*weaned*) or Adult ☐ Timed pregnant ☐ Dams w/ pups ☐ Other - *Specify*:		☐ Male ☐ Female ☐ Both		

*Max. Daily Census = approximate number of animals to be housed in the facility on any given day. Please note that this information is necessary to plan for housing space and should be expressed in whole numbers.
***Please add rows to these tables by placing cursor at end of last row and clicking return.*

Will embryonic and/or pre-weaning animals be used at any time during this protocol? ☐ YES ☒ NO
If yes, state species, age and number required for 3-year protocol (estimations based upon species characteristics or lab experience are acceptable).

Species	Age *(days)*	Total # *(for 3 yrs)*

Will additional animals be required for training current or new staff on proposed animal procedures? ☐ YES ☒ NO
If yes, state species, age and number required for 3-year protocol. Under 'Detailed Description of Animal Procedures' in the written protocol, please discuss training procedures.

Species	Age *(days)*	Total # *(for 3 yrs)*

V. HAZARDOUS AGENT(S) IDENTIFICATION

Please contact EHS for assistance as needed. *Link to Hazardous Agent Addendums*.

Will pathogenic organisms be utilized in live animals in this protocol? ☐ YES ☒ NO
If yes, complete a hazardous agent addendum for each pathogenic organism.

Will recombinant DNA be utilized in live animals in this protocol? ☐ YES ☒ NO
If yes, complete a hazardous agent addendum for each category, e.g., rDNA, plasmids, siRNA, viral vectors, recombinant cells, etc.

Will radioactive materials be utilized in live animals in this protocol? ☐ YES ☒ NO
If yes, complete a hazardous agent addendum for each radioactive material.

Will hazardous chemicals be utilized in live animals in this protocol? ☐ YES ☒ NO
If yes, complete a hazardous agent addendum for each chemical level, e.g., CL1 or CL2.
Please refer to EHS Hazardous Chemical Use in Animal Research website for guidance.

VI. METHOD OF EUTHANASIA

Please list each method of euthanasia to be used in this protocol. Please refer to the Recommended Methods of Euthanasia located on the OAWA Website under Guidelines. *Method and description can be cut and pasted into the below table.*

SPECIES	METHOD	DESCRIPTION
Mouse	Inhalant anesthetic overdose followed by exsanguination, perfusion with saline then paraformaldehyde and major organ harvest	Using a precision vaporizer with induction chamber and waste gas scavenger, Isoflurane, in oxygen, will be administered slowly at >4.5 % and continued until respiratory arrest occurs for > 60 seconds. Isoflurane is stopped, the chamber is flushed with oxygen only, the animal is removed from chamber, rapidly exsanguinated by cardiac puncture or thoracotomy and using a chemical fume hood, then perfused with saline followed by

		paraformaldehyde for brain harvest and immunohistochemical analysis. Excess perfusate will be collected and EHS contacted for disposal.
Mouse	Post-Ketamine/xylazine anesthesia followed by exsanguination	IP Ketamine (80-150 mg/kg), xylazine (8-15 mg/kg). Injectable anesthetic administered IP using a 25 gauge needle to a level of surgical anesthesia followed by exsanguination and organ harvest.

***Please add rows to this table by placing cursor at end of last row and clicking return.*

VII. LOCATION OF ANIMAL USAGE

BUILDING / RM # *List each location only once.*	LAB	ANIMAL FACILITY	LIST ALL PROCEDURES TO BE PERFORMED IN LIVE ANIMALS IN EACH LOCATION *(e.g., surgery, injections, behavior, imaging, euthanasia, etc.)*
BRB 4-015	☒	☐	Surgery, injections, decompression stress, behavior, euthanasia, perfusion
HH 685	☐	☒	Animal husbandry
HH645	☒	☐	MRI imaging studies
BRB 4-4039A	☒	☐	Behavioral studies
	☐	☐	

***Please add rows to this table by placing cursor at end of last row and clicking return.*

VIII. CONSIDERATION OF ALTERNATIVES TO POTENTIALLY PAINFUL / DISTRESSFUL PROCEDURES

Keep copies of computer database search results in your protocol file for a minimum of three years after the protocol has expired to demonstrate your compliance with the law if regulatory authorities or the IACUC should choose to audit your project. *Refer to instructions for further information regarding the completion of this section.*

1. Do any of the following criteria apply to this animal use protocol? ☒ YES ☒ NO
   If yes, check all that apply and complete question 2. If no, STOP questions 2-6 are not required!

   ☐ USDA Covered Species (*any species other than mice, rats, birds, fish, amphibians or reptiles*)
   ☐ Pain / Distress Category E
   ☐ Funded by the VA
   ☒ Funded by the DoD

2. Does this protocol include procedures that have the potential for producing more than momentary or slight pain or distress? ☒ YES ☐ NO *If yes, complete questions 3-6. If no, STOP questions 3-6 are not required!*

3. What are the potentially painful procedures or distressful conditions proposed in this animal use protocol?

   Survival surgery (ligation od lymphatic channels), decompression sickness

4. A literature search for alternatives to the potentially painful or distressful procedures (*listed above*) was performed. Search methods and sources are documented in table below:

Name of Database Searched: (*Minimum of 2*)	**Date search was performed:** (*mm/dd/yy*)	**Years covered by the search:** *(from yyyy to yyyy)*	**Search Strategies used:** (*including scientifically relevant terminology and Boolean operators*)	# of hits:	# of relevant hits:
FEDRIP	2/19/22	1085-2022	Decompression sickness and animal model	89	9

			Decompression sickness and alternative	0	
			Decompression sickness and glymphatics	0	
			Lymphatic ligation and glymphatics	14	6
			Lymphatic ligation and alternatives	0	
RePORTER	02/19/22	1985-2022	Decompression sickness and animal model	43	6
			Decompression sickness and alternative	0	
			Decompression sickness and glymphatics	0	
			Lymphatic ligation and glymphatics	12	4
			Lymphatic ligation and alternatives	0	

Database Resources: UMB HS-HSL Databases and AWIC

5. Did the literature search reveal less painful or distressful alternatives to the potentially painful or distressful procedures that are proposed?

   ☒ No alternatives, refinements, replacements or reduction methods were found.

   ☐Yes, but they cannot replace the procedures that are proposed. *See below explanation*.

   a. If yes, identify the painful or distressful procedures(s) and provide a BRIEF explanation why the alternatives found to this potentially painful or distressful procedure(s) were not acceptable alternatives.

      Click here to enter text.

6. List other sources of information used to refine this protocol, as applicable.

   Click here to enter text.

PRINCIPAL INVESTIGATOR'S ACKNOWLEDGEMENT OF RESPONSBILITY

I acknowledge and accept the responsibilities associated with serving as the principal investigator of an IACUC protocol as delineated in the OAWA Principal Investigator Manual.

I will conduct the project in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, USDA regulations (9 CFR parts 1, 2, 3), the Federal Animal Welfare Act (7 USC 2131 et. Seq.), and the *Guide for the Care and Use of Laboratory Animals*.

I authorize individuals listed on this application to conduct procedures involving animals and I accept responsibility for their oversight in the conduct of this proposal.

I confirm that all individuals working with animals have completed the IACUC required training and are participating in an appropriate Occupational Health & Safety program. Further, I certify that those individuals are properly trained, or will receive such training prior to working with animals, in all areas relevant to their assigned work with animals (e.g., biology, handling, and care of the species used; aseptic surgical methods and techniques; the concept, availability, and use of research or testing methods that limit the use of animals or minimize distress; the proper use of anesthetics & analgesics; and procedures for reporting animal welfare concerns).

For animals under this proposal, I understand that in cases of necessary medical treatment, Vet Resources veterinarians are authorized to provide any treatment required to sustain life; or if necessary, provide humane euthanasia to prevent unapproved distress and/or pain. I recognize that the veterinary staff will contact me as soon as possible using the emergency contact information that I provide in this application, but I understand that such contact may not always be possible prior to providing treatment or performing euthanasia.

I will notify the IACUC of unanticipated outcomes of animals use, including protocol or non-protocol disease or injury. Unanticipated outcomes are generally defined as negative impacts to animal welfare or well-being.

I recognize that veterinary consultation must occur when pain or distress is beyond the level IACUC approved in this protocol, or when my staff are unable to provide interventional control (i.e., euthanasia, immediate removal from the

study). I will notify VR veterinary staff when unanticipated pain or distress, unexpected morbidity, or unanticipated mortality occurs.

I will obtain approval from the IACUC before initiating any change in the study design or procedures by submitting an amendment request. I understand that work performed without IACUC approval cannot be published with certification of IACUC approval and may result in federally required reporting of non-compliance.

I am familiar with the University of Maryland Baltimore Environmental Health and Safety (EHS) policies and procedures relative to laboratory safety, biohazards, radiation safety, occupational health and safety, laser safety, chemical and biological waste management practices, select agents, and shipping of infectious materials and clinical specimens. I will abide by the regulations, policies and procedures that relate to research, testing or training at UM. I will ensure that all laboratory personnel engaged in this project will be informed of potential hazards and adequately trained in procedures of animal experimentation involving hazardous agents.

___via email _____ _3/8/2022_
Signature Date

__Stephen R Thom_____
Printed Name

_____
Signature – VA Attending Veterinarian *(if animal work is funded by the VAMC or conducted in the VAMC)*

WRITTEN PROTOCOL

Lay Summary *(limit response to ¼ - ½ page)*:

1. Using non-technical (lay) language, please describe the purpose of the proposed study.

Decompression sickness (DCS) presents a health risk for compressed air divers and caisson/tunnel workers, and it poses a constraint to provocative diving and submarine escape. Bubbles play a role in pathophysiological responses mediating DCS, however, they are often produced without symptoms. Therefore, additional etiological factors have been sought to explain DCS development. Microparticles (MPs, small vesicles present in the circulation) are elevated in association with simulated as well as *bona fide* underwater diving. These MPs carry inflammatory proteins that damage blood vessels throughout the body, including those in the brain. Maneuvers which decrease the incidence of DCS in the animal model also diminish MPs production in human divers. As MPs cause vascular damage and may also participate in gas bubble nucleation (a required stimulus to form bubbles in the body), their formation presents a feasible explanation for most aspects of DCS pathophysiology. Hence, further research focused on MPs has direct relevance to U.S. Navy operational diving, commercial, scientific and sport divers whose activities are constrained by DCS risk. The only meaningful intervention that has come from decades of traditional DCS research is staged decompression tables to limit symptom development. This project will take a novel approach to identify the role of neuroinflammation and MPs in DCS and further explore the potential use of a pharmaceutical agents that we have found to abrogate MPs injuries in other disorders. These might prove to be useful as clinical prophylactic and therapeutic interventions.

2. Describe the potential scientific benefit of the proposed study with respect to human and animal health, the advancement of knowledge, or the good of society.

These studies will improve understanding of decompression sickness and its treatment. While previous studies by us and others suggest that pressure exposure followed by decompression causes an adaptive immunological response, the results do not explain early activation of blood-borne white cells (neutrophils), the predilection of neutrophils for attacking the blood vessel lining of the brain and elsewhere, or why functional deficits are manifested over hours to days after the exposure. Another unexplained observation is the ability to transmit the decompression-induced insult to naïve mice by injections of microparticles isolated from the blood of decompressed mice. These are the specific points that will be addressed in this project.

Justification for the use of animals and species (*limit response to ¼ - ½ page*):

1. Please justify the use of animals in general. Discuss whether other alternatives (*e.g., cell culture, computer modeling/simulation*) to animal usage exist and if so, why they are not feasible, etc.

Currently, no non-animal model of decompression sickness can effectively reproduce the effects of brain injuries seen in animal and human exposures. Mice are well characterized in the laboratory and have been chosen to produce the most widely used animal brain injury models allowing adequate breeding, use of specific knock-out mice, surgical and post-surgical physiologic monitoring. Mice have been widely used in the studies of many brain injury including carbon monoxide, carbon dioxide, hypoxia and ischemia, making comparisons between our results and other studies possible. Our lab has well-characterized rat and mouse decompression sickness models. Per our database searches we found no alternative model using a less sentient animal, computer or cell culture model meeting the scientific aims of this submission.

2. Please document why a particular species was chosen for these studies (*cost is not acceptable as the sole justification*). Additionally, please justify the use of single sex / gender animals, e.g., experiments utilizing all males or all females.

We chose C57BL/6J mice because they are the most extensively used species/strain for studies of many brain injuries, including many of our prior studies. Experiments will be performed using equal numbers of male and female mice.

3. **Strain Identification** *(Experimental & Breeder Animals)*

Strain	Brief description of relevance to the proposed research
C57BL/6J	Mouse strain most often used in mouse decompression, CO, hypoxia, traumatic brain injury and stroke models.
B6.129S1-*Cd36*^{tm1Mfe}/J **[aka CD36 KO]**	CD36 knock-out mice exhibit resistance to some ischemia-reperfusion and hypoxia insults. The mice B6.129S1-***Cd36tm1Mfe***/J lack the CD36 protein, a cell-surface pattern recognition receptor, and have been found resistant to brain ischemia reperfusion. Prior work in our lab has shown these mice do not generate inflammatory microparticles because of the CD36 absence on endothelium, astrocytes and microglia. Therefore, we believe these animals will be of major use in this investigation.

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Do any of the above strains exhibit any known phenotypic variations that could potentially affect animal health, well-being or longevity? ☐ YES* ☒ NO
*If yes, please describe:

Do any of the above strains require phenotype induction, e.g., pharmacologically activated, siRNA, viral induced, etc.? ☐ YES* ☒ NO
*If yes, please include induction methods under *Detailed Description of Animal Procedures*.

Experimental Summary / Animal Usage (*limit response to ¼ - ½ page*):

1. **What are the objectives or underlying hypothesis of the planned experiments? Provide background information on the science. Information provided will be similar to the background section on a grant application but abbreviated to convey what has already been done in the field at large and why these experiments should be done.**

This research project is to evaluate the neuroinflammatory impact of high pressure and decompression using a murine model. It is specifically focused on neurological injuries that occur in approximately 30% of severe clinical decompression sickness (DCS) cases. Upwards of 60% of these manifest global changes that cannot be explained by other processes, such as formation of gas emboli within the blood vessels. The motivation for the project is to establish the molecular basis for neurological DCS. We have found that inflammatory microparticles (MPs) elevated in humans, and in animal models of DCS trigger dysfunction of the brain glial-based lymphatic (aka glymphatic) system in mice. We have identified a complex pathological interaction between brain-generated MPs and systemic inflammation that may resolve several unexplained clinical observations. We have also identified knock-out mice resistant to DCS pathology and a pharmaceutical in use in humans that protects against CNS injuries. Further, a recent population-based study demonstrated that those sustaining DCS have a 5.7-fold increased risk of developing a sleep disorder as a manifestation of CNS injury, Glymphatic dysfunction has been clearly shown to disturb sleep in multiple animal models.

Pharmaceuticals we will use are agents shown in prior work to eradicate circulating MPs and/or antagonize injuries mediated by the inflammatory cargo they carry. Therefore, studies will be done with an agent introduced into the mouse assess how they alter decompression-induced metabolic and glymphatic changes. Finally, we also plan to use knock-out (KO) mice that do not possess the CD36 protein, as we hypothesize that CD36 is required to allow inflammatory signals to be transmitted from blood to the brain. Others have shown that CD36 KO mice are resistant to brain ischemia-reperfusion damage, presumably because of diminished signaling. Pilot studies have shown CD36KO mice do not generate MPs and are resistant to decompression injuries. We hypothesize these KO mice will be useful to discern the

dynamics of communication between blood-borne MPs and transmission of inflammatory signals across the blood-brain barrier.

2. Does any of the proposed research duplicate previous work? ☐ YES ☒ NO

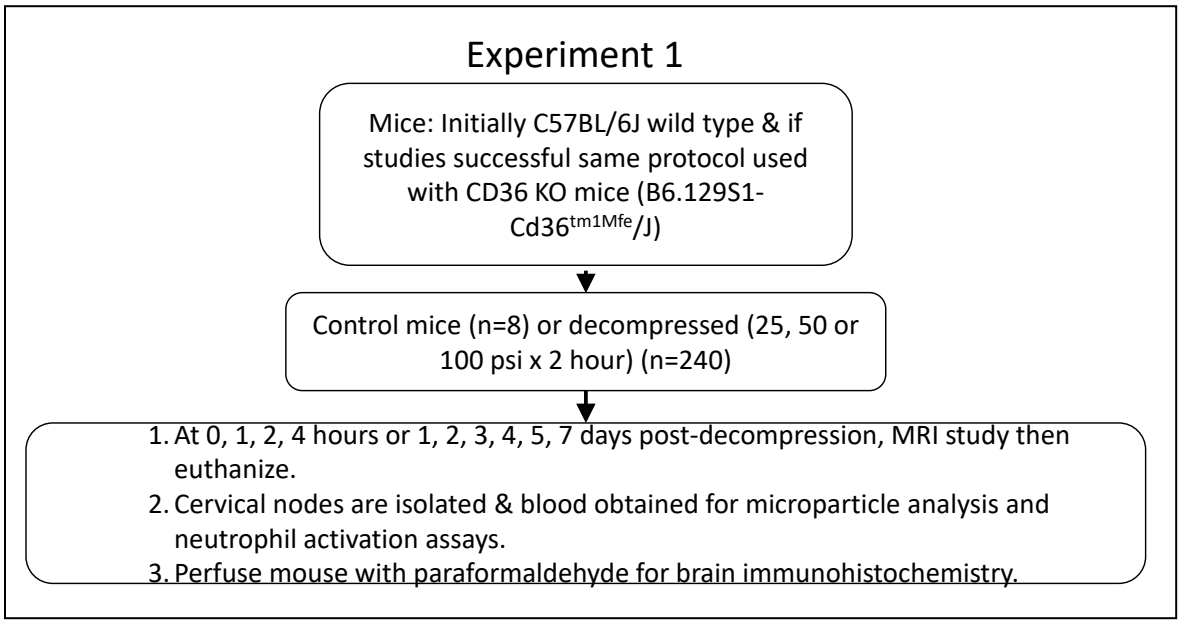
If yes, please discuss the rationale for this duplication.

Click here to enter text.

3. Planned Experiments. *The following information must be provided for <u>each</u> planned experiment.*

<u>Experiment 1</u> – Brain glymphatic flow assessment by Magnetic Resonance Imaging

Briefly describe the objective of this experiment. Studies will establish the time-course and pattern of glymphatic flow changes after mice are exposed to the standard



decompression stress (100 psi air pressure for 2 hours) as well as graded lower exposures more within established US Navy limits (25 or 50 psi for 2 hours). Note that in rodent studies since 1990 these exposures do not cause animal discomfort. The purpose of these experiments is to assess whether glymphatic dysfunction occurs at lower pressures. Times for studies were chosen based on knowledge of MPs production events. Glymphatic flow in small animal studies is typically assessed using cisterna magna injections of fluorescent tracers. MRI is a potential better method (fewer artifacts) and could be an adjunctive and/or alternative method but needs validation.

Describe what will happen to the animals from start to finish. Mice will be exposed to high pressure for 2 hours in the PI's lab, removed from the pressure chamber and studied at 0, 1, 2, 4 hours, or at 1, 2, 3, 4, 5 or 7 days post-exposure by placing a tail vein catheter for gadodiamide injection. If mice are to be studied more than 4 hours later they will be returned to the vivarium for standard housing until study. At the time the MRI study is to begin, the mouse will have a catheter placed in the tail vein in the PI's lab and then transported for the MRI study to the Core for Translational Research in Imaging @Maryland (C-TRIM), CIBR/Core Facilities, HH 645). The animal will be anesthetized in an animal chamber using a gas mixture of O_2 (1 L/min) and isoflurane (>3-4.5 % induction; >1-3% maintenance). The animal will then be placed prone in an animal holder with a nose cone and a bit bar. The surface RF array coil will be positioned over the head of the animal. The animal's eyes will be treated with a petrolatum based ophthalmic lubricant without antibiotics to prevent corneal desiccation. The animal holder will be moved to the center of the magnet and the isoflurane level will be changed to 1.5 to 2 %. The level of isofluorane will be further adjusted based on the respiration rate changes of the animal for the remainder of the experiment. A MR compatible small-animal monitoring and gating system (SA Instruments, Inc., New York, USA) will be used to monitor the animal respiration rate and body temperature. The animal body temperature will be maintained at 35-37°C using a warm water circulation. After moving the mouse on the imaging bed into the magnet, a system calibration will be performed followed by injection of a contrast agent

(gadodiamide) needed for detailed imaging. The IV injection of gadodiamide will be started (0.12 g/kg introduced at 2 µl/minute using an infusion pump) and a series of image acquisitions will followed for the next 2 hours to monitor contrast intake and efflux from the brain. After the study, the animal will be moved out from the magnet and the isoflurane will be stopped. The animal may be returned to the PI lab for euthanasia following the approved protocol. We then obtain brain tissue, blood cells, and cervical lymph nodes for analysis.

What is the longest that any one animal will be involved in an experiment? 1 week

List each experimental and control group, including the group size for each group (n=?). We anticipate that group sizes of 8 mice (4 male, 4 female) will be adequate to overcome variability and allow statistical analysis. There will be 10 experimental groups (0, 1, 2, 4 hours, or at 1, 2, 3, 4, 5 or 7 days post-exposure) and a control group. Therefore, as there are 3 pressure protocols [(3 X 10 groups X 8 mice/grp) + 8 controls] total of 248 wild type mice will be used. We expect that there will be important changes at many of these time points that require mechanistic investigation. Assuming this proves true, the same study pattern will be done repeated with CD36 knock-out mice, as outlined in the Experiment 1 figure.

Discuss what criteria were used to determine that the group size(s) proposed utilizes the minimal number of animals to generate statistically significant data. Our prior studies looking at MPs responses have required replicates of up to 8 per group to overcome variability and achieve statistical significance. Based on ANOVA we anticipated this number will achieve a 95 % confidence limit and significance level of $p<0.05$.

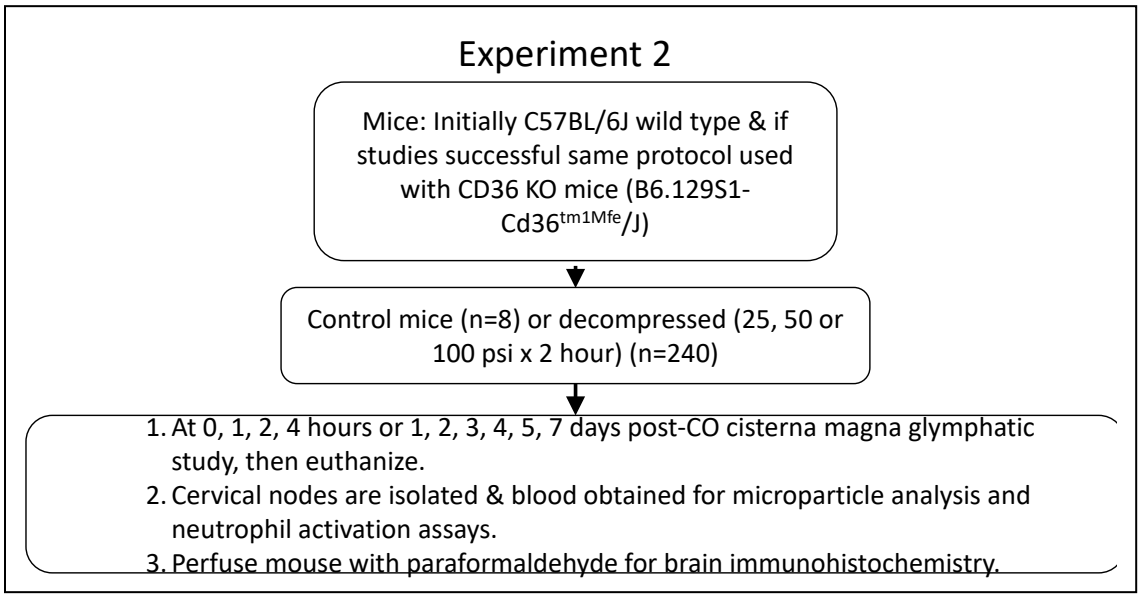
Indicate whether the experiment must be repeated and justify the number of repetitions. No repeats

Indicate the species to be used, and how many will be used *(e.g., X # mice per experiment x X # strains of mice).*
1. Wild type: group sizes of 8 mice (4 male, 4 female), 10 experimental groups and 3 pressure protocols plus a control group. Therefore, a total of 248 wild type mice will be used.

2. CD36 KO: We expect that there will be important changes at many of these time points that require mechanistic investigation. Assuming this proves true, the same study pattern will be done using the CD35 knock-out mice (n=248).

Experiment 2 – Glymphatic flow assessment by cisterna magna fluorescent tracer injections

Briefly describe the objective of this experiment. Glymphatic flow in small animal studies is typically assessed using cisterna magna injections of fluorescent tracers. Additionally this experiment is needed to validate the MRI results. As we need to do histochemical studies, we can couple the histochemical studies (*e.g.* astrocyte and microglial activation, alterations to myelin basic proteins) in the same mice. These studies will be performed entirely in the PI's lab. At the time for study after the pressure/decompression stress, mice will be anesthetized,



and a needle inserted into the cisterna magna. Contrast is injected and then the mouse euthanized at intervals for post-mortem brain analysis.

Describe what will happen to the animals from start to finish. Mice will be exposed to the high pressure/decompression stresses as described in experiment #1 and at time of study, anesthetized with isoflurane and placed in a stereotaxic apparatus that has been cleaned and equipped with a sterile 27guage needle for insertion. After cisterna magna catheter insertion, fluorescent contrast is injected at 2 µl/min for 5 minutes. The mouse will then be monitored continuously while under anesthesia for 15 to 120 minutes before euthanasia by exsanguination so that brain tissue, blood cells, and cervical lymphatics can be studied. The advantage is that using brain sections, fluorescent tracer is quantified and immunohistochemical assessments performed to evaluate perturbation/activation of cells involved with the glymphatic pathway by imaging astrocytes (AQP-4 and GFAP staining), microglia (ED-1 and CD-68 staining), oligodendroglia (native myelin basic protein [MBP] and degraded MBP) and determine their morphological changes as occur with activation and cell injury, and the relationship to cerebral blood vessels (collagen IV and smooth muscle actin staining) following our published methods.

What is the longest that any one animal will be involved in an experiment? 1 week

List each experimental and control group, including the group size for each group (n=?). We anticipate that group sizes of 8 mice (4 male, 4 female) will be adequate to overcome variability and allow statistical analysis. There will be 10 experimental groups (0, 1, 2, 4 hours, or at 1, 2, 3, 4, 5 or 7 days post-exposure) and a control group. Therefore, as there are 3 pressure protocols [(3 X 10 groups X 8 mice/grp) + 8 controls] total of 248 wild type mice will be used. We expect that there will be important changes at many of these time points that require mechanistic investigation. Assuming this proves true, the same study pattern will be done repeated with CD36 knock-out mice.

Discuss what criteria were used to determine that the group size(s) proposed utilizes the minimal number of animals to generate statistically significant data Our prior studies looking at MPs responses have required replicates of up to 8 per group to overcome variability and achieve statistical significance. Based on ANOVA we anticipated this number will achieve a 95 % confidence limit and significance level of $p<0.05$.

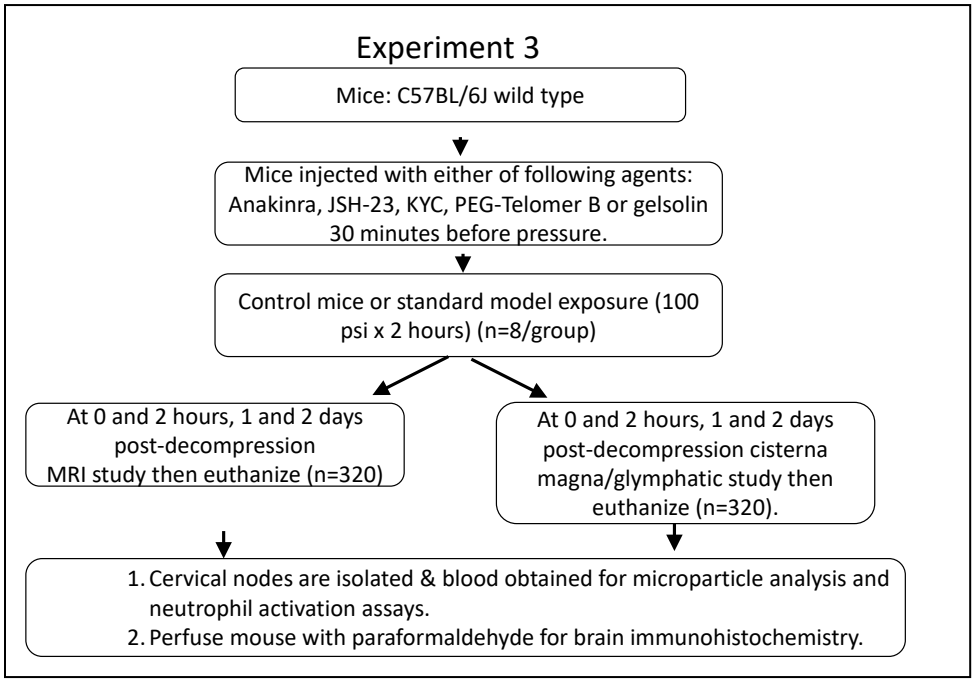
Indicate whether the experiment must be repeated and justify the number of repetitions. None

Indicate the species to be used, and how many will be used *(e.g., X # mice per experiment x X # strains of mice).*
1. Wild type: group sizes of 8 mice (4 male, 4 female), 10 experimental groups and 3 pressure protocols plus a control group. Therefore, a total of 248 wild type mice will be used.

2. CD36 KO: We expect that there will be important changes at many of these time points that require mechanistic investigation. Assuming this proves true, the same study pattern will be done using the CD35 knock-out mice (n=248).

Experiment 3 – Effect of pharmaceuticals on post-decompression glymphatic responses.

Briefly describe the objective of this experiment. The goal of this experiment is to determine whether agents which modify or eradicate MPs in blood will abrogate decompression-induced glymphatic responses. These include PEG-Telomer B, an FDA-approved surfactant that we have shown will lyse blood-borne MPs, human recombinant gelsolin that appears to lyse discretely the inflammatory types of MPs, and also agents that inhibit more classical aspects of inflammatory cascades including JSH-23 (4-methyl-N1-(3-phenyl-propyl)-benzene-1,2-diamine, an inhibitor of the NF-kB pathway), anakinra (an interleukin-1 receptor antagonist), and KYC (acetyl-lysyltyrosylcysteine, an inhibitor of myeloperoxidase). Mice will be injected with agents **30 minutes** ~~either just~~ before ~~or just after~~ exposure to 100 psi air pressure for 2 hours (standard model) to assess effects on inflammatory responses. Mice will be evaluated by MRI and cisterna magna injection studies (described in experiments #1 and #2) at times when neuroinflammatory events (assessed by immunohistochemistry and flow cytometry analysis of cervical node and blood microparticles) are documented. We predict we will need to do studies at times 0-hours, 2-hours post-decompression and days 1 and 2. After euthanasia, we will then obtain brain tissue, cervical lymph nodes and blood for complete analysis. We have based dosages for pharmaceuticals on rodent studies by us and others involved on other forms of neuroinflammation.



Describe what will happen to the animals from start to finish. Mice will be transported to the PI's lab and injected with one of 5 investigational agents. At 30 minutes after injection, they will be exposed to pressure (100 psi X 2 hours) or left as control (n=8). Mice will be studied by MRI or cisterna magna injection glymphatic assessment protocols at four time points: 0, 2 hours, 1 and 2 days post-exposure) and a control group. After the studies mice will be euthanized for brain histochemistry, lymph node and blood analyses. Therefore, a total of 320 mice will be used for each of two study types (MRI or cisterna magna).

What is the longest that any one animal will be involved in an experiment? 2 days

List each experimental and control group, including the group size for each group (n=?).

Control or decompression exposure after injections of one of 5 agents, then study by MRI or cisterna magna injections at 0, 2 hours, 1 day and 2 days post-exposure.

Discuss what criteria were used to determine that the group size(s) proposed utilizes the minimal number of animals to generate statistically significant data (*e.g., power analysis, reports in the literature, previous experience, etc.*). Our prior studies looking at MPs responses have required replicates of up to 8 per group to overcome variability and achieve statistical significance. Based on ANOVA we anticipated this number will achieve a 95 % confidence limit and significance level of $p<0.05$.

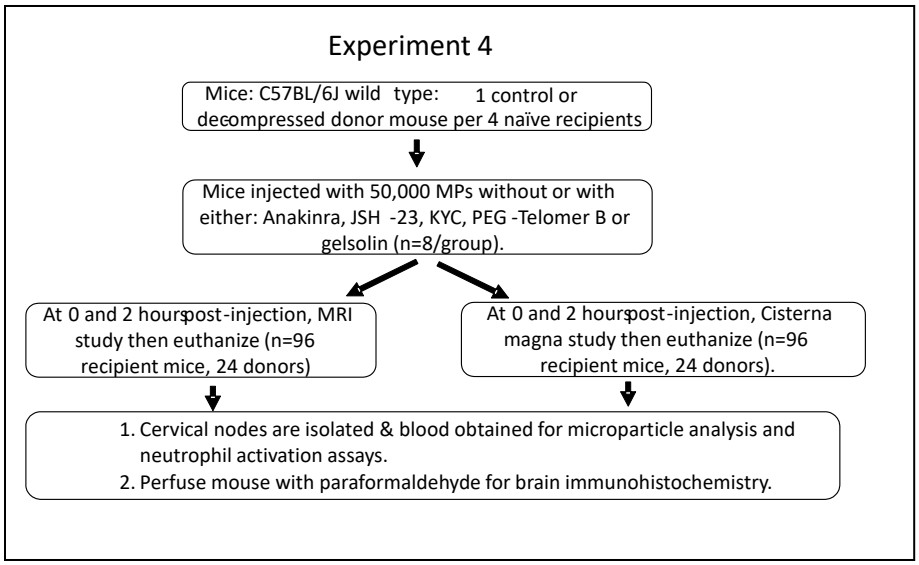
Indicate whether the experiment must be repeated and justify the number of repetitions. none

Indicate the species to be used, and how many will be used Wild type mice: group sizes of 8 mice (4 male, 4 female), control or decompression, study by MRI or cisterna magna injections at 0, 2 hours, 1 and 2 days post-

exposure. Therefore, 8 mice/group X 4 time points x 2 glymphatic analyses X control or decompression X 5 agents = 640 wild type mice will be used.

Experiment 4 – Assess glymphatic flow responses to purified MPs injections.

Briefly describe the objective of this experiment. We want to determine if MPs are the driving force for glymphatic/neurological damage post-decompression. We believe the most definitive approach to achieving this goal is to harvest MPs from blood of decompressed mice and inject them into naïve mice looking to recapitulate tissue injuries. Follow-on studies will then be performed to directly assess the pathological role for inflammatory mediators by co-injecting the MPs along with agents shown effective in experiment 3.



Describe what will happen to the animals from start to finish. Mice will be removed from the vivarium and brought to the lab. Some will be used as donors by exposing them to 100 psi air pressure for 2 hours (or control mice exposed to just air) and euthanizing them at the time of maximum MPs production (we suspect this will be 0 and 2 hours post exposure). MPs will be harvested from cervical lymph nodes, then re-suspended at 50,000 MPs/100 µl sterile PBS. Typically, 1 donor mouse provides sufficient MPs for 4 experimental mice. Recipient mice will be injected via a tail vein with 100 µl solution. Vascular and inflammatory responses to MPs have typically been seen to occur in 1-2 hours in our prior studies, so studies will be done at 0 and 2 hours post-injection (see Figure). These studies will be followed by performing the same experiment but including antagonists shown effective in experiment 3.

What is the longest that any one animal will be involved in an experiment? 1 day

List each experimental and control group, including the group size for each group (n=?). There will be 1 donor mouse for 4 recipient mice. Each time point (0 and 2 hours post-injection) will be done with 8 mice. There will be 6 groups, MPs alone or MPs with one of 5 agents); 8 x 6 = 48 mice x 2 (0 and 2 hours) = 96 recipient mice x study by MRI or cisterna magna injection (2 x 96 = 192 mice). For 192 mice we will need 48 MPs donors (24 control mice, 24 exposed to the 2 hour decompression protocol). Therefore 240 mice in total for experiment 4.

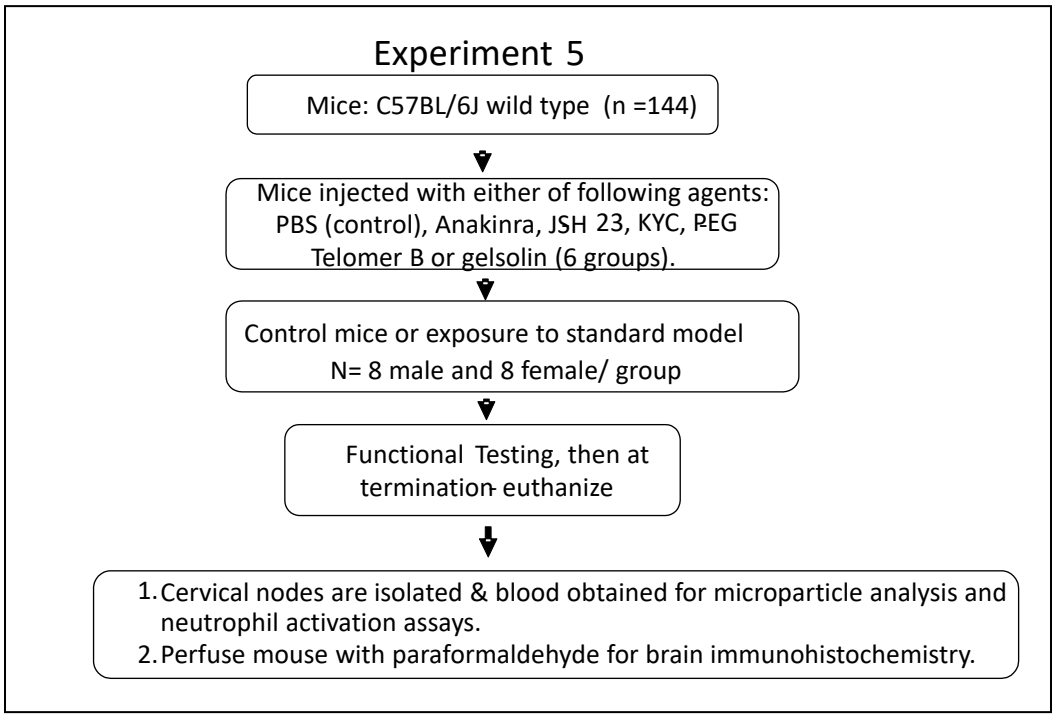
Discuss what criteria were used to determine that the group size(s) proposed utilizes the minimal number of animals to generate statistically significant data Our prior studies looking at MPs responses have required replicates of up to 8 per group to overcome variability and achieve statistical significance. Based on ANOVA we anticipated this number will achieve a 95 % confidence limit and significance level of $p<0.05$.

Indicate whether the experiment must be repeated and justify the number of repetitions. No repeats

Indicate the species to be used, and how many will be used. There will be 1 donor mouse for 4 recipient mice. Each time point (0 and 2 hours post-injection) will be done with 8 mice. There will be 6 groups, MPs alone or MPs with one of 5 agents); 8 x 6 = 48 mice x 2 (0 or 2 hours) = 96 recipient mice x study by MRI or cisterna magna injection (2 x 96 = 192 mice). For 192 mice we will need 48 MPs donors (24 control mice, 24 exposed to the pressure protocol). Therefore 240 mice in total for experiment 4.

Experiment 5 – Determine relationship of glymphatic dysfunction with functional deficits.

Briefly describe the objective of this experiment. We will perform functional testing to assess brain-related dysfunction in mice after decompression and determine whether any of the interventions described above that antagonize inflammatory changes mediated by microparticles abrogate the abnormalities. Most tests are simple tasks and we will observe mouse responses. The total time required for the battery of tasks will be **5** days following decompression. The list of tasks are as follows:



1. Novel Objects Recognition Test
2. Beam walking Test
3. Accelerated Roto-rod

Describe what will happen to the animals from start to finish. Each mouse will be utilized in a **5-day** protocol. First, on Monday **mice receive a test agent before pressure exposure (PBS, JSH-23, KYC, PEG Telomer B of gelsolin)** and then exposed to 100 psi for 2 hours. There will also be a control group that will have not received an agent or pressure/decompression. Each testing cycle group will involve 8 mice. Because a number of other studies have shown that sex can have a great impact on learning and behavioral assays, we will be doing studies in a manner to specifically assess responses in each sex. The first sequence will be only male mice: 4 control and 4 pressure-exposed mice. Ensuing cycles will each involve 8 mice, but there may then be all female mice, or comparisons of all pressure-exposed mice but with 4 undergoing one of the interventions (anakinra, JSH-23, KYC, PEG-telomer B, or gelsolin). After these exposures, mice will be taken to our testing room (Bressler 4-4039A) to initiate testing and after each day of testing returned to the vivarium overnight.

These tests are all performed in a quiet room, with indirect lighting inside a Plexiglass arena that will be cleaned with MB10 and manually scrubbed between testing trials. The arena will be given time to dry completely before the next mouse is placed for testing.

Novel Objects Testing will be in the following sequence:
Day 1 (Mon, after the pressure exposure): Acclimation to testing chamber 5 min per mouse
Day 2 (Tues): Acclimation to chamber 5 min per mouse
Day 3 (Wednesday): Novel object recognition test (1 hr retention test) - each mouse is allowed 5 minutes to explore 2 identical objects. They will then be removed to the holding cage for a 1 hour 'retention interval' and then replaced in the testing arena and allowed 5 minutes exploration on 1 old and 1 new object.
Day 4 (Thursday): Novel object recognition test (24 hr retention test).
Day 5 (Friday) each mouse will undergo the Novel object location test.

Beam Walk will be in the following sequence:
Day 1 (Mon, after the pressure exposure): Acclimation to testing room and beam walking apparatus (5 min/mouse).

Day 2 (Tues): Fine motor coordination will be evaluated. A soft padded surface will be placed at the base of the apparatus to cushion any mice that fall. The ability of the mouse to walk across a 1 m beam (3cm, 2cm, 1cm width) will be assessed, measuring distance and time.
Days 3-5: Testing will be repeated daily to evaluate persistence of deficits (recovery has been described in some studies).

Accelerating rotarod will be in the following sequence::
Day 1 (Mon, after the pressure exposure): Acclimation to testing room and apparatus (5 min/mouse).
Day 2 (Tues): Mouse will be placed in the room with rotarod apparatus. The rotarod will be set at a start speed of 4 rpm, acceleration rate 20 rpm/min. The mouse will be held by the tail, and then placed on the rotating rod, facing it away from the directions of rotating so it has to walk forward to remain upright. Acceleration will start at 10 sec after placing the mouse on the device. The speed at with the mouse falls will be recorded. If it falls off before 10 sec, the time of fall will be recorded, and will try again, up to three times in total. To allow recovery of muscular strength and a return to normal levels of arousal, mice will be rested by a return to the home cage after each motor test. Recording the speed at the first fall after the 10 sec point. The mean speed at fall is the datum. Bubble wrap is placed on the table under the rod to reduce chance of injury on displacement from rotating rod. The wrap is replaced after cleaning with soap water/ towel dried if soiled.
Days 3-5: Testing will be repeated daily to evaluate persistence of deficits (recovery has been described in some studies).

What is the longest that any one animal will be involved in an experiment? **5** days

List each experimental and control group, including the group size for each group (n=?).
Each group will involve 8 male and 8 female mice in 12 groups: Control or decompression, plus injection with PBS, or agents found effective in studies described above. We hypothesize that 3 agents will be found effective.

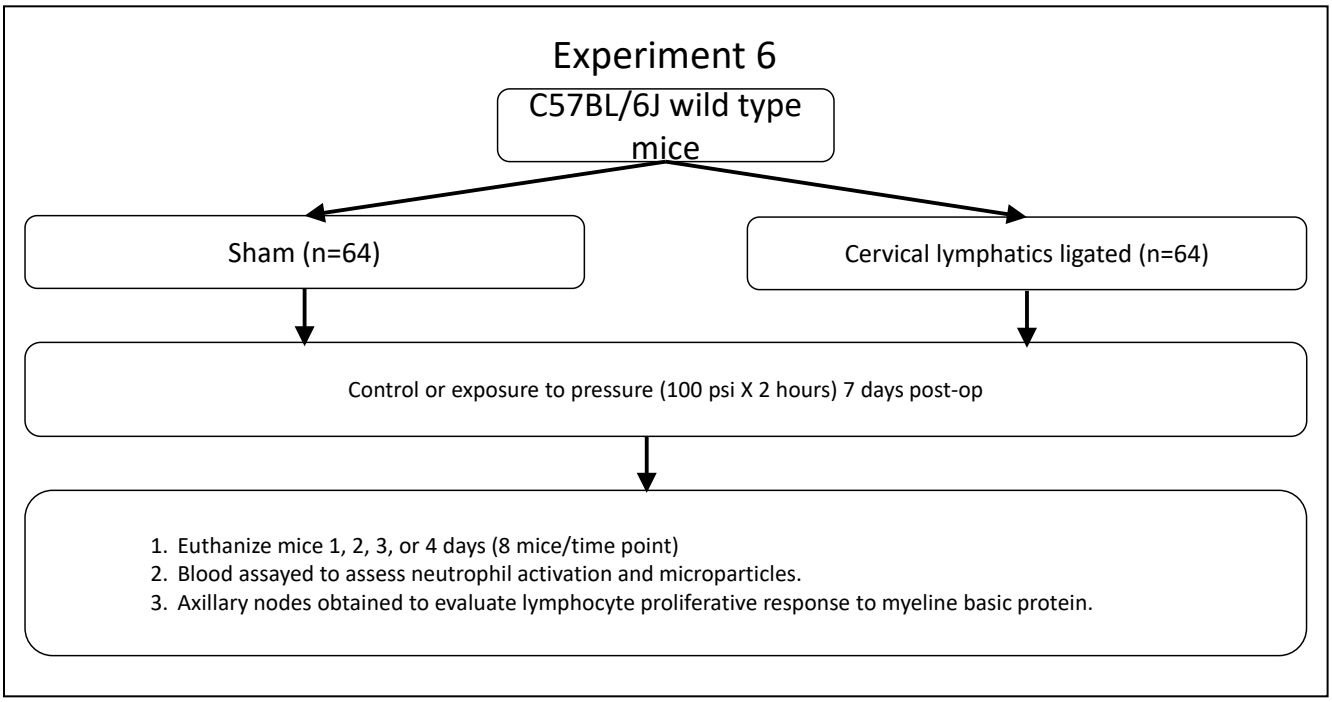
Discuss what criteria were used to determine that the group size(s) proposed utilizes the minimal number of animals to generate statistically significant data (*e.g., power analysis, reports in the literature, previous experience, etc.*). Prior studies looking at microparticle responses have required replicates of up to 8 per group to overcome variability and achieve statistical significance. With functional testing, we anticipate there will be greater variability versus the biochemical studies and there is also greater likelihood that sex will influence the results. Hence, we plan to study 8 male and 8 female per group. Based on ANOVA we anticipated this number will achieve a 95 % confidence limit and significance level of $p<0.05$.

Indicate whether the experiment must be repeated and justify the number of repetitions.
No repetitions are necessary.

Indicate the species to be used, and how many will be used *(e.g., X # mice per experiment x X # strains of mice).* 16 mice/group X 3 groups X 3 tests = 144 mice

Experiment 6 – Direct assessment of glymphatic outflow causing systemic immune response.

Briefly describe the objective of this experiment. We hypothesize that CNS-generated MPs that first lodge in cervical lymph nodes are responsible for establishing the decompression-induced inflammatory cycle by gaining access to the blood stream where they activate blood-borne neutrophils that then impinge on the blood brain barrier (likely via CD36 receptor). This is supported by preliminary data. The glymphatic system represents a



likely path for CNS-derived MPs to gain access to the peripheral circulation. Activated microglia can trigger neurotoxic T-cell activation via IL-1β (possibly via MPs) and reciprocally, stimulated T-cells activate microglia, and MBP tolerance induction diminishes T-cell mediated microglial activation. Studies show brain protein efflux via glymphatics following trauma, and obstructing lymphatic outflow can abrogate CNS autoimmune responses.

Describe what will happen to the animals from start to finish. We will directly evaluate the role of lymphatic efflux using methods described by van Zwam, *et al.* in their studies of experimental allergic encephalomyelitis (J Pathol 217: 543-551, 2009) [the point being that these procedures are well described in the literature]. The superficial and deep cervical lymph nodes are readily identified. Channels will be ligated, and nodes removed in parallel with sham-operated controls. One week later when mice have recovered from surgery, they will be exposed to pressure (100 psi X 2 hours). We hypothesize that after lymphadenectomy (versus sham) we will see decompression-induced astrocyte activation (*e.g.* NF-kB changes) but there will be no elevations of blood MPs that we believe cause progression of the decompression insult (*e.g.* neutrophil-derived MPs, loss of aquaporin 4 and other manifestations of neuroinflammation). We will also harvest lymphocytes from axillary nodes to assess proliferative response ("priming") to myelin basic protein following our published methods (see Thom, et al. PNAS 101: 13660-5, 2004).

What is the longest that any one animal will be involved in an experiment? 12 days

List each experimental and control group, including the group size for each group (n=?). Sham and lymphadenectomy groups (8 mice/group), control (air exposure only) or exposed to high pressure, euthanize mice at day 1,2,3 or 4 post-exposure.

Discuss what criteria were used to determine that the group size(s) proposed utilizes the minimal number of animals to generate statistically significant data. Our prior studies looking at MPs responses have required replicates of up to 8 per group to overcome variability and achieve statistical significance. Based on ANOVA we anticipated this number will achieve a 95 % confidence limit and significance level of $p<0.05$.

Indicate whether the experiment must be repeated and justify the number of repetitions. No repeats.

Indicate the species to be used, and how many will be used. 4 groups x 8 mice/group, 4 time points = 128 mice

4. **Animal Reuse – Will any of the proposed experiments utilize animals that have already undergone experimental procedures in this animal usage protocol <u>or</u> in a separate animal use protocol**? ☐ YES ☒ NO

<u>Detailed Descriptions of Animal Procedures</u>: *Identify <u>EACH</u> procedure to be performed on an animal. List as a subheading and provide a detailed description of how the work will be performed. <u>Please list procedures chronologically</u>. The following items must be addressed.*

<u>Animal Transport:</u> Mice in the vivarium will be transported according to procedures outlined in Veterinary Resources SOP #608.

<u>Exposure of mice in the hyperbaric chamber:</u> Typically, two mice but never more than four are placed in a steel chamber manufactured by Bethlehem Steel Corp. with a volume of ~ 2154 cm^3 (slightly smaller volume than a shoe box mouse cage) that is pressurized with air to up to 100 psi pressure. Chamber pressure is monitored by a sensitive gauge hydrostatically calibrated by the manufacturer. A lab worker is constantly monitoring the chamber pressure and intermittently looks into the chamber using the Plexiglas view port to monitor mouse activity. There is no national certification for animal use hyperbaric chambers but ours has been inspected by the Office of Naval Research grant program officer (who funds the study). Animals move around freely inside the chamber for the 2 hour exposure. Water is provided by a pressure-vented bottle. Air is flowed through the chamber at a rate of 5 liters/minute for the duration of the exposure to maintain a normal atmosphere without carbon dioxide build-up. Pressurization occurs slowly over a span of approximately 10 minutes (30 feet of sea water/5 minutes, same pressurization rate often used by human SCUBA divers) while mice are continually observed through a Plexiglas porthole. There is no notable discomfort experienced by the mice so long as pressurization does not occur at a rate that exceeds the ability of the animal to equalize pressure across their middle ear. Inadequate equalization is reflected by mice shaking their heads and exhibit excess activity during pressurization. If the chamber operator observes this behavior, pressurization is stopped for several minutes until the mouse appears calm and then pressurization is resumed as a slightly slower rate. Although we have never observed a mouse being intolerant to pressure, if a mouse appeared agitated with any pressurization this would suggest there is a mechanical obstruction rendering the mouse unable to equalize middle ear pressure. If that occurred, the mouse would be removed from the chamber and euthanized **.** Depressurization occurs at the same rate as pressurization (over ~ 10 minutes). This model has been used continuously for over 15 years and no mice have ever manifested evidence of decompression sickness such as unconsciousness, paralysis, dyspnea, change in behavior such as hyper- or hypo-activity, or hemoptysis. After each use, the pressure chamber is disinfected with 70% isopropyl alcohol and air dried.

<u>MRI studies:</u> After pressure exposures and placement of the tail vein catheter be done in the PI's lab, mice will be transported to Core for Translational Research in Imaging @Maryland (C-TRIM), CIBR/Core Facilities, HH 645). The animal will be anesthetized using isoflurane at a dose range of >3 - 4.5% for induction and >1 - 3% for maintenance via calibrated, precision vaporizer with scavenger system. The animal will then be placed prone in an animal holder with a nose cone and a bit bar. The surface RF array coil will be positioned over the head and the animal's eyes will be treated with a petrolatum based ophthalmic lubricant without antibiotics to prevent corneal desiccation. Typically, the scan is stopped at intervals of ~20 minutes when signal is checked and at these times the corneal desiccant will be re-applied, and level of anesthesia checked and recorded in a study log. The animal holder that is cleaned between uses with soap and water and manually scrubbed will be moved to the center of the magnet and the isoflurane level will be changed to ~ 3 %. The level of isoflurane will be further adjusted based on the respiration rate changes of the animal for the remainder of the experiment. A MR compatible small-animal monitoring and gating system (SA Instruments, Inc., New York, USA) will be used to monitor the animal respiration rate and body temperature. The animal body temperature will be maintained at 35-37°C using a warm water circulation blanket (scientific grade) with a disposable chux pad between mouse and blanket to prevent burns. After moving the imaging bed into the magnet, baseline calibrations will be done followed by injection of pharmaceutical-grade gadodiamide (Gd, 0.12 g/kg in a volume of 0.1 ml) via an IV catheter in the tail vein. When the injection finishes, image acquisitions will follow. After the whole data acquisition, which will be take not more than 2 hours, the animal will be moved out from the magnet and the isoflurane will be stopped. The catheter will

be removed, skin swabbed with 70% alcohol. The injection site will be observed for 5-10 minutes while the mouse is awakening from anesthesia to be assured there is no hemorrhage (if hemorrhage occurred, the site would be held gently with gauze to stop the bleeding). Animals will be provided thermal support during anesthesia recovery and then taken to the PI's lab for euthanasia.

Intra-cisternal injections for fluorescent imaging: This is a non-survival surgical procedure with a maximum duration of 2 hours. Mice will be placed in a precision vaporizer with charcoal scavenging waste system will be used. Mice will be placed in an induction chamber and using 100% oxygen as the delivery gas, anesthesia will be established with an induction dose of >3 to 4.5% isoflurane. Once the animal is recumbent the chamber will be flushed with oxygen and the animal placed in a stereotactic apparatus that was pre-washed with soap, water and then dried. The apparatus has a heating plate (scientific grade) to maintain mouse temperature. A lubricated tip pf a rectal probe is inserted to monitor temperature and maintain at 36oC during the procedure. Ocular lubricant is applied and re-applied every 20 minutes. A lack of toe pinch response is verified prior to proceeding. The scalp will be prepared by clipping with a #40 blade or smaller. The site will be prepped with a surgical scrub soap rinsed with 70% alcohol swabbing (alternating washes repeated for a total of 3 times) and then after a ~0.7 cm longitudinal scalp incision with clean, soap-washed/rinsed instruments and scalp skin is gently retracted using a sterile cotton swab to expose the skull. Using predefined stereotaxic coordinates at 1 mm posterior and 3.5 mm lateral to the bregma the skull is marked as the intended injection site. A dental drill is used to place a burr hole piercing to the inner cortical layer of the skull at these coordinates using a 0.45 mm clean soap-washed/rinsed burr drill bit using refined circular movements. The drill is then removed and a syringe injector in the stereotactic apparatus is used to insert a 33-gauge needle to 1.5 mm below the surface of the brain. At that point 0.5 µl fluorescent tracer is injected. The mouse is removed from the apparatus, kept under isoflurane anesthesia and scalp skin closed over the skull with a single 4-0 nylon suture. At times from 15 to 120 minutes mice will be euthanized by exsanguination. During this time anesthesia checks based on toe pinch are performed at 15-minute intervals. At the designated time euthanasia is carried out. After blood is obtained, the right heart will be opened, a cannula placed in the left ventricle and mouse perfused first with saline until clear effluent returns to the right heart blood followed by gravity-perfusion for 15 minutes with 10% neutral buffered formalin (NBF). Mice will be constantly observed through this procedure. Throughout the observation period mice will be kept on a warming pad (scientific grade) to maintain thermal support.

Blood collections: All blood collection will be performed under anesthesia at time of euthanasia and at no other times.

Routes of Administration:
Intravenous IV (tail vein) – For injections the mouse will be placed in a plastic restrainer that allows access to the tail vein. The Tail vein injection site is wiped with 70% alcohol and a sterile 29G (purchased from SAI Infusion Technologies, Inc.) catheter inserted into the tail vein for IV administration.

SC – Mice will be restrained by scruffing, with the head slightly tilted down. The injection site will be prepped with 70% alcohol. The skin/fur on the back of the neck will be pulled back (i.e,. "tented) to allow insertion of the needle subcutaneously and the contents of the syringe delivered under the skin. The injection volume will not exceed 0.6 ml and needle gauge will be 26 G or smaller.

IP - Mice will be placed in a plastic restraint device that allows free access to the abdomen. After the fur and abdominal wall are wiped with 70% alcohol, a sterile 27 gauge needle will be inserted along the lateral aspect of the mid-abdomen for agent injection. The total volume of solution will not exceed 0.2 ml.

Functional testing protocols:

Novel Object Recognition Test

Normal rodents naturally examine novel objects longer than familiar ones. This natural tendency permits assessment of perirhinal cortex dependent memory without stressful conditions, or high task demands. Memory of familiar objects can be tested at both short and long intervals, to detect deficits in short and long-term memory. Briefly, mice are placed in an arena housing 2 identical objects that are cleaned between uses with soap and water, manually scrubbed and dried prior to repeat usage. Mice are permitted to explore freely. This and subsequent sessions are video recorded for offline analysis. After a retention interval, the mouse is returned to the arena and one of the objects is replaced with a new one. It is expected that mice with an intact memory for the familiar object will spend more time examining the novel object.

For the short (1 hr retention) test, each mouse is allowed 5 minutes to explore 2 identical objects. They will then be removed to the holding cage for a 1 hour 'retention interval' and then replaced in the testing arena and allowed 5 minutes exploration on 1 old and 1 new object. The animals will be monitored continuously during this activity and total testing time for each mouse is estimated to be 1 hour and 15 minutes to allow for lag-times with animal transfer. Mice will be returned to the vivarium at the test session.

For the 'long-term' (24 hr or more retention) test, after taken from the vivarium and returned to the testing site each mouse will be placed in the testing arena after the 24 hour 'retention interval' and allowed 5 minute exploration on the 1 old and 1 new object. The animals will be monitored continuously during this activity and total testing time for each mouse is estimated to be 15 minutes to allow for lag-times with animal transfer.

Beam Walk Test
This study will assess motor and balance functions, as are often impaired in DCS. Mice will be acclimatized to the apparatus after the pressure exposure and daily testing conducted starting 24 hours later. Fine motor coordination will be evaluated by assessing distance and time that mice can ambulate across a 1 m beam (3cm, 2cm, 1cm width). The area beneath the beam has a soft padded surface to cushion any mice that fall. The ability of the mouse to walk will be assessed, measuring distance and time. After each use, the beam and padding are disinfected with 70% isopropyl alcohol and air dried.

Accelerating rotarod Test
Mice will be acclimatized to the apparatus after the pressure exposure and daily testing conducted starting 24 hours later. The mouse is placed in the room with rotarod apparatus and the rotarod set at a start speed of 4 rpm. The mouse is held by the tail and placed on the rotating rod, facing it away from the directions of rotating so it has to walk forward to remain upright. After ambulation starts, acceleration starts at 10 sec after placing the mouse on the device. The speed at with the mouse falls will be recorded. If it falls off before 10 sec, the time of fall will be recorded, and will try again, up to three times in total. To allow recovery of muscular strength and a return to normal levels of arousal, mice will be rested by a return to the home cage after each motor test. Recording the speed at the first fall after the 10 sec point. The mean speed at fall is the datum. Bubble wrap is placed on the table under the rod to reduce chance of injury on displacement from rotating rod. The wrap is replaced after cleaning with soap water/ towel dried if soiled. Testing will be repeated daily to evaluate persistence of deficits (recovery has been described in some studies). After each use, the beam and padding are disinfected with 70% isopropyl alcohol and air dried.

Video recording of cognitive tasks: For each of the tasks described above, the mice will be recorded on video to aid with recording of time to achieve each of the tasks. We will comply with all UMB SOM IACUC guidelines on image recording. Specifically, we do not anticipate including image / recording in publications or exhibitions. However, if ever we do think this is a reasonable activity, we will obtain IACUC approval of the image / recording prior to use. Further, with capturing all image / recording, we will be careful to limit the visibility of the animal facility and research staff. Moreover, all images / recordings will be removed from the recording device and stored on a secured device.

Cervical node MPs procurement: This is a non-survival surgical procedure in which mice are anesthetized (Ketamine/xylazine) and the neck and chest are fully clipped and then swabbed with 8-12% solution of either betadine or chlorhexidine sudsing surgical scrub applied with clean cotton gauze, then rinsed with 70% alcohol on gauze and both steps repeated two more times. The last application of the alcohol is allowed to air dry. Once a level of surgical anesthesia is reached, the neck and thorax are aseptically opened and exsanguination performed followed node harvesting. The neck is dissected, cervical lymph nodes identified and harvested for *ex vivo* mincing and microparticle purification. This is achieved by centrifugation in sterile tubes for injections into the tail vein of recipient mice (as described above under IV tail vein injection). This procedure is performed in the PI's lab

Survival surgery – ligation of cervical lymphatics: Prior to pressure exposure, mice will have deep cervical lymphatic chains ligated to prevent release of CNS-derived MPs from gaining access to the blood stream. Isoflurane anesthesia will be induced via induction chamber and continued post induction via a face mask. Post skin prep, surgery will be initiated as described in detail below and blood flow will be monitored (to be assured we do not disturb flow during the surgery). Cerebral blood flow will be monitored by Laser Doppler Flowmetry (LDF) (Moor Instruments, Inc.

Wilmington, DE) during the procedure. Servo controlled thermal support is provided to maintain steady by temperature. Sham animals will undergo the same procedure except that lymphatic ligation will not be done. To mitigate the pain and distress, we will use a local anesthetic (bupivacaine) at surgery to reduce immediate post op pain and will euthanize mice early if exhibiting signs stated in the 'Alternative Endpoints' section below.

Surgical Area Prep: In order to maintain aseptic conditions, the surgical desk and other accessories will be free of clutter, cleaned with 10 % dilution of household bleach, wiped dry with a clean towel, and covered with a clean drape. Washed and cleaned instruments will be autoclaved at 121 º C for 20 min.

Surgeon prep: The surgeon will don a facemask, wash hands with antiseptic soap, dry hands on clean paper towels and don a surgical gown, and then sterile gloves. Gloves will be changed between animals or if grossly soiled or break in aseptic technique occurs (e.g., glove accidentally touches non-sterile objects).

Instrument Prep: For first use on any day all instruments will be autoclaved. When conducting multiple surgeries on the same day, between use on animals, the instrument tips will be rinsed clean of any organic debris and the distal 1/3 of the instruments will be re-sterilized in a bead sterilizer and placed on sterile drape or roll of sterile cotton gauze to air cool a minimum of 5-10 minutes until next use.

Anesthesia: Mice are weighed, and weights recorded on their anesthesia records. A precision vaporizer with charcoal scavenging waste system will be used to delivery isoflurane. Mice will be placed in an induction chamber and using 100% oxygen as the delivery gas, anesthesia will be established with an induction dose of >3 to 4.5% isoflurane. Once the animal is recumbent and respiration noted to slow, the chamber will be flushed with oxygen and the animal moved to the to an area a few feet from the 'surgery table. Anesthesia will be maintained via nose cone using Isoflurane at >1 - 3 % in 100 % O_2 and monitored continuously during and after the procedure, as described below. In any rare cases of a non-functioning isoflurane system, Ketamine (80-150mg/kg)/Xylazine (8-15mg/kg) will be administered intraperitoneally post manual scruffing of mice by grasping them at the nape of the neck. Injection will be done at a mid-abdomen injection site after prep (70 % alcohol on clean cotton gauze,) using a 25 G or smaller bore needle to induce for surgical prep and this surgery and surgical plane of anesthesia confirmed as described in above. If top off is needed at around 25 minutes post-induction, ½ the initial dose of ketamine will be administered, and if at 45-50 minutes if top off is needed then ½ doses of ketamine and xylazine will be administered.

Surgical Site Prep: In an area located approximately 1-2 feet from the surgery table, fur is removed from both sides of the neck of the anesthetized mouse by using electric clippers with a # 40-50 clipper blade, fur is removed to provide 1 cm margins around the planned incision lines. The clipping margins will be from brow to back of skull, and from ventral to dorsal midline. The clipped areas will be wiped with a gauze wet with 70% ethanol to remove any loose fur. Post removal of lose fur, animals are moved to the surgery table.

Thermal Support: During the ~ 60 min surgical procedure, animals will be maintained at a temperature of 37.0±0.5 degree Celsius with a homoeothermic-heating pad (Physio Suite Mouse STAT Right Temp, Kent Scientific Co., Torrington, CT). Note that a disposable chux pad will be placed between the mouse and the heating pad to prevent burns.

Ocular Protectant: Protective ocular ointment (Para-lube or Lacri-lube) is placed in both eyes to reduce chances of corneal desiccation. Application will be repeated every 20-30 minutes post induction of anesthesia.

Aseptic Prep: The Clipped skin area is prepped with and 8-12% solution of either betadine or chlorhexidine sudsing surgical scrub applied with clean cotton gauze, then rinsed with 70% alcohol on gauze and both steps repeated two more times. The last application of the alcohol is allowed to air dry.

Surgical Procedure:

1) Depth of the anesthesia will be confirmed by a lack of corneal reflex and/or no response to deep tail or foot pinch. Anesthetic depth will be re-assessed at least every 10-15 minutes throughout the surgery and noted on anesthesia record (adequate, increased, decreased etc).

2) Local analgesia will be provided by injecting approximately 0.2 ml of bupivacaine (0.25% - 0.5%, diluted 1:1 with saline, max 6 mg/kg) subcutaneously in the incision sites with a 25 G or smaller bore needle, which will provide local anesthesia for approximately 6-8 hrs.

3) After skin prep a small incision (3-4 mm) will be made with sterilized scissors between the ear and the eye.
4) The muscles under the skin will be separated with the same scissors to expose the temporal bone for inserting a needle laser Doppler probe (diam. 2 mm, or 20G) which will allow monitoring of cerebral blood flow throughout the procedure. Note that the Laser Doppler is an instrument dedicated for animal use.
5) The Doppler probe will be cleaned thoroughly with soap and water then rinsed with 70% ethanol and cold sterilized in Cidex overnight each day. It is rinsed with sterile water to remove Cidex prior to placement in the animal.
6) The probe is inserted at the bone above the middle cerebral artery territory and signal monitored throughout the surgical procedure.
7) Bilateral ventral cervical incisions, approximately 1-1.5 cm long will be made and the major vessels in the carotid sheath will be identified.
8) Lymphatic vessels and nodes are found in this region and easily identified. Nodes will be removed, and vessels ligated with a sterile 7-0 nylon monofilament surgical suture.
9) The ventral cervical incisions and incision for the LDF probe will be closed with FDA approved surgical glue (Nexaban, Vet Bond) cyanoacrylate tissue adhesive applied with TB syringe and 25 G needle. If bulging at the incision is noted at this point, 5-0 nylon simple sutures will be added.
10) The entire time for surgery will not exceed 60 minutes under any circumstances. After incision closure, animals will be placed in a recovery cage for the recovery period and the body temperature will be maintained at 37.0±0.5°C through use of Stryker T/Pump and warm water recirculating pad. The heating pad will be only be placed under half of the cage bottom to allow the animal to move away from the heat source if desired. After surgical completion mice will receive sub-cutaneous buprenorphine that will be re-administered twice daily for the one-week recovery period.
11) Once the animals are ambulatory, they will be returned to their home cages that have a supplemental heat source on one side and clean dry bedding. Once fully ambulatory with no signs of abnormal behavior, the supplemental heat source will be removed, and the mouse returned to the Animal Facility.
12) The laboratory staff will monitor the mice twice daily for the rest of the week. The animals will be visual inspected daily for any clinical signs, including on weekends. At any sign of redness or edema, a comparative medicine veterinarian will be contacted to evaluate the animal and make further recommendations. If the animal is exhibiting signs of being in pain (reluctance to move, eat or drink and vocalizations with handling), a veterinarian will be consulted. If the animal is too sick to recover, it will be immediately euthanized.
13) A green "Small Animal Surgery/Procedure" card obtained from VR animal care staff will be placed on the cage of each animal undergoing surgery. The front of the card will indicate date and type of procedure, as well as the contact person with both cell and lab numbers.
14) At 1 week post-op well appearing mice will undergo pressure exposure protocol and then studied at times up to 4 days later. If findings from Experiments 1-7 suggest that observation periods need to be longer than 4 days we will submit an addendum to IACUC to modify the procedure.

Exogenous Substances: *(any substance administered to a live animal)*

Substance	**Dose Range** *(mg/kg)*	**Route**	**pharmaceutical grade*** *(yes / no)*	**Chemical Hazard?** *(No, CL1, CL2)*	**Purpose** *(anesthetic, analgesic, paralytic, experimental, etc.)*
Gadodiamide	0.12 mg/kg	IV	Yes	No	**Gadolinium contrast agent**
Gelsolin	1 ug/kg	IV	Yes	No	Experimental
PEG-Telomer B	0.002 mg/kg	IV	Yes	No	Experimental
Anakinra	100 mg/kg	IP	Yes	No	Experimental
JSH (4-methyl-N1-(3-phenyl-propyl)-benzene-1,2-diamine)	3 mg/kg	IP	Yes	No	Experimental
KYC (acetyl-lysyltyrosylcysteine)	0.3 mg/kg	IP	Yes	No	Experimental
PBS saline	10 ml/kg or 10ml	IV	Yes	No	Solvent, perfusion
isoflurane	>3 - 4.5% for ind and >1 - 3% for maintenance; > 4.5% for euthanasia.	Inhalation	Yes	No	Anesthesia – delivered by calibrated, precision vaporizer with scavenger system

Ocular protectant (lacrilube)	n/a	Each eye	yes	No	Eye protection
Fluorescent cadaverine	0.1 mg/kg	Intra-cisternal	No –but sterile and filtered	No	Brain glymphatic imaging
Fluorescent ovalbumin	0.1 mg/kg	Intra-cisternal	No –but sterile and filtered	No	Brain glymphatic imaging
ketamine	80-150 mg/kg	IP	Yes	No	Anesthetic
xylazine	8-15 mg/kg	IP	Yes	No	Anesthetic
Buprenorphine	0.05-0.1 mg/kg	SQ	Yes	No	Post-op analgesic
bupivicaine	0.2 ml (0.25% - 0.5%, diluted 1:1 with saline, max 6 mg/kg)	SC	Yes	No	Local analgesia
*Scientifically justify use of non-pharmaceutical grade compounds: Non-pharmaceutical grade agents will be used only when pharmaceutical grade agents are not available.					
☒ *I confirm any non-pharmaceutical grade compounds will be reconstituted and stored per the *IACUC Guidelines on the use of Non-pharmaceutical Grade Compounds in Animal Use Protocols*.					

CL1 – Chemical Level 1; CL2 – Chemical Level 2; Refer to EHS Hazardous Chemical Use in Animal Research website for guidance.

Identification of Animals: *Please provide details on the method of identification (e.g., ear tagging, tattooing, etc.) used for all animals in the study and the age of animals when procedures are performed.*

Mice do not have to be tagged or tattooed. We will use ~20 gram, 8 week old mice. Mice will be identified at the cage level by **marking the ears or tail with a color using an ink pen**.

General Monitoring: *Please provide details to the general monitoring of animals throughout the course of these experiments. Define parameters to be monitored, frequency of monitoring (at least 3 times a week), and identify the individual(s) responsible for monitoring.*

Mice are under observation daily Monday through Friday and provided with extra food and water over weekends. All animals will have free mobility and access to food and water. During the study, we will monitor for general signs of animal distress. If mice were found to be behaving as if in pain based on abnormal behavior (hyperactive, or not moving at all), exhibiting piloerection, or porphyrin deposits from eyes they would be immediately euthanized.

Alternative Endpoints: *Please specify criteria to be used to determine whether early euthanasia (prior to the experimental endpoint) is warranted. Please refer to the Endpoint Guidelines available on the OAWA website and include only those that are applicable to the proposed research.*

There are no specific manifestations to adverse effects from decompression, or other manipulations planned in this project. That is, one would look for general signs of animal distress. If mice were found to be behaving as if in pain based on abnormal behavior (hyperactive, or not moving at all), exhibiting piloerection, or porphyrin deposits from eyes they would be immediately euthanized (ketamine/xylazine and then exsanguination). None of the agents planned for use should cause animals to lose weight or suffer any other sort of stress in the short time they will be under observation until euthanasia.

Regarding animals subjected to survival surgery, if mice appear moribund as defined below they will be euthanized.

(i) 15 % weight loss over 24 hours: The percentage of weight loss = (Pre surgery weight minus weight on any day) divided by pre-surgery weight x 100. For animals after surgery, body weight will be assessed every other day starting on day 1 post op.
(ii) Inability to rise or ambulate.
(iii) Inability to eat/ drink for 24 hrs.
(iv) Recumbent mice that are unresponsive to gentile finger prodding.
(v) Evidence of CNS coma signs (*e.g.* no pupillary reflex).
(vi) Diarrhea for more than 3 days.
(vii) Self-induced trauma.

(viii) Incision line infection (red/ swelling, discharge or dehiscence).
(ix) Bleeding from any orifice.
Animals with any one of these conditions will be considered as moribund and euthanized by ketamine/xylazine followed by exsanguination for tissues collection/ analysis.

Research Personnel Qualifications, Experience or Training: *Please provide the following information for each person working with live animals under this protocol. List the PI information first, then any Co-PIs, etc.*

Name, Email & Phone #	**Indicate role in project and list procedures he/she will be performing.**	**Indicate years' experience working with the *species* of animals proposed for use in this protocol.**	**Indicate years' experience performing the *proposed procedures in the species* utilized in this protocol.**	**If the individual is not trained in either the procedures or species (*or both*), please indicate how he/she will be trained.**
Stephen R. Thom (b) (6) [redacted]	Principal Investigator; IV & IP pharmaceutical administration, cervical lymph node ligation/survival surgery, behavioral protocol, **high pressure exposures/decompression**, mouse euthanasia	33	26	Trained person
(b) (6) [redacted]	Lab manager, IV & IP pharmaceutical administration, **high pressure exposures/decompression,** behavioral protocol, mouse euthanasia	27	16	Trained person
(b) (6) [redacted]	Research Associate, IV & IP pharmaceutical administration, cervical lymph node ligation/survival surgery, behavioral protocol, **high pressure exposures/decompression,** mouse euthanasia, **colony maintenance and lab animal monitoring.**	7	3	Trained person
(b) (6) [redacted]	Post-doctoral fellow, IV & IP pharmaceutical administration, cervical lymph node ligation/survival surgery, behavioral protocol, **high pressure exposures/decompression**, mouse euthanasia	3	2 months	Trained person

***Please add rows to this table by placing cursor at end of last row and clicking return.*

☒ As PI, I confirm that no minors will be working with live vertebrate animals per UMB Policy VI-99.01(A).

☒ As PI, I acknowledge that a COI Exemption must be filed with the UMB Office of Accountability & Compliance should my spouse or a family member work under this animal use protocol per UMB Policy.

From:	Dean, Jay
To:	Chapman, Sandra E CIV (USA)
Subject:	[Non-DoD Source] FW: USDA Inspection Report Reminder Notice - H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, US
Date:	Friday, February 12, 2021 1:49:54 PM
Attachments:	USDA inspection report-12-2020-IR_930_USF_03DEC20.pdf

Hello Sandra,
Sorry to bother you with this, but I keep getting this message from the army about not submitting the most recent USDA report; however, I submit the most recent report each time. This is the 2nd notice I've received in two weeks. Who do I contact about this. I think there is an error in their database.
Thanks,
Jay Dean

From: Animal Care and Use Review Office
Sent: Friday, February 12, 2021 12:14 PM
To: Dean, Jay ; Engelman, Robert (robert.engelman@moffitt.org) ; Engelman, Robert (robert.engelman@moffitt.org) ; Moulvi, Farah
Subject: USDA Inspection Report Reminder Notice - H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, US
Dear Dr. Jay Dean and Dr. Robert Engelman and Dr. Farah Moulvi,

As described below, DOD regulations require ACURO to review the USDA inspection report for your facility annually. To date, we have not received your report. Failure to provide this information could result in a risk to your PIs' DOD funding.

Your institution has been approved to conduct animal studies that are supported by the Department of Defense (DOD) and overseen by the Animal Care and Use Review Office (ACURO). In accordance with DOD Instruction 3216.01, Use of Animals in DOD Conducted and Supported Research and Training, ACURO is required to review the annual USDA inspection report for your facility each year. Please submit your institution's most recent USDA inspection report to ACURO's USDA report email box (usarmy.detrick.medcom-usamrmc.mbx.acuro-usda@mail.mil)).
If your institution is exempt from USDA inspection, please notify our office of that as well as the reason for the exemption. If you have any questions or need any additional information,
Please don't hesitate to contact our office.

Best Regards,

Animal Care and Use Review Office (ACURO)
usarmy.detrick.medcom-usamrmc.mbx.acuro-usda@mail.mil

[EXTERNAL EMAIL] DO NOT CLICK links or attachments unless you recognize the sender and know the content is safe.

BUMED Animal Use Appendix for Research Involving Animals
ABBREVIATED VERSION

*****NOTE: BUMED will ONLY review protocols that have been approved by your IACUC.*****
****Animal work MAY NOT be initiated until the awardee receives BUMED approval.****
Animal work initiated without BUMED approval is noncompliant and may not be funded.

Institutions using DoD funds to support the use of animals in research, product development, testing and education projects must provide electronic copies of the following documents to the Department of the Navy (DON) Bureau of Medicine and Surgery (BUMED) Veterinary Affairs Office for review and approval prior to initiation:

a. A copy of their **IACUC-approved institutional protocol(s)** (BUMED will ONLY review **approved** protocols) and documentation of IACUC approval

b. A copy of all existing IACUC-approved protocol amendments or modifications and documentation of IACUC approval (**future modifications or amendments** must be reviewed and approved by BUMED **PRIOR** to implementation)

c. A completed Appendix for each IACUC-approved protocol.

This requirement also applies to all subcontractors using animals in support of DoD-funded projects or programs.

Specific information requested in the following animal use Appendix is derived from requirements in the Animal Welfare Regulations (AWRs), the *Guide for the Care and Use of Laboratory Animals*, and other applicable Federal and DoD regulations. The DoD policies and requirements for the use of animals in DoD-supported research, development, testing and evaluation are described in DoD Instruction 3216.01, dated September 13, 2010 and SECNAVINST 3900.38C, *The Care and Use of Laboratory Animals in DOD Programs*, dated February 16, 2005. These requirements differ from those of other funding agencies. Use of the Appendix is intended to meet the requirements of these documents.

Questions concerning animal use and review should be directed to BUMED Veterinary Affairs:

Phone: 301-619-9241 or 301-619-9224
Email: DON – VRPP, usn.ncr.bumedfchva.mbx.don---vrpp@mail.mil
Mail: Department of the Navy Bureau of Medicine and Surgery
ATTN: Director for Veterinary Affairs
Bldg 1564 Room 122
1564 Freedman Drive
Fort Detrick, MD 21702

~~~~~~

Each section of this Appendix must be completed. To assist you in completing this appendix, instructions and explanations are provided as *hidden, red and italicized text*. To view the instructions and/or examples for each section, select the "**Show/Hide ¶**" button on your tool bar (the button itself appears as the ¶ symbol). To print the hidden text, select "Print Hidden Text" in the print options section. It is important that you carefully **read the instructions** for each paragraph to ensure you provide a comprehensive response. Begin typing responses after the colon (":") for each section to ensure your typing is not within the hidden text. Submit electronic copies of the appendix only; please do not submit printed copies to BUMED. Any section of the Appendix that is not applicable to your proposal, e.g., no surgery or no prolonged restraint, should be marked "**No**" or "**N/A**". There are no space limitations for the responses.

It is essential that only animal studies or procedures documented in an IACUC–approved protocol or amendment be performed at your facility. BUMED will collect animal usage information for end-of-year DoD animal use reports separate from the grant annual progress reporting requirement throughout the life of your award. For this reason, Principal Investigators or other delegated research personnel should keep accurate records and be able to provide an audit trail of all animal use that correlates to their approved protocol.
~~~~~~

X	Yes		No	All animal studies described in the attached IACUC-approved protocol are funded by this DOD award/contract. If no, STOP ! You must use the **full version** of the Animal Use Appendix.

1. Administrative Data: (Provide Attending Vet, IACUC, and Research Office info for the work site.)

DoD/Contract PI Name:	Jay B. Dean, PhD		
Grant/Contract PI Email:	jaydean@usf.edu	**Phone:**	813-974-1547
Protocol PI Name:	Jay B. Dean, PhD		
Protocol PI Email:	jaydean@usf.edu	**Phone:**	813-974-1547
Animal Research Site (RS):	University of South Florida, Tampa, FL		
RS Attending Veterinarian:	Dr. Norman Wiltshire, DVM, DACLAM, Assoc. Dir. Comp. Med.		
Attending Vet Email:	wiltshire@usf.edu	**Phone:**	(813) 974-3673
RS IACUC Point of Contact (POC):	Dr. Shari Pilon-Thomas (Vice-Chair USF IACUC); [Note: Dr. Jay B. Dean (P.I.) is the Chair of the USF IACUC]		
IACUC POC Email:	Shari.Pilon-Thomas@moffitt.org	**Phone:**	(813) 745-4247
RS Grants Office Point of Contact (POC):	Mariah Curry		
Grants Office POC Email:	currym1@usf.edu	**Phone:**	(813) 974-5044
Animal Protocol Title:	Mitigation of CNS oxygen toxicity: targeted antioxidant therapy		

2. Total Number of Animals Used (by Species) and USDA Pain/Distress Category:

SPECIES	HIGHEST USDA PAIN/DISTRESS CATEGORY (B, C, D, E*) See table below for definitions	TOTAL NUMBER
Sprague-Dawley rat (Aim 1)	E	90

USDA Pain/Distress Category Definitions:

- ***Column B:*** *Animals being bred, and animals being held for use in research, testing, teaching, experiments or surgery but not yet used for those purposes.*
- ***Column C:*** *List the number of animals that will experience no more than slight or momentary pain or distress as a result of experimental manipulations or procedures on this protocol.*
- ***Column D:*** *List the number of animals that will potentially experience more than momentary or slight pain or distress that **WILL** be alleviated through the use of anesthetics and/or analgesics.*
- ***Column E:*** *List the number of animals that will experience more than momentary or slight pain or distress that **WILL NOT** be alleviated or relieved with anesthetics or analgesics.* **If any animals are listed in USDA Column E (Unalleviated Pain or Distress), the PI must provide a scientifically valid justification for withholding pain relieving medication:**. .
    - USDA Pain Category E (= pain category C in USF IACUC animal use protocol): Animals listed in USDA Pain Category E will be exposed to hyperbaric oxygen +/-

CO_2 until onset of seizure, which may cause discomfort. The potential for discomfort during and following seizure, which is an experimental endpoint (i.e., CNS oxygen toxicity), is why these animals are placed in USDA pain category E. The animals are unanesthetized in these experiments so that their visible behaviors can be used as one of the criteria to score onset of a seizure; we use a six-point scale of behavior to evaluate the type of seizures. Sensitivity to hyperbaric oxygen is blunted in an anesthetized animal and thus the latency time to seizures is severely lengthened plus a higher oxygen pressure is required. After the seizure occurs the breathing atmosphere is changed from 100% O_2 (or ~97%O_2, balance CO_2) to air and the animal chamber is decompressed at a rate of ~1 atmosphere/minute. After recovery at room pressure, the animal appears normal based on its behavior.

3. Literature Search for Unnecessary Duplication: This search is required for all animal use proposals. Note the DoD-specific database requirements in subparagraph a.

a. Literature Source(s) Searched:

http://www.ntis.gov/products/fedrip.aspxhttp://projectreporter.nih.gov/reporter.cfm

[X] Federal Research in Progress (FEDRIP) http://www.ntis.gov/products/fedrip.aspx

or

[ ] Research Portfolio Online Reporting Tool Expenditures and Results (RePORTER) http://projectreporter.nih.gov/reporter.cfm

b. Date of Search:
1 December, 2022
c. Years Covered By Search:
1997-2022

d. Key Words and/or Search Strategy:
Hyperbaric oxygen seizure genesis, hyperbaric oxygen sensitivity, hyperbaric oxygen toxicity, hyperbaric brainstem nuclei/neurons, oxygen sensitive neurons, CO_2 sensitive neurons, hyperbaric seizures, seizure genesis (non-epileptic), rat brain slice, seizure radio telemetry, rat radio telemetry, cranial nerve nuclei, oxygen sensitive cranial nuclei, CO_2 sensitive cranial nuclei, alternatives, anesthesia, pain, LNAME, Mito-Q, TPMP, ketone, ketone ester, CNS oxygen toxicity

e. Results of Search:
There were no relevant projects &/or publications even with altering the amount of words searched and the order the search was conducted.

****Questions 4-10 refer to the Research Site and Protocol Principal Investigator.****

Information and/or documents required in questions 4-8 should be obtained from the research site's IACUC or veterinary staff. Documents may be provided directly to BUMED by the Protocol Principal Investigator (PI) or institution staffs. If the Protocol PI prefers that BUMED staff contact the institution to obtain this information and/or documents, the PI must specifically request this action in writing. The Protocol PI should contact BUMED (see contact information on cover page) to arrange for submission of this written request.

4. Institutional Animal Care and Use Committee(s) (IACUC) Approval(s):

Institutional Animal Use Protocol Number: IS00011501

IACUC (initial) approval date:	December 6, 2022	Protocol expiration (rewrite) date:	December 6, 2025

5. Veterinary Care: Provide a brief description of the veterinary care plan at the research site. Describe routine care; weekend, holiday, and emergency care; and identify whether the attending veterinarian is on staff full-time or by contract.

USF MCOM Comparative Medicine is fully accredited by AAALAC International as program #000434, is managed in accordance with the Guide for the Care and Use of Laboratory Animals, the Animal Welfare Regulations, the PHS Policy, the FDA Good Laboratory Practices, and the IACUC Principles and Procedures of Animal Care and Use, has an assurance #D-16-00589 (A4100-01) on file with OLAW/PHS and maintains registration #58-R-0015 with USDA/APHIS/AC.

Four (4) Comparative Medicine veterinarians are full-time staff, Florida-licensed, members of the AVMA, and are specialty board certified by ACLAM, and/or have research doctorates. Comparative Medicine maintains institutional membership with AALAS. All program staff are AALAS-certified, and many are either registered with the Society of Quality Assurance, and/or certified by the Academy of Surgical Research or are State of Florida-certified veterinary technicians.

Daily routine, weekend, holiday, and emergency animal care is provided by animal care program staff, all of whom are certified by the American Association for Laboratory Animal Science (AALAS), and more staff are certified at the highest, technologist level {LATG) than any other level. Cage changes occur twice weekly. Health surveillance occurs twice daily and assesses: food and water intake, fecal output, assurance of water supply and patency, and general appearance of animals (e.g. presence of lesions/injuries, coat condition, disposition). If at any time there is a medical emergency, the clinical veterinarian is notified immediately.

6. Institutional Accreditation / Assurances:

a. Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) Accreditation (do **NOT** provide AAALAC correspondence)**:**

[X] Yes [ ] No Animal work is being performed at an AAALAC International-accredited facility.

b. Public Health Service Animal Welfare Assurance Statement:

[X] Yes [ ] No Animal work is being performed at a PHS-assured facility.

c. Non-accredited, Unassured Facilities: If neither 6.a. nor 6.b. above apply to the facility where animal work is being performed, submit a statement signed by the Institutional Official that states the care and use of animals will be conducted in accordance with the National Research Council's *Guide for the Care and Use of Laboratory Animals* and applicable Federal and DoD regulations.

7. Animal Procurement:

[X] Yes [ ] No If the protocol involves Animal Welfare Act-regulated species, are the animals obtained legally from suppliers licensed by the USDA? If the supplier claims exemption from USDA licensing, provide confirmation from the research site's IACUC that the exemption criteria have been met. If work is conducted outside the US, have animals been obtained legally in accordance with national policy? If

wildlife are used, provide IACUC assurance that animals have been obtained legally and provide copies of applicable state, federal and/or international capture and use permits.

8. Overseas / Foreign Country Animal Work:

[ ] Yes [X] No Animal work will be performed outside the United States.

If "Yes," answer the following questions.

a. What is the law or regulation governing the use of animals in research in the research site's host country? Please provide a copy or link to this law or regulation in English.

b. Does the research site's host country adhere to European Union (EU) Directive 86/609 or EU Directive 2010/63 standards of animal housing and care?

c. If the research site is in Canada, does the institution hold a Canadian Council on Animal Care (CCAC) certificate?

d. Does the research site adhere to any national or international standards of animal housing and care that are more stringent than the host country's laws or regulations (such as AAALAC or CCAC)? If so, please describe below or provide a document, in English, that describes these standards.

e. Does the research site's host country or local institute require a local ethical committee review or Animal Care and Use Committee review? If so, please describe below or provide a document, in English, describing the committee's membership, purpose, authority and function.

9. Site Visits

[ ] Yes [**X**] No Does animal work involve at least one of the following species: dogs, cats, nonhuman primates, marine mammals? If yes, provide a planned start date for work in these species and point of contact for site visit coordination. Based on accreditation status, species used, and type of research conducted, a site visit to the performance site may be required.

10. Protocol Principal Investigator Assurances:

The law specifically requires several written assurances from the P.I. Please read and sign the assurances as indicated (this page may be photocopied and signed).

As the Principal Investigator on this protocol, I acknowledge my responsibilities and provide assurances for the following:

A. Painful Procedures: I assure that discomfort and injury to animals will be limited to that which is unavoidable in the conduct of scientifically valuable research and that analgesic, anesthetic, and/or tranquilizing drugs will be used where indicated and appropriate to minimize pain and/or distress to animals.

B. Animal Use: The animals authorized for use in this protocol will be used only in the activities and in the manner described herein, unless a modification is specifically approved by the IACUC and the DoD Department of the Navy **Bureau of Medicine and Surgery** (BUMED) Veterinary Affairs Office prior to its implementation.

C. Duplication of Effort: I have made a reasonable, good faith effort to ensure that this protocol is not an unnecessary duplication of previous experiments.

D. Statistical Assurance: I assure that I have consulted with a qualified individual who evaluated the experimental design with respect to the statistical analysis, and that the minimum number of animals needed for scientific validity will be used.

E. Training: I verify that the personnel performing the animal procedures/manipulations/ observations described in this protocol are technically competent and have been properly trained to ensure that no unnecessary pain or distress will be caused to the animals as a result of the procedures/manipulations.

F. Responsibility: I acknowledge the inherent moral, ethical and administrative obligations associated with the performance of this animal use protocol, and I assure that all individuals associated with this project will demonstrate a concern for the health, comfort, welfare, and well-being of the research animals. Additionally, I pledge to implement animal use alternatives where feasible, and conduct humane and lawful research.

G. Scientific Review: This proposed animal use protocol has received appropriate peer scientific review, and is consistent with good scientific research practice.

Jay B. Dean (b) (6)

(Protocol Principal Investigator Printed Name) (Protocol Principal Investigator Signature and Date)

NOTE: In accordance with SECNAVINST 3900.38C, the DON BUMED Veterinary Affairs Officer (or designee thereof) will conduct a site visit to all sites using nonhuman primates, dogs, cats or marine mammals in the proposal, or where a site visit is deemed warranted.

United States Department of Agriculture
Animal and Plant Health Inspection Service

DBARKSDALE
INS-0000814996

Inspection Report

UNIVERSITY OF SOUTH FLORIDA
12901 BRUCE B DOWNS BLVD MDC 20
TAMPA, FL 33612

Customer ID: **930**
Certificate: **58-R-0015**
Site: 001
UNIVERSITY OF SOUTH FLORIDA

Type: ROUTINE INSPECTION
Date: 20-SEP-2022

No non-compliant items identified during this inspection.

This inspection and exit interview were conducted with the facility representatives .

Prepared By: DAWN BARKSDALE
USDA, APHIS, Animal Care
Title: VETERINARY MEDICAL OFFICER
Date: 21-SEP-2022

Received by Title: IACUC Representative
Date: 21-SEP-2022

United States Department of Agriculture
Animal and Plant Health Inspection Service

Customer: 930
Inspection Date: 20-Sep-2022

Species Inspected

Cust No	Cert No	Site	Site Name	Inspection
930	58-R-0015	001	UNIVERSITY OF SOUTH FLORIDA	20-SEP-2022

Count	**Scientific Name**	**Common Name**
000080	*Heterocephalus glaber*	NAKED MOLE-RAT
000040	*Acomys cahirinus*	CAIRO SPINY MOUSE
000120	**Total**	

UNIVERSITY of SOUTH FLORIDA

MEMORANDUM

TO:	Jay Dean,
	(b) (6)
FROM:	Farah Moulvi, MSPH, IACUC Coordinator Institutional Animal Care & Use Committee Research Integrity & Compliance
DATE:	12/6/2022
PROJECT TITLE:	Mitigation of CNS oxygen toxicity: targeted antioxidant therapy Mitigation of CNS oxygen toxicity: mitochondria targeted antioxidant therapy and aquaporin gas channels
FUNDING SOURCE:	Federal government or major agency that awards grants based on peer-reviewed proposals (NIH, NSF, DOD, AHA, ACS, etc.) Office of Naval Research
IACUC PROTOCOL #:	R IS00011501
PROTOCOL STATUS:	**APPROVED**

The Institutional Animal Care and Use Committee (IACUC) reviewed your application requesting the use of animals in research for the above-entitled study. The IACUC **APPROVED** your request to use the following animals in your **protocol for a three-year period beginning 12/6/2022 and expiring on 12/6/2025:**

Rat: Sprague-Dawley (rats for MitoQ, L-NAME & Ketone ester (KE) dosing, dives & telemetry) (5-12wk/150-350g/male)	90

Please take note of the following:

- **After three years, all continuing studies must be completely re-described in a new electronic application and submitted to IACUC for review.**
- **All modifications to the IACUC-Approved Protocol must be approved by the IACUC prior to initiating the modification.**
- **All costs invoiced to a grant account must be allocable to the purpose of the grant.**

UNIVERSITY OF SOUTH FLORIDA
USF RESEARCH & INNOVATION

Hello, Jay Dean

Compare

15 - Substance Administration

15.2 Substance - Chemical Agents

15.3 Substance - Conventional Chemical

15.5 Substance - Vehicle/Diluent/Solvent

15.13 Substance - Final Page

16 - Specimen Collection

16.1 Specimen Collection - Ante Mortem

17 - Surgery

17.1 Surgery

18 - Other Experimental Procedures

18.8 Other - Other Experimental

Investigator Assurance

Investigator's Assurance

Reading: IS00011501

Go to forms menu | Print | Icons | Help

Study Identification

1.1

The title and narrative of this IACUC application must match the title and narrative of the grant that supports this research.

Submit a separate IACUC application for each federal or major agency grant.

1.1.1 * **Main Title** >>Get Help
Mitigation of CNS oxygen toxicity: targeted antioxidant therapy

1.1.2 **Additional Titles:** >>Get Help
Mitigation of CNS oxygen toxicity: mitochondria targeted antioxidant therapy and aquaporin gas channels

1.1.3 * **Principal Investigator:**
The applicant Principal or Secondary Investigator must have a faculty appointment with the University or an appropriate appointment with the James A. Haley Veteran's Administration Medical Center, H. Lee Moffitt Cancer Center and Research Institute, or New College of Florida.
Jay Dean

1.1.4 **Secondary Investigator(s):**

First Name	Last Name	Organization	IACUC Cert Expiration	Profile
There are no items to display				

1.1.5
Research Staff:
List all IACUC-certified personnel that will contribute to the study. >>Get Help

Last Name	First Name	Organization	IACUC Cert Expiration	Profile
Moss	Sara	Cell Biology, Microbiology and Molecular Biology (CMMB)	12/22/2023	00019174
Rogers	Christopher	Molecular Pharmacology and Physiology	2/5/2026	00009982
Stavitzski	Nicole	Integrative Biology	7/20/2027	00013453

1.1.6
* **List Secondary Study Contact(s):**
Secondary study contacts can manage the correspondence of a protocol in the absence of the PI. >>Get Help

Person	Organization
Nicole Stavitzski	Integrative Biology

1.1.7 * **Please Select:**
- ○ New Project
- ● **3rd year renewal replacing a previously approved protocol**

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Protocol Continuations

1.2

Exit

1.2.1 * **Please enter the number of the previous protocol this application is replacing.**

Compare

15 - Substance Administration

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15.13 Substance - Final Page

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17.1 Surgery

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18.8 Other - Other Experimental

Investigator Assurance

Investigator's Assurance

IS00007438

1.2.1a **Upload the previous protocol:**

Name	Modified	Version
Mitigation of CNS oxygen toxicity: mitochondria-targeted antioxidant therapy and aquaporin gas channels(0.01)	11/29/2022 8:06 AM	0.01

1.2.2 * **For research applications ONLY, summarize work accomplished during the prior three-year approval period and how this proposal extends those findings. (Indicate Not Applicable, N/A, for Murine Colony, Antisera Production or Tissue applications).**
This protocol continues work unfinished in the current ONR-funded project (Award N00014-15-1-2572). To date, we have used 171 rats. We have completed most of Experiments 1-3 in Sprague Dawley rats (in vivo). This new protocol requests animals to complete experiment set 3. Based on our results to date, we abandoned brain slice experiments 4&5. Regarding Experiments 6-8, we have used 51 mice. We completed experiments 6&7, and based on our results we abandoned Experiment 8. Our reduced animal use from that originally estimated is due to canceled experiments based on results using MitoQ in intact rats and results in AQP4-KO vs. WT mice.

eiacuc

Study Personnel Certification Information

1.3

The following individuals' certification information must be current before IACUC approval can occur. Please Note: The information on this form is read-only. An individual's certification information can be updated on his or her Researcher Profile. Verification dates are granted by the IACUC following review of submitted information

Principal Investigator:

Name	CV	Animal Experience		Documentation of Online Training Completion	Date Verified	Link to Profile
Jay Dean	CV August 2022_Jay B Dean(0.01)	Rat	41	Dean, Jay USF ARO 2012 05-24.pdf(0.03)	8/30/2021	00006346
		Snake	2			
		Cat	5			
		Mouse	2			

Secondary Investigators:

Name	CV	Animal Experience	Documentation of Online Training Completion	Date Verified	Link to Profile
There are no items to display					

Research Staff:

Name	CV	Animal Experience		Documentation of Online Training Completion	Date Verified	Link to Profile
Sara Moss	C.V. .docx(0.02)	Mouse	<1	CEUCertificate.pdf(0.01)	12/22/2017	00019174
Christopher Rogers	2021.05.05.CQR.CV.docx(0.02)	Mouse	16	AALAS(0.01)	2/5/2020	00009982
		Rat	7			
Nicole Stavitzski	Nicole Stavitzski - Resume 2-10-15 (1).pdf(0.01)	Rat	6	AALAS Certificate.jpg(0.04)	7/20/2021	00013453
		Mouse	1.5			

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Compare

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17 - Surgery

17.1 Surgery

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18.8 Other - Other Experimental

Investigator Assurance

Investigator's Assurance

Funding Information

2.1

2.1.1 * **Select the funding source which best describes the sponsor of this research (check all that apply):** >>Get Help

Federal government or major agency that awards grants based on peer-reviewed proposals (NIH, NSF, DOD, AHA, ACS, etc.)

2.1.2 * **Does the funding source/sponsor of this study require compliance with 21 CFR 58 Good Laboratory Practices (GLP) Standards?** >>Get Help

○ Yes ● **No**

2.1.3 * **Will this protocol use any VA resources?**

○ Yes ● **No**

2.1.4 * **Will this protocol be supported by funds administered by Moffitt Cancer Center?**

○ Yes ● **No**

eiacuc

Funding - Federal/Major Agency

2.2

2.2.1 **Indicate the federal agencies that award grants based on peer-reviewed proposals:** >>Get Help

Office of Naval Research

2.2.1a **Other federal or major agency:**

2.2.2 * **Please enter the grant or contract number:**

ONR N00014-20-1-2434; USF#6143120800

2.2.3 * **Upload the federal or major agency grant narrative, or portions that include the face page, research plan and vertebrate section which describes the specific aims and methods involving vertebrate animal use.** >>Get Help

Name		Modified	Version
Award notice title page ff.(0.01)	•••	11/22/2022 4:52 PM	0.01
ONR grant 2019-2022(0.01)	•••	11/29/2022 8:55 AM	0.01

eiacuc

Protocol Type

3.1

3.1.1 * **Select the option that best describes your protocol type:** >>Get Help

Type
● **Research or Teaching**
○ Wildlife Research
○ Murine Colony Only
○ Antisera Production (Rabbit Only)
○ Tissue Use Only

Compare

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17.1 Surgery

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18.8 Other - Other Experimental

Investigator Assurance

Investigator's Assurance

eiacuc

Alternatives and Literature Search

4.1

4.1.1

*** On this page indicate that a search for alternatives and alternative methods has been conducted, including a search of at least two relevant databases (e.g., PubMed), covering the dates searched (i.e. at least the last ten years), using the indicated search term(s), and that the search was conducted on the indicated date. Two entries must be made to section 4.1.1, then respond to the following three sections.** >>Get Help

	Name of Database	Dates Covered	Key Words and Search Strategy	Date Search Conducted
View	PubMed	1980-2022	mitochondria-targeted antioxidant therapy, MitoQ or Mitoquinone, L-NAME, hyperbaric oxygen or HBO or HBO2, ketone ester, seizure, oxygen toxicity, rat, alternative anesthesia, pain, radio-telemetry, local field potentials, nitric oxide synthase inhibitor	11/18/2022
View	Google Scholar	1980-2022	mitochondria-targeted antioxidant therapy, MitoQ or Mitoquinone, L-NAME, hyperbaric oxygen or HBO or HBO2, ketone ester, seizure, oxygen toxicity, rat, alternative anesthesia, pain, radio-telemetry, local field potentials, nitric oxide synthase inhibitor	11/18/2022

4.1.2 *** Indicate in a narrative below that in the search that non-animal alternatives were considered, that less sentient species were considered, and that none were found suitable. (The narrative must demonstrate that alternatives to the use of animals as described in this application are not available.)** >>Get Help

A search for non-animal alternatives and a less sentient species was conducted. However, this is the first study of the effects of MitoQ on the latency time to seizure in CNS-OT. Moreover, it is the first study of the effects of various nitric oxide synthase inhibitors on local field potentials in the brain in conscious animals using deep intracerebral radio telemetry. It would be premature to employ alternative non-animal models. Computer simulation and reduced neural tissue preparations would be inappropriate until the temporal patterns and locations of the hyperoxia-sensitive neural networks are identified.

4.1.3 *** Indicate in a narrative below that alternatives to painful procedures were considered, none were found suitable, and that methods described will be continuously refined so as to reduce animal discomfort. (The narrative must demonstrate that alternatives to these procedures as described in this application which may cause pain or discomfort to animals are not available or suitable.)** >>Get Help

Alternative to painful procedures have been considered but none were found suitable. Our experiments used freely behaving, awake rats to study the effects of anti-oxidant therapies on the sensitivity of the CNS to hyperbaric oxygen (HBO) which manifests as generalized seizures. Thus, the end-point of the experiment--seizure--is required, which may cause some discomfort. Animals that are instrumented for deep electrode radio telemetry prior to HBO exposures are anesthetized for sterile surgery and treated with analgesics pre- and post-surgically to minimize pain from telemetry module and recording probe implants.

4.1.4 *** Indicate in a narrative below that this animal use does not unnecessarily duplicate previous research or existing studies:** >>Get Help

Our search revealed that this research does not duplicate prior research or ongoing studies. Only 3 hits occurred when combining "mitochondria-targeted antioxidant therapy" and "hyperbaric oxygen" and these dealt with Parkinson's Disease, high altitude exposure, and a review article on the role of oxidative species in cell signaling; that is, there was no scientific overlap. Searching "ketone ester" and "HBO or HBO2" gave hits that were our own published research studies and 4 from external sites but not in the same context as the present study. In the present study, exogenous ketone ester will be used to determine its effects on a neurological measurement that we have never made before during exposure to HBO. All other combinations of searches did not provide evidence of overlapping research.

eiacuc

Compare

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18.8 Other - Other Experimental

Investigator Assurance

Investigator's Assurance

Characteristics of Animals and Pain Category of Research

5.1

List and describe the animals to be used. Indicate the anticipated number of animals to be used in each Pain Category of Research and the total number of animals involved during the 3-year approval period of this protocol. Indicate strain or line designation if rodents are requested. Indicate the number of each species/strains that will be involved in planned procedures that are anticipated to produce momentary, slight, or no pain, discomfort or distress (USDA Pain Category C); more than momentary or slight pain, discomfort or distress which is alleviated by the use of appropriate anesthetics and/or analgesics (USDA Pain Category D); or pain, discomfort, or distress, which cannot, or is not alleviated by the administration of appropriate anesthetics and/or analgesics (USDA Pain Category E). (Note: Refer to IACUC Principles & Procedures XII.)

5.1.1 * **For each animal group, click +Add and complete the form.**

	Species / Stock/Strain / Characteristics	# Pain Category C	# Pain Category D	# Pain Category E	Total Number
View	Rat: Sprague-Dawley (rats for MitoQ, L-NAME & Ketone ester (KE) dosing, dives & telemetry) (5-12wk/150-350g/male)	0	0	90	90

If any additional strains have been added at Amendment, they will be shown below:

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Housing and Use Sites

6.1

6.1.1 * **Will animals be housed in a University-managed animal facility?**

● **Yes** ○ No

6.1.2 * **Will animals be transferred from the housing room to any animal research/procedural/testing areas outside of the University-managed animal facility?**

● **Yes** ○ No

6.1.3 * **Does this proposal involve an inter-institutional collaboration or will be supported by a subcontract from another institution, or will involve animals housed at, or on behalf of another institution?**

○ Yes ● **No**

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Housing Facilities

6.2

6.2.1

* **For each HOUSING FACILITY where animals will be housed, click +Add and complete the form.** >>Get Help

	Location	Species
View	MDC – College of Medicine	Rat: Sprague-Dawley (rats for MitoQ, L-NAME & Ketone ester (KE) dosing, dives & telemetry) (5-12wk/150-350g/male)

Compare

15 - Substance Administration

15.2 Substance - Chemical Agents

15.3 Substance - Conventional Chemical

15.5 Substance - Vehicle/Diluent/Solvent

15.13 Substance - Final Page

16 - Specimen Collection

16.1 Specimen Collection - Ante Mortem

17 - Surgery

17.1 Surgery

18 - Other Experimental Procedures

18.8 Other - Other Experimental

Investigator Assurance

Investigator's Assurance

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Outside Housing and Use Sites

6.3

6.3.1

*** Enter the animal research/procedural/testing location where animals will be taken during the course of study, whether survival surgery will be performed, whether animals will be maintained/housed for >12 hours at this site, whether animals will need to be returned to a Comparative Medicine-managed facility after the procedure or that the procedure will be terminal, and provide a scientific or logistical justification for why animals must be removed from the facility:** >>Get Help

	Building	Room	Species	Survival Surgery	Housed >12 Hours	Return to Housing	Justification	Transport Method
View	MDL	1026 Hyperbaric Lab	Rat: Sprague-Dawley (rats for MitoQ, L-NAME & Ketone ester (KE) dosing, dives & telemetry) (5-12wk/150-350g/male)	yes	no	yes	Animals are transported to PI's lab to undergo recovery surgery; that is, to implant epidural and deep intracerebral recording electrodes & a radio-telemetry module that is connected to epidural and deep intracerebral electrodes. Equipment in the lab is used to establish the electrodes are working while anesthetized before the animal recovers and is returned to the vivarium for later use in an experiment (at least 1 wk later). Rats are also brought to the PI's lab to be dived in the hyperbaric chamber to assess their sensitivity to HBO w/wo anti-oxidant therapy for neuroprotection against CNS-OT.	Rats are transported from COM vivarium across the courtyard to MDL Bldg in a vivarium-supplied cage atop a smoothly rolling cart. The animal cage is covered by 1-2 lab coats. The trip takes ~1.5 minutes.

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Study Summary and Animal Use Rationale

7.1

7.1.1 *** Very briefly summarize (less than 150 words) in lay terms that are intelligible to a nonscientist (1) the purpose of the study, (2) the sequential procedures involved (*i.e., in general terms*), and (3) why the study is important to human or animal health, the**

Compare

15 - Substance Administration

15.2 Substance - Chemical Agents

15.3 Substance - Conventional Chemical

15.5 Substance - Vehicle/Diluent/Solvent

15.13 Substance - Final Page

16 - Specimen Collection

16.1 Specimen Collection - Ante Mortem

17 - Surgery

17.1 Surgery

18 - Other Experimental Procedures

18.8 Other - Other Experimental

Investigator Assurance

Investigator's Assurance

advancement of knowledge, or the good of society: >>Get Help
The research project is in its final year of funding and continues work to be finished based on the original goals and results to date. The purpose of this study is to determine if mitochondria-targeted antioxidant therapy combined with other neuroprotective strategies decreases risk for central nervous system oxygen toxicity (CNS-OT; i.e., seizures) during exposure to hyperbaric oxygen (HBO with/without hypercapnia). Mitoquinone (MitoQ) was the antioxidant we've been testing in combination with or in addition to exogenous ketone ester (KE) and nitric oxide synthase (NOS) inhibitor (L-NAME). Methods used include survival surgery to implant telemetric deep intracerebral electrodes; simulated “dives” in HBO +/-CO2 using a hyperbaric pressure chamber; and local field potential brain activities. The study is important to determine if these strategies mitigate risk of CNS-OT for neuroprotection during exposure to HBO and thus potentially longer, safer dives by Special Operations warfighters.

7.1.2 * **Provide a narrative that indicates the design of this study considered the impact of the proposed procedures on the animals' well-being and balance that with the specific aims of research. (Explain how the scientific benefits of the intended research outweighs harm to the animals):**
The design of this study considered the impact of the proposed procedures on the animals' well being relative to the scientific benefits. Proposed experiments require stereotaxic neurosurgery & body cavity surgery in anesthetized animals (sterile, w/recovery) to implant radio-telemetry modules that are connected to deep intracerebral and epidural recording electrodes to measure brain local field potentials in the brain stem and forebrain in awake animals (>1wk recovery from surgery). These types of procedures and experiments are necessary at this time because very little is known about the the sites for seizure genesis in the brain during exposure to HBO. Moreover, it is unknown if the proposed cocktail of antioxidant therapy provides neuroprotection against CNS-OT seizures. Thus, these experiments provide important, new information as the U.S. Navy works to develop mitigation strategies for using HBO in the undersea soldier for longer and safer diving operations. Research done in small animal studies at USF has been/will be used to provide guidance to U.S. Navy researchers using more sentient species (20 kg swine) and ultimately, U.S. Navy SEALs.

7.1.3 * **Describe the characteristics of each species/strain requested that justify its use in this protocol. If a specific sex is required, explain why:**
We will use the male Sprague Dawley rat. All of our prior research on O2, CO2 sensitivity and mechanisms of CNS-OT has been done in this animal model. Adult animals are used in radio telemetry experiments because they are better suited for implanting the large 4ET telemetry modules. Males are used for all HBO diving experiments because males are more resistant to HBO induced seizures compared to females. Once our studies in males are concluded, we envision future experiments in new projects also including female rats.

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Experimental Design

8.1

8.1.1 * **Provide a clear and concise description of the specific experimental manipulations and treatments of animals in chronological order so that IACUC reviewers understand exactly what will be done to all animals from entry into the study to its endpoints:** >>Get Help
Note: the proposed experiments are based on work required to finish the goals of the original project in its final year of funding (plus no cost extension to be requested).

Mitigation strategies for neuroprotection against CNS-OT seizures.

Experiment set 1)
Antioxidant therapies in un-instrumented rats. Rationale: our experiments to date indicate that MitoQ (22mg/kg) while breathing air at sea level is safe. During exposure to HBO, this same dose delays seizures (good!) but becomes toxic within tens of minutes of exposure to HBO and after decompression (bad!). Decreasing [MitoQ] down to 13 mg/kg continues to block seizures but remains toxic during exposure to HBO. We want to do two remaining experiments to finish the MitoQ story. First, does combining MitoQ + L-NAME prevent MitoQ toxicity in HBO; our rationale is for using L-NAME is that inhibition of superoxide by MitoQ allows run away nitric oxide production, which is otherwise normally consumed by its reaction with superoxide to yield peroxynitrite. Thus, we want to decrease both superoxide (MitoQ) and nitric oxide (L-NAME). Second, is the toxicity of MitoQ in HBO due to mitoquinone per se or TPMP (the molecule that allows MitoQ to easily penetrate the cell and mitochondria membranes)?

Approach 1: expose freely behaving rats to HBO +/-CO2 plus either:
a) triphenylphosphonium cation (= TPMP, a control for MitoQ; 15-22mg/kg, i.p.). TPMP is bonded to coenzyme Q to render MitoQ highly permeable to the cell membrane and mitochondrial membrane but it does not inhibit superoxide. Mitoquinone, by contrast, is what inhibits mitochondrial superoxide. Each rat serves as its own control (i.p., vehicle) and is dived twice (i.p. TPMP), 3-7 days between dives; agents randomized. 10 rats.

b) MitoQ plus L-NAME (= N(gamma)-nitro-L-arginine methyl ester). L-NAME inhibits nitric oxide synthase (NOS) and thus nitric oxide (.NO) production during exposure to HBO. Our results indicate that 30 mg/kg L-NAME alone delays onset of seizures in HBO. Two L-NAME doses (30 & 60 mg/kg, i.p.) will be tested in combination with 15 mg/kg MitoQ (i..p.). Each rat is dived once. 30 rats (= 10 controls; 2 treatment groups, 10/group).

c) KE (7.5g/kg) +/- L-NAME (30 & 60 mg/kg). Rationale. Both agents delay seizures during exposures to HBO +/-CO2. Are their neuroprotective effects additive, multiplicative, or neither? When testing KE vs. vehicle, the day of the dive, pre- and post-dive blood samples are taken to measure ketone and glucose levels. Each rat serves as it own control (i.p., vehicle) and is dived 3-4 times with treatments randomized every 3-7 days. 20 rats.

Compare

15 - Substance Administration

15.2 Substance - Chemical Agents

15.3 Substance - Conventional Chemical

15.5 Substance - Vehicle/Diluent/Solvent

15.13 Substance - Final Page

16 - Specimen Collection

16.1 Specimen Collection - Ante Mortem

17 - Surgery

17.1 Surgery

18 - Other Experimental Procedures

18.8 Other - Other Experimental

Investigator Assurance

Investigator's Assurance

The end point of each "dive" in 1.a-1.c is the animal's latency time to onset of first and second bouts of seizure (= LSz1 & LSz2) or 120 min of HBO +/-CO2, which ever comes first. In dives with significantly delayed seizures, any signs of pulmonary oxygen toxicity (tachypnea, foaming around mouth) will cause the dive to be terminated before 90min.

Experiment Set 2)
Deep intracerebral and epidural radio telemetric electrode recordings of local field potentials in freely behaving rat during exposure to HBO +/-CO2. Our goal is to determine so called "ox tox trigger zones" where seizures originate during exposure to HBO and then determine the effects of anti-oxidant therapies on this neural activity based on experiment 1.

Approach 2:
Under deep anesthesia (isoflurane), 2-4 intracerebral recording electrodes (tungsten; FHC, Inc.) are implanted using the stereotaxic frame/manipulator into motor cortex and deep subcortical nuclei that are implicated in seizure genesis, including brain stem cardiorespiratory nuclei and cranial nerve nuclei) and seizure amplification/propagation nuclei (CA1 hippocampus). Recording and reference electrodes are secured to the skull using dental adhesive and stainless steel screws. The telemetry module (4ET, Data Sciences International, DSI Inc.) is implanted in the abdomen and the four pairs of biopotential leads are tunneled under the skin, exposed at the skull, and attached to the recording electrodes and epidural reference electrodes. We have successfully developed these techniques during the previous protocol. In some cases, only two deep electrodes are implanted and the remaining two electrodes are implanted in the respiratory muscles and chest muscles to measure respiratory electromyogram and electrocardiogram activities, respectively.

After recovery from surgery (at least 1 wk), each animal is dosed with either vehicle or the most effective treatment from Experiment 1 and dived to 3-5 ATA O2 (HBO +/-CO2) until onset of 1st and 2nd bouts of seizures. After the first dive, at least one to seven days later, the animal is treated with one of the other test agents and dived again until onset of 1st & 2nd bouts of seizures. Up to 4 dives may be done, using HBO alone (2 dives) vs. HBO + CO2 (2 dives). The order of treatments is randomized across dive series in each animal.

All experiments are done in unrestrained, freely behaving animals. The length of time spent in the animal chamber inside the hyperbaric chamber is no longer than 3.5 hours. Each animal has access to hydrogel; however, no food is provided to reduce chewing activity and activation of brainstem circuits that otherwise confound our experimental recordings. Either before or after the "dive" in HBO +/-CO2, the animal's hypercapnic (air --> 5, 7 or 10% CO2 in air, 5-15 min) and hypoxic (air --> 8% O2 in N2, 5-10 min) ventilatory responses will be tested at room pressure. If testing KE effects, the day of the dive, pre- and post-dive blood samples are taken to measure ketone and glucose levels.

If needed, two control experiments to separate out the effects of normal behaviors that activate brain stem neural activity (e.g., grooming, scratching ear) from changes in neural activity induced by exposure to HBO +/-CO2 are as follows: 1) The rat is dived in HBO +/-CO2 with <1.4% isoflurane added to breathing atmosphere (1.4% is the minimum level of anesthesia) to calm the rat and prevent excessive grooming, etc. The low dose isoflurane would be administered once the animal reaches maximum HBO "depth" and sustained until onset of CNS ox tox seizures. 2) An alternative control is i.p. injection 6-9 mg/kg xylazine prior to diving to calm the rat for the duration of the "dive" (0.5-1 hr). A total of 30 rats are required: (10 rats/group) x 3 electrode configurations. Each rat is dived 2-4 times with 1-7 days between each dive.

8.1.2

For complicated experimental designs, upload a detailed flowchart, diagram, or table, which depicts the experiments or sequence of events with a timeline, and indicates the number of animals needed for each experimental group and the number of groups required: >>Get Help

Name	Modified	Version
There are no items to display		

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Statistical Analysis and Rationale

9.1

The following species/strains/lines were requested in Section 5. Use this table to complete your response to the questions below: >>Get Help

Species / Stock/Strain / Characteristics	# Pain Category C	# Pain Category D	# Pain Category E	Total Number
Rat: Sprague-Dawley (rats for MitoQ, L-NAME & Ketone ester (KE) dosing, dives & telemetry) (5-12wk/150-350g/male)	0	0	90	90

9.1.1 * **Describe the statistical analyses and rationale used to determine experimental group sizes and the total number of each species of animals requested:** >>Get Help

Compare

15 - Substance Administration

15.2 Substance - Chemical Agents

15.3 Substance - Conventional Chemical

15.5 Substance - Vehicle/Diluent/Solvent

15.13 Substance - Final Page

16 - Specimen Collection

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18.8 Other - Other Experimental

Investigator Assurance

Investigator's Assurance

For Expts. 1a-1b and 2: based on our past studies of exposing rats to HBO +/-CO2, power analysis indicated a power = 0.8-0.9 for n=10 rats per group.

Expt. 1a. expose freely behaving rats to HBO +/-CO2 plus TPMP, a control for MitoQ; 15-22mg/kg, i.p.). Each rat serves as its own control (i.p., vehicle) and is dived twice (i.p. TPMP), 3-7 days between dives; agents randomized. Thus, for this experiment, we are requesting: [(10 rats where each rat serves as its own control (vehicle) plus one additional dive on TPMP) = 10 rats

Expt. 1b. expose freely behaving rats to HBO +/-CO2 plus L-NAME plus MitoQ. Two L-NAME doses (30 & 60 mg/kg, i.p.) will be tested in combination with 15 mg/kg MitoQ (i..p.). Each rat is dived once. (10 rats/group) x (3 groups: controls & 2 [L-NAME]) = 30 rats.

Expt. 1c. expose freely behaving rats to HBO +/-CO2 plus KE (7.5g/kg) +/- L-NAME (30 & 60 mg/kg). Each rat serves as it own control (i.p., vehicle) and is dived 3-4 times with treatments randomized every 3-7 days. Thus, (10 rats/group) x (2 groups: KE +/- 30 & 60 L-NAME) = 20 rats. Each rat is dived 4 times (randomized order) in i) vehicle, ii) KE, iii) L-NAME, and iv) KE + L-NAME-dose.

Experiment 2) Telemetric Deep intracerebral microelectrode recordings from different brain regions in unrestrained, freely behaving rats during exposure to HBO -/+CO2. 30 rats are requested = (10 rats/group) x (3 telemetric electrode configurations).

9.1.2 * **Provide an explanation/rationale for assigning the specific number animals to the different pain categories of research declared above:**
Rats in pain category E (are there because they will experience potential distress from seizures caused by protracted exposure to HBO +/-CO2.

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Experimental Endpoints

10.1

10.1.1 * **Is animal death (excluding death from euthanasia) an intentional endpoint in this protocol?** >>Get Help

○ Yes ● **No**

10.1.1a **If Yes, explain why an earlier endpoint is not possible:**

10.1.2 * **Will animals be involved in procedures that are anticipated to produce more than momentary or slight pain, discomfort, or distress which cannot, or will not be alleviated by the administration of appropriate anesthetics or analgesics?** >>Get Help

● **Yes** ○ No

10.1.2a **If Yes, describe the potential consequences of experimental manipulations, and the precise clinical criteria that will be used to ensure timely intervention:**
During HBO +/- CO2 exposure, the rat is visualized in real time continuously via video feed for telltale signs of CNS oxygen toxicity, including generalized tonic-clonic seizures of the upper limbs, neck and head coincident with increased brain wave activity (when measured, e.g., Expt. 2). Once the 2nd bout of seizures begins (the 1st is often minor and short lived lasting only a few seconds) the inspired [O2] is decreased to air and chamber decompression initiated. Typically, seizures stop immediately or within 1-2 minutes. If animals exhibit evidence of pain and discomfort at any time following surgery and during exposure to one of the treatments during HBO +/-CO2 they will be removed and euthanatized; e.g., failure to respond to stimuli, inactivity, hunched or abnormal posture, altered gait, ruffled/matted/or soiled fur, piloerection, circling, head tilt, seizures, vocalization, head pressing, and/or rapid/shallow/labored breathing.

10.1.2b **If Yes, describe the frequency of animal observation:**
During HBO +/-CO2 exposures, the animal is visualized in real time continuously via video feed for telltale signs of oxygen toxicity of brain (10.1.2a). All animals are checked daily while in the vivarium by a member of CompMed. Following surgery, animals are checked by a member of the P.I.'s lab for the next 1-3 days.

10.1.2c **If Yes, describe the intervention (e.g., treatment) used upon reaching the clinical endpoint, or indicate that animals reaching clinical endpoints will be euthanatized (but do not describe methods of euthanasia here, as these will be requested under item 11.1.1b):** >>Get Help
Any animal exhibiting abnormal physiology or behavior will be removed and euthanatized with CO2 per SOP.

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Compare

15 - Substance Administration

15.2 Substance - Chemical Agents

15.3 Substance - Conventional Chemical

15.5 Substance - Vehicle/Diluent/Solvent

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18.8 Other - Other Experimental

Investigator Assurance

Investigator's Assurance

Euthanasia

11.1

11.1.1 * **Are animals euthanatized?**

● **Yes** ○ No

11.1.1a **If No, for each affected species, describe the final disposition of the animals:**

11.1.1b **If Yes, for each affected species, describe the method of euthanasia and means of ensuring death, indicating dose and route if a chemical agent or provide justification if a physical method:** >>Get Help

Rats with telemetry modules and epidural and deep intracerebral electrodes are anesthetized with 5% isoflurane followed by exsanguination via transcardial perfusion with phosphate buffered saline (PBS) followed by 4% paraformaldehyde in PBS. The carcass is decapitated postmortem and the head placed in PFA fixative to be imaged for electrode tip locations using co-registered CT and MRI scans at SAIL. Rats used in Expt series 1 are placed in the CO2 gassing chamber (not precharged with CO2) and slowly filled with CO2 from a compressed gas tank per AVMA guidelines and CM SOP 401. After cessation of breathing the animals are observed in room air for at least 10 minutes, or by employing a secondary method of euthanasia such as cervical dislocation, decapitation, or bilateral thoracotomy. The CO2 gassing chamber is cleaned and dried between each use.

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Procedure Classifications

12.1

Indicate the procedures you will employ in this study from the list below.

* **Select all that apply:**

Section 13: **Husbandry & Caging:**

- [ ] Special Husbandry (Non-standard) or Housing at Satellite Locations
- [x] **Food and/or Water Manipulation**
- [ ] Special Caging (Non-standard)
- [x] **Exemption from Required Species-specific Enrichment and/or Socially Compatible Group Housing**
- [ ] Dog Exercise
- [ ] NHP Enrichment

Section 14: **Drugs Administered for Clinical Care:**

- [x] **Anesthetic, Analgesic, or Veterinary Therapeutic Administration (include drugs used during surgery)**

Section 15:

Test Substances Administered for Experimentation: >>Get Help

- [ ] Biological Toxins/Infectious Agent
- [x] **Chemical Agent: Vehicle/Diluent/Solvent**
- [ ] Pharmacological Agent
- [ ] Radioactive Isotope
- [ ] Adjuvant/Antigen
- [ ] Tumor Cell Lines
- [ ] rDNA
- [ ] Primary Human Explants-Tissue, Cells, Fluids

Compare

15 - Substance Administration

15.2 Substance - Chemical Agents

15.3 Substance - Conventional Chemical

15.5 Substance - Vehicle/Diluent/Solvent

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Investigator Assurance

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- [ ] Other Agents

Section 16: **Specimen Collection:**

- [x] **Specimen Collection Ante mortem**
- [x] **Specimen Collection Post mortem (PI acknowledges that animals will be confirmed dead before tissues are collected.)**

Section 17: **Surgery:**

- [x] **Surgery (check Drug Administration above to account for drugs used during surgery)**

Section 18: **Other Experimental Procedures:**

- [ ] Irradiation
- [ ] Diagnostic Imaging & Microscopy
- [ ] Use of Human Patient Procedural Areas
- [ ] Breeding (Not Colony Production)
- [ ] Prolonged Restraint
- [ ] Stress Paradigms
- [ ] Behavioral Testing
- [x] **Other Experimental Procedure (Not listed above)**

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Food and/or Water Manipulation

13.2

13.2.1 * **Name each affected species and describe all non-standard food and/or water manipulations (e.g. special dietary formulations, fluid supplements, food or water deprivation, calorie restrictions):** >>Get Help
During exposure to HBO +/-CO2 in the pressure chambers, rats will be provided hydrogel for fluid intake.

13.2.2 * **Justify the implementation of this non-standard food and/or water manipulation:**
The study requires the use of a hyperbaric pressure chamber for HBO diving (up to 3.5 hours) to study CNS-OT mitigation strategies.

13.2.3 * **Describe the methods used to monitor animal condition during the procedure and methods used to avoid and alleviate discomfort, if anticipated:**
During HBO dives, the animal is under constant digital camera and visual observation.

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Exemption from Required Species-specific Enrichment

13.4

13.4.1 * **Justify why animals in this study (except for dogs or nonhuman primates which are addressed elsewhere in this application) either (A) require exemption from the animal program's routine species-specific environmental enrichment plan and indicate if alternatives to the plan will be provided; or (B) require exemption from group housing, the default standard housing for social species, and provide a justification for this request:**

Compare

15 - Substance Administration

15.2 Substance - Chemical Agents

15.3 Substance - Conventional Chemical

15.5 Substance - Vehicle/Diluent/Solvent

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Investigator Assurance

Investigator's Assurance

>>Get Help

Sprague Dawley rats implanted with telemetry modules and electrodes are individually housed. This prevents a cage mate from chewing the wound clips post surgery. Rats are checked daily by CompMed staff and frequently by members of the PI's lab.

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Drug Administration

14.1

14.1.1 * **Indicate the type(s) of drugs you will administer to animals during the course of this study.**

Anesthetics, analgesics and/or tranquilizers

Conventional Veterinary Therapeutics

14.1.2 * **Please press +Add and complete the chart below for each drug used in this protocol. (Please note that you will be asked to provide further information for each drug on subsequent pages.)** >>Get Help 2

	Drug	Pharmaceutical Grade?	Non Pharmaceutical Explanation	DEA Controlled?
View	isotonic saline	yes		no
View	Isoflurane (Forane)	yes		no
View	Meloxicam Sustained Release	yes		no
View	xylazine	yes		no
View	Buprenorphine Sustained Release	yes		yes

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Anesthetics, Analgesics, Tranquilizers and Paralytics

14.2

14.2.1 * **Please press +Add and complete the chart below for each drug used as an anesthetic, analgesic, tranquilizer, paralytic or reversal agent. (Do not include drugs used for therapeutic purposes):** >>Get Help

	Drug	Species	Dose	Route	Administration Frequency	Treatment Duration	Monitoring Efficacy
View	Buprenorphine Sustained Release	Rat: Sprague-Dawley (rats for MitoQ, L-NAME & Ketone ester (KE) dosing, dives & telemetry) (5-12wk/150-350g/male)	1mg/kg	s.c.	1x post op while waking up	72 hours	pain relief postof
View	Isoflurane (Forane)	Rat: Sprague-Dawley (rats for MitoQ, L-	0.25-1.4% in oxygen	breathing atmosphere	1x	30-120 min, duration of HBO +/- CO2 dive	to calm the rat and prevent exce movements t activate brair neural activit

Compare

15 - Substance Administration

15.2 Substance - Chemical Agents

15.3 Substance - Conventional Chemical

15.5 Substance - Vehicle/Diluent/Solvent

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Investigator Assurance

Investigator's Assurance

	Drug	Species	Dose	Route	Administration Frequency	Treatment Duration	Monitoring Efficacy
		NAME & Ketone ester (KE) dosing, dives & telemetry) (5-12wk/150-350g/male)					independently of that caused by exposure to HBO
View	Isoflurane (Forane)	Rat: Sprague-Dawley (rats for MitoQ, L-NAME & Ketone ester (KE) dosing, dives & telemetry) (5-12wk/150-350g/male)	3-5% induction; 1-3% during surgery (telemetry module & CNS electrodes)	inhalation	1x	1-2 hours	Induce and maintain anesthesia during sterile, survival surgery to implant radio telemetry module and epidural and deep intracranial electrodes to record local field potentials in CNS
View	Meloxicam Sustained Release	Rat: Sprague-Dawley (rats for MitoQ, L-NAME & Ketone ester (KE) dosing, dives & telemetry) (5-12wk/150-350g/male)	4 mg/kg	s.c.	pre op, 1x	72 hours	Meloxicam will be given as a preemptive analgesic in sterile saline prior to initiating surgery at any site. It is administered 1x/day and then p.r.n. at 24 hr intervals until 48 hours. Analgesic efficacy will be monitored post surgery by watching for signs of pain and discomfort; e.g., inactivity, hunched or abnormal posture, altered gait, ruffled/matted/or soiled fur, piloerection, circling, head tilt, vocalization, head pressing, and/or rapid/shallow/labored breathing.
View	xylazine	Rat: Sprague-Dawley (rats for MitoQ, L-NAME & Ketone ester (KE) dosing, dives & telemetry) (5-12wk/150-350g/male)	6-9 mg/kg	i.p.	1x	seconds	xylazine is used to calm the rat and reduce various behaviors that activate brain stem neural activity independently of that caused by exposure to hyperbaric oxygen

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Conventional Veterinary Therapeutics

14.4

14.4.1

* **Please press +Add and complete the chart below for each therapeutic drug used:**
>>Get Help

	Drug	Species	Dose	Route	Administration Frequency	Treatment Duration	Monitoring Efficacy
View	isotonic saline	Rat: Sprague-Dawley (rats for MitoQ, L-NAME & Ketone ester (KE) dosing, dives &	10 ml/kg/hr	sc	3-5x boluses over duration of surgery	15-30sec/treatment	to rehydrate animal during surgery as needed

Compare

15 - Substance Administration

15.2 Substance - Chemical Agents

15.3 Substance - Conventional Chemical

15.5 Substance - Vehicle/Diluent/Solvent

15.13 Substance - Final Page

16 - Specimen Collection

16.1 Specimen Collection - Ante Mortem

17 - Surgery

17.1 Surgery

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18.8 Other - Other Experimental

Investigator Assurance

Investigator's Assurance

Drug	Species	Dose	Route	Administration Frequency	Treatment Duration	Monitoring Efficacy
	telemetry) (5-12wk/150-350g/male)					

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Chemical Agents - Vehicle - Diluent - Solvent

15.2

15.2.1 * **Indicate the type of Chemical Agent you will administer to animals in this study:**

- [x] **Conventional Chemical Name**
- [ ] Novel Chemical Name or Structure
- [x] **Vehicle/Diluent/Solvent**

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Conventional Chemical Details

15.3

15.3.1 * **Please press +Add and complete the chart below for each Conventional Chemical used.** >>Get Help

View

Agent	hypoxia (low oxygen)
Species	Rat: Sprague-Dawley (rats for MitoQ, L-NAME & Ketone ester (KE) dosing, dives & telemetry) (5-12wk/150-350g/male)
Dose/Volume/Route	8-10% O2 in nitrogen / inhaled from atmosphere
Frequency/Interval	1x per experimental run; 2x total
Duration	5-10 min
Pharmaceutical Grade?	No
Non Pharmaceutical Explanation	specialty gas mixture supplied by Airco; 8-10% O2, balance N2
Purpose of Substance	We are recording neural activity from brain stem cardiorespiratory centers using telemetric deep electrodes and in some cases measuring respiratory muscle EMG. We want to test the animal's hypoxic ventilatory response (hyperventilation) to determine the electrode's functional location in the nucleus tractus solitarius, the first central synapse of the hypoxia peripheral chemoreceptors. After euthanasia, the electrode's position is also confirmed anatomically.
Expected Effect	Breathing 8-10% O2 in nitrogen will stimulate breathing and increase heart rate but since its exposure is limited to <10 min no adverse effects are expected. The animal will experience respiratory alkalosis during exposure to hypoxia through hyperventilation of CO2. This is a routine test used to activate the hypoxic ventilatory response in rodents.

View

Agent	hypercapnia (high carbon dioxode)
Species	Rat: Sprague-Dawley (rats for MitoQ, L-NAME & Ketone ester (KE) dosing, dives & telemetry) (5-12wk/150-350g/male)

Compare

15 - Substance Administration

15.2 Substance - Chemical Agents

15.3 Substance - Conventional Chemical

15.5 Substance - Vehicle/Diluent/Solvent

15.13 Substance - Final Page

16 - Specimen Collection

16.1 Specimen Collection - Ante Mortem

17 - Surgery

17.1 Surgery

18 - Other Experimental Procedures

18.8 Other - Other Experimental

Investigator Assurance

Investigator's Assurance

Dose/Volume/Route	5, 7, or 10% CO2 / inhaled from atmosphere
Frequency/Interval	1x per experimental run; 2x total
Duration	5-15 minutes
Pharmaceutical Grade?	No
Non Pharmaceutical Explanation	specialty gas mixture requested and supplied by Airco; CO2 in air
Purpose of Substance	In experiment 3, we are recording neural activity from brain stem cardiorespiratory centers using telemetric deep electrodes while measuring respiratory muscle EMG. We want to test the animal's hypercapnic ventilatory response (hyperventilation) to determine the electrode's functional location in a CO2 chemoreceptor region/respiratory region of CNS. After euthanasia, the electrode's position is also confirmed anatomically.
Expected Effect	Breathing 5-10% CO2 in air will stimulate breathing and increase heart rate but since its exposure is limited to <15 min no adverse effects are expected. The animal will experience respiratory acidosis during exposure to hypercapnia.

View

Agent	Hyperbaric oxygen (HBO) +/-hypercapnia
Species	Rat: Sprague-Dawley (rats for MitoQ, L-NAME & Ketone ester (KE) dosing, dives & telemetry) (5-12wk/150-350g/male)
Dose/Volume/Route	3 ATA total pressure (=2.93-2.95 ATA O2 in 0.05-0.07 ATA CO2); and 5 ATA O2 without CO2
Frequency/Interval	1-4x
Duration	3 ATA (HBO +CO2) x 2 hr; 5 ATA O2 only x 2 hr
Pharmaceutical Grade?	No
Non Pharmaceutical Explanation	specialty gas mixture requested and supplied by Airco; CO2 in O2
Purpose of Substance	HBO is used to induce CNS-OT seizures in rats. 5 ATA O2 (without CO2) and 2.93-2.95 balance CO2 at 3 ATA barometric pressure have similar latency time to seizures; CO2 retention accelerates onset of seizures. Thus, we can use a lower level of HBO (2.93-2.95 ATA vs. 5 ATA).
Expected Effect	HBO induces non-convulsive S/Sx that ultimately lead to tonic-clonic seizures. Non-convulsive S/Sx including bradycardia followed by tachycardia and hypertension and hyperventilation. Onset of seizures induces cardiogenic pulmonary edema. Physiological changes due to seizures will reverse with varying time courses from minutes (cardiorespiration) to hours (pulmonary edema).

View

Agent	triphenylphosphonium cation (TPMP)
Species	Rat: Sprague-Dawley (rats for MitoQ, L-NAME & Ketone ester (KE) dosing, dives & telemetry) (5-12wk/150-350g/male)
Dose/Volume/Route	15-22mg/kg / 0.1-0.3ml / i.p.
Frequency/Interval	1x
Duration	momentary
Pharmaceutical Grade?	Yes
Non Pharmaceutical Explanation	*No Value Entered*
Purpose of Substance	TPMP is the molecule that is covalently bonded to the antioxidant coenzymeQ to produce MitoQ and make it permeable to the cell and mitochondrial membrane.
Expected Effect	No adverse side effects are anticipated after up-and-down acute toxicity testing. Toxicity in mice, when reported, was not characterized other than "toxic". TPMP and MitoQ are usually given in similar doses in animal experiments. TPMP is used as a control for MitoQ.

View

Agent	Mitoquinone (MitoQ)
Species	Rat: Sprague-Dawley (rats for MitoQ, L-NAME & Ketone ester (KE) dosing, dives & telemetry) (5-12wk/150-350g/male)
Dose/Volume/Route	15-22mg/kg / 0.1-0.3ml / i.p.
Frequency/Interval	1x

Compare

15 - Substance Administration

15.2 Substance - Chemical Agents

15.3 Substance - Conventional Chemical

15.5 Substance - Vehicle/Diluent/Solvent

15.13 Substance - Final Page

16 - Specimen Collection

16.1 Specimen Collection - Ante Mortem

17 - Surgery

17.1 Surgery

18 - Other Experimental Procedures

18.8 Other - Other Experimental

Investigator Assurance

Investigator's Assurance

Duration	momentary
Pharmaceutical Grade?	Yes
Non Pharmaceutical Explanation	*No Value Entered*
Purpose of Substance	MitoQ is a supplement sold for human consumption. It is a mitochondria-targeted antioxidant.
Expected Effect	No adverse side effects were found during up-and-down acute toxicity testing and breathing room air. Toxicity in mice, when reported, was not characterized other than "toxic". However, when MitoQ is combined with HBO it becomes toxic and can result in death. This was an unexpected in our initial experiments. It is why we want to now combine MitoQ with L-NAME to offset the possible harmful effects of unbridled NO production in the absence of superoxide production. If rats exhibit adverse end points as defined above then the dive will be halted and the animal euthanatized.

View

Agent	Ketone ester, 75:25 mixture of (R,S)-1,3-butanediol monoaceto-acetate and (R,S)-1,3-butanediol diacetoacetate (food additive)
Species	Rat: Sprague-Dawley (rats for MitoQ, L-NAME & Ketone ester (KE) dosing, dives & telemetry) (5-12wk/150-350g/male)
Dose/Volume/Route	7.5 g/kg bw / oral gavage, approx 1-3 ml
Frequency/Interval	1x
Duration	30-120 min prior to HBO dive
Pharmaceutical Grade?	No
Non Pharmaceutical Explanation	unavailable in USP grade. Synthesized by biochemist working with our lab. Currently, KE is undergoing FDA GRAS testing
Purpose of Substance	Ketone ester is used to reduce the risk of CNS oxygen toxicity. The (R,S)-1,3-butanediol-acetoacetate monoesters and diester are nonionized sodium-free ketogenic precursors to ketone bodies beta-hydroxybutyrate and acetoacetate. They represent a convenient form of ketone body administration for parenteral and enteral nutrition.
Expected Effect	No adverse effects are expected; however, KE is expected to delay onset of seizures during exposure to HBO2.

View

Agent	N-nitro-L-arginine-methylester (L-NAME)
Species	Rat: Sprague-Dawley (rats for MitoQ, L-NAME & Ketone ester (KE) dosing, dives & telemetry) (5-12wk/150-350g/male)
Dose/Volume/Route	30, 60 & 82.5mg/kg / 0.2-0.4 ml / i.p.
Frequency/Interval	1x per experimental run; 2x total
Duration	30-120min prior to HBO dive
Pharmaceutical Grade?	Yes
Non Pharmaceutical Explanation	*No Value Entered*
Purpose of Substance	L-NAME is a non-specific inhibitor of nitric oxide synthase (NOS) and will blunt nitric oxide production during exposure to HBO.
Expected Effect	We postulate that L-NAME will offset the adverse effects of MitoQ + HBO that we have observed in our first experiments. Up and down acute toxicity with L-NAME from 30-82.5 mg/kg (i.p.) indicates is not toxic when breathing 1 ATA air.

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Compare

15 - Substance Administration

15.2 Substance - Chemical Agents

15.3 Substance - Conventional Chemical

15.5 Substance - Vehicle/Diluent/Solvent

15.13 Substance - Final Page

16 - Specimen Collection

16.1 Specimen Collection - Ante Mortem

17 - Surgery

17.1 Surgery

18 - Other Experimental Procedures

18.8 Other - Other Experimental

Investigator Assurance

Investigator's Assurance

Vehicle/Diluent/Solvent Details

15.5

15.5.1 * **Please press +Add and complete the chart below for each Vehicle/Diluent/Solvent used.**
>>Get Help

View

Agent	0.9% saline
Species	Rat: Sprague-Dawley (rats for MitoQ, L-NAME & Ketone ester (KE) dosing, dives & telemetry) (5-12wk/150-350g/male)
Dose/Volume/Route	1 ml/kg
Frequency/Interval	1x per experimental run; 2x total
Duration	momentary
Pharmaceutical Grade?	Yes
Non Pharmaceutical Explanation	*No Value Entered*
Purpose of Substance	vehicle with L-NAME, TPMP and MitoQ
Expected Effect	none

View

Agent	water
Species	Rat: Sprague-Dawley (rats for MitoQ, L-NAME & Ketone ester (KE) dosing, dives & telemetry) (5-12wk/150-350g/male)
Dose/Volume/Route	oral gavage, 1-3ml
Frequency/Interval	1x
Duration	30-120min prior to HBO dive
Pharmaceutical Grade?	No
Non Pharmaceutical Explanation	water from lab still
Purpose of Substance	control vehicle for ketone ester experiments
Expected Effect	none

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Substance Administration Final Page

15.13

15.13.1 * **Is there a possibility that any of the test substance(s) could cause more than momentary or slight pain, discomfort, or distress to the animals, either immediately following substance administration, or as a consequence long after administering the test substance(s)?**

● **Yes** ○ No

15.13.1a **If Yes, for each such substance, describe the consequences of administration that have a potential to cause animal discomfort, pain, or distress, and how discomfort, pain or distress will be anticipated, minimized or alleviated (e.g., nude mice administered tumor cells will be humanely euthanatized if induced tumors become >1.0 cm in diameter, or if tumors ulcerate, or if tumors interfere with posture, locomotion or feeding). If discomfort, pain, or distress will not be alleviated, then justify how treatments interfere**

Compare

15 - Substance Administration

15.2 Substance - Chemical Agents

15.3 Substance - Conventional Chemical

15.5 Substance - Vehicle/Diluent/Solvent

15.13 Substance - Final Page

16 - Specimen Collection

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Investigator Assurance

Investigator's Assurance

with the procedures or the interpretation of results:
HBO will stimulate breathing and cause bradycardia initially. Without antioxidant therapy, the rat will seize, on average, after ~20 min HBO +/-CO2. With MitoQ, if L-NAME does not offset MitoQ's toxicity in HBO then the rat may exhibit lethargy as we observed in our initial experiments. If other agents delay seizures some animals may develop signs of pulmonary oxygen toxicity as evidenced by frothy fluid accumulation around oronasal region. In any of these cases, we will terminate the dive.

15.13.2 * **Are any of the test substances listed above considered a regulated or potentially hazardous material to research or animal care personnel?**

○ Yes ● **No**

15.13.2a **If Yes, (1) list those substances in the space below, (2) describe procedures to ensure these substances and/or animals to which these substances have been administered will be handled safely (e.g., BSL-2, USF Chemical Hygiene Plan, Universal Precautions), and (3) provide an assurance that laboratory and animal care management and staff will be notified when such substances are in use:** >>Get Help

15.13.2b **Please upload any additional information regarding substances to be administered to animals (e.g., characterization of rodent biologics as pathogen-free, a detailed chemical structure for unique test substances that will allow their analysis for potential occupational hazards):**

Name	Modified	Version
There are no items to display		

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Specimen Collection *Ante Mortem*

16.1

16.1.1 * **For each specimen collected ante mortem (i.e., collections prior to euthanasia), press +Add and complete the form.**

	Species	Tissue or Fluid Collected	Amount collected	Frequency	Method of Collection
View	Rat: Sprague-Dawley (rats for MitoQ, L-NAME & Ketone ester (KE) dosing, dives & telemetry) (5-12wk/150-350g/male)	venous blood	10 uL, KE vs. water vehicle; analyze ketone body level	1x before & after HBO dive (2x total)	saphenous vein via 23G or 18G needle

16.1.2 **For specimen collection procedures that DO NOT have the potential of causing more than momentary pain or discomfort, describe the method of restraint and the method and site of collection:** >>Get Help
manual restraint using forearm, unanesthetized.

16.1.3 **For specimen collection procedures that DO have the potential of causing more than momentary pain or discomfort, describe the method of anesthesia including dose and route, the method of restraint and the method and site of collection:** >>Get Help

16.1.4 **For specimen collections procedures that DO have the potential of causing more than momentary pain or discomfort, indicate whether post-procedural analgesics will be used, their dose, route, and frequency of administration, and if not used, a justification for their omission. Provide a description of the post-procedural methods of monitoring:**

eiacuc

Surgery

17.1

Section 17 must be completed separately for each species used in surgical procedures. Select a species and all applicable strains/lines involved. If multiple species will be used in surgical procedures, answer "Yes" to 17.1.10 and complete an additional Surgery section.

17.1.1 * **Species:**

Rat: Sprague-Dawley (rats for MitoQ, L-NAME & Ketone ester (KE) dosing, dives & telemetry) (5-12wk/150-350g/male)

17.1.2 * **Select all that apply:**
Survival

Compare

15 - Substance Administration

15.2 Substance - Chemical Agents

15.3 Substance - Conventional Chemical

15.5 Substance - Vehicle/Diluent/Solvent

15.13 Substance - Final Page

16 - Specimen Collection

16.1 Specimen Collection - Ante Mortem

17 - Surgery

17.1 Surgery

18 - Other Experimental Procedures

18.8 Other - Other Experimental

Investigator Assurance

Investigator's Assurance

17.1.2a **Please list personnel that will conduct survival surgical procedures. If delegated to Comparative Medicine surgeons, these surgeons need not be individually named:** >>Get Help

Name	AALAS Aseptic Training	Aseptic Wet Lab Certificate	Link to Profile
Jay Dean	ExamCertificate_aseptic surgery_Jay Dean.pdf(0.01)	Jay Dean Aseptic Surgical Training Wet Lab Certificate(0.01)	00006346
Nicole Stavitzski	AAALAS Certificate 12-11-2019.pdf(0.02)	trainingdocs.zip(0.01)	00013453

PI: Jay Dean ExamCertificate_aseptic surgery_Jay Dean.pdf(0.01) •••

Jay Dean Aseptic Surgical Training Wet Lab Certificate(0.01) ••• 00006346

17.1.3 * **Describe pre-operative medications, including pre-emptive analgesia, tranquilizers, sedatives, pre-anesthetics, and general anesthetics, and their dose and route:** >>Get Help

SURVIVAL SURGERY, DEEP INTRACRANIAL MICROELECTRODES & RADIO TRANSMITTER IMPLANT IN RATS. Each rat will undergo only one survival surgery to implant a 4ET telemetry transmitter. Sterile surgery will be conducted under general anesthesia. Rats will be anesthetized with isoflurane in an induction chamber using 3-5% isofluorane in O2 delivered via a scavenger-equipped calibrated vaporizer. After chamber induction of anesthesia, the rat is removed from the chamber and placed on a rodent-specific nose cone apparatus and maintained anesthetized using 1-3% isoflurane with oxygen. Body temperature will be servocontrolled at 36-38degC using a heating pad and rectal temperature probe. A preemptive analgesic will be given (Meloxicam SR) in sterile saline prior to initiating surgery. The level of anesthesia is determined by continuous monitoring of reflexes (eye blink, rate of breathing). If the animal becomes light during surgery (responds to tactile stimulation: eye blink), then an increase in % isoflurane be given until adequate anesthesia is achieved. Fluid replacement is given i.v. as needed.

17.1.4 * **Describe pre-operative procedures, including the withholding of food or water, (for what period of time), shaving of hair with enough border to prevent contamination of the operative site, three surgical germicidal scrubs of site, draping with sterile drapes, and the placement of leads, probes, or catheters.** >>Get Help

Food and water are NOT withheld before surgery.
For radio-transmitter implantation the abdominal, neck and head regions are clipped. All fur clipping is done away from the surgical table. The shaved site has enough border to prevent contamination of the operative site. The surgical sites are prepared by scrubbing thrice with the antiseptic chlorohexidine and rinsed with sterile saline. The surgical site is then draped with a sterile Bioclusive cloak. Surgical instruments to be used for surgery are autoclaved beforehand. The surgeon and assistant wear lab coats and sterile gloves during surgery. In multiple, same-day surgeries the surgical instruments are steam sterilized prior to use in the initial surgery. Prior to use the next surgery, surgical items are cleaned with sterile saline and sterilized using a hot-bead sterilizer or an alternate set of sterile instruments is used. If the radio telemetry transmitter is to be re-used we will follow the manufacturer's instructions for resterilization. The same sterilization process is used for the FHC tungsten electrode before it is trimmed to length using a pair of sterile scissors. Resterilization documentation is uploaded below: Actril Resterilization (392-0027-020).pdf

17.1.5 * **Describe each surgical procedure in chronological order and in sufficient detail so that the IACUC will be able to determine what is being performed on each animal:** >>Get Help

After the rat is anesthetized and the abdomen is opened to place the telemetry module and battery inside the animal. The abdomen is open for no more than 10 to 15 minutes. The following procedures are done with no electrocautery necessary; i.e., the tissue dissection is not extensive and amounts to minimal and gentle displacement of organs for placement of telemetry module. 1) A sharp trocar is passed through the abdominal wall for passage of 4 pairs of wire leads through one opening. These leads will eventually be tunneled subcutaneously upwards along the back and neck to terminate at the opening in the skin over the skull. 2) The battery and telemetry module are inserted into the abdomen and the body cavity closed using absorbable sutures through the muscle wall. Absorbable sutures material suitable for internal use in mammals include polydioxanone (PDS®), polybutester (Novafil®), polypropylene (Surgilene®, Prolene®), and polygalactin 910 (Vicryl®). 3) An opening is cut in the skin over the skull and then the wire leads for the telemetry module are tunneled upwards subcutaneously to the opening in the skin over the skull. 4) The rat is secured in a stereotaxic frame using non-perforating ear bars. 5) Holes are drilled in the skull at up to four positions defined by coordinates given in the rat stereotaxic atlas for the brain nucleus targeted for recording microelectrode tip placement. Each tungsten microelectrode is attached to its telemetry lead as instructed in Papazoglou et al. 2016, J. Vis. Exp. (112): 54216. The electrode is cut to the required length and positioned stereotaxically using the micromanipulator. Likewise, the reference electrodes for each lead are positioned epidurally over the cerebellar surface. 6) Once all four recording and reference electrodes are positioned, they are fixed in place using dental epoxy. 7) The skin covering the skull is closed by nonabsorbable suture or wound clips. 8) The animal is removed from the stereotaxic frame and placed in dorsal recumbency and the abdominal skin closed with wound clips, which are removed a week later. The following is a general synopsis of the sequence of surgical procedures. Typically, within 30 minutes of ending the surgery the rat will eat Cheerios when offered. A new trainee can do this surgery in no more than 2 hrs and the experienced surgeon routinely does this in 1 hr.

17.1.6 * **Describe pre- and intra-operative monitoring procedures of depth of anesthesia (e.g., rate & depth of respiration, inter-digital pinch or palpebral blink reflex), and describe all intra-operative medications and/or support:** >>Get Help

Isoflurane at 3-5% in O2 is used for induction of anesthesia. 1-3% isoflurane in O2 will be used throughout all the surgical procedures. The level of anesthesia is determined by continuous

Compare

15 - Substance Administration

15.2 Substance - Chemical Agents

15.3 Substance - Conventional Chemical

15.5 Substance - Vehicle/Diluent/Solvent

15.13 Substance - Final Page

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17.1 Surgery

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Investigator Assurance

Investigator's Assurance

monitoring of rate of breathing and periodic checks (every few minutes) of reflexes (toe & tail pinch, eye blink). If the animal becomes light during surgery (responds to toe & tail pinch, etc.) then an increase in %isoflurane will be given until adequate anesthesia is achieved.

17.1.7 * **Describe immediate post-operative monitoring procedures. Animals recovering from general anesthesia must be monitored at least until they are sternally recumbent and capable of purposeful movement.**
The animal will be watched until it is fully recovered. Rats will be assessed visually for respiratory rate and depth. Body temperature will be supported with a heating pad placed under the cage in a manner that allows the animal to move away from the heat source as they recover. Animals will be monitored continuously until they are able to exhibit purposeful movement and maintain sternal recumbency.

17.1.8 * **Describe post-operative monitoring and care after animals are returned to housing, including the administration of analgesics, medications, fluids, and any other support methods (dose, route and frequency of post-operative analgesics and medications must be described), and indicate that skin sutures or staples will be removed at approximately 10 - 14 days post-operatively:** >>Get Help
Survival for at least 7 days in the COM vivarium will be required to allow recovery from surgery (telemetry unit & intracranial microelectrodes implantation) so the animal can be studied in the awake state. Post-operative Buprenorphine SR will be given as animal begins to wakeup from surgery (14.2.1). Until the day of the experiment, each operated rat will be checked daily by a member the P.I.'s lab or CompMed staff. As already stated, an animal exhibiting abnormal physiology or behavior will be removed and euthanized with CO2; e.g., failure to respond to stimuli, inactivity, hunched or abnormal posture, altered gait, ruffled/matted/or soiled fur, piloerection, circling, head tilt, seizures, vocalization, head pressing, and/or rapid/shallow/labored breathing. Sutures will be removed by day 10 if animal has not been exposed to HBO yet.

17.1.9 * **Will more than one major survival surgical procedure be performed on a single animal? (Respond "No" if a survival surgical procedure is followed by a non-survival surgical procedure.)** >>Get Help
○ Yes ● **No**

17.1.9a **If Yes, name the procedures and provide scientific rationale/justification:**

17.1.10 **For complicated surgical procedures/design/equipment etc., please upload a detailed diagram or SOPs:**

Name	Modified	Version
Actril Resterilization (392-0027-020).pdf(0.01)	11/8/2019 4:34 PM	0.01

17.1.11 * **Within this protocol, will another animal species be used in surgical procedures?**
○ Yes ● **No**

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Other Experimental Procedures

18.8

18.8.1

* **Please press +Add and provide details for each other experimental procedure used:**
>>Get Help

View

Species	Rat: Sprague-Dawley (neonates & weaned rats used in research to harvest brain slices/M&F)
Name of procedure:	tattoo on tail
Describe the procedure:	Before a rat recovers from survival surgery, an identifying number will be tattooed on its tail using equipment borrowed from CompMed.
How long will the procedure last?	<1min
Methods to minimize pain:	the animal will be anesthetized
Describe monitoring:	same as those described for its survival surgery
Describe post-procedure monitoring:	same as those described for its survival surgery
Will a log of observations be kept?	Yes

Compare

15 - Substance Administration

15.2 Substance - Chemical Agents

15.3 Substance - Conventional Chemical

15.5 Substance - Vehicle/Diluent/Solvent

15.13 Substance - Final Page

16 - Specimen Collection

16.1 Specimen Collection - Ante Mortem

17 - Surgery

17.1 Surgery

18 - Other Experimental Procedures

18.8 Other - Other Experimental

Investigator Assurance

Investigator's Assurance

eiacuc

IS

Instructions for Submission

You have reached the final page of this application.
Submission State: 18. Approved

If your submission state is "Pre Submission", please follow the steps below to submit this application for review.

1. Click the **Finish** button below to exit this form and return to the workspace.
2. Locate the **Submit for Veterinarian Review** activity on the left side of the workspace; click it, and follow any additional instructions to complete your submission.

Please Note: Following Veterinarian Review, the PI will be required to affirm the following upon submission to the IACUC:

- This description is complete and accurate.
- Written IACUC approval will be obtained prior to implementing changes to this protocol.
- Current biomedical supplies will be used.
- Complete animal procedural/surgical/testing records will be maintained.
- Conduct will be in accordance with the PHS Policy, AWR, The Guide, AAALAC Guidelines, DEA Regulations, and IACUC Principles and Procedures.
- Research personnel will abide by all relevant, universal precautions regarding blood-borne pathogens, appropriate biosafety level precautions, radiation safety procedures, and the chemical hygiene plan.
- All vertebrate animals or animal tissues will be acquired by lawful means, used judiciously, and disposed of appropriately.
- Personnel/students are adequately trained and experienced, or supervised to perform these procedures.
- Personnel/students are aware of occupational health & safety practices associated with the use of animals or animal tissues.
- IACUC applications describing teaching or training laboratories must assure the IACUC in writing that they or their designated IACUC-certified staff will continually directly supervise the students, and that prior to the lab commencing, will discuss with the students the potential risks and hazards associated with their involvement in the laboratory involving animals. All attending students will then complete a Student Safety in Teaching Laboratories Involving Animals form, which the PI will sign and provide to Comparative Medicine before the lab commences available at http://www.research.usf.edu/dric/iacuc/occupational-health-safety.asp

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Compare

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15.2 Substance - Chemical Agents

15.3 Substance - Conventional Chemical

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16.1 Specimen Collection - Ante Mortem

17 - Surgery

17.1 Surgery

18 - Other Experimental Procedures

18.8 Other - Other Experimental

Investigator Assurance

Investigator's Assurance

From:	Jay Dean
To:	Kaminski, Sarah R CTR (USA)
Cc:	Chapman, Sandra E CIV (USA); Nicole Stavitzski
Subject:	[Non-DoD Source] New approved USF animal protocol w/BUMED appendix, USDA report, and USF protocol: for ongoing ONR project
Date:	Tuesday, December 6, 2022 1:02:15 PM
Attachments:	BUMED_Animal Protocol_Mitigation of CNS OT 12-6-2022.pdf Delivered_IR_930_UNIVOFSOUTHFLORIDA_2022SEPT20.pdf IACUC Letter for Study IS00011501.pdf New animal use protocol JBDean USF_IS00011501.pdf
Importance:	High

Sarah,

My current ONR grant, now in its 3rd year, needed an new USF animal protocol since the current approved protocol ends on Dec. 10th 2022. The replacement protocol to complete the work in the MitoQ project was approved today. Attached are the following files: BUMED appendix form; approved USF animal protocol; approval letter; and most recent USDA inspection report.

Let me know what other information you need.

Regards,

Jay Dean

From: Dean, Jay
To: Chapman, Sandra E CIV (USA); Jeffress, Jenny V CTR (USA)
Cc: Mothershed, Victoria; Landon, Carol; D"Agostino, Dominic; Wesley, William
Subject: [Non-DoD Source] ONR research, Jay Dean"s Lab
Date: Wednesday, January 29, 2020 10:12:29 AM
Importance: High

Dear Sandra and Jenny,

I'm writing to update you on the status of my lab at USF. My current ONR grant on CNS ox tox and ketones is on its 2nd no cost extension through April, but on Feb 20th it will have insufficient funds to cover the salaries based on % effort for my lab manager, Ms. Carol Landon, my grad student, Mr. Chris Hinojo, and myself. While my salary is not a problem, I'm concerned about support for Carol and Chris who are working on ONR-related research. They will both be funded in the new ONR grant on CNS ox tox and mitochondrial targeted antioxidant therapy, but that grant is delayed in being processed and starting because the approved USF animal protocol has not been reviewed by BUMED. I can cover their salaries and some operating costs after Feb 20th by one of two possible mechanisms:

1. If the animal protocol was approved and an official letter of support beyond the email you sent me was sent to USF, then USF would consider doing an underwrite to cover us until the grant is sent to USF at which point USF would be reimbursed using ONR funds since we would start working on the project; or
2. Dr. Dominic D'Agostino can support Carol and Chris using funds he has in various accounts (non-ONR) to support his ketogenic research. Dom's "loan", however, can only last for a few months at best.

Is there anything you can do expedite BUMED's review of my animal protocol so that the grant can be processed sooner than later? If so, that would be appreciated very much.

Thank you for considering my request,

Jay Dean, PhD
Professor of Mol Pharm & Physiol
Dir., USF Hyperbaric Biomed. Res. Lab.
Morsani College of Medicine
USF Tampa

From: Dean, Jay
To: Chapman, Sandra E CIV (USA); Jeffress, Jenny V CTR (USA)
Cc: Landon, Carol; Wesley, William
Subject: [Non-DoD Source] question about money transfer
Date: Thursday, April 16, 2020 1:33:31 PM

Sandra,
I just received the update below from our department office regarding the status of our budgets. My question is this: can the $596 remaining in the ox tox study that ends 4/30/2020 (2nd NCE) be transferred over to the electrodermal resistance project (w/Ki Chon) and used towards the purchase of animals for the skin resistance/HBO project with Ki Chon? With ONR permission, can that be done inhouse or do we need another NCE to use the $596?
Thanks,
Jay
****
"Jay
Attached are your latest financial projections. Here are the important notes –

- Payroll is posted thru 04/02/20. Wages are accrued thru the end of each grant year but not on 113200.
- Animals are posted or encumbered thru **Feb.** only.
- **113200 Oxygen Toxicity** - Projection goes out to grant end of **04/30/20**. **There are no more wages posting here. A NCE has not been approved yet. Only $596 remain for supplies & future animals.**
- **118700 Rat Study -** You only **have $1,500 of budget available** for supplies/animals and this is **now over 7/14/20** .

Please see me with any questions.
Bill"

From:	Dean, Jay
To:	Becker, Christopher E CTR USN CNR ARLINGTON VA (USA); Chapman, Sandra E CIV (USA)
Cc:	Landon, Carol; Wesley, William; Glover, Ethel M CIV USN CNO (USA)
Subject:	[Non-DoD Source] RE: Dr. Dean - 06/17/20 Financial update attached
Date:	Wednesday, July 29, 2020 12:14:39 PM

Well, there ya go! Thanks! I learn something new every day. Thanks for the info and update. Welcome back Chris.
Jay

From: Becker, Christopher E CTR USN CNR ARLINGTON VA (USA)
Sent: Wednesday, July 29, 2020 12:08 PM
To: Chapman, Sandra E CIV (USA) ; Dean, Jay
Cc: Landon, Carol ; Wesley, William ; Glover, Ethel M CIV USN CNO (USA)
Subject: RE: Dr. Dean - 06/17/20 Financial update attached
Jay,
Unbeknownst to Sandra or me, your grant had already received a 12 month NCE as part of ONR's COVID relief to universities. Current end date is 14 July 2021. Modification is attached.
Both you and Brenda Simmons, listed as business office contact, should have received email notification of this modification on 22 April. The emails are somewhat cryptic, but you should not ignore them. They let you know that a document (grant modification) is available to download in EDA.
-Chris
Chris Becker
Contractor support, ONR Warfighter Performance Department
Phone: 571.239.2868
Chris.Becker.ctr@navy.mil

From: Chapman, Sandra E CIV (USA) <sandra.chapman@navy.mil>
Sent: Wednesday, July 29, 2020 11:53 AM
To: 'Dean, Jay' <jaydean@usf.edu>; Becker, Christopher E CTR USN CNR ARLINGTON VA (USA) <chris.becker.ctr@navy.mil>
Cc: Landon, Carol <clandon@usf.edu>; Wesley, William <wwesley@usf.edu>
Subject: RE: Dr. Dean - 06/17/20 Financial update attached
Hi Jay,
Jenny is no longer supporting the program and we are very fortunate to have the assistance of Chris Becker at this time.
I will check on the status and get back you.
Best,
Sandra

From: Dean, Jay <jaydean@usf.edu>
Sent: Wednesday, July 29, 2020 11:50 AM
To: Chapman, Sandra E CIV (USA) <sandra.chapman@navy.mil>; Jeffress, Jenny V CTR USN CNR ARLINGTON VA (USA) <jenny.jeffress.ctr@navy.mil>
Cc: Landon, Carol <clandon@usf.edu>; Wesley, William <wwesley@usf.edu>
Subject: [Non-DoD Source] FW: Dr. Dean - 06/17/20 Financial update attached
Hello Sandra and Jenny,
We have not heard anything about the no cost extension you requested on July 10th. Any news?
Thanks,
Jay Dean

From: Chapman, Sandra E CIV (USA) <sandra.chapman@navy.mil>
Sent: Friday, July 10, 2020 6:15 PM
To: Glover, Ethel M CIV USN CNO (USA) <ethel.glover@navy.mil>; ONR_Atlanta <ONR_Atlanta.fcm@navy.mil>

Cc: Wesley, William <wwesley@usf.edu>; Becker, Christopher E CTR USN CNR ARLINGTON VA (USA) <chris.becker.ctr@navy.mil>; Dean, Jay <jaydean@usf.edu>; Landon, Carol <clandon@usf.edu>
Subject: RE: Dr. Dean - 06/17/20 Financial update attached
Hi,
I would like to request a NCE for Award N000141912314 to Jay Dean at University of South Florida through Nov, 2020 due to COVID-related delays in laboratory access.
Best,
Sandra

From: Landon, Carol <clandon@usf.edu>
Sent: Friday, July 10, 2020 9:33 AM
To: Chapman, Sandra E CIV (USA) <sandra.chapman@navy.mil>; Dean, Jay <jaydean@usf.edu>; Becker, Christopher E CTR USN CNR ARLINGTON VA (USA) <chris.becker.ctr@navy.mil>
Cc: Wesley, William <wwesley@usf.edu>
Subject: [Non-DoD Source] Re: Dr. Dean - 06/17/20 Financial update attached
Good morning,
Dr. Dean asked me to respond to your question.
He would like to request that the NCE extend to the end of November, 2020, if possible.
All the best,
Carol Landon

From: Chapman, Sandra E CIV (USA)
Sent: Friday, July 10, 2020 9:16 AM
To: Dean, Jay; Becker, Christopher E CTR USN CNR ARLINGTON VA (USA)
Cc: Landon, Carol; Wesley, William
Subject: RE: Dr. Dean - 06/17/20 Financial update attached
Jay,
How long do you need the extension for?
Best,
Sandra

From: Dean, Jay <jaydean@usf.edu>
Sent: Thursday, July 9, 2020 4:27 PM
To: Jeffress, Jenny V CTR USN CNR ARLINGTON VA (USA) <jenny.jeffress.ctr@navy.mil>; Chapman, Sandra E CIV (USA) <sandra.chapman@navy.mil>
Cc: Landon, Carol <clandon@usf.edu>; Wesley, William <wwesley@usf.edu>
Subject: [Non-DoD Source] RE: Dr. Dean - 06/17/20 Financial update attached
Importance: High
Hello,
It just came to my attention that the electrodermal project nce ends on July 14. I'm still working on this project due to delays caused by COVID-19 and failure of our air compressor used to pressurize the chamber. I still need the remaining funds for the animals we are studying.
My apology for this late notice, but is it possible to get another nce?
Regards,
Jay Dean
ONR, Undersea Medicine Program; Electrodermal activity during hyperbaric oxygen exposure—rat study. (N000141912314)

From: Landon, Carol <clandon@usf.edu>
Sent: Thursday, July 9, 2020 4:19 PM
To: Dean, Jay <jaydean@usf.edu>
Subject: Fw: Dr. Dean - 06/17/20 Financial update attached
Jay,
I just noticed that the NCE for the electraderm project ends on July 14.....that's in 5 days.

<<>>
We now have about $900 in the acct since we just spent $102 to pay for the May animal bill. There are 3 implanted rats on that skin protocol. I think the animal protocol is good until Dec 2020
Carol

From: Wesley, William <wwesley@usf.edu>
Sent: Thursday, June 18, 2020 1:02 PM
To: Dean, Jay <jaydean@usf.edu>
Cc: Landon, Carol <clandon@usf.edu>; Mothershed, Victoria <vmothershed@usf.edu>
Subject: Dr. Dean - 06/17/20 Financial update attached
Dr. Dean and Carol –
Attached are your latest financial projections. Here are the important notes –

- Payroll is posted thru 05/28/20. Wages are accrued thru the end of each grant year but not on 113200.
- Animals are posted or encumbered thru **April** only.
- **113200 Oxygen Toxicity** - **Only $123 remain for supplies & future animals.**
- **18300 RIA** -
- **118700 Rat Study - now over 7/14/20. Only a little more than $1,000 remain.**
- **120800 New ONR** - Wages are projected thru year 1.

Please see me with any questions.
Bill

From: Dean, Jay
To: Jeffress, Jenny V CTR (USA)
Cc: Chapman, Sandra E CIV (USA); Mothershed, Victoria; Wesley, William; Landon, Carol
Subject: [Non-DoD Source] RE: Dr. Dean request for another NCE for N00014-16-1-2537 CNS ox tox project
Date: Friday, April 17, 2020 12:21:58 PM

Hello Jenny,
I just finished speaking with Sandra about our current status at USF and how ONR can help us. Sandra has verbally approved us requesting another NCE for the N00014-16-1-2537 CNS ox tox project, which ends April 30th, 2020. Sorry for the late request. Once we get the NCE then we can internally rebudget the remaining funds in travel to be used towards purchase and maintenance of animals for needed experiments in N00014-19-1-2314. See my email below. Let me know how we (USF) can assist you with this process. Assuming the pandemic quiets down in the next few weeks, how about an 3 month NCE from May 1-July 31, 2020?
Thanks,
Jay Dean
(P.S. and thank you for getting the new ONR grant pushed through!)

From: Dean, Jay
Sent: Friday, April 17, 2020 10:28 AM
To: Chapman, Sandra E CIV (USA) ; Jeffress, Jenny V CTR (USA)
Cc: Landon, Carol ; Wesley, William ; Mothershed, Victoria
Subject: RE: Dr. Dean - 04/15/20 Financial update attached
N00014-19-1-2314
N00014-18-1-2701 Dom

From: Dean, Jay
Sent: Friday, April 17, 2020 10:12 AM
To: Wesley, William <wwesley@usf.edu>
Cc: Landon, Carol <clandon@usf.edu>; Victoria Mothershed <noreply@box.com>
Subject: RE: Dr. Dean - 04/15/20 Financial update attached
Hi Sandra,
Is the following possible? And I apologize for any inconvenience this causes you and Jenny. N00014-16-1-2537 CNS ox tox project in my lab ends its 2nd NCE on April 30th. As it turns out, there also were unused travel funds ($700) that I had not realized when I wrote you yesterday. We had not traveled as much the past year as I was trying to conserve funds for operating costs in lab. Thus, there are $700 + $ 596 (= $1296) left in the project that would help if we could

1. Get another NCE for this project for 2-3 months (allowing for the pandemic to quiet down);
2. Get ONR permission to re-budget travel funds left in account for animal costs;
3. Get ONR permission to use remaining funds in N00014-16-1-2537 ($1296) and use towards N00014-19-1-2314, the HBO_2 & electrodermal project that is currently in a NCE. That would give us $1296 + $1500 = $2796 towards animals and cover any issues we run into finishing the study.

Jay

From: Wesley, William <wwesley@usf.edu>
Sent: Friday, April 17, 2020 7:35 AM
To: Dean, Jay <jaydean@usf.edu>

Cc: Landon, Carol <clandon@usf.edu>; Victoria Mothershed <noreply@box.com>
Subject: RE: Dr. Dean - 04/15/20 Financial update attached
Dr. Dean –
I better clarify a couple things –

- 113200 - You have $700 encumbered in travel. This travel needs to be completed by 4/30. Unless this needs to be voided. If it needs to be voided you have $1,296 available to spend & **PCard charges NEED TO POST BY 4/30**.
- 118700 – I have wages ending this week. If they are extended, the money will go fast. **End or keep going**?
- 113200 – Animal charges for March & April can still post, even late.

Thanks & be safe!
Bill

From: Wesley, William
Sent: Thursday, April 16, 2020 12:58 PM
To: Dean, Jay <jaydean@usf.edu>
Cc: Landon, Carol <clandon@usf.edu>; Victoria Mothershed <noreply@box.com>
Subject: Dr. Dean - 04/15/20 Financial update attached
Dr. Dean and Carol –
Attached are your latest financial projections. Here are the important notes –

- Payroll is posted thru 04/02/20. Wages are accrued thru the end of each grant year but not on 113200.
- Animals are posted or encumbered thru **Feb.** only.
- **113200 Oxygen Toxicity** - Projection goes out to grant end of **04/30/20**. **There are no more wages posting here. A NCE has not been approved yet. Only $596 remain for supplies & future animals.**
- **18300 RIA** - RIA funds from last year have been deposited here.
- **118700 Rat Study -** You only **have $1,500 of budget available** for supplies/animals and this is **now over 7/14/20** .

Please see me with any questions.
Bill

From:	Jay Dean
To:	Chapman, Sandra E CIV (USA)
Subject:	[Non-DoD Source] RE: Image request
Date:	Sunday, May 1, 2022 7:51:31 PM
Attachments:	image001.png Ciarlone et al 2019 CNS function and dysfunction during exposure to hyperbaric oxygen in operational and clinical settings.pdf

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Hi Sandra, You said: "How will this be integrated into divers training/operations to improve their missions? Do we see them being able to have deeper depths when breathing 100% O2- what would that improve? What would them implications be for the 'savings' for dive profiles? "

I've always viewed the question of CNS ox tox as not necessarily deeper depths, but longer, safer bottom times without concern of seizures when breathing oxygen. Currently, 10min at 50 fsw is the limit on oxygen; however, as shown in our 2019 article (attached, Fig. below), many divers could remain longer than 10min at 50 fsw given their inherent tolerance of HBO2. Each dot in Fig. 1 is an individual diver who developed "early toxic indications" of CNS ox tox (= nonconvulsive S/Sx; in this case, lip twitching, vertigo/nausea) or full blown seizures without this early S/Sx. Based on this work (Royal Navy) and USN (WW2 and afterwards), the limits are set based on those most sensitive to HBO_2; i.e., to the left of the blue vertical dashed line ("USN O_2 limit 50 FSW"). If, however, a therapeutic intervention could extend tolerance to HBO_2 and delay or prevent seizures and pulmonary ox tox then longer operational bottom times could be possible. Whether or not this also meant deeper dives would be a separate question. My thoughts… Jay

Fig. 1. The effect of breathing 2.5 ATA O_2 (50 fsw) while submerged either quietly or exercising on the latency to onset of either an early non-convulsive toxic end-point (lip twitching or vertigo/nausea) or seizures. Each data point represents a dive that was terminated early due to onset of an acute toxic indication of CNS-OT. Data are listed from shortest to longest latent period prior to onset of toxic endpoint. Divers that did not develop any toxic indications are not represented. For comparison, the current USN limit for breathing HBO_2 at 50 fsw (10 min) is indicated by the blue vertical, dashed line and diamond. In these dive trials, seizures (red symbols) occurred without any prior noticeable non-convulsive toxic indication. Experience showed that in cases when a non-convulsive S/Sx was missed or ignored

that the diver developed seizures. Data are redrawn and average latencies are calculated by the authors using data sets in Appendix 2, Tables 2, 7, and 21 in Donald [87]. The maximum limit of the dive without an acute toxic indication was 30 min (Table 2: 100 dives, resting/working), ~60 min (Table 7: 40 dives, resting) and 120 min (Table 21: 46 dives, working). LT, lip twitching (black symbols); Sz, seizures (red symbols); and V/N, vertigo/nausea (green symbols).

Jay

From: Chapman, Sandra E CIV (USA) <sandra.e.chapman2.civ@us.navy.mil>
Sent: Sunday, May 1, 2022 6:15 PM
To: Jay Dean <jaydean@usf.edu>
Subject: RE: Image request

Jay,

Thanks for your quick response and explanation and materials! This is helpful. The one slide you provided still does leave me with some questions- I'm not sure I am clear on the labels- are the black circles surviving rats? Red dots Dead?? Believe it or not- this is still way too complex for my leadership- they would stare for days and not understand – and to some degree me as well since I am not familiar with the labeling and even the axis since I am not clear on the dosing profile of the MitoQ. I do not want to overcomplicate the slide I use for my leadership with L-NAME at this point since this is the first I am even learning of it so certainly isn't reflective of my strategic directions.

It is interesting to see the outcome of the MitoQ studies and I do think valuable- but I do think it would have been good if we could have had a strategic meeting to discuss together since a new direction was the wise choice. I think L-NAME is a reasonable direction for the reasons you indicate of chasing down the NO production, though I am not sure would have been the direction I would have selected since from our bigger picture perspective at ONR, I am having questions now about this overall direction since it seems to be opening more cans of worms than we can resolve- you yourself indicate that there will always be the pulm OT lingering as a co-occurring threat. Let's see if you get some feedback from others in the program review but after that I think we should plan a meeting with you, Eckmann, Gasier, Florian, Hall, and any others you may recommend to assess the strategic future research priorities for OT. It is true that we now have pressure at ONR to only produce positive results, but one thing I think might not be clear is that my strategic direction with OT has been different than Bill's. His interpretation from our leadership was that we need to chase down active treatments for OT. I don't want to push interventions without knowing the underlying mechanisms to OT. The question we all need to ask is "How will this be integrated into divers training/operations to improve their missions? " Do we see them being able to have deeper depths when breathing 100% O2- what would that improve? What would them implications be for the 'savings' for dive profiles?

I am not interested in continually throwing a wet noodle against the wall- this is why I have stopped ketone/ketosis research and in my mind will be the end of this direction as well. I see this as being back to the drawing board- and before we brainstorm ways forward we need to look at bigger picture.

Sandra

From: Jay Dean <jaydean@usf.edu>
Sent: Sunday, May 1, 2022 4:11 PM
To: Chapman, Sandra E CIV (USA) <sandra.e.chapman2.civ@us.navy.mil>
Subject: [Non-DoD Source] RE: Image request

Hello Sandra,
The MitoQ did delay Sz during exposure to 5 ATA HBO2 but it also made the animals lethargic (ruffled fur, quiet) or caused death within tens of minutes to hours after decompression (slides . My thoughts are that doses of MitoQ that are non-toxic in 1 ATA air, and delay Sz during exposure to HBO2, subsequently become lethal during exposure to HBO2 (slides 4-6). Postulated mechanism: unbridled ·NO production in the face of diminished production of mitochondrial superoxide, which normally reacts with ·NO and thus is attenuated by its consumption in that reaction. It's possible the animals are suffering pulmonary O2 toxicity as a result due to excessive NO production, or another yet to be identified mechanisms that is out of control due to excessive NO production (see slide 9). We did try a few experiments combining MitoQ with L-NAME to decrease NO production but those animals also died (slide 6).
As for next steps, I decided to not pursue MitoQ further since ONR was interested in positive results; i.e., neuroprotection against seizure without impairing performance. The question of why HBO + MitoQ is toxic is interesting but I thought it would take us down a path that ONR would not want us to pursue. My apology if I was wrong in that regard. So I started testing L-NAME, which has been shown to block seizures in anesthetized animals but never studied in freely behaving, unanesthetized animals. So, we've had good luck with that in recent months. The attached slides show the MitoQ data and the L-NAME data (slides 7 & 8); the L-NAME data is what I was proposing to pursue to figure out an optimal dose. At 30mg/kg ip we get some animals that don't seizure after 75 min. The question is will a higher dose increase that number of animals without impairing their health otherwise.
Let me know if you need more information.
You can call me at (b) (6) [redacted] too or we could video chat if I need to explain the slides to you.
Jay

From: Chapman, Sandra E CIV (USA) <sandra.e.chapman2.civ@us.navy.mil>
Sent: Sunday, May 1, 2022 10:07 AM
To: Jay Dean <jaydean@usf.edu>
Subject: Image request

Hi Jay,

I look forward to hearing your talk next week.

I am writing to request an image from you for my department review slides which are due tomorrow. The images provided for the program review don't relate to the MitoQ research. I will reporting your findings on the MitoQ in a department brief and need a SIMPLE (my leadership needs this to be straightforward and uncomplicated) graphic to accompany the description: Mitoquinone (MitoQ) is used to decrease mitochondrial superoxide production during exposure to hyperbaric oxygen in freely behaving rats. Explore the potential for MitoQ to reduce the risk of CNS-OT in freely

behaving rats by lowering mitochondrial superoxide production, decreasing the oxygen-sensitivity of neurons, and increase latency time to seizure. Findings show that MitoQ delays/blocks seizure, but MitoQ plus hyperbaric oxygen is toxic.

Do you have thoughts on why you see the toxicity and it is hard to follow the next steps in the materials provided because I don't recall l-name from the proposal. Thanks,
Sandra

From: Dean, Jay
To: Makayan, Caitlin J CTR USN (USA); Chapman, Sandra E CIV (USA)
Subject: [Non-DoD Source] RE: Invitation to Attend the 2020 Undersea Medicine Program Review Series - October 2020
Date: Friday, August 14, 2020 12:35:01 PM
Attachments: image001.png

Hello Sandra,
The last request I received for an Abstract and Quad chart was in January for a program review in April. This was cancelled and I just received the request today for the virtual Oct meeting. The new grant on Mitochondria Targeted Antioxidant Therapy for CNS ox tox just started in April and we have only recently returned to the lab due to the pandemic. In preparing to work on the new grant and the electrodermal grant we discovered that our air compressor had died, which is used to compress the dive chamber in parallel with the animal chamber (100% oxygen). We have ordered a new air compressor which will be installed, hopefully, in a few weeks. In the meantime, I've hooked up a series of compressed air cylinders to get the chamber back online. Unfortunately, I'm having surgery next week on Tuesday and will on leave for 2-4 weeks (total knee replacement).
I am now working on the two reports that I just learned of an hour ago. I will try to put together an abstract and quad chart too before surgery. There's not too much to report yet however.
Jay

From: Makayan, Caitlin J CTR USN (USA)
Sent: Friday, August 14, 2020 12:01 PM
To: gerardo.bosco@unipd.it; koopmanh@uncw.edu; peter.lindholm@ki.se; richard.moon@duke.edu; vp407@email.unc.edu; (b) (6) [redacted] umaryland.edu; swb@uoregon.edu; mason@chemistry.harvard.edu; Gallagher, Dennis G CIV USN NAVSURFWARCEN PNC FL (USA) ; Nikola.Miskovic@fer.hr; dnocera@fas.harvard.edu; dnocera@fas.harvard.edu; dino@utep.edu; brandon.m.casper4.civ@mail.mil; marta.hatzell@me.gatech.edu; melissailardo@gmail.com; rebecca.kramer@yale.edu; cmajidi@andrew.cmu.edu; Sorensen, Kara C CIV SPAWARSYSCEN-PACIFIC, 71750 ; wfb2@case.edu; wfb2@case.edu; (b) (6) [redacted] @gmail.com; malmstad@usc.edu; dnocera@fas.harvard.edu; prangamani@ucsd.edu; prangamani@ucsd.edu; zjschlad@buffalo.edu; tbroderick@ihmc.us; afahlman@whoi.edu; dkernagis@ihmc.us; dkernagis@ihmc.us; rachel.r.markwald.civ@mail.mil; sj.mitchell@auckland.ac.nz; (b) (6) [redacted] umaryland.edu; ki.chon@uconn.edu; D'Agostino, Dominic ; Dean, Jay ; Aaron.a.hall6.civ@mail.mil; blairjoh@buffalo.edu; Klemp, Alexander O CIV USN NAVEXDIVINGU PNC FL (USA) ; claude.piantadosi@duke.edu
Cc: Chapman, Sandra E CIV (USA)
Subject: Invitation to Attend the 2020 Undersea Medicine Program Review Series - October 2020

Dear Undersea Medicine Program and STEM Principal Investigators,
I am contract support working with Dr. Sandra Chapman for her Undersea Medicine Program. Dr. Chapman would like to invite you to attend the upcoming 2020 Undersea Medicine Program Review Series that will be held the first week of October. Please provide your availability for the week of 5 October (10/5/2020-10/9/2020) and indicate whether you would be available in the AM (9:00AM-12:00PM) and/or PM (1:00PM-4:00PM) timeframe. Please submit your availability to me at caitlin.makayan.ctr@navy.mil by NLT COB 21 August 2020.
This year's Undersea Medicine Program Review will be restructured in a working group format to encourage discussion and future directions on issues relevant to the diving community. Each Principal Investigator will have a total of 10 minutes for their presentation (including 8 minutes for presentations and 2 minutes devoted to questions). If your project is new, Dr. Chapman would love for you to share goals of the project and preliminary or early data.

The review series topics will include:

- Diving Technology
- Gas/Hyperbaric Physiology
- Decompression Sickness
- Oxygen Toxicity
- Neurocognitive Distress and Assessment
- Environmental Protection
- STEM Education

Please see attached spreadsheet to identify which session you have been assigned to brief your project in. An agenda with the final times for each session will be sent out with login information. All are welcome to join any session. This year, there will be a new session added for the STEM program and we welcome you to participate if you're interested.

Thank you for those of you who have already submitted your quads and abstracts in preparation for the upcoming program review! For those of you who have not already submitted your quads and abstracts, this is a reminder that they are due and must be submitted as soon as possible to me at caitlin.makayan.ctr@navy.mil by NLT COB Friday, 21 August 2020.

Action Item Deadlines

- 21 August 2020: Provide Quads and Abstracts
- 21 August 2020: Provide Availability for 10/5/2020-10/9/2020; AM/PM

If you have any questions or concerns, please contact me for more information.

Best Regards,

Caitlin

Caitlin Makayan
SETA Support Contractor
Allegient Defense, Inc.
caitlin.makayan.ctr@navy.mil
caitlin.makayan.ctr@navy.smil.mil
Work: (703) 696-2580
Telework: (571) 445-4763

Office of Naval Research
Warfighter Performance Department
Warfighter Protection & Applications Division
875 North Randolph Street
Arlington, VA 22203-1995

Outlook-q1mnq3kp

From: Dean, Jay
To: Chapman, Sandra E CIV (USA); Makayan, Caitlin J CTR USN (USA)
Subject: [Non-DoD Source] RE: Invitation to Attend the 2020 Undersea Medicine Program Review Series - October 2020
Date: Friday, August 14, 2020 2:28:21 PM
Attachments: image002.jpg
image003.png

Oh boy! Thank you! I might even have to step in and teach your kids for a day or two as a thank you!

Jay

From: Chapman, Sandra E CIV (USA)
Sent: Friday, August 14, 2020 2:26 PM
To: Dean, Jay ; Makayan, Caitlin J CTR USN (USA)
Subject: RE: Invitation to Attend the 2020 Undersea Medicine Program Review Series - October 2020

Yes, actually Caitlin also raised my attention to this after I wrote you and so yes, you are good! Don't worry about creating quads/abstracts for the new award- you have enough on your plate. But I would ask that you brief them when you present for the Program Review.

Best,

Sandra

From: Dean, Jay <jaydean@usf.edu>
Sent: Friday, August 14, 2020 2:12 PM
To: Chapman, Sandra E CIV (USA) <sandra.chapman@navy.mil>; Makayan, Caitlin J CTR USN (USA) <caitlin.makayan.ctr@navy.mil>
Subject: [Non-DoD Source] RE: Invitation to Attend the 2020 Undersea Medicine Program Review Series - October 2020

Sandra,

Okay, I'm going crazy and clearly suffering COVID dementia or 'old timers' disease...I sent you (and Chris Becker) abstracts and quad charts back on May 21st for my two ONR projects that are on NCE: the CNS ox tox project and the electrodermal project. Those are the two project I am writing the reports for now. However, I did not send the abstract and quadchart for the new Mitochondria targeted antioxidant therapy project. I will work on that between now and my surgery Tuesday and send it before then. I just submitted one report and should be able to finish the second report later today.

Sorry for the confusion—on my part...

Stay safe and good luck with home schooling. I do NOT envy you.

Jay

From: Chapman, Sandra E CIV (USA) <sandra.chapman@navy.mil>
Sent: Friday, August 14, 2020 12:56 PM
To: Dean, Jay <jaydean@usf.edu>; Makayan, Caitlin J CTR USN (USA) <caitlin.makayan.ctr@navy.mil>
Subject: RE: Invitation to Attend the 2020 Undersea Medicine Program Review Series - October 2020

Jay- when the meeting was cancelled I noted that the abstracts and quads would still be due but gave an extension, so I am afraid it is inaccurate to say that the last mention of it was in January. I have been collecting quads and abstracts for new projects because I believe it is important for you to be included in the discussions we have in the program review and for all to be informed of your project. It is perfectly acceptable to report goals of the projects for new awards.

I do apologize that the progress reporting system notifications went to your junk mail and I wish I could give you an extension. Unfortunately, it

is not a system I control. I will attempt to do a better job of sending my own notices of the PR deadlines earlier. I just got quite behind this year trying to juggle my full-time job AND teaching my two school-age children!
Yes, COVID has not been kind to many of us.
Sandra

From: Dean, Jay <jaydean@usf.edu>
Sent: Friday, August 14, 2020 12:34 PM
To: Makayan, Caitlin J CTR USN (USA) <caitlin.makayan.ctr@navy.mil>; Chapman, Sandra E CIV (USA) <sandra.chapman@navy.mil>
Subject: [Non-DoD Source] RE: Invitation to Attend the 2020 Undersea Medicine Program Review Series - October 2020
Hello Sandra,
The last request I received for an Abstract and Quad chart was in January for a program review in April. This was cancelled and I just received the request today for the virtual Oct meeting. The new grant on Mitochondria Targeted Antioxidant Therapy for CNS ox tox just started in April and we have only recently returned to the lab due to the pandemic. In preparing to work on the new grant and the electrodermal grant we discovered that our air compressor had died, which is used to compress the dive chamber in parallel with the animal chamber (100% oxygen). We have ordered a new air compressor which will be installed, hopefully, in a few weeks. In the meantime, I've hooked up a series of compressed air cylinders to get the chamber back online. Unfortunately, I'm having surgery next week on Tuesday and will on leave for 2-4 weeks (total knee replacement).
I am now working on the two reports that I just learned of an hour ago. I will try to put together an abstract and quad chart too before surgery. There's not too much to report yet however.
Jay

From: Makayan, Caitlin J CTR USN (USA) <caitlin.makayan.ctr@navy.mil>
Sent: Friday, August 14, 2020 12:01 PM
To: gerardo.bosco@unipd.it; koopmanh@uncw.edu; peter.lindholm@ki.se; richard.moon@duke.edu; vp407@email.unc.edu; (b) (6) umaryland.edu; swb@uoregon.edu; mason@chemistry.harvard.edu; Gallagher, Dennis G CIV USN NAVSURFWARCEN PNC FL (USA) <dennis.g.gallagher@navy.mil>; Nikola.Miskovic@fer.hr; dnocera@fas.harvard.edu; dnocera@fas.harvard.edu; dino@utep.edu; brandon.m.casper4.civ@mail.mil; marta.hatzell@me.gatech.edu; (b) (6) @gmail.com; rebecca.kramer@yale.edu; cmajidi@andrew.cmu.edu; Sorensen, Kara C CIV SPAWARSYSCEN-PACIFIC, 71750 <sorensek@spawar.navy.mil>; wfb2@case.edu; wfb2@case.edu; (b) (6) @gmail.com; malmstad@usc.edu; dnocera@fas.harvard.edu; prangamani@ucsd.edu; prangamani@ucsd.edu; zjschlad@buffalo.edu; tbroderick@ihmc.us; afahlman@whoi.edu; dkernagis@ihmc.us; dkernagis@ihmc.us; rachel.r.markwald.civ@mail.mil; sj.mitchell@auckland.ac.nz; (b) (6) umaryland.edu; ki.chon@uconn.edu; D'Agostino, Dominic <ddagosti@usf.edu>; Dean, Jay <jaydean@usf.edu>; Aaron.a.hall6.civ@mail.mil; blairjoh@buffalo.edu; Klemp, Alexander O CIV USN NAVEXDIVINGU PNC FL (USA) <alex.o.klemp@navy.mil>; claude.piantadosi@duke.edu
Cc: Chapman, Sandra E CIV (USA) <sandra.chapman@navy.mil>
Subject: Invitation to Attend the 2020 Undersea Medicine Program Review Series - October 2020
Dear Undersea Medicine Program and STEM Principal Investigators,
I am contract support working with Dr. Sandra Chapman for her Undersea Medicine Program. Dr. Chapman would like to invite you to attend the upcoming 2020 Undersea Medicine Program Review Series that will be held the first week of October. Please provide your availability for the week of 5 October (10/5/2020-10/9/2020) and indicate whether you would be available in the AM (9:00AM-12:00PM) and/or PM (1:00PM-4:00PM) timeframe. Please submit your availability to me at caitlin.makayan.ctr@navy.mil by NLT COB 21 August 2020.
This year's Undersea Medicine Program Review will be restructured in a working group format to encourage discussion and future directions on issues relevant to the diving community. Each Principal Investigator will have a total of 10 minutes for their presentation (including 8 minutes for presentations and 2 minutes devoted to questions). If your project is new, Dr. Chapman would love for you to share goals of the project and preliminary or early data.
The review series topics will include:

- Diving Technology
- Gas/Hyperbaric Physiology
- Decompression Sickness
- Oxygen Toxicity
- Neurocognitive Distress and Assessment
- Environmental Protection
- STEM Education

Please see attached spreadsheet to identify which session you have been assigned to brief your project in. An agenda with the final times for each session will be sent out with login information. All are welcome to join any session. This year, there will be a new session added for the STEM program and we welcome you to participate if you're interested.
Thank you for those of you who have already submitted your quads and abstracts in preparation for the upcoming program review! For those of you who have not already submitted your quads and abstracts, this is a reminder that they are due and must be submitted as soon as possible to me at caitlin.makayan.ctr@navy.mil by NLT COB Friday, 21 August 2020.

Action Item Deadlines

- 21 August 2020: Provide Quads and Abstracts
- 21 August 2020: Provide Availability for 10/5/2020-10/9/2020; AM/PM

If you have any questions or concerns, please contact me for more information.
Best Regards,
Caitlin
Caitlin Makayan
SETA Support Contractor

Allegient Defense, Inc.
caitlin.makayan.ctr@navy.mil
caitlin.makayan.ctr@navy.smil.mil
Work: (703) 696-2580
Telework: (571) 445-4763

Office of Naval Research
Warfighter Performance Department
Warfighter Protection & Applications Division
875 North Randolph Street
Arlington, VA 22203-1995
Outlook-q1mnq3kp

From: Dean, Jay
To: Chapman, Sandra E CIV (USA)
Cc: Mothershed, Victoria; Wesley, William; Landon, Carol
Subject: [Non-DoD Source] RE: N00014-19-1-2314: Expiring Grant With Unspent Funds
Date: Friday, March 13, 2020 2:17:03 PM

N00014-19-1-2314: Expiring Grant With Unspent Funds
Project: Electrodermal activity in rats during exposure to HBO
P.I.: Jay B. Dean, USF Tampa

Dear Dr. Chapman,
I'm writing to request a no-cost extension of the above referenced project through June 30, 2020, so that we can complete the experiments in our small animal model. The initial setup of the equipment and this new system took a longer time than originally planned.
Thank you for considering my request.
Respectfully,
Jay B. Dean, PhD
Professor of Mol Pharm and Physiol.
USF Tampa

-----Original Message-----
From: Chapman, Sandra E CIV (USA) <sandra.chapman@navy.mil>
Sent: Friday, March 13, 2020 1:37 PM
To: Dean, Jay <jaydean@usf.edu>
Cc: Jeffress, Jenny V CTR (USA) <jenny.jeffress.ctr@navy.mil>
Subject: RE: N00014-19-1-2314: Expiring Grant With Unspent Funds

This email originated from outside of USF. Do not click links or open attachments unless you recognize the sender or understand the content is safe.

From: Dean, Jay
To: Jeffress, Jenny V CTR (USA)
Cc: Chapman, Sandra E CIV (USA); Mothershed, Victoria; Wesley, William; Landon, Carol
Subject: [Non-DoD Source] RE: USF update, Jay Dean"s lab
Date: Monday, March 30, 2020 11:27:18 AM

Hi Jenny,
Thank you for checking on the status of our new project. I understand completely, these are challenging times as we all try to adapt to our new abnormal normal. Keep me posted, and you and yours please stay safe.
Regards,
Jay

From: Jeffress, Jenny V CTR (USA)
Sent: Monday, March 30, 2020 6:20 AM
To: Dean, Jay
Cc: Chapman, Sandra E CIV (USA)
Subject: RE: USF update, Jay Dean's lab
Good morning, Jay –
Thank you for the update – it sounds like you guys are definitely keeping busy while on lockdown!
I just wanted to follow up quickly on the status of your Mitochondrial Targeted Anti-Oxidant Therapy grant. I initiated the funding action over a month ago, and it's made its way through ONR's approval chain pretty quickly, but has been with our Grants folks for a while now. I expect that—like all of us up here at ONR—they may still be trying to dig out from under the chaos of transitioning to telework status almost overnight. They often provide updates to us on the status of outstanding grant proposals at the end of each week, but I don't yet see one in my inbox from Friday, which is unusual. I'll give it today/tomorrow to see if get any requests for additional info from them, or—better yet—see if it gets awarded, and then I'll reach out and gently ask for an status check.
Thanks for your patience, Jay – and take care!

Jenny V. Jeffress
Contract Support Program Analyst
ONR/Code 34: Warfighter Performance
703.588.2932
jenny.jeffress.ctr@navy.mil

From: Dean, Jay <jaydean@usf.edu>
Sent: Friday, March 27, 2020 10:54 AM
To: Chapman, Sandra E CIV (USA) <sandra.chapman@navy.mil>; Jeffress, Jenny V CTR (USA) <jenny.jeffress.ctr@navy.mil>
Subject: [Non-DoD Source] USF update, Jay Dean's lab
Hello Sandra and Jenny,
I hope this message finds you and your families both safe and healthy... In response to the Coronavirus, USF has transitioned to remote instruction for the duration of the spring semester. Likewise, all research staff are encouraged to work remotely and only go into the lab only when necessary. In our lab, Carol, my lab manager, and I are the only two who can enter our building as needed on a limited basis. Consequently, we ceased lab work two weeks ago and remain at home working on data organization, data analysis, and manuscript writing. I meet almost daily with my lab members online using Microsoft Teams Meeting, and communicate daily via texting and email... And

finally, is there any update on the new ONR grant to USF on mitochondrial targeted antioxidants to mitigate onset of CNS ox tox? I'm out of funds to run any more experiments pertaining to this work. Carol is currently being supported by one of Dr. D'Agostino's nonONR projects and Chris is being supported by the department; however, neither of these options will last long term. My effort is being carried by the department.
In the meantime, please stay safe.
Regards,
Jay Dean

Whitley, Daylyn C CTR (USA)

To: Walter Boron
Subject: RE: [Non-DoD Source] RE: Boron subcontract

From: Walter Boron <wfb2@case.edu>
Sent: Thursday, April 9, 2020 4:08 PM
To: Chapman, Sandra E CIV (USA) <sandra.chapman@navy.mil>; Dean, Jay <jaydean@usf.edu>
Cc: Jeffress, Jenny V CTR (USA) <jenny.jeffress.ctr@navy.mil>; Morley Schwebel <mxs86@case.edu>; Gerald Babcock <gerald.babcock@case.edu>; Lesa Goodman <lag116@case.edu>
Subject: RE: [Non-DoD Source] RE: Boron subcontract

Hi Sandra,
Sorry for the confusion.
The only thing that CWRU is doing with the mice in Cleveland is to breed them. The mice never leave the breeding facility except for shipment to Florida.
Of course, there are costs associated with housing the mice, planning and executing the breeding (including genotyping), maintaining records, and shipping the mice to Florida.
Although neither Dr. Boron nor staff will perform any "animal testing" in Cleveland, Dr. Boron is overseeing the planning and execution of the breeding in Cleveland. Also, Dr. Boron is engaged with Dr. Dean in the design, execution, and interpretation of the experiments in Florida, and will also participate in paper writing.
If the above explanation is satisfactory and we do not need IACUC approval for Dr. Boron, then how do we obtain an "exemption letter" and from whom?
Best regards and stay safe!
Walter

Walter F. Boron, M.D., Ph.D.
Myers/Scarpa Professor and Chair
Dept. Physiology and Biophysics
Case Western Reserve University
Cleveland, OH 44106 USA

Medical Physiology (the textbook), Co-Editor

From: Chapman, Sandra E CIV (USA) <sandra.chapman@navy.mil>
Sent: Thursday, April 9, 2020 3:17 PM
To: Walter Boron <wfb2@case.edu>; Dean, Jay <jaydean@usf.edu>
Cc: Jeffress, Jenny V CTR (USA) <jenny.jeffress.ctr@navy.mil>
Subject: RE: [Non-DoD Source] RE: Boron subcontract

Drs. Boron and Dean,

I have received the following feedback from the review of your proposal submission with regard to Dr. Boron's role as co-PI and developing the mice, which then would constitute as animal use research and therefore we would need an animal use approval or exemption for his role in the project. See comments:

Discussion for item #1 above.

I'm still unclear on the extent of Dr. Boron's role in the research to be done under this grant. Although you say CWRU is only providing the mice, the proposal suggests a broader role for Dr. Boron of CWRU. At the very least, the extent of his participation in the research project funded under this grant is unclear from the proposal. We need to have a clear statement that he is not participating in the research besides supplying the mice, if that's the case. You said he's only supplying the supplying the mutant and control mouse/mice. If he is supplying mice in any way modified by experimentation before being supplied, then we would need an animal use approval package for that as well.

The proposal from U of Southern Florida (USF) also suggests he has a broader role, because it lists him as a "co-PI." It also requests $12,692 for his work, and it indicates that he will collaborate on "Objective 4." From the proposal:

"Objective 4 will be done in collaboration with Dr. Walter Boron at Case Western Reserve University (CWRU), Cleveland, Ohio. We plan to use AQP4-knockout (AQP4-KO) and wild-type (WT) mice, which are supplied by CWRU, to test the contribution of AQP4 in O2 & CO2 uptake across the BBB during exposure to normocapnic & hypercapnic HBO2 (i.e., HBO2 ±CO2)." If this just means that Dr. Boron will supply the mice, then I think what we have is sufficient (other than having the checklist corrected to include CSWU as a sub), but it isn't clear.

If Dr. Boron is doing animal testing, then I think we need more, because the USF IACUC approved protocol does not list Dr. Boron or any other secondary investigator. See paragraph 1.1.4. of the protocol. Para. 8.1.1. of the protocol (also on a separate pdf) states that "AQP4-KO and WT control mice will be shipped to USF Tampa from Case Western Reserve University, Cleveland, Ohio, Dept. of Physiology and Biophysics," which confirms that CSWU will supply the mice but does not mention Dr. Boron or rule out whether Dr. Boron will be involved in the research itself.

Please let me know if you will be able to provide an exemption letter or if you have the IUCUC approval. Unfortunately, if it is deemed animal use this would mean it would have to get approved by the Navy Vet…

Sandra

From: Walter Boron <wfb2@case.edu>
Sent: Monday, April 6, 2020 3:03 PM
To: Chapman, Sandra E CIV (USA) <sandra.chapman@navy.mil>; Dean, Jay <jaydean@usf.edu>
Cc: Jeffress, Jenny V CTR (USA) <jenny.jeffress.ctr@navy.mil>
Subject: [Non-DoD Source] RE: Boron subcontract

Hi Sandra and Jay,
I will be sure to alert our team here in case you need information from us.
Best regards and most of all, stay safe!
Walter

Walter F. Boron, M.D., Ph.D.
Myers/Scarpa Professor and Chair
Dept. Physiology and Biophysics
Case Western Reserve University
Cleveland, OH 44106 USA

Medical Physiology (the textbook), Co-Editor

From: Chapman, Sandra E CIV (USA) <sandra.chapman@navy.mil>
Sent: Monday, April 6, 2020 2:54 PM
To: Dean, Jay <jaydean@usf.edu>; Boron (wfb2@case.edu) <wfb2@case.edu>
Cc: Jeffress, Jenny V CTR (USA) <jenny.jeffress.ctr@navy.mil>
Subject: Boron subcontract

Hi Jay,

Can you confirm that Boron as a subcontractor on your proposal does not need to be included in the animal use approval because his contribution is not considered animal use research (supplying the mutant lines is not considered animal use). If this is not correct and it is included in the scope of animal use for this proposal animal use- do you have the animal use approval docs for Boron?

Thank you,
Sandra

AWARD/ MODIFICATION

3a. AWARDING OFFICE:
Office of Naval Research
875 N. Randolph Street, Suite 1425
Arlington, VA 22203-1995

1. INSTRUMENT TYPE: Grant Award

3b. CFDA: 12.300

2. AUTHORITY: 10 USC 2358 and 31 USC 6304, as amended

3c. DUNS NUMBER: 069687242

4. AWARD NO.: N00014-19-1-2314

5. MODIFICATION NO.: A00002

6. MODIFICATION TYPE: ADM

7. PR NO.: 4720005810

8. ACTIVITY/AGENCY PROPOSAL NO.: GRANT12788416

9. RECIPIENT PROPOSAL NO.:

10. PROPOSAL DATE: 02082019

11. ACTIVITY TYPE: R&D

12. PROGRAM TYPE: ONR

13. ISSUED TO
13a. ADDRESS:
UNIVERSITY OF SOUTH FLORIDA
4202 E FOWLER AVE
TAMPA FL 33620-9951
UNITED STATES OF AMERICA

13b. CAGE: 1F202

13c. EDI/EFT NUMBER: N/A

13d. BUSINESS OFFICE CONTACT: Brenda Simmons

13e. TELEPHONE NUMBER: 813-974-8778

13f. EMAIL ADDRESS: bsimmons@usf.edu

14. REMITTANCE ADDRESS (IF DIFFERENT FROM BLOCK 13):
Same as Block # 13

15. RESEARCH TITLE AND/OR DESCRIPTION OF PROJECT AND/OR PROPOSAL TITLE:
Electrodermal activity during hyberbaric oxygen exposure: rat study

16. FUNDING

	ACTIVITY/AGENCY SHARE	RECIPIENT SHARE	TOTAL
PREVIOUSLY OBLIGATED:			$24,977.00
OBLIGATED BY THIS ACTION:			$0.00
TOTAL OBLIGATED ON AWARD:			$24,977.00
FUTURE FUNDING:			$0.00
GRANT TOTAL:			$24,977.00

17. CURRENT FUNDING PERIOD
N/A THROUGH N/A

18. PERIOD OF PERFORMANCE
04/15/2019 THROUGH 07/14/2021

19. ACCOUNTING AND APPROPRIATION DATA:
See Attached Financial Accounting Data Sheet(s)

20a. PRINCIPAL INVESTIGATOR/RECIPIENT TECHNICAL REPRESENTATIVE:
Jay Dean

20b. TELEPHONE NUMBER: 813-974-1547

20c. EMAIL ADDRESS: jdean@health.usf.edu

21. TECHNICAL REPRESENTATIVE
21a. NAME: SANDRA CHAPMAN

21b. CODE: 0342

21c. ADDRESS:
ONR WARFIGHTER PROTECTION & APPLICATIONS
875 N. Randolph Street
Arlington VA 22203-1995

21d. TELEPHONE NUMBER:

21e. EMAIL ADDRESS: SANDRA.CHAPMAN@NAVY.MIL

22. POC FOR THIS ACTION
22a. NAME: ETHEL M GLOVER

22b. CODE: 0243

22c. ADDRESS:
Office of Naval Research
100 Alabama Street SW Suite 4R15
Atlanta GA 30303-3104

22d. TELEPHONE NUMBER: 404-562-1625

22e. EMAIL ADDRESS: ETHEL.GLOVER@NAVY.MIL

23a. ADMINISTRATIVE OFFICE:
ONR REG Office Atlanta
Telephone: (404) 562-1600
Email:ONR_Atlanta@navy.mil
100 Alabama Street, SW Suite 4R15
ATLANTA GA 30303

23b. CODE: N66020

24. SUBMIT PAYMENT REQUEST TO:
Same as block 23a

25a. PAYING OFFICE
DFAS-CO/SOUTH ENTITLEMENT OPERATIONS
HQ0338
PO Box 182264
COLUMBUS OH 43218

25b. CODE: HQ0338

26a. PATENT OFFICE:
Office of Naval Research
ATTN: ONR Office of Counsel
Intellectual Property Section
One Liberty Center
875 North Randolph Street, Suite 1425
Arlington, VA 22203-1995

26b. CODE: N00014

ONR AWARD FORM (10/99) - version 1.1

AWARD NO.	AWARD/MODIFICATION	MODIFICATION NO.	
N00014-19-1-2314		A00002	

27. SPECIAL INSTRUCTIONS:

See Special Requirements on Page 5

28. DELEGATIONS: The administration duties listed below have been delegated to the administrative office (block 23a). Upon request the awarding office contact (block 22) will make their full text available. Please direct questions to the contacts @: http://www.onr.navy.mil/Contracts-Grants/Regional-Contacts.aspx

This award provides full delegation to the administrative office in Block 23 of the grant. Full delegation includes the functions described in the DoDGARs at 32 CFR 22.715 and the administrative grants officer functions related to payments described in 32 CFR 22.810.

29. TERMS AND CONDITIONS: The following terms and conditions are incorporated herein by reference with the same force and effect as if they were given in full text. Upon request the awarding office contact named in block 22 will make their full text available, or they can be found at the specified URL.

DOCUMENT	URL

The following documents may be found at:
https://www.onr.navy.mil/work-with-us/manage-your-award/manage-grant-award/grants-terms-conditions
DOD RESEARCH AND DEVELOPMENT GENERAL TERMS AND CONDITIONS (JULY 2018)
UAWA AWARD A (SEP 2017)
ADDENDUM TO THE DOD R&D GENERAL TERMS AND CONDITIONS AND ONR PROGRAMMATIC REQUIREMENTS - DOMESTIC RESEARCH (JAN 2019)

30. OPTIONS

	OPTION NO.	AMOUNT	PERIOD
(1)			
(2)			
(3)			
(4)			

31. REPORTS: The reporting requirements under this award are specified in the articles of part 5 (Financial, Programmatic, and Property Reporting) to the General Terms and Conditions and in any additional language for Part 5 in an ONR Addendum to the General Terms and Conditions specified in Block 29.

32. FOR THE RECIPIENT		33. FOR THE UNITED STATES OF AMERICA	
32a. SIGNATURE OF PERSON AUTHORIZED TO SIGN		33a. SIGNATURE OF AWARDING OFFICER Digitally signed by ETHEL GLOVER 1060290039 Dated: 2020.04.22 08:40:41 EST	
32b. NAME AND TITLE OF SIGNER	32c. DATE SIGNED	33b. NAME AND TITLE OF AWARD OFFICER ETHEL GLOVER	33c. DATE SIGNED 04/22/2020

ONR AWARD FORM (10/99) - version 1.1

FINANCIAL ACCOUNTING DATA SHEET - NAVY

1. CONTRACT NUMBER (CRITICAL)	2. SPIIN (CRITICAL)	3. MOD (CRITICAL)	4. PR NUMBER
N00014-19-1-2314		A00002	4720005810

CLIN/SLIN	6. LINE OF ACCOUNTING													7.	NAVY INTERNAL USE ONLY REF DOC/ACRN
	A. ACRN (CRITICAL)	B. APPROPRIATION (CRITICAL)	C. SUBHEAD (CRITICAL)	D. OBJ CLA	E. PARM	F. RFM	G. SA	H. AAA (CRITICAL)	I. IT	J. PAA	K. COST CODE			AMOUNT (CRITICAL)	
											PROJ UNIT	MCC	PDLI & SUF		
													PAGE TOTAL	$0.00	
													GRAND TOTAL	$0.00	

PREPARED/AUTHORIZED BY:

DATE:

COMPTROLLER APPROVAL:
FOR FISCAL DATA AND SIGNATURE
BY _____ for COMPTROLLER, ONR CONTRACT REVIEWED
DATE:

ONR AWARD FORM (10/99) - version 1.1

FINANCIAL ACCOUNTING DATA SHEET – NON-NAVY DoD ACTIVITIES

1. CONTRACT NUMBER (CRITICAL)	2. SPIIN (CRITICAL)	3. MOD (CRITICAL)	4. PR NUMBER	
N00014-19-1-2314		A00002	4720005810	

5. CLIN/SLIN	6. ACRN (CRITICAL)	7. ACCOUNTING CITATION	8. AMOUNT (CRITICAL)	NAVY INTERNAL USE ONLY REF DOC/ACRN
		PAGE TOTAL		
		GRAND TOTAL		

PREPARED/AUTHORIZED BY:	COMPTROLLER APPROVAL: FOR FISCAL DATA AND SIGNATURE
DATE:	BY _____ for COMPTROLLER, ONR CONTRACT REVIEWED DATE:

ONR AWARD FORM (10/99) - version 1.1

AWARD NO. N00014-19-1-2314	SPECIAL REQUIREMENTS	MODIFICATION NO. A00002	

The purpose of this modification is to provide a twelve-month extension to the period of performance, at no additional cost to the government. The new end date for this award is 07/14/2021.

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This modification is in accordance with Under Secretary of Defense for Research & Engineering DoD-wide supplemental Terms & Conditions (T&Cs) for the COVID-19 Crisis for Current and Prospective Awardees Impacted by Novel COVID-19.

.

All other terms and conditions remain unchanged.

From: Becker, Christopher E CTR USN CNR ARLINGTON VA (USA)
To: Chapman, Sandra E CIV (USA); "Dean, Jay"
Cc: Landon, Carol; Wesley, William; Glover, Ethel M CIV USN CNO (USA)
Subject: RE: Dr. Dean - 06/17/20 Financial update attached
Date: Wednesday, July 29, 2020 12:07:57 PM
Attachments: N000141912314_A00002.pdf

Jay,
Unbeknownst to Sandra or me, your grant had already received a 12 month NCE as part of ONR's COVID relief to universities. Current end date is 14 July 2021. Modification is attached.
Both you and Brenda Simmons, listed as business office contact, should have received email notification of this modification on 22 April. The emails are somewhat cryptic, but you should not ignore them. They let you know that a document (grant modification) is available to download in EDA.
-Chris

Chris Becker
Contractor support, ONR Warfighter Performance Department
Phone: 571.239.2868
Chris.Becker.ctr@navy.mil

From: Chapman, Sandra E CIV (USA) <sandra.chapman@navy.mil>
Sent: Wednesday, July 29, 2020 11:53 AM
To: 'Dean, Jay' <jaydean@usf.edu>; Becker, Christopher E CTR USN CNR ARLINGTON VA (USA) <chris.becker.ctr@navy.mil>
Cc: Landon, Carol <clandon@usf.edu>; Wesley, William <wwesley@usf.edu>
Subject: RE: Dr. Dean - 06/17/20 Financial update attached

Hi Jay,

Jenny is no longer supporting the program and we are very fortunate to have the assistance of Chris Becker at this time.

I will check on the status and get back you.

Best,
Sandra

From: Dean, Jay <jaydean@usf.edu>
Sent: Wednesday, July 29, 2020 11:50 AM
To: Chapman, Sandra E CIV (USA) <sandra.chapman@navy.mil>; Jeffress, Jenny V CTR USN CNR ARLINGTON VA (USA) <jenny.jeffress.ctr@navy.mil>
Cc: Landon, Carol <clandon@usf.edu>; Wesley, William <wwesley@usf.edu>
Subject: [Non-DoD Source] FW: Dr. Dean - 06/17/20 Financial update attached

Hello Sandra and Jenny,

We have not heard anything about the no cost extension you requested on July 10th. Any news?
Thanks,
Jay Dean

From: Chapman, Sandra E CIV (USA) <sandra.chapman@navy.mil>
Sent: Friday, July 10, 2020 6:15 PM
To: Glover, Ethel M CIV USN CNO (USA) <ethel.glover@navy.mil>; ONR_Atlanta <ONR_Atlanta.fcm@navy.mil>
Cc: Wesley, William <wwesley@usf.edu>; Becker, Christopher E CTR USN CNR ARLINGTON VA (USA) <chris.becker.ctr@navy.mil>; Dean, Jay <jaydean@usf.edu>; Landon, Carol <clandon@usf.edu>
Subject: RE: Dr. Dean - 06/17/20 Financial update attached

Hi,

I would like to request a NCE for Award N000141912314 to Jay Dean at University of South Florida through Nov, 2020 due to COVID-related delays in laboratory access.

Best,
Sandra

From: Landon, Carol <clandon@usf.edu>
Sent: Friday, July 10, 2020 9:33 AM
To: Chapman, Sandra E CIV (USA) <sandra.chapman@navy.mil>; Dean, Jay <jaydean@usf.edu>; Becker, Christopher E CTR USN CNR ARLINGTON VA (USA) <chris.becker.ctr@navy.mil>
Cc: Wesley, William <wwesley@usf.edu>
Subject: [Non-DoD Source] Re: Dr. Dean - 06/17/20 Financial update attached

Good morning,

Dr. Dean asked me to respond to your question.

He would like to request that the NCE extend to the end of November, 2020, if possible.

All the best,

Carol Landon

From: Chapman, Sandra E CIV (USA)
Sent: Friday, July 10, 2020 9:16 AM
To: Dean, Jay; Becker, Christopher E CTR USN CNR ARLINGTON VA (USA)
Cc: Landon, Carol; Wesley, William

Subject: RE: Dr. Dean - 06/17/20 Financial update attached

Jay,

How long do you need the extension for?

Best,
Sandra

From: Dean, Jay <jaydean@usf.edu>
Sent: Thursday, July 9, 2020 4:27 PM
To: Jeffress, Jenny V CTR USN CNR ARLINGTON VA (USA) <jenny.jeffress.ctr@navy.mil>; Chapman, Sandra E CIV (USA) <sandra.chapman@navy.mil>
Cc: Landon, Carol <clandon@usf.edu>; Wesley, William <wwesley@usf.edu>
Subject: [Non-DoD Source] RE: Dr. Dean - 06/17/20 Financial update attached
Importance: High

Hello,
It just came to my attention that the electrodermal project nce ends on July 14. I'm still working on this project due to delays caused by COVID-19 and failure of our air compressor used to pressurize the chamber. I still need the remaining funds for the animals we are studying.
My apology for this late notice, but is it possible to get another nce?
Regards,
Jay Dean

ONR, Undersea Medicine Program; Electrodermal activity during hyperbaric oxygen exposure—rat study. (N000141912314)

From: Landon, Carol <clandon@usf.edu>
Sent: Thursday, July 9, 2020 4:19 PM
To: Dean, Jay <jaydean@usf.edu>
Subject: Fw: Dr. Dean - 06/17/20 Financial update attached

Jay,

I just noticed that the NCE for the electraderm project ends on July 14.....that's in 5 days.
<<<see below>>>
We now have about $900 in the acct since we just spent $102 to pay for the May animal bill.
There are 3 implanted rats on that skin protocol. I think the animal protocol is good until Dec 2020

Carol

From: Wesley, William <wwesley@usf.edu>
Sent: Thursday, June 18, 2020 1:02 PM
To: Dean, Jay <jaydean@usf.edu>
Cc: Landon, Carol <clandon@usf.edu>; Mothershed, Victoria <vmothershed@usf.edu>
Subject: Dr. Dean - 06/17/20 Financial update attached

Dr. Dean and Carol –

Attached are your latest financial projections. Here are the important notes –

- Payroll is posted thru 05/28/20. Wages are accrued thru the end of each grant year but not on 113200.
- Animals are posted or encumbered thru **April** only.
- **113200 Oxygen Toxicity** - **Only $123 remain for supplies & future animals.**
- **18300 RIA** -
- **118700 Rat Study -** **now over 7/14/20. Only a little more than $1,000 remain.**
- **120800 New ONR** - Wages are projected thru year 1.

Please see me with any questions.

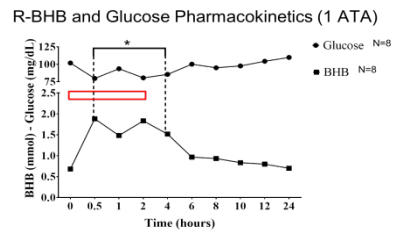
Bill

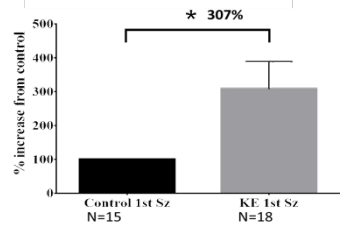
Optimizing Ketone Metabolic Therapy and Identifying Biomarkers for Mitigation and Predicting CNS Oxygen Toxicity: Animal Studies

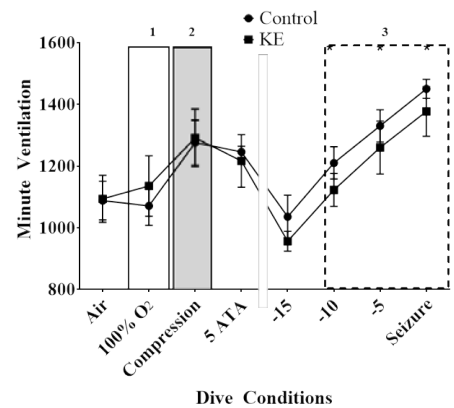
Dominic P. D'Agostino, PhD (PI)
Jay B. Dean (Co-I); Angela M. Poff (Co-I)

Summary: Ketogenic metabolic therapy (KMT) is an effective mitigation strategy to delay Central Nervous System Oxygen Toxicity (CNS-OT) seizures in rats, and has a proven safety in humans. The current study tests the most promising ketogenic agents (KAs) and lower dose on CNS-OT, cognition, behavior and motor performance. The diverse neuropharmacological and signaling mechanisms of KMT remain under investigation, but the current study supports a KMT-induced increase in antioxidant capacity.

Naval Applications: Naval Special Warfare and Special Operations diving and submarine operations are limited by the risk CNS-OT. KMT is a promising mitigation strategy for CNS-OT, enhanced safety and performance resilience.







Aims: Our 1st aim was to understand the dose-dependence of KE adminstration for delaying CNS-OT without impairing cognitive or motor performance. 2nd aim was to determine the effects of HBO ± KE on presumptive physiological markers (e.g. body temperature, heart rate, respiratory rate) that can be monitored in real time to predict an impending Sz. 3rd aim was to study the anti-convulsant mechanisms of therapeutic ketosis by measuring redox potential of blood from animals pre-and post- Sz with and without KE treatment. As designed, the proposed studies answer crucial questions needed for advancing the science and future human application of KMT for CNS-OT and warfighter performance resilience.

Issues: No issues

FY22 Accomplishments, Discoveries, & Inventions

- KMT delays Sz_1 by 307% overall
- Cognition and performance remain unchanged; anxiolytic effect
- Hyperoxic hyperventilation & bradycardia precedes seizures
- Antioxidant capacity is increased with KMT
- 4 Manuscripts and 2 Book Chapters
  - Stavitzski NM, et.al. *AJP Reg.* 2021 PMID: 34132115
  - Brunner et.al (2021). *Nutrients*,13(11),4082. PMID: 34836344
  - Prins PJ.(2021) *Frontiers in Physiology*, 12, 780755(2021).
  - Poff, A.M et.al.(2021) *Frontiers in Nutrition*, 8, 783659.
  - 2 book chapters on KMT in Ketogenic Diet and Metabolic Therapies (2nd Edition) Oxford University Press

FY23 Goals

- Test the safety and efficacy of new Glycerol-AcAc Triester

PI Contact Email: ddagosti@usf.edu

OPTIMIZING KETONE METABOLIC THERAPY AND IDENTIFYING BIOMARKERS FOR MITIGATION AND PREDICTION OF CNS OXYGEN TOXICITY: ANIMAL STUDIES

Dominic P. D'Agostino, PhD (PI); Angela Poff, PhD (Co-I); Jay B. Dean, PhD (Co-I)
ddagosti@usf.edu
813-396-9628

Background

Central Nervous System-Oxygen Toxicity (CNS-OT) seizures (Sz) remain a limitation to Naval Special Warfare and Special Operations diving and submarine operations. Ketogenic metabolic therapy (KMT) is a strategy to induce therapeutic ketosis and is an effective mitigation strategy to delay CNS-OT. Thus, KMT has the potential to enhance warfighter safety, resilience and readiness. Prior work with ketone ester (KE; R,S-1,3-butanediol diacetoacetate) demonstrated a significant delay in CNS-OT by 575% in rats, and this neuroprotective effect was observed without the need for dietary restriction. Since this particular study only tested a single large dose (10g/kg), the optimal dosing protocol for KE that provides neuroprotection without hindering cognitive or motor performance remained to be elucidated. Further work is needed, especially since newer compounds and formulations are being developed that have a more favorable pharmacokinetic profile. Additionally, little is known regarding the multiple mechanisms underlying KMT-induced effect on delaying CNS-OT and behavior. The current study examines the effect of KMT on CNS-OT seizures, behavior and antioxidant capacity.

Objectives

The primary goal of this work was to understand the effect of KE adminstration at a dose range that delays CNS-OT without impairing cognitive or motor performance. Secondly, there remained a crucial need to identify early physiological predictors of CNS-OT onset, both within and outside the context of ketogenic intervention to avert Sz onset. Thus, a 2nd goal was to determine the effects of HBO ± KE on presumptive physiological markers (e.g. body temperature, heart rate, respiratory rate) that can be monitored in real time to predict an impending Sz. The 3rd goal was to study the anti-convulsant mechanisms of KMT by measuring redox potential of blood from animals pre-and post- Sz with and without KE treatment. As designed, the proposed studies answer crucial questions to benefit the US Navy and hyperbaric medicine, thereby advancing the science and future human application of KMT for CNS-OT and warfighter performance resilience.

Methods

In FY 2021-2022, we completed the remaining Aim 1 experiments, validating the anticonvulsant effects of KE (at a lower) dose as a form of KMT for preventing CNS-OT. This work was published (Stavitzski NM, et.al. *AJP Reg*. 2021 PMID: 34132115) and helps to define the performance aspects of the KMT. In this study Sprague Dawley rats were implanted with radio-telemetry modules, allowing for real-time monitoring of body temperature, EEG, ECG, and respiratory EMG during an HBO dive protocol, and were monitored for 1st and 2nd seizure. Body temp, HR, and respiratory rate data was collected continuously throughout the dive and analyzed various timepoints of the protocol to look for potential changes prior to Sz onset (Aim 2). Additionally, blood was collected from the saphenous vein pre- and post-dive for analysis of redox potential. Serum was separated from the blood samples by centrifuge, and analyzed with the RedoxSYS system which measures static and capacity oxidation-reduction potential (Aim 3).

Results

Pharmacokinetics of KE on blood BHB and glucose levels is shown in **Figure 1**. KMT delivered as KE (7.5 g/kg) by single oral gavage significantly increased blood ketone levels (R-BHB) and reduced glucose. KE also significantly elevates acetoacetate and acetone, which are important for mitigating CNS-OT. During therapeutic ketosis, 0.5 – 4hrs, glucose levels were decreased and BHB increased ($p<0.0001$). Prior to KE adminstration the rats had been fed standard rodent chow, and thus the ketosis was independent of diet. **Figure 2** shows KE delayed CNS-OT (5 ATA O_2) seizures. Data represented was normalized to account for known variability in seizure latency (LSz) of control animals. Average LSz_1 (in min.) for control rats was 6.42 ± 0.44 versus 22.16 ± 5.10 in KE-treated rats, a 307% increase. **Figure 3** depicts three phases of "The Compound Ventilatory Response to Hyperoxia". Phase 1 is due to normobaric hyperoxia, a transient reduction in minute ventilation, due to peripheral chemoreceptor effect. Phase "2" (in gray), arises from increased pressure and brain PO2. Phase "3" (dotted line) develops just prior to the onset of CNS-OT. There was a significant difference between the groups starting at ~15-10 minutes before seizure consistent with previous findings, confirming that respiration can be used as a physio-marker of an impending seizure. **Figure 4** shows a significant difference at all time points ($p<0.0001$) in temperature for Control vs KE group. The mechanism of KE-induced decrease in temperature is unknown, but evidence suggests BHB can decrease sympathetic tone via GPR41 antagonism. **In summary**, the present findings demonstrate that a single dose of KE prior to 5 ATA O2 increases LSz without impairing cognition or motor performance. Furthermore, this acute dose induced and maintained a state of therapeutic ketosis for up to 4-6hrs, independent of diet. This information can be used to provide guidance on resilience strategies for delaying CNS-OT to enable deeper, longer, and safer dives for US Navy and recreational divers and in current treatment protocols used in HBO2 therapy.

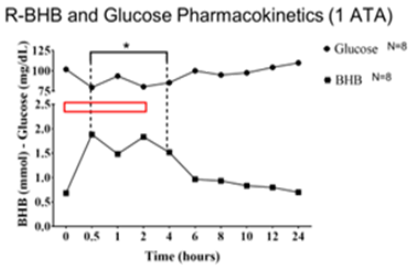


Figure 1. Pharmacokinetic Effect of KE. Oral gavage of KE was administered immediately after baseline. BHB and glucose was measured at baseline, 0.5, 1, 2, 4, 6, 8, 10, 12, 24hr post-gavage. Top line graph (•) shows glucose and the bottom-line graph (■) shows the levels of BHB.

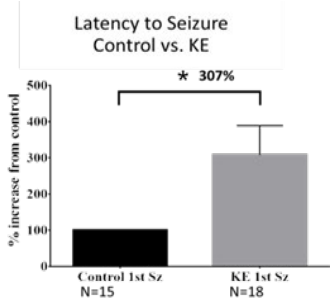


Figure 2. KE Prolongs Latency to CNS-OT Seizure. The average LSz1 was increased 307% with KMT. Normalized to Controls (n=14; One-way Anova).

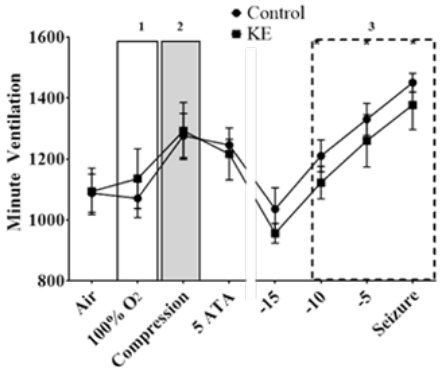


Figure 3. Ventilatory Response to Hyperoxia. Phase 1 is marked with black solid outline, phase 2 is the gray box, and phase 3 is dotted box.

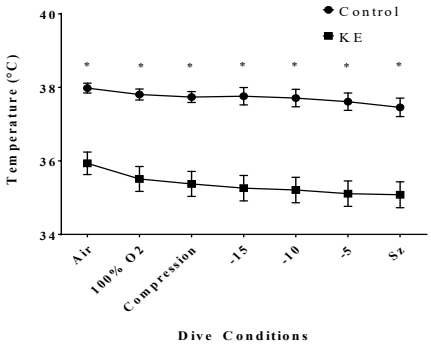


Figure 4. Temperature Changes to KMT. Control (closed circles) and KE (closed squares) were significantly different when compared at each dive condition ($p<0.05$).

From: Chapman, Sandra E CIV (USA)
To: Walter Boron; heath.gasier@duke.edu
Cc: Kaminski, Sarah R CTR (USA)
Subject: Full proposal invitation
Date: Monday, August 8, 2022 12:51:00 PM
Attachments: FY23 INSTRUCTIONS FOR APPLYING FOR FUNDING FROM THE ONR_Aug 2022.docx

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Dr. Boron,

I would like to formally invite you to submit a full proposal to the Undersea Medicine program (instructions for submission re attached). I am inviting a proposal on the Pilot effort and expect the proposal will provide answers to the conditional KO of AQP5 and/or AQP1 for reducing oxidative damage without compromise of physical performance to enable follow-on transitional studies by NIH or others (JPC5) to transition to drug development and testing in swine and humans.

The deadline for the complete package to include Human Use Approvals (as you might recall this is required prior to award by Oct. 15th, 2022. Instructions for human use can be found here: Human Subject Research Documentation Requirements | Office of Naval Research (navy.mil)

Please let me know if you have additional questions. I will be away from Aug. 10-20th, but just got Sarah Kaminski's email, cc'd here, and you can direct questions her way - she's very new, but also very capable and so I believe she will be able to find a way to answer your questions ☺

Best,
Sandra

From: Walter Boron
To: Chapman, Sandra E CIV (USA); Chapman, Sandra E CIV (USA)
Cc: Rossana Occhipinti; Morley Schwebel
Subject: [Non-DoD Source] Pilot project on Pulmonary oxygen toxicity
Date: Monday, May 23, 2022 4:09:22 PM

Hi Sandra,
I hope that you are doing well.
I am writing to get your sense of how to proceed with the AQP5/Pulmonary Oxygen Toxicity project. As I indicated in my Abstract, we got off to a slow start because of (a) the low fertility rate of the AQP5-KO mice, and (b) the switch from Claude to Heath and thus the time to hone in on which assays. In addition, (c) the hyperoxic mice are not eating/drinking as much as they should, which threw off the wet weight/dry weight study that we were doing to assess pulmonary edema.
We have now overcome 'a' and 'b' and are on a bit of a roll. Even though the N's are still low, I am cautiously optimistic because the LDH (marker of cell leakiness) and protein (marker of alveolar blood-gas barrier leakiness) values are strikingly low in AQP5-KO and AQP5/AQP1 double KO (dKO) mice. We have now expanded our AQP5-KO and dKO colonies to the point where we can supply large numbers of mice to Heath. However, we are both out of money for this project, and this hurts Heath especially hard …
Do you have any funds available to keep this Pilot Project on life support for the remainder of FY22?
I know that the Whitepaper deadline is coming up. What are your thoughts?
Should we suggest another Pilot to build up the N's with the current protocol, and/or to explore other POT protocols?
Should we apply for a regular research grant with the preliminary we have (or could potentially get during the summer)?
I would be happy to chat via Zoom, for example.
Best regards,
Walter
Walter F. Boron, M.D., Ph.D.
Distinguished University Professor
Myers/Scarpa Professor and Chair
Dept. Physiology and Biophysics
Case Western Reserve University
Cleveland, OH 44106 USA
Medical Physiology (the textbook), Co-Editor

From: Walter Boron
To: Walter Boron; Chapman, Sandra E CIV (USA); Chapman, Sandra E CIV (USA)
Cc: Rossana Occhipinti; Morley Schwebel
Subject: [Non-DoD Source] RE: Pilot project on Pulmonary oxygen toxicity
Date: Friday, June 3, 2022 2:28:56 PM

Hi Sandra,
I sent the note below while you were out of town. In case it is buried under a mountain of other e-mails, I am resending.
Best regards,
Walter
Walter F. Boron, M.D., Ph.D.
Distinguished University Professor
Myers/Scarpa Professor and Chair
Dept. Physiology and Biophysics
Case Western Reserve University
Cleveland, OH 44106 USA
Medical Physiology (the textbook), Co-Editor

From: Walter Boron <wfb2@case.edu>
Sent: Monday, May 23, 2022 4:09 PM
To: Chapman, Sandra E CIV (USA) <sandra.e.chapman2.civ@us.navy.mil>; Chapman, Sandra E CIV (USA) <sandra.chapman@navy.mil>
Cc: Rossana Occhipinti <rxo22@case.edu>; Morley Schwebel <mxs86@case.edu>
Subject: Pilot project on Pulmonary oxygen toxicity
Hi Sandra,
I hope that you are doing well.
I am writing to get your sense of how to proceed with the AQP5/Pulmonary Oxygen Toxicity project. As I indicated in my Abstract, we got off to a slow start because of (a) the low fertility rate of the AQP5-KO mice, and (b) the switch from Claude to Heath and thus the time to hone in on which assays. In addition, (c) the hyperoxic mice are not eating/drinking as much as they should, which threw off the wet weight/dry weight study that we were doing to assess pulmonary edema.
We have now overcome 'a' and 'b' and are on a bit of a roll. Even though the N's are still low, I am cautiously optimistic because the LDH (marker of cell leakiness) and protein (marker of alveolar blood-gas barrier leakiness) values are strikingly low in AQP5-KO and AQP5/AQP1 double KO (dKO) mice. We have now expanded our AQP5-KO and dKO colonies to the point where we can supply large numbers of mice to Heath. However, we are both out of money for this project, and this hurts Heath especially hard …
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Walter

Walter F. Boron, M.D., Ph.D.
Distinguished University Professor
Myers/Scarpa Professor and Chair
Dept. Physiology and Biophysics
Case Western Reserve University
Cleveland, OH 44106 USA
Medical Physiology (the textbook), Co-Editor

From: Walter Boron
To: Chapman, Sandra E CIV (USA); sandra.e.chapman2@us.navy.mil
Subject: [Non-DoD Source] RE: Undersea Medicine program updates
Date: Tuesday, August 2, 2022 9:27:53 AM

Hi Sandra,
Too bad that we're losing Pooja, but Sarah is absolutely terrific!
Walter

Walter F. Boron, MD, PhD
Distinguished University Professor
Myers/Scarpa Professor and Chair
Department of Physiology and Biophysics
School of Medicine
Case Western Reserve University
Cleveland, OH 44106 USA
Medical Physiology (the textbook), Co-Editor

From: Chapman, Sandra E CIV (USA) <sandra.e.chapman2.civ@us.navy.mil>
Sent: Tuesday, August 2, 2022 9:24 AM
To: sandra.e.chapman2@us.navy.mil
Subject: Undersea Medicine program updates

Greetings,
I am writing to let you know about some staffing changes for the Undersea Medicine program.
A couple weeks ago, Pooja resigned from her role as a SETA supporting the Undersea Medicine program. While certainly sad to lose such talent, I am excited for her as she embarks on a new journey in her career. When we have a stable replacement, I will let everyone know. Just before she left, those of you with active grants (6-1/6-2) should have received a message about the annual reports, RPPR, from Pooja announcing that they will now be due in November. If you did not receive a message, please let me know and I will make sure to get the information to you.
The good news is that Undersea Medicine will now be joined by Sarah Kaminski, who some of you may know since she supported the Undersea Medicine program years ago. We are blessed to have her back as a Program Analyst (PA)- I hear nothing but rave reviews from all those who know her. This is a very welcomed change for the program and will help alleviate some of the turbulence we have had lately without a stable and permanent PA. I encourage everyone to start cc'ing Sarah on all administrative issues (NCE's, human/animal use, proposal submission, etc.) once I am able to provide her email address, which she is still waiting on.
Finally, please note that I have a new email address as we have transitioned to a new IT infrastructure through Microsoft known as Flankspeed, which has brought both benefits and growing pains.
Please let me know if you have any questions.
Best,
Sandra

From: Dean, Jay
To: Chapman, Sandra E CIV (USA); Walter Boron
Cc: Jeffress, Jenny V CTR (USA); Morley Schwebel; Gerald Babcock; Lesa Goodman
Subject: RE: [Non-DoD Source] RE: Boron subcontract
Date: Thursday, April 9, 2020 4:32:19 PM

Sandra,
Walter will need to confirm that.
Jay

From: Chapman, Sandra E CIV (USA)
Sent: Thursday, April 9, 2020 4:12 PM
To: Walter Boron ; Dean, Jay
Cc: Jeffress, Jenny V CTR (USA) ; Morley Schwebel ; Gerald Babcock ; Lesa Goodman
Subject: RE: [Non-DoD Source] RE: Boron subcontract
Can you confirm that the experimentation to generate these mutant strains was already done under a previous award?
Thank you,
Sandra

From: Walter Boron <wfb2@case.edu>
Sent: Thursday, April 9, 2020 4:08 PM
To: Chapman, Sandra E CIV (USA) <sandra.chapman@navy.mil>; Dean, Jay <jaydean@usf.edu>
Cc: Jeffress, Jenny V CTR (USA) <jenny.jeffress.ctr@navy.mil>; Morley Schwebel <mxs86@case.edu>; Gerald Babcock <gerald.babcock@case.edu>; Lesa Goodman <lag116@case.edu>
Subject: RE: [Non-DoD Source] RE: Boron subcontract
Hi Sandra,
Sorry for the confusion.
The only thing that CWRU is doing with the mice in Cleveland is to breed them. The mice never leave the breeding facility except for shipment to Florida.
Of course, there are costs associated with housing the mice, planning and executing the breeding (including genotyping), maintaining records, and shipping the mice to Florida.
Although neither Dr. Boron nor staff will perform any “animal testing” in Cleveland, Dr. Boron is overseeing the planning and execution of the breeding in Cleveland. Also, Dr. Boron is engaged with Dr. Dean in the design, execution, and interpretation of the experiments in Florida, and will also participate in paper writing.
If the above explanation is satisfactory and we do not need IACUC approval for Dr. Boron, then how do we obtain an “exemption letter” and from whom?
Best regards and stay safe!
Walter
Walter F. Boron, M.D., Ph.D.
Myers/Scarpa Professor and Chair
Dept. Physiology and Biophysics
Case Western Reserve University
Cleveland, OH 44106 USA
Medical Physiology (the textbook), Co-Editor

From: Chapman, Sandra E CIV (USA) <sandra.chapman@navy.mil>

Sent: Thursday, April 9, 2020 3:17 PM
To: Walter Boron <wfb2@case.edu>; Dean, Jay <jaydean@usf.edu>
Cc: Jeffress, Jenny V CTR (USA) <jenny.jeffress.ctr@navy.mil>
Subject: RE: [Non-DoD Source] RE: Boron subcontract

Drs. Boron and Dean,

I have received the following feedback from the review of your proposal submission with regard to Dr. Boron's role as co-PI and developing the mice, which then would constitute as animal use research and therefore we would need an animal use approval or exemption for his role in the project. See comments:

Discussion for item #1 above.

I'm still unclear on the extent of Dr. Boron's role in the research to be done under this grant. Although you say CWRU is only providing the mice, the proposal suggests a broader role for Dr. Boron of CWRU. At the very least, the extent of his participation in the research project funded under this grant is unclear from the proposal. We need to have a clear statement that he is not participating in the research besides supplying the mice, if that's the case. You said he's only supplying the supplying the mutant and control mouse/mice. If he is supplying mice in any way modified by experimentation before being supplied, then we would need an animal use approval package for that as well.

The proposal from U of Southern Florida (USF) also suggests he has a broader role, because it lists him as a "co-PI." It also requests $12,692 for his work, and it indicates that he will collaborate on "Objective 4." From the proposal:

"Objective 4 will be done in collaboration with Dr. Walter Boron at Case Western Reserve University (CWRU), Cleveland, Ohio. We plan to use AQP4-knockout (AQP4-KO) and wild-type (WT) mice, which are supplied by CWRU, to test the contribution of AQP4 in O2 & CO2 uptake across the BBB during exposure to normocapnic & hypercapnic HBO2 (i.e., HBO2 ±CO2)." If this just means that Dr. Boron will supply the mice, then I think what we have is sufficient (other than having the checklist corrected to include CSWU as a sub), but it isn't clear.

If Dr. Boron is doing animal testing, then I think we need more, because the USF IACUC approved protocol does not list Dr. Boron or any other secondary investigator. See paragraph 1.1.4. of the protocol. Para. 8.1.1. of the protocol (also on a separate pdf) states that "AQP4-KO and WT control mice will be shipped to USF Tampa from Case Western Reserve University, Cleveland, Ohio, Dept. of Physiology and Biophysics," which confirms that CSWU will supply the mice but does not mention Dr. Boron or rule out whether Dr. Boron will be involved in the research itself.

Please let me know if you will be able to provide an exemption letter or if you have the IUCUC approval. Unfortunately, if it is deemed animal use this would mean it would have to get approved by the Navy Vet…

Sandra

From: Walter Boron <wfb2@case.edu>
Sent: Monday, April 6, 2020 3:03 PM
To: Chapman, Sandra E CIV (USA) <sandra.chapman@navy.mil>; Dean, Jay <jaydean@usf.edu>
Cc: Jeffress, Jenny V CTR (USA) <jenny.jeffress.ctr@navy.mil>

Subject: [Non-DoD Source] RE: Boron subcontract

Hi Sandra and Jay,

I will be sure to alert our team here in case you need information from us.

Best regards and most of all, stay safe!

Walter

Walter F. Boron, M.D., Ph.D.
Myers/Scarpa Professor and Chair
Dept. Physiology and Biophysics
Case Western Reserve University
Cleveland, OH 44106 USA
Medical Physiology (the textbook), Co-Editor

From: Chapman, Sandra E CIV (USA) <sandra.chapman@navy.mil>
Sent: Monday, April 6, 2020 2:54 PM
To: Dean, Jay <jaydean@usf.edu>; Boron (wfb2@case.edu) <wfb2@case.edu>
Cc: Jeffress, Jenny V CTR (USA) <jenny.jeffress.ctr@navy.mil>
Subject: Boron subcontract

Hi Jay,

Can you confirm that Boron as a subcontractor on your proposal does not need to be included in the animal use approval because his contribution is not considered animal use research (supplying the mutant lines is not considered animal use). If this is not correct and it is included in the scope of animal use for this proposal animal use- do you have the animal use approval docs for Boron?

Thank you,

Sandra

From: Walter Boron
To: Chapman, Sandra E CIV (USA); Chapman, Sandra E CIV (USA)
Cc: Rossana Occhipinti; Morley Schwebel
Subject: RE: [Non-DoD Source] RE: Pilot project on Pulmonary oxygen toxicity
Date: Saturday, June 4, 2022 10:27:03 AM

Hi Sandra,

After all the investment that ONR has made in gas-channel research, it seems to me that we are at the edge of a breakthrough with pulmonary oxygen toxicity (POT). The progress, at first, was slow with the AQP5-KOs because litter size is low (but we now have expanded these colonies), and because (as you say) a lot of the effort fell on Heath who was overtaxed. Although we are out of dollars, we keep pushing ahead because the outcome is potentially so important.

The most recent preliminary data on AQP5-KOs and AQP5/1 double KOs shows a striking decrease in LDH (i.e., reduced lysis of the type I cells lining the alveoli) and a striking decrease in Albumin (i.e., reduced leakiness of the blood-gas wall, which includes the type I cells and the capillary endothelial cells).

Most recently, we analyzed the mortality data during hyperoxia (you have not seen these data previously), and these results (although the N's are small for the double KOs) are striking:

- In our/Heath experiments, % mortality with 60-72 h >99% O_2 was:
  - WT: 35%
  - $AQP1^{-/-}$: 21%
  - $AQP5^{-/-}$: 9%
  - dKO: 0%

The preliminary LDH, Albumin, and Mortality data all support the hypothesis that blocking these channels improves outcome in POT.

One of the thoughts that we have had (not yet shared with Heath) is for us to take the project more or even entirely within house here at CWRU, with Heath as a Co-PI who could visit Cleveland to teach/oversee technique, advise on assays, and monitor progress. We have a sophisticated Mouse Physiological Phenotyping Core (MPPC) in the Department of Physiology and Biophysics but had never done bronchiolar alveolar lavage (BAL, which generates the materials for the LDH, Albumin, etc. assays). Our MPPC, which is immediately adjacent to the Animal Resources Center that houses mouse colonies and behind the "wall" (which means mice can move back and forth between the MPPC and ARC), has the facilities to do the hyperoxia experiments. I am certain that we could master BAL with Heath's help. Doing the animal work entirely in Cleveland would eliminate the strain (on the PIs and mice) and cost of shipping.

I would argue that, with a modest investment, this is the time to nail down the genetic proof of principle that blocking AQP5 (blocking AQP1 is probably not as important alone, but appears to bolster the effect of blocking AQP5) will ameliorate POT in divers and submariners. The next step could be to develop an AQP5 blocker. A company I co-founded has experience developing blockers for another AQP (AQP4, in stroke). An inhaled inhibitor(s) could be a game changer.

Best regards,
Walter

Walter F. Boron, MD, PhD
Distinguished University Professor
Myers/Scarpa Professor and Chair
Department of Physiology and Biophysics

School of Medicine
Case Western Reserve University
Cleveland, OH 44106 USA
Medical Physiology (the textbook), Co-Editor

From: Chapman, Sandra E CIV (USA) <sandra.e.chapman2.civ@us.navy.mil>
Sent: Friday, June 3, 2022 7:25 PM
To: Walter Boron <wfb2@case.edu>; Chapman, Sandra E CIV (USA) <sandra.chapman@navy.mil>
Cc: Rossana Occhipinti <rxo22@case.edu>; Morley Schwebel <mxs86@case.edu>
Subject: RE: [Non-DoD Source] RE: Pilot project on Pulmonary oxygen toxicity

HI Walter,

I presume you are inquiring about FY23 funds. You may submit a white paper, which as I indicated in my Program Review are due June 15th. This is a difficult project though because the majority of the research analysis falls on Heath and he has indicated to me that these are difficult studies for them to run due to the animal facilities restrictions in maintaining mice which has resulted in Heath having to complete these studies during the weekends. I think we may need to think about alternative strategies so that this doesn't strain Heath and their time/resources.

Moving forward I can say that the gas channel research is not in my strategic directions for planned research due to the slow progress and intense investment needs for this basic research that has to date received considerable investment by ONR.

Best,

Sandra

From: Walter Boron <wfb2@case.edu>
Sent: Friday, June 3, 2022 2:29 PM
To: Walter Boron <wfb2@case.edu>; Chapman, Sandra E CIV (USA) <sandra.e.chapman2.civ@us.navy.mil>; Chapman, Sandra E CIV (USA) <sandra.chapman@navy.mil>
Cc: Rossana Occhipinti <rxo22@case.edu>; Morley Schwebel <mxs86@case.edu>
Subject: [Non-DoD Source] RE: Pilot project on Pulmonary oxygen toxicity

Hi Sandra,

I sent the note below while you were out of town. In case it is buried under a mountain of other e-mails, I am resending.

Best regards,

Walter

Walter F. Boron, M.D., Ph.D.
Distinguished University Professor
Myers/Scarpa Professor and Chair
Dept. Physiology and Biophysics
Case Western Reserve University
Cleveland, OH 44106 USA
Medical Physiology (the textbook), Co-Editor

From: Walter Boron <wfb2@case.edu>
Sent: Monday, May 23, 2022 4:09 PM
To: Chapman, Sandra E CIV (USA) <sandra.e.chapman2.civ@us.navy.mil>; Chapman, Sandra E CIV (USA) <sandra.chapman@navy.mil>
Cc: Rossana Occhipinti <rxo22@case.edu>; Morley Schwebel <mxs86@case.edu>

Subject: Pilot project on Pulmonary oxygen toxicity

Hi Sandra,

I hope that you are doing well.

I am writing to get your sense of how to proceed with the AQP5/Pulmonary Oxygen Toxicity project. As I indicated in my Abstract, we got off to a slow start because of (a) the low fertility rate of the AQP5-KO mice, and (b) the switch from Claude to Heath and thus the time to hone in on which assays. In addition, (c) the hyperoxic mice are not eating/drinking as much as they should, which threw off the wet weight/dry weight study that we were doing to assess pulmonary edema.

We have now overcome 'a' and 'b' and are on a bit of a roll. Even though the N's are still low, I am cautiously optimistic because the LDH (marker of cell leakiness) and protein (marker of alveolar blood-gas barrier leakiness) values are strikingly low in AQP5-KO and AQP5/AQP1 double KO (dKO) mice. We have now expanded our AQP5-KO and dKO colonies to the point where we can supply large numbers of mice to Heath. However, we are both out of money for this project, and this hurts Heath especially hard …

Do you have any funds available to keep this Pilot Project on life support for the remainder of FY22?

I know that the Whitepaper deadline is coming up. What are your thoughts?

Should we suggest another Pilot to build up the N's with the current protocol, and/or to explore other POT protocols?

Should we apply for a regular research grant with the preliminary we have (or could potentially get during the summer)?

I would be happy to chat via Zoom, for example.

Best regards,

Walter

Walter F. Boron, M.D., Ph.D.
Distinguished University Professor
Myers/Scarpa Professor and Chair
Dept. Physiology and Biophysics
Case Western Reserve University
Cleveland, OH 44106 USA
Medical Physiology (the textbook), Co-Editor

From:	Chapman, Sandra E CIV (USA)
To:	Walter Boron; Dr Heath Gasier, Ph.D.
Cc:	Rossana Occhipinti; Fraser Moss; Chapman, Sandra E CIV (USA)
Subject:	RE: [Non-DoD Source] Response to ONR Tracking Number: 22-000002868 from Applicant: Case Western Reserve University, Principal Investigator: Walter Boron
Date:	Thursday, June 16, 2022 3:48:00 PM

Thanks- understood.

From: Walter Boron <wfb2@case.edu>
Sent: Thursday, June 16, 2022 2:44 PM
To: Dr Heath Gasier, Ph.D. <heath.gasier@duke.edu>
Cc: Rossana Occhipinti <rxo22@case.edu>; Fraser Moss <fraser.moss@case.edu>; Chapman, Sandra E CIV (USA) <sandra.chapman@navy.mil>
Subject: RE: [Non-DoD Source] Response to ONR Tracking Number: 22-000002868 from Applicant: Case Western Reserve University, Principal Investigator: Walter Boron

Hi Heath,
A few minutes ago, I sent you the glitched *Pilot* file … don't know how, because I didn't even have that file on my computer! Google Drive (which is where our files are stored) at Case has been acting weird all day, and maybe even yesterday, when the problem first arose).
Anyway, attached now is the correct *Pilot* file and the *Research* Grant file, which was always correct.
W

Walter F. Boron, MD, PhD
Distinguished University Professor
Myers/Scarpa Professor and Chair
Department of Physiology and Biophysics
School of Medicine
Case Western Reserve University
Cleveland, OH 44106 USA

Medical Physiology (the textbook), Co-Editor

From: Walter Boron <wfb2@case.edu>
Sent: Thursday, June 16, 2022 2:30 PM
To: 'Dr Heath Gasier, Ph.D.' <heath.gasier@duke.edu>
Subject: FW: [Non-DoD Source] Response to ONR Tracking Number: 22-000002868 from Applicant: Case Western Reserve University, Principal Investigator: Walter Boron

Hi Heath,
Sorry for not sending these files to you yesterday … maybe you would have caught my mistake with the glitched *Pilot Grant* file. The corrected on is attached.
I am also attaching the merged *Research Grant* file. I took your suggestions to heart when finishing it up.
Best regards,

Walter

Walter F. Boron, MD, PhD
Distinguished University Professor
Myers/Scarpa Professor and Chair
Department of Physiology and Biophysics
School of Medicine
Case Western Reserve University
Cleveland, OH 44106 USA

Medical Physiology (the textbook), Co-Editor

-----Original Message-----
From: Chapman, Sandra E CIV (USA) <sandra.e.chapman2.civ@us.navy.mil>
Sent: Wednesday, June 15, 2022 3:17 PM
To: Walter Boron <wfb2@case.edu>
Cc: Patel, Pooja CTR USN COMNAVDIST WASH DC (USA) <pooja.patel8.ctr@us.navy.mil>
Subject: FW: [Non-DoD Source] Response to ONR Tracking Number: 22-000002868 from Applicant: Case Western Reserve University, Principal Investigator: Walter Boron

Confirming receipt.

-----Original Message-----
From: notifier@fedconnect.net <notifier@fedconnect.net>
Sent: Wednesday, June 15, 2022 3:03 PM
To: Chapman, Sandra E CIV (USA) <sandra.e.chapman2.civ@us.navy.mil>
Subject: [Non-DoD Source] Response to ONR Tracking Number: 22-000002868 from Applicant: Case Western Reserve University, Principal Investigator: Walter Boron

Please review the referenced submission

ONR Tracking Number: 22-000002868
Type of Submission: White Paper - New for Grant/Coop Agreement
BAA/Special Notice/Funding Opportunity Number: N00014-22-S-B001
ONR Program Officer/Point of Contract (POC) Name: Sandra Chapman
ONR Program Officer/POC Code: Code 342 - Warfighter Protection and Applications
Project Title: Pilot: AQP5 as a Target for Prophylactic Countermeasures for Pulmonary Hyperbaric Oxygen Toxicity
Performer's Tracking Number:
Performer Name: Case Western Reserve University
Performer Address: 10900 Euclid Avenue, Cleveland, OH
Performer Cage Code: 4B566
Performer UEI Number: HJMKEF7EJW69

Performer EFT Segment: 0000
Type of Business: Educational Institution
Performer Business POC Name: Erin Fogarty
Performer Business POC Email: medres@case.edu
Performer Business POC Phone Number: 216-368-4432
Principal Investigator Name: Walter Boron
Principal Investigator Email: walter.boron@case.edu
Principal Investigator Phone Number: 216-368-5298
Co-Principal Investigator Name: Heath Gasier

BUMED Animal Use Appendix for Research Involving Animals
ABBREVIATED VERSION

*****NOTE: BUMED will ONLY review protocols that have been approved by your IACUC.*****
****Animal work MAY NOT be initiated until the awardee receives BUMED approval.****
Animal work initiated without BUMED approval is noncompliant and may not be funded.

Institutions using DoD funds to support the use of animals in research, product development, testing and education projects must provide electronic copies of the following documents to the Department of the Navy (DON) Bureau of Medicine and Surgery (BUMED) Veterinary Affairs Office for review and approval prior to initiation:

a. A copy of their **IACUC-approved institutional protocol(s)** (BUMED will ONLY review **approved** protocols) and documentation of IACUC approval

b. A copy of all existing IACUC-approved protocol amendments or modifications and documentation of IACUC approval (**future modifications or amendments** must be reviewed and approved by BUMED **PRIOR** to implementation)

c. A completed Appendix for each IACUC-approved protocol.

This requirement also applies to all subcontractors using animals in support of DoD-funded projects or programs.

Specific information requested in the following animal use Appendix is derived from requirements in the Animal Welfare Regulations (AWRs), the *Guide for the Care and Use of Laboratory Animals*, and other applicable Federal and DoD regulations. The DoD policies and requirements for the use of animals in DoD-supported research, development, testing and evaluation are described in DoD Instruction 3216.01, dated September 13, 2010 and SECNAVINST 3900.38C, *The Care and Use of Laboratory Animals in DOD Programs*, dated February 16, 2005. These requirements differ from those of other funding agencies. Use of the Appendix is intended to meet the requirements of these documents.

Questions concerning animal use and review should be directed to BUMED Veterinary Affairs:

Phone: 301-619-9241 or 301-619-9224
Email: DON – VRPP, usn.ncr.bumedfchva.mbx.don---vrpp@mail.mil
Mail: Department of the Navy Bureau of Medicine and Surgery
ATTN: Director for Veterinary Affairs
Bldg 1564 Room 122
1564 Freedman Drive
Fort Detrick, MD 21702

~~~~~~

Each section of this Appendix must be completed. To assist you in completing this appendix, instructions and explanations are provided as *hidden, red and italicized text*. To view the instructions and/or examples for each section, select the "**Show/Hide ¶**" button on your tool bar (the button itself appears as the ¶ symbol). To print the hidden text, select "Print Hidden Text" in the print options section. It is important that you carefully **read the instructions** for each paragraph to ensure you provide a comprehensive response. Begin typing responses after the colon (":") for each section to ensure your typing is not within the hidden text. Submit electronic copies of the appendix only; please do not submit printed copies to BUMED. Any section of the Appendix that is not applicable to your proposal, e.g., no surgery or no prolonged restraint, should be marked "**No**" or "**N/A**". There are no space limitations for the responses.

It is essential that only animal studies or procedures documented in an IACUC–approved protocol or amendment be performed at your facility. BUMED will collect animal usage information for end-of-year DoD animal use reports separate from the grant annual progress reporting requirement throughout the life of your award. For this reason, Principal Investigators or other delegated research personnel should keep accurate records and be able to provide an audit trail of all animal use that correlates to their approved protocol.
~~~~~~

x	Yes		No	All animal studies described in the attached IACUC-approved protocol are funded by this DOD award/contract. If no, STOP! You must use the **full version** of the Animal Use Appendix.

1. Administrative Data: (Provide Attending Vet, IACUC, and Research Office info for the work site.)

DoD/Contract PI Name:	Peter Lindholm		
Grant/Contract PI Email:	plindholm@health.ucsd.edu	**Phone:**	6508230735
Protocol PI Name:	Peter Lindholm		
Protocol PI Email:	plindholm@health.ucsd.edu	**Phone:**	6508230735
Animal Research Site (RS):	University of California San Diego		
RS Attending Veterinarian:	Keith Jenne		
Attending Vet Email:	kjenne@ucsd.edu	**Phone:**	858-822-5687
RS IACUC Point of Contact (POC):	Kristen Anderson-Vicino		
IACUC POC Email:	kca002@ucsd.edu	**Phone:**	858-534-1594
RS Grants Office Point of Contact (POC):	Elizabeth Tran-Nguyen, Contract and Grant Officer		
Grants Office POC Email:	ttrannguyen@ucsd.edu	**Phone:**	(858)246-2383
Animal Protocol Title:	Nitrogen gas uptake and excretion during compression/decompression to simulate diving		

2. Total Number of Animals Used (by Species) and USDA Pain/Distress Category :

SPECIES	HIGHEST USDA PAIN/DISTRESS CATEGORY (B, C, D, E*) See table below for definitions	TOTAL NUMBER
Rattus Norvegicus	D	58

USDA Pain/Distress Category Definitions:

- ***Column B:*** *Animals being bred, and animals being held for use in research, testing, teaching, experiments or surgery but not yet used for those purposes.*
- ***Column C:*** *List the number of animals that will experience no more than slight or momentary pain or distress as a result of experimental manipulations or procedures on this protocol.*
- ***Column D:*** *List the number of animals that will potentially experience more than momentary or slight pain or distress that **WILL** be alleviated through the use of anesthetics and/or analgesics.*

- ***Column E:*** *List the number of animals that will experience more than momentary or slight pain or distress that* ***WILL NOT*** *be alleviated or relieved with anesthetics or analgesics.* **If any animals are listed in USDA Column E (Unalleviated Pain or Distress), the PI must provide a scientifically valid justification for withholding pain relieving medication:**. .

3. Literature Search for Unnecessary Duplication: This search is required for all animal use proposals. Note the DoD-specific database requirements in subparagraph a.

a. Literature Source(s) Searched:
http://www.ntis.gov/products/fedrip.aspxhttp://projectreporter.nih.gov/reporter.cfm

☐ Federal Research in Progress (FEDRIP) http://www.ntis.gov/products/fedrip.aspx

or

☒ Research Portfolio Online Reporting Tool Expenditures and Results (RePORTER)
http://projectreporter.nih.gov/reporter.cfm

b. Date of Search: March 11, 2020

c. Years Covered By Search: 1995-2020

d. Key Words and/or Search Strategy: 13N2, Decompression, Nitrogen

e. Results of Search: nothing relevant.

******Questions 4-10 refer to the Research Site and Protocol Principal Investigator.******
Information and/or documents required in questions 4-8 should be obtained from the research site's IACUC or veterinary staff. Documents may be provided directly to BUMED by the Protocol Principal Investigator (PI) or institution staffs. If the Protocol PI prefers that BUMED staff contact the institution to obtain this information and/or documents, the PI must specifically request this action in writing. The Protocol PI should contact BUMED (see contact information on cover page) to arrange for submission of this written request.

4. Institutional Animal Care and Use Committee(s) (IACUC) Approval(s):

Institutional Animal Use Protocol Number:	S19154		
IACUC (initial) approval date:	11/26/2019	Protocol expiration (rewrite) date:	11/26/2022

5. Veterinary Care: Provide a brief description of the veterinary care plan at the research site. Describe routine care; weekend, holiday, and emergency care; and identify whether the attending veterinarian is on staff full-time or by contract.

6. Institutional Accreditation / Assurances:

a. Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) Accreditation (do **NOT** provide AAALAC correspondence)**:**

☒ Yes ☐ No Animal work is being performed at an AAALAC International-accredited facility.

b. Public Health Service Animal Welfare Assurance Statement:

[x] Yes [ ] No Animal work is being performed at a PHS-assured facility.

c. Non-accredited, Unassured Facilities: If neither 6.a. nor 6.b. above apply to the facility where animal work is being performed, submit a statement signed by the Institutional Official that states the care and use of animals will be conducted in accordance with the National Research Council's *Guide for the Care and Use of Laboratory Animals* and applicable Federal and DoD regulations.

7. Animal Procurement:

[x] Yes [ ] No If the protocol involves Animal Welfare Act-regulated species, are the animals obtained legally from suppliers licensed by the USDA? If the supplier claims exemption from USDA licensing, provide confirmation from the research site's IACUC that the exemption criteria have been met. If work is conducted outside the US, have animals been obtained legally in accordance with national policy? If wildlife are used, provide IACUC assurance that animals have been obtained legally and provide copies of applicable state, federal and/or international capture and use permits.

8. Overseas / Foreign Country Animal Work:

[ ] Yes [x] No Animal work will be performed outside the United States.

If "Yes," answer the following questions.

a. What is the law or regulation governing the use of animals in research in the research site's host country? Please provide a copy or link to this law or regulation in English.

b. Does the research site's host country adhere to European Union (EU) Directive 86/609 or EU Directive 2010/63 standards of animal housing and care?

c. If the research site is in Canada, does the institution hold a Canadian Council on Animal Care (CCAC) certificate?

d. Does the research site adhere to any national or international standards of animal housing and care that are more stringent than the host country's laws or regulations (such as AAALAC or CCAC)? If so, please describe below or provide a document, in English, that describes these standards.

e. Does the research site's host country or local institute require a local ethical committee review or Animal Care and Use Committee review? If so, please describe below or provide a document, in English, describing the committee's membership, purpose, authority and function.

9. Site Visits

[ ] Yes [x] No Does animal work involve at least one of the following species: dogs, cats, nonhuman primates, marine mammals? If yes, provide a planned start date for work in these species and point of contact for site visit coordination. Based on accreditation status, species used, and type of research conducted, a site visit to the performance site may be required.

10. Protocol Principal Investigator Assurances:

The law specifically requires several written assurances from the P.I. Please read and sign the assurances as indicated (this page may be photocopied and signed).

As the Principal Investigator on this protocol, I acknowledge my responsibilities and provide assurances for the following:

A. Painful Procedures: I assure that discomfort and injury to animals will be limited to that which is unavoidable in the conduct of scientifically valuable research and that analgesic, anesthetic, and/or tranquilizing drugs will be used where indicated and appropriate to minimize pain and/or distress to animals.

B. Animal Use: The animals authorized for use in this protocol will be used only in the activities and in the manner described herein, unless a modification is specifically approved by the IACUC and the DoD Department of the Navy **Bureau of Medicine and Surgery** (BUMED) Veterinary Affairs Office prior to its implementation.

C. Duplication of Effort: I have made a reasonable, good faith effort to ensure that this protocol is not an unnecessary duplication of previous experiments.

D. Statistical Assurance: I assure that I have consulted with a qualified individual who evaluated the experimental design with respect to the statistical analysis, and that the minimum number of animals needed for scientific validity will be used.

E. Training: I verify that the personnel performing the animal procedures/manipulations/ observations described in this protocol are technically competent and have been properly trained to ensure that no unnecessary pain or distress will be caused to the animals as a result of the procedures/manipulations.

F. Responsibility: I acknowledge the inherent moral, ethical and administrative obligations associated with the performance of this animal use protocol, and I assure that all individuals associated with this project will demonstrate a concern for the health, comfort, welfare, and well-being of the research animals. Additionally, I pledge to implement animal use alternatives where feasible, and conduct humane and lawful research.

G. Scientific Review: This proposed animal use protocol has received appropriate peer scientific review, and is consistent with good scientific research practice.

Peter Lindholm — March 11/2020 (b) (6) [redacted]

(Protocol Principal Investigator Printed Name) — (Protocol Principal Investigator Signature and Date)

NOTE: In accordance with SECNAVINST 3900.38C, the DON BUMED Veterinary Affairs Officer (or designee thereof) will conduct a site visit to all sites using nonhuman primates, dogs, cats or marine mammals in the proposal, or where a site visit is deemed warranted.

From: Bullock, Sevgi E CIV USN ONR (USA)
To: Chapman, Sandra E CIV (USA)
Cc: McKee, Lauren G CIV USN (USA); Jeffress, Jenny V CTR USN CNR ARLINGTON VA (USA)
Subject: RE: Lindholm Animal Use
Date: Wednesday, February 19, 2020 1:03:09 PM

Sandra,

Per our discussion. This being a grant ONR will not award with animal use work without the DoD Vet approval. So the PI will need to submit a proposal/budget removing all animal use work. Once he's ready and had DoD Vet approval the PI can submit an expansion proposal/budget.

Sevgi

Ms. Sevgi Bullock
Business Financial Manager
ONR/ONRG Human Research Protection Official
ONR/ONRG Animal and rDNA Use Administrator
Office of Naval Research
Warfighter Performance S&T Department, Code 34
875 North Randolph Street, Room 1027
Arlington, VA 22203
703-696-4046
sevgi.bullock@navy.mil

From: Chapman, Sandra E CIV (USA) <sandra.chapman@navy.mil>
Sent: Wednesday, February 19, 2020 12:36 PM
To: Bullock, Sevgi E CIV USN ONR (USA) <sevgi.bullock@navy.mil>
Cc: McKee, Lauren G CIV USN (USA) <lauren.mckee@navy.mil>; Jeffress, Jenny V CTR (USA) <jenny.jeffress.ctr@navy.mil>
Subject: Lindholm Animal Use

Hi Sevgi,

I wanted to circle back with you about how we proceed with Lindholm's proposal. His proposal has three staged aims and the third and last that will happen after the first two include sheep animal use that the PI does not yet have approval for. My question is if we can proceed with the proposal as is making a note that the third aim is contingent on animal use approval- and in essence the third year becomes an option year (which is always the case). Or do we need to have him resubmit the proposal to take out the third aim and make that an expansion when he has the approval?

Thank you!
Sandra

Sandra Chapman, Ph.D.
Program Officer, Undersea Medicine

Warfighter Protection & Applications Division Office of Naval Research
☎703.588.2429
(b) (6) [redacted]
sandra.chapman@navy.mil

From: (b) (6) [redacted]
To: Chapman, Sandra E CIV (USA)
Subject: [Non-DoD Source] budgets - follow up
Date: Wednesday, November 4, 2020 11:21:05 AM

Hi Sandra,

Thanks for the phone conversation yesterday. I understand the limitations regarding charges to animal versus human studies. I want to just give you an update. The Glymphatics grant year 1 ended at the end of October. The administration here is working on reconciling the various costs, but in total I'm about $21,000 over budget due exclusively to the MRI charges. My plan is to use another departmental account to cover this cost. When I do lectures for various groups or odd activities like that, I have them submitted my 'pay' to the department to give me a 'wiggle room' account. Fortunately, this will cover the short-fall.

Steve

Stephen R. Thom, M.D., Ph.D.
Professor of Emergency Medicine

From: (b) (6) [redacted]
To: Chapman, Sandra E CIV (USA)
Subject: [Non-DoD Source] Question about grant budgets
Date: Tuesday, November 3, 2020 8:54:30 AM

Dear Sandra,

When you have a few minutes, I'd like to talk with you about my budgets. Rest assured; I am not asking for more money. I want to know if I can re-budget some of N00014-20-1-2641 for animal and MRI studies. I have over-spent funds budgeted for MRI in the glymphatics grant N00014-20-1-2000. However, we have found gelsolin treatment prevents the glymphatic abnormalities triggered by decompression. As gelsolin studies are part of ..2641, I think I am still within the aims outlined for the grant.

Please just let me know a good time to call (or you can call me - cell (b) (6) [redacted]).

Thanks,

Steve

Stephen R. Thom, M.D., Ph.D.
Professor of Emergency Medicine

From: (b) (6) [redacted]
To: Chapman, Sandra E CIV (USA)
Cc: Becker, Christopher E CTR (USA)
Subject: [Non-DoD Source] Re: budgets - follow up
Date: Friday, November 6, 2020 11:47:15 AM

Thank you, Sandra.

I am confused too. Since getting the MRI charges I have been working on getting a clear assessment of the grant status here. I am now waiting on my grants administrator to clarify where we are and funds remaining. The immediate problem, it turns out, is that they had not been charging salary support to the grant account. Therefore, they need to go through and get all this straight.

I will let you know once I am given some answers. Thank you for your support.

Steve

Stephen R. Thom, M.D., Ph.D.
Professor of Emergency Medicine

From: Chapman, Sandra E CIV (USA)
Sent: Friday, November 6, 2020 10:32 AM
To: Thom, Stephen
Cc: Becker, Christopher E CTR USN CNR ARLINGTON VA (USA)
Subject: RE: budgets - follow up

Stephen,
I am a little confused. I am not sure how or why the year 1 funds expire. This is generally not the case and you should have flexibility to spend the funds within the PoP. I am sure you are not saying that you have spent all the glymphatic award. If it is a matter of the way the grant was written, you may simply request from me to shift funds from one activity to another. This is standard and we do this frequently at ONR. You would send me a request to shift funds with an explanation and I would forward my approval to the regional office.
Sandra

From: Thom, Stephen
Sent: Wednesday, November 4, 2020 11:21 AM
To: Chapman, Sandra E CIV (USA)
Subject: [Non-DoD Source] budgets - follow up
Hi Sandra,
Thanks for the phone conversation yesterday. I understand the limitations regarding charges to animal versus human studies. I want to just give you an update. The Glymphatics grant year 1 ended at the end of October. The administration here is working on reconciling the various costs, but in total I'm about $21,000 over budget due exclusively to the MRI charges. My plan

is to use another departmental account to cover this cost. When I do lectures for various groups or odd activities like that, I have them submitted my 'pay' to the department to give me a 'wiggle room' account. Fortunately, this will cover the short-fall.
Steve
Stephen R. Thom, M.D., Ph.D.
Professor of Emergency Medicine

OPTIMIZING KETONE METABOLIC THERAPY AND IDENTIFYING BIOMARKERS FOR MITIGATION AND PREDICTION OF CNS OXYGEN TOXICITY: ANIMAL STUDIES

Dominic P. D'Agostino, PhD (PI); Angela Poff, PhD (Co-I); Jay B. Dean, PhD (Co-I)
ddagosti@usf.edu
813-396-9628

Background

Central Nervous System-Oxygen Toxicity (CNS-OT) seizures (Sz) remain a limitation to Naval Special Warfare and Special Operations diving and submarine operations. Ketogenic metabolic therapy (KMT) is a strategy to induce therapeutic ketosis and is an effective mitigation strategy to delay CNS-OT. Thus, KMT has the potential to enhance warfighter safety, resilience and readiness. Prior work with ketone ester (KE; R,S-1,3-butanediol diacetoacetate) demonstrated a significant delay in CNS-OT by 575% in rats, and this neuroprotective effect was observed without the need for dietary restriction. Since this particular study only tested a single large dose (10g/kg), the optimal dosing protocol for KE that provides neuroprotection without hindering cognitive or motor performance remained to be elucidated. Further work is needed, especially since newer compounds and formulations are being developed that have a more favorable pharmacokinetic profile. Additionally, little is known regarding the multiple mechanisms underlying KMT-induced effect on delaying CNS-OT and behavior. The current study examines the effect of KMT on CNS-OT seizures, behavior and antioxidant capacity.

Objectives

The primary goal of this work was to understand the effect of KE adminstration at a dose range that delays CNS-OT without impairing cognitive or motor performance. Secondly, there remained a crucial need to identify early physiological predictors of CNS-OT onset, both within and outside the context of ketogenic intervention to avert Sz onset. Thus, a 2nd goal was to determine the effects of HBO ± KE on presumptive physiological markers (e.g. body temperature, heart rate, respiratory rate) that can be monitored in real time to predict an impending Sz. The 3rd goal was to study the anti-convulsant mechanisms of KMT by measuring redox potential of blood from animals pre-and post- Sz with and without KE treatment. As designed, the proposed studies answer crucial questions to benefit the US Navy and hyperbaric medicine, thereby advancing the science and future human application of KMT for CNS-OT and warfighter performance resilience.

Methods

In FY 2021-2022, we completed the remaining Aim 1 experiments, validating the anticonvulsant effects of KE (at a lower) dose as a form of KMT for preventing CNS-OT. This work was published (Stavitzski NM, et.al. *AJP Reg*. 2021 PMID: 34132115) and helps to define the performance aspects of the KMT. In this study Sprague Dawley rats were implanted with radio-telemetry modules, allowing for real-time monitoring of body temperature, EEG, ECG, and respiratory EMG during an HBO dive protocol, and were monitored for 1st and 2nd seizure. Body temp, HR, and respiratory rate data was collected continuously throughout the dive and analyzed various timepoints of the protocol to look for potential changes prior to Sz onset (Aim 2). Additionally, blood was collected from the saphenous vein pre- and post-dive for analysis of redox potential. Serum was separated from the blood samples by centrifuge, and analyzed with the RedoxSYS system which measures static and capacity oxidation-reduction potential (Aim 3).

Results

Pharmacokinetics of KE on blood BHB and glucose levels is shown in **Figure 1**. KMT delivered as KE (7.5 g/kg) by single oral gavage significantly increased blood ketone levels (R-BHB) and reduced glucose. KE also significantly elevates acetoacetate and acetone, which are important for mitigating CNS-OT. During therapeutic ketosis, 0.5 – 4hrs, glucose levels were decreased and BHB increased ($p<0.0001$). Prior to KE adminstration the rats had been fed standard rodent chow, and thus the ketosis was independent of diet. **Figure 2** shows KE delayed CNS-OT (5 ATA O_2) seizures. Data represented was normalized to account for known variability in seizure latency (LSz) of control animals. Average LSz_1 (in min.) for control rats was 6.42 ± 0.44 versus 22.16 ± 5.10 in KE-treated rats, a 307% increase. **Figure 3** depicts three phases of "The Compound Ventilatory Response to Hyperoxia". Phase 1 is due to normobaric hyperoxia, a transient reduction in minute ventilation, due to peripheral chemoreceptor effect. Phase "2" (in gray), arises from increased pressure and brain PO2. Phase "3" (dotted line) develops just prior to the onset of CNS-OT. There was a significant difference between the groups starting at ~15-10 minutes before seizure consistent with previous findings, confirming that respiration can be used as a physio-marker of an impending seizure. **Figure 4** shows a significant difference at all time points ($p<0.0001$) in temperature for Control vs KE group. The mechanism of KE-induced decrease in temperature is unknown, but evidence suggests BHB can decrease sympathetic tone via GPR41 antagonism. **In summary**, the present findings demonstrate that a single dose of KE prior to 5 ATA O2 increases LSz without impairing cognition or motor performance. Furthermore, this acute dose induced and maintained a state of therapeutic ketosis for up to 4-6hrs, independent of diet. This information can be used to provide guidance on resilience strategies for delaying CNS-OT to enable deeper, longer, and safer dives for US Navy and recreational divers and in current treatment protocols used in HBO2 therapy.

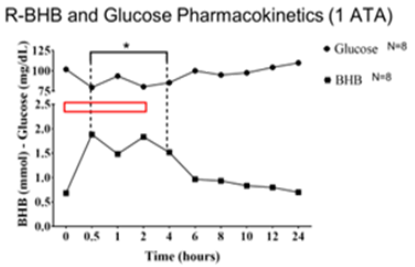


Figure 1. Pharmacokinetic Effect of KE. Oral gavage of KE was administered immediately after baseline. BHB and glucose was measured at baseline, 0.5, 1, 2, 4, 6, 8, 10, 12, 24hr post-gavage. Top line graph (•) shows glucose and the bottom-line graph (■) shows the levels of BHB.

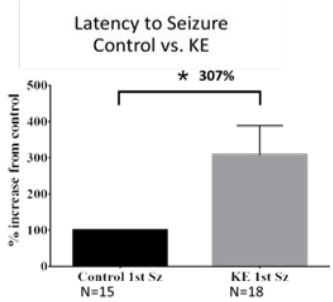


Figure 2. KE Prolongs Latency to CNS-OT Seizure. The average LSz1 was increased 307% with KMT. Normalized to Controls (n=14; One-way Anova).

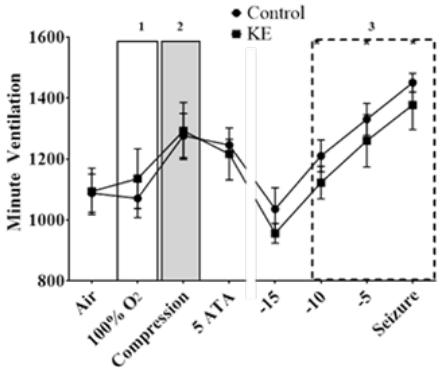


Figure 3. Ventilatory Response to Hyperoxia. Phase 1 is marked with black solid outline, phase 2 is the gray box, and phase 3 is dotted box.

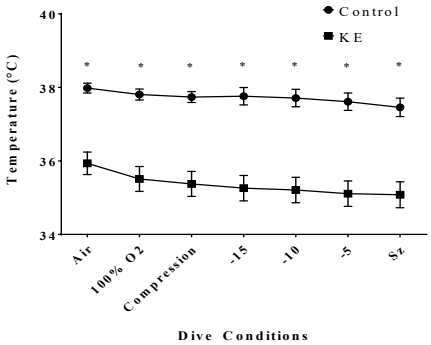


Figure 4. Temperature Changes to KMT. Control (closed circles) and KE (closed squares) were significantly different when compared at each dive condition ($p<0.05$).

MITIGATION OF CNS OXYGEN TOXICITY: MITOCHONDRIA-TARGETED ANTIOXIDANT THERAPY AND AQUAPORIN GAS CHANNELS

Jay B. Dean, PhD, P.I.

University of South Florida, Tampa, FL 33612
jaydean@usf.edu; (b) (6) [redacted] *(cell), 813-974-1547 (USF)*

Background

This 6.2 project focuses on mitigation of CNS oxygen toxicity (CNS-OT) by targeting two, subcellular targets in the brain that are postulated to contribute to the neuropathogenesis of seizure (Sz) genesis during exposure to HBO_2: **1)** mitochondrial superoxide ($\cdot O_2^-$); and **2)** O_2-/CO_2-permeabilities through aquaporin-4 (AQP4) at the blood-brain barrier (BBB).

Mitoquinone (MitoQ) is used to decrease mitochondrial $\cdot O_2^-$ production during exposure to HBO_2 in freely behaving rats. We hypothesize that MitoQ reduces the risk of CNS-OT in freely behaving rats by ↓$\cdot O_2^-$ production, ↓O_2-sensitivity of neurons, and ↑ latency time to seizure (LSz).

The **gas channel AQP4** is permeable to CO_2 and presumably O_2. We hypothesize that AQP4-knockout (KO) mice will exhibit a longer LSz as compared to their wild-type (WT) controls during HBO_2 ±CO_2.

The U.S. Naval significance of this project is that exposure to HBO_2 & CO_2 rebreathing are limiting factors in USN SPECWAR/SPECOPS diving and submarine operations. These experiments are designed to identify molecular targets of seizure genesis in potential mitigation strategies for CNS-OT and CO_2 retention.

Objectives

1. Determine effects of HBO_2 ± MitoQ on the **i)** LSz and **ii)** the temporal pattern of neural activation across the brain stem, subcortical nuclei, and motor cortices during the safe latent period that precedes initiation of Sz in rats.

2. Determine the effects of HBO_2 ±MitoQ on neuronal excitability, $\cdot O_2^-$ production, and mitochondrial membrane potential; these experiments (expts) were canceled based on negative results in Objective 1. A new set of experiments were initiated to study the neuroprotective effects of L-NAME therapy on LSz during HBO_2 ±CO_2 in freely behaving rats and incorporated in Objective 1 in place of MitoQ.

3. Determine the role of AQP4 in CNS-OT by testing the effects of HBO_2 ±CO_2 on the LSz in mice with decreased AQP4 expression (AQP4-KO), and WT mice. Experiments to upregulate AQP4 in WT mice were canceled due to results of first set of experiments.

Methods

1.i Determine optimal dose/route for MitoQ via **a)** up-and-down acute toxicity testing; **b)** temporal analysis of brain and blood levels of MitoQ using liquid chromatography/mass spec/mass spec (LC/MS/MS); **c)** dive rats ±MitoQ in 5 ATA O_2 and measure LSz. L-NAME therapy: 30mg/kg(ip), 30min before dive (Demchenko et al., 2001 Brain Res 917(2):253-261).

1.ii Record cortical surface & deep intracerebral brain activities using radio telemetry in freely behaving rats in 5 ATA O_2 ±MitoQ and measure temporal pattern of neural activation in various CNS regions prior to and during Sz.

2. Cancelled rat brain slice expts due to negative results with MitoQ in Objective 1.

3. (b) (4) [redacted] that are exposed to HBO_2 (5 & 4 ATA O_2; & 2.925 ATA O_2 +0.075 ATA CO_2).

Results

1.i (rat, MitoQ Therapy) MitoQ (ip) maximum safe dose=22mg/kg while breathing 1 ATA air. 22mg/kg MitoQ (ip) 30min before dive delayed/prevented Sz during exposure to 5 ATA O_2; however, all rats became stressed during HBO_2, and 3/5 rats died ≤24 hr. A 2nd up-down toxicity test was conducted combing 11-22mg/kg +5 ATA O_2. Rats treated with 13-22mg/kg MitoQ+HBO_2 either did not Sz by 1hr (n=9/15) or had delayed Sz (n=4/15). Thus, 13/15 rats had increased tolerance to HBO_2 *per se*; however, 7/15 rats died ≤24hr. Conversely, rats treated with 11mg/kg MitoQ +HBO_2 did not die, but LSz was no different from control (EtOH) rats. We conclude that doses of MitoQ that are non-toxic in 1 ATA air, and delay Sz genesis during exposure to HBO_2, ultimately turn lethal when combined with HBO_2 and decompression.

1.ii (rat, deep brain electrode telemetry) Simultaneous cortical surface (ECoG) & 1-2 deep

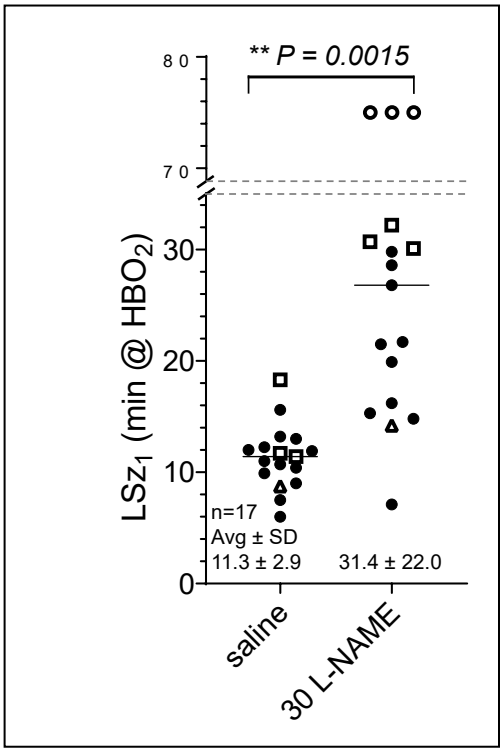


Fig.1. 30mg/kg L-NAME (ip) 30min prior to HBO_2 significantly delays the latency time to Sz (LSz_1). ●, 5 ATA O_2; O, 5 ATA O_2 but did not Sz by 75 min (dive terminated); □, 2.95 ATA O_2 + 0.05 ATA CO_2 or 2.93 ATA O_2 + 0.07 ATA CO_2; Δ 4.88 ATA O_2 + 0.12 ATA CO_2. Both uninstrumented and instrumented (telemetry) rats are included.

intracerebral telemetry electrode recordings (e.g., CA1 hippocampus & dorsal medulla) concurrent with respiratory EMG and EKG are feasible during exposure to HBO_2 in freely behaving rat. Initial findings show that ECoG activity remains quiet until onset of generalized Sz. In contrast, activity in the medulla oblongata and cerebellum is intermittent or continuous and either begins during O_2 compression, later during HBO_2 exposure before ↑ECoG, or just prior to ↑ECoG activity (1st Sz). During 2nd Sz, neural activities are coordinated and more intense. Hypercapnic HBO_2 accelerates Sz genesis and intensifies the neurological and physical Sz and L-NAME delays onset of ictal activity (see 1.iii, next).

1.iii (rat, L-NAME Therapy) Exposure to 30mg/kg L-NAME (ip) 30min before dive delays Sz in 5 ATA O_2 and 2.925 ATA O_2/0.075 ATA CO_2 (**Fig. 1:** control = 11.3±2.9 *vs.* L-NAME = 31.4±22min, n=17, P=0.0015). In L-NAME treated rats (n=3), the dive was terminated after 75 min (5 ATA O_2) because rat did not seize. Planned expts will test optimal dose of L-NAME the delays Sz genesis.

1.iv (rat/mouse, hypercapnic HBO_2 model of CNS-OT) The LSz_1 in rodents (male Sprague-Dawley rat, Envigo; and male C57BL/6 mice, (b) (4) is the same (P=0.4364) whether breathing 2.5%CO_2 in O_2 (97.5%) at **P_B=3 ATA** (12.0 ±2.8min, n=16) *vs.* 100% O_2 at **P_B=5 ATA** (11.0 ±2.5min, n=25). Thus, high levels of HBO_2 (5 ATA) typically used to study CNS-OT in unanesthetized rodents (rationale: accelerate Sz genesis and avert confounding effects of pulmonary ox tox) can be reduced using 2.925 ATA O_2 with 0.075 ATA CO_2. Hypercapnic HBO_2 also models CO_2 rebreathing/retention, which is a comorbidity in undersea medicine. Planned expts will determine effects of graded CO_2 retention during HBO_2 on LSz_1 in rat (uninstrumented & deep electrode telemetry dives) to optimize hypercapnic HBO_2 rat model.

3.i (b) (4)

[redacted]

Future expts would benefit from telemetric measurements of ECoG during exposure to hypercapnic HBO_2 (P_B=3 ATA). Hypercapnia and intermittent hypoxia/reoxygenation at P_B=1 ATA should be tested in event that HBO_2 diffusion gradient overwhelms contribution of AQP4 channel to O_2/CO_2 uptake at BBB.

ONR N00014-20-1-2434

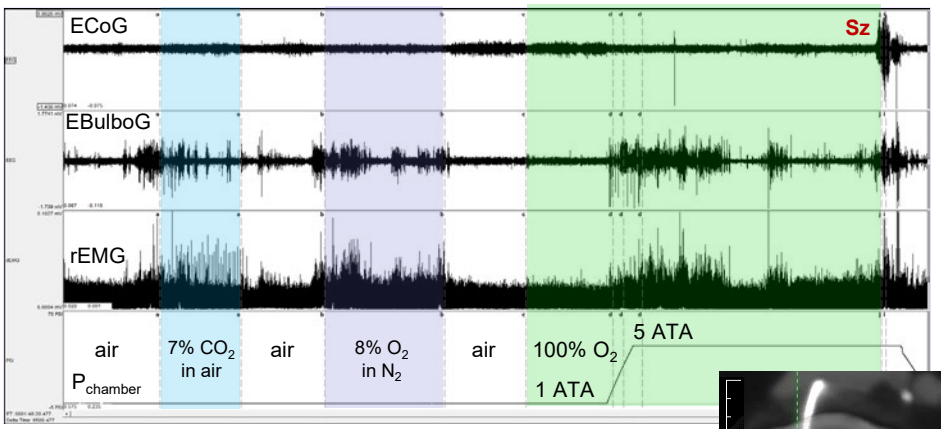
Notes:

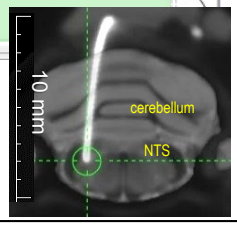
MITIGATION OF CNS O_2 TOXICITY: MITOCHONDRIA-TARGETED ANTIOXIDANT THERAPY & AQUAPORIN GAS CHANNELS

P.I. / Jay B. Dean; Co-I. (b) (4) [redacted]

Background: This 6.2 project focuses on mitigation of CNS oxygen toxicity (CNS-OT) by targeting two, subcellular targets in the brain that are thought to contribute to seizure (Sz) genesis during exposure to HBO_2: 1) mitochondrial superoxide ($\cdot O_2^-$) generation; and 2) O_2-/CO_2-permeabilities through aquaporin-4 (AQP4) at the blood-brain barrier (BBB).

Naval & Scientific Benefits: Exposure to HBO_2 and CO_2 are limiting factors in USN SPECWAR/SPECOPS diving and sub-marine operations. These experiments will identify sites of Sz genesis for targeting potential mitigation strategies for CNS-OT and CO_2 retention. Mitoquinone (MitoQ), which inhibits mitochondrial $\cdot O_2^-$, will be tested in rats. The role of AQP4 in O_2/CO_2 uptake at the BBB will be tested in mice in which AQP4 expression is down regulated & up regulated.

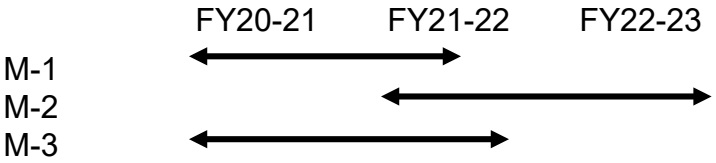




(Fig.1) 1.8hr records of cortical (ECoG), medullary (EBulboG) & respiratory (rEMG) activities while breathing air, hypercapnia, hypoxia & hyperoxia (1&5ATA O_2) in unrestrained rat. ↑EBulboG activity during hypercapnia, hypoxia & during compression (33.6 min *before* Sz) and remained intermittently active until onset of Sz (↑ECoG); LSz=31.7min. ↑EBulboG activity correlated with ↑rEMG during exposure to ↑CO_2, ↓O_2 & HBO_2. **(Right inset)** EBulboG electrode tip in nucleus tractus solitarius (NTS)/ parvicellular reticular n. (medulla oblongata); co-registered CT-MRI scans.

Milestones. Identify effects of HBO_2 ±MitoQ on: **M1)** the LSz & temporal pattern of neural activation in brain stem, subcortical nuclei & motor cortices prior to Sz in freely behaving rats (**Fig.1**). **M2)** proposed brain slice experiments (expts) to study effects of MitoQ±HBO_2 on neurons were abandoned due its combined toxicity in rat. New expts in rat were initiated using L-NAME therapy (inhibits NOS → ↓·NO). **M3)** determine the effects of HBO_2 ±CO_2 on LSz in AQP4-KO *vs.* WT mice.

Milestone Tracker:



FY21 Accomplishments, Discoveries, & Inventions

- MitoQ delays/blocks Sz, but MitoQ *plus* HBO_2 is toxic.
- developed deep brain telemetric recordings in rat **(Fig.1)**; medullary & cerebellar activity precedes cortical Sz activity.
- initiated new (HBO_2±CO_2)±L-NAME expts in rats: 30 mg/kg L-NAME (ip) ↑LSz_1 from 11.3±2.9 to 31.4±22min (n=17, P=0.0015); 3 rats did not seize (75 min × 5 ATA O).
- (b) (4) [redacted]
- hypercapnic HBO_2: you can ↓[HBO_2] from 5 to 2.9 ATA and model CO_2-rebreathing/retention

FY23 Goals: continue deep electrode telemetry expts: 1) dose response L-NAME during HBO_2±CO_2; and 2) identify sites of Sz genesis. **P.I. Contact Email:** jaydean@usf.edu

OMB Number: 4040-0001
Expiration Date: 12/31/2022

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE

State Application Identifier MD

1. TYPE OF SUBMISSION

☐ Pre-application ☒ Application ☐ Changed/Corrected Application

4. a. Federal Identifier N00014

b. Agency Routing Identifier 342 [Chapman, Sandra]

c. Previous Grants.gov Tracking ID

2. DATE SUBMITTED 09/30/2021

Applicant Identifier 63998

5. APPLICANT INFORMATION

Organizational DUNS: 188435911

Legal Name: University of Maryland, Baltimore

Department: Emergency Medicine

Division: SOM Clinical Departments

Street1: Office of Research and Development

Street2: 620 W.Lexington St.

City: Baltimore

County / Parish: Baltimore City

State: MD: Maryland

Province:

Country: USA: UNITED STATES

ZIP / Postal Code: 21201-1508

Person to be contacted on matters involving this application

Prefix:

First Name: (b) (6)

Middle Name:

Last Name: (b) (6)

Suffix:

Position/Title: Specialist Contract/Grants Sr

Street1: (b) (6)

Street2: (b) (6)

City: Baltimore

County / Parish:

State: MD: Maryland

Province:

Country: USA: UNITED STATES

ZIP / Postal Code: 21201-1508

Phone Number: (b) (6)

Fax Number:

Email: (b) (6)

6. EMPLOYER IDENTIFICATION *(EIN) or (TIN)*: 526002033

7. TYPE OF APPLICANT: H: Public/State Controlled Institution of Higher Education

Other (Specify):

Small Business Organization Type ☐ Women Owned ☐ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION:

☒ New ☐ Resubmission

☐ Renewal ☐ Continuation ☐ Revision

If Revision, mark appropriate box(es).

☐ A. Increase Award ☐ B. Decrease Award ☐ C. Increase Duration ☐ D. Decrease Duration

☐ E. Other (specify):

Is this application being submitted to other agencies? Yes ☐ No ☒ What other Agencies?

9. NAME OF FEDERAL AGENCY: Office of Naval Research

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER: 12.300

TITLE: Basic and Applied Scientific Research

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT:

Neuroinflammation and glymphatic dysfunction in decompression sickness

12. PROPOSED PROJECT:

Start Date: 06/01/2022

Ending Date: 05/31/2025

13. CONGRESSIONAL DISTRICT OF APPLICANT MD-007

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: First Name: Stephen Middle Name: R.
Last Name: Thom Suffix:
Position/Title: Professor
Organization Name: University of Maryland, Baltimore
Department: Emergency Medicine Division: SOM Clinical Departments
Street1: (b) (6) [redacted]
Street2: (b) (6) [redacted]
City: Baltimore County / Parish:
State: MD: Maryland Province:
Country: USA: UNITED STATES ZIP / Postal Code: 21201-1642
Phone Number: (b) (6) [redacted] Fax Number:
Email: (b) (6) [redacted]

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested	981,996.45
b. Total Non-Federal Funds	0.00
c. Total Federal & Non-Federal Funds	981,996.45
d. Estimated Program Income	0.00

16. IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?

a. YES ☐ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:

DATE:

b. NO ☒ PROGRAM IS NOT COVERED BY E.O. 12372; OR

☐ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious. or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

☒ **I agree**

The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.

18. SFLLL (Disclosure of Lobbying Activities) or other Explanatory Documentation

Add Attachment | Delete Attachment | View Attachment

19. Authorized Representative

Prefix: First Name: (b) (6) [redacted] Middle Name:
Last Name: (b) (6) [redacted] Suffix:
Position/Title: Specialist Contract/Grants Sr
Organization: University of Maryland, Baltimore
Department: Division:
Street1: (b) (6) [redacted]
Street2: (b) (6) [redacted]
City: Baltimore County / Parish:
State: MD: Maryland Province:
Country: USA: UNITED STATES ZIP / Postal Code: 21201-1508
Phone Number: (b) (6) [redacted] Fax Number:
Email: (b) (6) [redacted]

Signature of Authorized Representative: (b) (6) [redacted]

Date Signed: 09/30/2021

20. Pre-application Add Attachment | Delete Attachment | View Attachment

21. Cover Letter Attachment Add Attachment | Delete Attachment | View Attachment

OMB Number: 4040-0001
Expiration Date: 12/31/2022

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: * First Name: Stephen Middle Name: R.
* Last Name: Thom Suffix:
Position/Title: Professor Department: Emergency Medicine
Organization Name: University of Maryland, Baltimore Division: SOM Clinical Departments
* Street1: (b) (6) [redacted]
Street2: (b) (6) [redacted]
* City: Baltimore County/ Parish:
* State: MD: Maryland Province:
* Country: USA: UNITED STATES * Zip / Postal Code: 21201-1642
* Phone Number: (b) (6) [redacted] Fax Number:
* E-Mail: (b) (6) [redacted]

Credential, e.g., agency login:

*** Project Role:** PD/PI **Other Project Role Category:**

Degree Type: Medical Doctor
Degree Year: 1981

***Attach Biographical Sketch** (b) (6) [redacted] Add Attachment Delete Attachment View Attachment
Attach Current & Pending Support Add Attachment Delete Attachment View Attachment

PROFILE - Senior/Key Person 1

Prefix: * First Name: (b) (6) [redacted] Middle Name:
* Last Name: (b) (6) [redacted] Suffix:
Position/Title: Associate Professor Department: Diagnostic Radiology
Organization Name: University of Maryland, Baltimore Division: SOM Clinical Departments
* Street1: (b) (6) [redacted]
Street2: (b) (6) [redacted]
* City: Baltimore County/ Parish:
* State: MD: Maryland Province:
* Country: USA: UNITED STATES * Zip / Postal Code: 21201-1519
* Phone Number: (b) (6) [redacted] Fax Number:
* E-Mail: (b) (6) [redacted]

Credential, e.g., agency login: (b) (6) [redacted]

*** Project Role:** Other (Specify) **Other Project Role Category:** Co-Investigator

Degree Type: Master of Philosophy
Degree Year: 1995

Attach Biographical Sketch (b) (6) [redacted] Biosketch.pdf Add Attachment Delete Attachment View Attachment
Attach Current & Pending Support Add Attachment Delete Attachment View Attachment

RESEARCH & RELATED Other Project Information

OMB Number: 4040-0001
Expiration Date: 12/31/2022

1. Are Human Subjects Involved? ☐ Yes ☒ No

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ☐ Yes ☐ No

If yes, check appropriate exemption number. ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8

If no, is the IRB review Pending? ☐ Yes ☐ No

IRB Approval Date:

Human Subject Assurance Number:

2. Are Vertebrate Animals Used? ☒ Yes ☐ No

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ☒ Yes ☐ No

IACUC Approval Date:

Animal Welfare Assurance Number: A3200-01

3. Is proprietary/privileged information included in the application? ☐ Yes ☒ No

4.a. Does this Project Have an Actual or Potential Impact - positive or negative - on the environment? ☐ Yes ☒ No

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ☐ Yes ☐ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place? ☐ Yes ☒ No

5.a. If yes, please explain:

6. Does this project involve activities outside of the United States or partnerships with international collaborators? ☐ Yes ☒ No

6.a. If yes, identify countries:

6.b. Optional Explanation:

7. Project Summary/Abstract Add Attachment Delete Attachment View Attachment

8. Project Narrative Narrative.pdf Add Attachment Delete Attachment View Attachment

9. Bibliography & References Cited Add Attachment Delete Attachment View Attachment

10. Facilities & Other Resources Add Attachment Delete Attachment View Attachment

11. Equipment Add Attachment Delete Attachment View Attachment

12. Other Attachments Add Attachments Delete Attachments View Attachments

OMB Number: 4040-0019
Expiration Date: 02/28/2022

Project Abstract Summary

This Project Abstract Summary form must be submitted or the application will be considered incomplete. Ensure the Project Abstract field succinctly describes the project in plain language that the public can understand and use without the full proposal. Use 4,000 characters or less. Do not include personally identifiable, sensitive or proprietary information. Refer to Agency instructions for any additional Project Abstract field requirements. If the application is funded, your project abstract information (as submitted) will be made available to public websites and/or databases including USAspending.gov.

Funding Opportunity Number

N00014-21-S-B001

CFDA(s)

Applicant Name

Dr. Stephen Thom

Descriptive Title of Applicant's Project

Neuroinflammation and glymphatic dysfunction in decompression sickness

Project Abstract

This research project is to evaluate the neuroinflammatory impact of high pressure and decompression using a murine model. The motivation is to establish the molecular basis for neurological decompression sickness (DCS). We have found that inflammatory microparticles (MPs) elevated in humans, and in animal models of DCS trigger dysfunction of the brain glial-based lymphatic (aka glymphatic) system in mice. We have identified a complex pathological interaction between brain-generated MPs and systemic inflammation that may resolve several unexplained clinical observations. We have also identified knock-out mice resistant to DCS pathology and a pharmaceutical in use in humans that protects against CNS injuries. Specific Aims are: (1) Assess glymphatics function in mice by MRI and fluorescence microscopy; (2) Examine MPs elevations, astrocyte and microglial activation and evidence of neuroinflammation in mice; (3) Assess role of glymphatic MPs outflow in glymphatic dysfunction and neuroinflammation.

RESEARCH & RELATED BUDGET - Budget Period 1

OMB Number: 4040-0001
Expiration Date: 12/31/2022

ORGANIZATIONAL DUNS: 188435911 **Enter name of Organization:** University of Maryland, Baltimore

Budget Type: ☒ Project ☐ Subaward/Consortium **Budget Period: 1** **Start Date:** 06/01/2022 **End Date:** 05/31/2023

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Stephen	R.	Thom		0.00	1.80	0.00	0.00	(b) (4)	(b) (4)	(b) (4)
Project Role: PD/PI											
	(b) (6)		(b) (6)		0.00	1.20	0.00	0.00	(b) (4)	(b) (4)	(b) (4)
Project Role: Co-Investigator											

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

Total Funds requested for all Senior Key Persons in the attached file 0.00

Total Senior/Key Person (b) (4)

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
0	Post Doctoral Associates	0.00	0.00	0.00	0.00	0.00	0.00
0	Graduate Students	0.00	0.00	0.00	0.00	0.00	0.00
0	Undergraduate Students	0.00	0.00	0.00	0.00	0.00	0.00
0	Secretarial/Clerical	0.00	0.00	0.00	0.00	0.00	0.00
0	Other	0.00	0.00	0.00	0.00	0.00	0.00
1	Other Professionals	12.00	0.00	0.00	(b) (4)	(b) (4)	(b) (4)
0	Allocated Admin Support	0.00	0.00	0.00	0.00	0.00	0.00
1	**Total Number Other Personnel**					**Total Other Personnel**	(b) (4)

Total Salary, Wages and Fringe Benefits (A+B) 171,936.22

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	Funds Requested ($)

Additional Equipment: Add Attachment | Delete Attachment | View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	

D. Travel

		Funds Requested ($)
1.	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	1,000.00
2.	Foreign Travel Costs	0.00
	Total Travel Cost	1,000.00

E. Participant/Trainee Support Costs

		Funds Requested ($)
1.	Tuition/Fees/Health Insurance	0.00
2.	Stipends	0.00
3.	Travel	0.00
4.	Subsistence	0.00
5.	Other: Other	0.00
0	**Number of Participants/Trainees** — **Total Participant/Trainee Support Costs**	0.00

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	17,900.00
2.	Publication Costs	2,100.00
3.	Consultant Services	0.00
4.	ADP/Computer Services	0.00
5.	Subawards/Consortium/Contractual Costs	0.00
6.	Equipment or Facility Rental/User Fees	0.00
7.	Alterations and Renovations	0.00
8.	Other Direct Costs	9,500.00
9.	All Other Costs	0.00
10.		
	Total Other Direct Costs	29,500.00

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	202,436.22

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
MTDC-On or Off-Adjacent	(b) (4)	(b) (4)	(b) (4)
		Total Indirect Costs	(b) (4)

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

Department of Health and Human Services, Lucy Siow 301-492-4855

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	312,763.96

J. Fee

Funds Requested ($)

K. Total Costs and Fee

	Funds Requested ($)
Total Costs and Fee (I + J)	312,763.96

L. Budget Justification

(Only attach one file.) BudgetJustification.pdf

Add Attachment | Delete Attachment | View Attachment

RESEARCH & RELATED BUDGET - Budget Period 2

OMB Number: 4040-0001
Expiration Date: 12/31/2022

ORGANIZATIONAL DUNS: 188435911 **Enter name of Organization:** University of Maryland, Baltimore

Budget Type: ☒ Project ☐ Subaward/Consortium **Budget Period: 2** **Start Date:** 06/01/2023 **End Date:** 05/31/2024

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Stephen	R.	Thom		0.00	1.80	0.00	0.00	(b) (4)	(b) (4)	(b) (4)
Project Role: PD/PI											
	(b) (6)		(b) (6)		0.00	1.20	0.00	0.00	(b) (4)	(b) (4)	(b) (4)
Project Role: Co-Investigator											

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

Total Funds requested for all Senior Key Persons in the attached file 0.00

Total Senior/Key Person (b) (4)

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
0	Post Doctoral Associates	0.00	0.00	0.00	0.00	0.00	0.00
0	Graduate Students	0.00	0.00	0.00	0.00	0.00	0.00
0	Undergraduate Students	0.00	0.00	0.00	0.00	0.00	0.00
0	Secretarial/Clerical	0.00	0.00	0.00	0.00	0.00	0.00
0	Other	0.00	0.00	0.00	0.00	0.00	0.00
1	Other Professionals	12.00	0.00	0.00	(b) (4)	(b) (4)	(b) (4)
0	Allocated Admin Support	0.00	0.00	0.00	0.00	0.00	0.00
1	**Total Number Other Personnel**					**Total Other Personnel**	(b) (4)

Total Salary, Wages and Fringe Benefits (A+B) 181,964.40

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	Funds Requested ($)

Additional Equipment: Add Attachment | Delete Attachment | View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	

D. Travel

		Funds Requested ($)
1.	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	1,030.00
2.	Foreign Travel Costs	0.00
	Total Travel Cost	1,030.00

E. Participant/Trainee Support Costs

		Funds Requested ($)
1.	Tuition/Fees/Health Insurance	0.00
2.	Stipends	0.00
3.	Travel	0.00
4.	Subsistence	0.00
5.	Other: Other	0.00
0	**Number of Participants/Trainees** — **Total Participant/Trainee Support Costs**	0.00

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	18,437.00
2.	Publication Costs	2,163.00
3.	Consultant Services	0.00
4.	ADP/Computer Services	0.00
5.	Subawards/Consortium/Contractual Costs	0.00
6.	Equipment or Facility Rental/User Fees	0.00
7.	Alterations and Renovations	0.00
8.	Other Direct Costs	9,785.00
9.	All Other Costs	0.00
10.		
	Total Other Direct Costs	30,385.00

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	213,379.40

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
MTDC-On or Off-Adjacent	(b) (4)	(b) (4)	(b) (4)
		Total Indirect Costs	(b) (4)

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

Department of Health and Human Services, Lucy Siow 301-492-4855

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	329,671.19

J. Fee

Funds Requested ($)

K. Total Costs and Fee

	Funds Requested ($)
Total Costs and Fee (I + J)	329,671.19

L. Budget Justification

(Only attach one file.) BudgetJustification.pdf

Add Attachment | Delete Attachment | View Attachment

RESEARCH & RELATED BUDGET - Budget Period 3

OMB Number: 4040-0001
Expiration Date: 12/31/2022

ORGANIZATIONAL DUNS: 188435911 **Enter name of Organization:** University of Maryland, Baltimore

Budget Type: ☒ Project ☐ Subaward/Consortium **Budget Period: 3** **Start Date:** 06/01/2024 **End Date:** 05/31/2025

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Stephen	R.	Thom		0.00	1.80	0.00	0.00	(b) (4)	(b) (4)	(b) (4)
Project Role: PD/PI											
	(b) (6)		(b) (6)		0.00	1.20	0.00	0.00	(b) (4)	(b) (4)	(b) (4)
Project Role: Co-Investigator											

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

Total Funds requested for all Senior Key Persons in the attached file 0.00

Total Senior/Key Person (b) (4)

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
0	Post Doctoral Associates	0.00	0.00	0.00	0.00	0.00	0.00
0	Graduate Students	0.00	0.00	0.00	0.00	0.00	0.00
0	Undergraduate Students	0.00	0.00	0.00	0.00	0.00	0.00
0	Secretarial/Clerical	0.00	0.00	0.00	0.00	0.00	0.00
0	Other	0.00	0.00	0.00	0.00	0.00	0.00
1	Other Professionals	12.00	0.00	0.00	(b) (4)	(b) (4)	(b) (4)
0	Allocated Admin Support	0.00	0.00	0.00	0.00	0.00	0.00
1	**Total Number Other Personnel**					**Total Other Personnel**	(b) (4)

Total Salary, Wages and Fringe Benefits (A+B) 187,423.33

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	Funds Requested ($)

Additional Equipment: Add Attachment | Delete Attachment | View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	

D. Travel

		Funds Requested ($)
1.	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	1,060.90
2.	Foreign Travel Costs	0.00
	Total Travel Cost	1,060.90

E. Participant/Trainee Support Costs

		Funds Requested ($)
1.	Tuition/Fees/Health Insurance	0.00
2.	Stipends	0.00
3.	Travel	0.00
4.	Subsistence	0.00
5.	Other: Other	0.00
0	**Number of Participants/Trainees** — **Total Participant/Trainee Support Costs**	0.00

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	18,990.11
2.	Publication Costs	2,227.89
3.	Consultant Services	0.00
4.	ADP/Computer Services	0.00
5.	Subawards/Consortium/Contractual Costs	0.00
6.	Equipment or Facility Rental/User Fees	0.00
7.	Alterations and Renovations	0.00
8.	Other Direct Costs	10,078.55
9.	All Other Costs	0.00
10.		
	Total Other Direct Costs	31,296.55

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	219,780.78

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
MTDC-On or Off-Adjacent	(b) (4)	(b) (4)	(b) (4)
		Total Indirect Costs	(b) (4)

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

Department of Health and Human Services, Lucy Siow 301-492-4855

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	339,561.30

J. Fee

Funds Requested ($)

K. Total Costs and Fee

	Funds Requested ($)
Total Costs and Fee (I + J)	339,561.30

L. Budget Justification

(Only attach one file.) BudgetJustification.pdf

Add Attachment | Delete Attachment | View Attachment

RESEARCH & RELATED BUDGET - Cumulative Budget

		Totals ($)
Section A, Senior/Key Person		(b) (4)
Section B, Other Personnel		(b) (4)
Total Number Other Personnel	3	
Total Salary, Wages and Fringe Benefits (A+B)		541,323.95
Section C, Equipment		
Section D, Travel		3,090.90
1. Domestic	3,090.90	
2. Foreign	0.00	
Section E, Participant/Trainee Support Costs		0.00
1. Tuition/Fees/Health Insurance	0.00	
2. Stipends	0.00	
3. Travel	0.00	
4. Subsistence	0.00	
5. Other	0.00	
6. Number of Participants/Trainees	0	
Section F, Other Direct Costs		91,181.55
1. Materials and Supplies	55,327.11	
2. Publication Costs	6,490.89	
3. Consultant Services	0.00	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	0.00	
6. Equipment or Facility Rental/User Fees	0.00	
7. Alterations and Renovations	0.00	
8. Other 1	29,363.55	
9. Other 2	0.00	
10. Other 3		
Section G, Direct Costs (A thru F)		635,596.40
Section H, Indirect Costs		(b) (4)
Section I, Total Direct and Indirect Costs (G + H)		981,996.45
Section J, Fee		
Section K, Total Costs and Fee (I + J)		981,996.45

Personnel:
Stephen R. Thom, MD, PhD, principal investigator will devote 15 % effort, 1.8 calendar months to this research effort. He will be responsible for the overall supervision and have primary responsibility for data analysis and coordinating experimental design among the members of the research team.

(b) (6), (b) (6) co-investigator, will devote 10 % effort, 1.2 calendar months to the research effort. She will apply her expertise with magnetic resonance imaging (MRI) and spectroscopy (MRS) to the challenge of obtaining reliable, quantifiable *in vivo* data using the murine model. She will also participate in data analysis and preparation of manuscripts with the PI.

(b) (6), (b) (6) research associate, will devote 100 % effort, 12 calendar months, to this project under Dr. Thom's supervision. She will have day-to-day responsibility for flow cytometry analysis of blood and lymph node samples for microparticles. She has experience with the stereotactic surgery for glymphatic flow fluorescence assays.

Equipment
None.

Animal Purchase – Aims 1-3 involve vertebrate animal use. The current cost for mice from Jackson Laboratories is $29/mouse.

Supplies & services

There is an extensive need for antibodies, biochemicals, as well as other laboratory supplies for all specific aims.

Plastics and glassware costs cover all items to be used in standard biochemical and cell analysis studies.

Publications- The cost of publications, particularly with publication of color flow scattergrams, continues to escalate. Funds are requested to assist with these costs.

Flow supplies: Flow cytometry is a major portion of the research effort and funds to purchase of agents to maintain accuracy and daily operation are requested.

Core use: Funds for University MRI and imaging (confocal microscopes) core usage is requested. The current cost for MRI time is $200/hour.

Travel - Principal Investigator and co-investigator to attend annual scientific meetings to present results.

Animal Care – Costs for husbandry of lab animals is requested.

Equipment service agreements – Costs for annual service agreements to maintain the ultracentrifuge, cell counter, flow cytometer, Odyssey plate reader and cryostat are requested.

All expenses are increased by 3% per annum. Facilities and administrative cost are calculated at (b) (4)%. Fringe benefits for faculty are (b) (4)% for FY22.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: (b) (6)

eRA COMMONS USER NAME (credential, e.g., agency login): (b) (6)

POSITION TITLE:

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Shanghai Jiaotong University	B.S.	06/1985	Biomedical Engineering
University of Illinois at Urbana-Champaign	Ph.D.	05/1995	Biophysics
National Institutes of Health	Visiting Fellow	02/1998	MRI in Medicine
Memorial Sloan-Kettering Cancer Center	Research Associate	02/2000	MRI/MRS in cancer
National Institute of Mental Health	Research Fellow	07/2009	MRI/MRS in Medicine

A. Personal Statement

We plan to use *in vivo* DOTA-Gd enhanced T_1-weighted MRI to evaluate the neuroinflammatory impact of high pressure and decompression in mouse brains in this proposed research. I have the expertise, leadership and motivation necessary to successfully carry out the proposed work. I have a broad background in neuroimaging technology, with specific training and expertise in key research areas for this application. I have over 26 years of expertise in the area of magnetic resonance imaging (MRI). I have applied advanced MR imaging techniques to animal models and clinical applications at the University of Maryland, NIH, Memorial Sloan-Kettering Cancer Center, and published 80 peer-reviewed original journal papers. I will provide my expertise in MR experiment design, optimization, and acquisition.

B. Positions, Scientific Appointments, and Honors

2020-present Professor, Department of Diagnostic Radiology and Nuclear Medicine, University of Maryland School of Medicine
2009-present Manager, MRI operations, C-TRIM, University of Maryland School of Medicine
2014-2020 Associate Professor, Department of Diagnostic Radiology and Nuclear Medicine, University of Maryland School of Medicine
2009-2014 Assistant Professor, Department of Diagnostic Radiology and Nuclear Medicine, University of Maryland School of Medicine
2003-2009 Research Fellow, Molecular Imaging Branch, NIMH, NIH
2000-2002 Instructor, Department of Medical Physics, Memorial Sloan-Kettering Cancer Center, New York
2000-2002 Manager, In Vivo NMR imaging core facility, Memorial Sloan-Kettering Cancer Center
1998-2000 Research Associate, Department of Medical Physics, Memorial Sloan-Kettering Cancer Center, New York

1995-1998 Visiting Fellow, Laboratory of Diagnostic Radiology Research, NIH

C. Contributions to Science

1. I contributed significantly in the development and application of diffusion-weighted MRI, three-dimensional *in vivo* MR microimaging, functional MRI, functional MRS, T_1-weighted MRI, *in vivo* ^{19}F MRI, superparamagnetic iron nanoparticle enhanced high resolution MRI, magnetization-transfer-enhanced MRI, radiofrequency coil construction, as well as *in vivo* high resolution localized MR spectroscopy techniques on ^{1}H, ^{31}P, and ^{13}C. My early contribution was on developing a novel spectral localization by imaging (SLIM) technique and applied it to the biological system under the leadership of Prof. Paul Lauterbur, who received a 2003 Nobel Prize in Medicine for developing MRI.

   a. (b) (6) [redacted] (1997) Biochemical Heterogeneity in Normal and Abnormal Human Uterus Measured by ^{31}P SLIM Localization. Magn Reson Med 37: 736-743. PMID: 9126948
   b. (b) (6) [redacted] (1997) Diffusion Measurement in Phantoms and Tissues Using SLIM Localization. J Magn Reson 129: 130-134. PMID: 9441880

2. Working with small animals at high magnetic field strength, high spatial resolution on MRI and MRS becomes very essential. I involved in developing techniques that met this goal, and applied the techniques to many disease rodent models.

   a. (b) (6) [redacted] (2011) Early Microstructural and Metabolic Changes Following Controlled Cortical Impact Injury in rat: A Magnetic Resonance Imaging and Spectroscopy Study. J Neurotrauma 28:2091-2102. PMID: 217619629
   b. (b) (6) [redacted] (2014) Olanzapine antipsychotic treatment of adolescent rats causes long term changes in glutamate and GABA levels in the nucleus accumbens, *Schizophr. Res*. 161: 452-457. PMID: 25487700
   c. (b) (6) [redacted] (2015) In vivo longitudinal proton magnetic resonance spectroscopy on neonatal hypoxic-ischemic rat brain injury – Neuroprotective effects of acetyl-L-carnitine. *Magn. Reson. Med*. 74:1530-1542. PMID: 25461739
   d. (b) (6) [redacted] (2020) Abnormalities in Brain and Muscle Microstructure and Neurochemistry of the DMD Rat Measured by *in vivo* Diffusion Tensor Imaging and High Resolution Localized ^{1}H MRS. Front Neurosci. 14:739. doi: 10.3389/fnins.2020.00739. PMID: 32760246;

3. Functional MRI (fMRI) is a functional neuroimaging procedure using MRI technology that measures brain activity by detecting associated changes in blood flow. I started my interest in this field during my postdoc training (2000). Since then I have involved in both human and animal studies. Especially, working on both functional MRI (fMRI) and functional MRS (fMRS), I was the first to demonstrate an unambiguous reduction in phosphocreatine during focal activation using the high sensitivity of 11.7 Tesla magnet, suggesting an energy demand associated with functional hyperemia requiring additional ATP provided by the creatine kinase reaction (NeuroImage 28: 401-9, 2005).

   a. (b) (6) [redacted] (2012) Thalamocortical Asynchrony in Conditions of Spinal Cord Injury Pain in Rats. J Neurosci 32:15843–15848. PMID: 23136423
   b. (b) (6) [redacted]. (2018) Effects of Early Alcohol Exposure on Functional Organization and Microstructure of a Visual-Tactile Integrative Circuit. Alcohol Clin Exp Res.42:727-734. PubMed PMID: 29438595
   c. (b) (6) [redacted] (2019) Altered Forebrain Functional Connectivity and Neurotransmission in a Kinase-Inactive Met Mouse Model of Autism. Mol Imaging. 18:1536012118821034. PubMed PMID: 30799683

d. (b) (6) [redacted]. (2019) Functional Connectivity and Metabolic Alterations in Medial Prefrontal Cortex in a Rat Model of Fetal Alcohol Spectrum Disorder: A Resting-State Functional Magnetic Resonance Imaging and in vivo Proton Magnetic Resonance Spectroscopy Study. Dev Neurosci. 41:67-78. doi: 10.1159/000499183. PubMed PMID: 30999297.

4. My work on *in vivo* ^{13}C MR spectroscopy provided an important approach to detect brain metabolites in a dynamic and functional way with high sensitivity and reliability. For example, I demonstrated, for the first time, that the exchange between pyruvate and lactate catalyzed by lactate dehydrogenase can be detected *in vivo* using carbon-13 magnetization transfer, a dynamic and functional effect. Since lactate dehydrogenase is an undisputed biomarker for cancer, this MRS technique is very useful for cancer research (Magn Reson Med 57:258-64, 2007). To improve the sensitivity of carbon-13 magnetization (saturation) transfer effect, I demonstrated an experiment using a carbon-to-proton, spatially localized, heteronuclear polarization transfer technique with significant enhancement of the sensitivity (Magn Reson Imaging 26:413-9, 2008). During infusion of uniformly carbon-13 labeled glucose, I found that both glutamate and glutamine C4 signal underwent a doublet to triplet transition with a temporal lag for glutamine. This observation gives a direct demonstration of the precursor-product relationship between neuronal glutamate and astroglial glutamine and a significant glutamate-glutamine cycling flux between neurons and astroglia (J Magn Reson 182: 221-8, 2006).

   a. (b) (6) [redacted] (2006). In vivo dynamic turnover of cerebral ^{13}C isotopomers from [U-^{13}C]glucose. J Magn Reson 182: 221-8. PMCID: 16859940
   b. (b) (6) [redacted] (2007). In vivo ^{13}C saturation transfer effect of the lactate dehydrogenase reaction. Magn Reson Med 57: 258-64. PMCID: 17260357
   c. (b) (6) [redacted] (2008) Inverse polarization transfer for detecting in vivo ^{13}C magnetization transfer effect of specific enzyme reactions in ^{1}H spectra. Magn Reson Imaging 26:413-419. PMID: 18063339
   d. (b) (6) [redacted] (2009). Studying enzymes by in vivo ^{13}C magnetic resonance spectroscopy. Prog. NMR Spectrosc. 55:266-283. PMCID: 20161496

5. I facilitated *in vivo* ^{19}F MRI research on campus of University of Maryland and demonstrated the very first *in vivo* ^{19}F MR image of a mouse gall bladder. Working with a team of collaborators, we establish and tested MRI detectable artificial bile acids for clinical investigations using ^{19}F MRI.

   a. (b) (6) [redacted] (2011) In Vivo magnetic resonance imaging to detect biliary excretion of 19F-labeled drug in mice. Drug Metab Dispos. 39:736-739. PMID: 21270105
   b. (b) (6) [redacted] (2013) Design and Characterization of a Novel Fluorinated Magnetic Resonance Imaging Agent for Functional Analysis of Bile Acid Transporter Activity. Pharmaceutical Research 30(5), 1240-1251. PMID:23319170
   c. (b) (6) [redacted] (2014) In Vivo Performance of a Novel Fluorinated Magnetic Resonance Imaging Agent for Functional Analysis of Bile Acid Transport. *Mol Pharm.* 11:1575-1582. PMID:24708306
   d. (b) (6) [redacted] (2018) Attenuated Accumulation of Novel Fluorine (^{19}F)-Labeled Bile Acid Analogues in Gallbladders of Fibroblast Growth Factor-15 (FGF15)-Deficient Mice *Mol Pharm.* 15:4827-4834. PMID:30247920

Complete List of Published Work in My Bibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/1PcF0rMW2pGAC/bibliography/48605617/public/?sort=date&direction=descending

Curriculum Vitae

Stephen R. Thom, M.D., Ph.D.
Professor, Department of Emergency Medicine
University of Maryland

September, 2021

Office Address: (b) (6) [redacted]

Phone Number: (b) (6) [redacted]
Email: (b) (6) [redacted]

Education:

1975 B.A., Biology, University of Rochester, Rochester, N.Y.
1981 M.D., University of Rochester
1981 Ph.D., Microbial Physiology, University of Rochester

Postgraduate Education and Training:

1981 - 1982 Internship, Internal Medicine, University of Maryland, Baltimore, MD
1982 - 1984 Residency, Emergency Medicine, UCLA Hospital and Clinics, Los Angeles, CA
1982 - 1984 Fellowship, Undersea & Hyperbaric Medicine, Memorial Medical Center Long Beach, CA

Certificates:

1987 Diplomate, American Board of Emergency Medicine (recertified through 2026)
2000 Additional Certification–Undersea & Hyperbaric Medicine (recertified through 2029)

Medical Licensures:

Inactive California
Inactive Colorado
Inactive Pennsylvania
Active Maryland

Academic Appointments:

1984 - 1986 Assistant Professor, Dept. of Microbiology, Univ. of Colorado Health Sciences Center
1986 - 1994 Assistant Professor, Dept. of Medicine, University of Pennsylvania
1994 - 1995 Assistant Professor, Emergency Medicine, University of Pennsylvania
1995 - 2003 Associate Professor, Emergency Medicine, University of Pennsylvania
1997 - 2003 Associate Professor, Emergency Med. in Pediatrics, Children's Hospital of Philadelphia
2003 - 2013 Professor, Emergency Medicine, University of Pennsylvania
2003 - 2013 Professor, Emergency Medicine in Pediatrics, Children's Hospital of Philadelphia

2013 - Professor of Emergency Medicine, University of Maryland

Professional Society Memberships:

1981-present	American Society for Microbiology
1982-present	Undersea and Hyperbaric Medical Society
1984-present	American College of Emergency Physicians
1986-present	American Federation for Clinical Research
1986-present	Society for Academic Emergency Medicine
1986-present	American Physiological Society
1990-presnet	American Medical Association
1988-present	Society for Free Radical Biology and Medicine
1992-present	Air Medical Physicians Association
1996-present	International Society for Stem Cell Research
2013-present	American Society for Biochemistry and Molecular Biology

Honors and Awards:

1980	Sherman Award - N.Y. American Society for Microbiology outstanding graduate student research
1981	Sigma Xi
1988	Edgar End Award - from the Gulf Coast Chapter of the Undersea and Hyperbaric Medical Society, "for outstanding research in hyperbaric medicine"
1996	Albert R. Behnke Award from the Undersea and Hyperbaric Medical Society, for "outstanding scientific contributions to advances in the undersea or hyperbaric medical field - carbon monoxide vascular injury and HBO_2 effects".
1998	C. Longoni Award from the Italian Undersea and Hyperbaric Medical Society, for "outstanding scientific contributions to undersea and hyperbaric medicine"
2007	Paul Bert Award from the Undersea and Hyperbaric Medical Society, for "excellence in the practice of hyperbaric medicine and basic research"
2008	Hyperbaric Research Prize from an international scientific panel of the Baromedical Research Foundation, for "basic science contributions that have provided a vital foundation for the practice of hyperbaric medicine"
2008	Albert R. Behnke Award from the Undersea and Hyperbaric Medical Society, for "outstanding scientific contributions to advances in the undersea or hyperbaric medical field - bone marrow stem cell HBO_2 effects"
2011	President's Award, Undersea & Hyperbaric Med. Society – scientific presentation.
2012	President's Award, Undersea & Hyperbaric Med. Society – scientific presentation.
2013	President's Award, Undersea & Hyperbaric Med. Society – scientific presentation.
2021	President's Award, Undersea & Hyperbaric Med. Society – scientific presentation

Clinical Activities:

Emergency Department Attending

Clinical Expertise:

Board certified in Emergency Medicine
Additional board certification in Undersea and Hyperbaric Medicine

Scope of Clinical Practice:

Site of Primary Practice:	Emergency Medicine
Responsibilities with Practice	Leadership/administration and primary clinical care
Total Time Spent:	~10% FTE

Development of any Clinical Programs:

Developed University of Pennsylvania Undersea and Hyperbaric Medicine Program
Established ACGME-certified fellowship in Undersea and Hyperbaric Medicine Program

Administrative Service:

1984 – 1986	Medical Director, Porter Regional Baromedicine Center, Porter Memorial Hospital, Denver, CO
1986 - 2013	Chief of Hyperbaric Medicine, Institute for Environmental Medicine, University of Pennsylvania, Philadelphia, PA.
1992 - 2013	Medical Director, Pennstar Flight Program, Hospital of the University of Pennsylvania, Philadelphia, PA
2013 -	Research Director, Department of Emergency Medicine. University of Maryland.
2013 -	Chairman, Department of Emergency Medicine Appointments & Promotions Committee

Institutional Service:

1987-1999	Penn Star, Trauma Service Subcommittee of Medical Affairs Committee
1995-2010	Penn Teaching Evaluation Committee, Medical School Committee on Appointments and Promotions
1996-1999	Penn Combined Degree (M.D.- Ph.D.) Program Admissions Committee
1999-2012	Chairman, Penn Dept. Emergency Medicine, Committee on Appointments and Promotions
2001-2002	Penn Dean's Committee to Review the General Clinical Research Center
2013 -	University of Maryland School of Medicine Admissions Committee interviewer
2019 -	University of Maryland Medical Scientist Training Program interviewer
2019 -	Alternate, School of Medicine Council, University of Maryland School of Medicine

National Service:

Ad hoc reviewer: American Journal of Physiology; Archives of Biochemistry and Biophysics; Archives of Pediatrics & Adolescent Medicine; Biochemica Biophysica Acta; Clinical Toxicology; Critical Care Medicine; Free Radical Biology & Medicine; Nature - Medicine; Journal of Applied Physiology; Journal of Clinical Investigation; Journal of Leukocyte Biology; Journal of Neurology, Neurosurgery, Psychiatry; Journal of Toxicology and Environmental Health; The Lancet, Proceedings of the National Academy of Sciences USA.

1986-present	Undersea and Hyperbaric Medical Society Hyperbaric Oxygen Therapy Committee

1987-1990 National American College of Emergency Physicians Research Committee
1998-2000 U.S. Environmental Protection Agency Advisory Committee on Air Quality Criteria for Carbon Monoxide

2001-2002 Study Section, National Institutes of Health, NIEHS Program on Advanced Research Cooperation in Environmental Health Sciences. Special Emphasis Panel
2001-2002 Curriculum Review Committee for Department of Military and Emergency Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD
2001-2002 Office of Naval Research, Medical Science and Technology Division Grant reviewer
2003-2004 Study Section, National Institutes of Health, NIEHS Program on Advanced Research Cooperation in Environmental Health Sciences. Special Emphasis Panel
2003-2004 Office of Naval Research Program Review
2008-2011 U.S. Environmental Protection Agency Advisory Committee on Air Quality Criteria for Carbon Monoxide
2007-2008 Study Section, National Institutes of Health, NIDCR Special Emphasis Panel ZDE1
2008-2010 EPA Clean Air Scientific Advisory Committee Carbon Monoxide Review Panel
2010-2011 Study Section, National Institutes of Health, Molecular, Cellular and Developmental Neurosciences Integrated Review Group
2018- Study Section, NIH – NIDDK Special Emphasis Panel ZDK1 GRB 1

Local Service:

1994-1998 Educational content advisor - board review courses; Pennsylvania ACEP

Teaching Service:

Medical Student Teaching:

1989 - 2013 Penn Respiratory Module, Medical School Curriculum, "O_2 transport physiology" 2nd year students, 2 contact hours/year

Graduate Student Teaching:

2008 - 2013 Penn Pharmacology 570, "Vascular permeability and edema" 2nd year students, 2 contact hours/year
2008 - 2013 Penn Pharmacology 600, homework module, 2nd year students

2018 - Member Graduate faculty of University of Maryland, Baltimore
-Biochemistry and Molecular Biology Graduate Group and Graduate Group in Molecular Medicine

Resident and Fellow Teaching:

1994 - 2013 Penn Emergency Medicine Didactic lectures 1st thru 4th year residents, 4 hours/year
2004 - 2013 Penn Undersea and Hyperbaric Medicine fellows, 5-10 lectures/year
2013 - U. Maryland Emergency Medicine residents and medical student bedside teaching

PhD Defense Thesis Committees:

2000 - (b) (6) - Norwegian University of Science and Technology
2011 - (b) (6) - Copenhagen University Hospital, Rigshospitalet, Denmark;

2013 - (b) (6) - Norwegian University of Science and Technology
2013 - (b) (6) - University of Pennsylvania
2015 - (b) (6) - Aarhus University, Aarhus, Denmark
2016 – (b) (6) – University of Maryland Balt. Campus
2017 – (b) (6) – University of Maryland Balt. Campus
2020 - (b) (6) – University of Maryland Balt. Campus

Mentoring:

2015 – 2021 Emergency Medicine Residency, University of Maryland Medical Center
1-2, 1st year resident (longitudinal), 3-5 contact hours/year

Active Grants:

6/19/20 – 6/18/23 (PI: 20% effort) "Microparticles, inflammation and decompression sickness" Office of Naval Research N00014-20-1-2641
Annual Direct Cost: $294,302
Total Direct Cost: $909,659

10/25/19 – 09/30/21 (PI: 20% effort) "Glymphatic function in extreme environments".
Office of Naval Research N00014-20-2000
Annual Direct Cost: $291,799
Total Direct Cost: $592,353

06/1/18 – 05/31/22 (co-PI: 20% effort) *"NOS1AP and capon associated impaired healing in those with diabetic foot ulcers"*
National Institute of Diabetes and Digestive and Kidney Diseases
RO1 DK116199
Sub-award annual direct: $185,895
Sub-award total direct: $616,402

6/22/20 – 6/21/23 (co-I: 10% effort) "*Harnessing splenic adaptations to improve diving capabilities"*
Office of Naval Research N00014-20-2593/Primary to U Indiana.
Sub-award annual direct: $28,492
Sub-award total direct: $135,896

7/1/20 – 6/30/22 (co-I: 1% effort) *"Breath-Hold Diving: Mechanisms of Hypoxemia and Decompression Stress"*
Office of Naval Research N00014-20-2579/Primary to U Padova.
Sub-award annual direct: $11,419
Sub-award total direct: $35,815

7/1/21 – 6/30/23 (PI-2%)" *Real-time Monitoring for Decompression Sickness*"
Office of Naval Research N21A-T013-0112/SBIR with Dynaflow, Inc.
Sub-award annual direct: $30,000
Sub-award total direct: $60,000

Completed Grants:

08/01/16 – 06/30/20 (PI: 15% effort)
"Microparticles, platelet-neutrophil aggregation and decompression sickness"
Office of Naval Research N00014-16-0613
Sub-award annual direct: $286,016
Sub-award total direct: $753,492

04/05/16 – 03/31/20 (Co-I: 5% effort)
"Biomarkers and treatment of hypobaria-exacerbated trauma"
U.S. Air Force FA8650-16-2-6H02 ([redacted], PI)
Annual direct: $893,213
Sub-award annual direct: $13,289

09/01/12 – 08/31/17 (co-PI 20% effort)
"Stem cell mobilization in diabetes"
National Institute of Diabetes and Digestive and Kidney Diseases
RO1 DK094260
Sub-award annual direct: $483,031
Sub-award total direct: $1,427,410

10/1/13 – 09/30/16 (PI: 15% effort)
"Pharmacological inhibition of neutrophil adherence by nitric oxide"

Office of Naval Research N00014-12-10363
Annual Direct Cost: $ 240,646
Total Direct Cost: $626,272

07/01/13 - 06/30/16 (PI: 15% effort)
"Microparticles, platelet-neutrophil aggregation and decompression sickness in a murine model"
Office of Naval Research N00014-13-10613
Annual Direct Cost: $ 208,913
Total Direct Cost: $1,253,755

07/01/13 - 06/30/16 (PI: 15% effort)
"*Microparticle production with decompression stress"*
Office of Naval Research N00014-13-10614
Annual Direct Cost: $195,821

Total Direct Cost: $587,634

03/01/12 – 02/28/16 (Co-investigator: 5% effort) PI: (b) (6) [redacted]
"Microbiome responses to oxidative stress"
National Institute of General Medical Science
RO1 GM094260
Annual Direct Costs: $ 14,434 (sub-award)
Total Direct Costs: $ 57,736

04/01/11 – 03/31/14 (Co-investigator: 5% effort) PI: (b) (6) [redacted]
"Comparative Effectiveness of Adjunctive Devices for Prevention of Amputation in Diabetes"
Agency for Health Care Quality
Annual Direct Costs: $280,950
Total Direct Costs: $607,604

03/01/12 – 06/30/13 (PI: 20% effort)
"Pharmacological Inhibition of Neutrophil Adherence"
Office of Naval Research N00014-12-1-0420
Annual Direct Cost: $205,899
Total Direct Cost: $629,389

04/15/06 – 05/01/12 (PI: 30% effort)
"Hyperoxia and neutrophil adhesion"
Office of Naval Research N00014-06-01-0363
Annual Direct Cost: $ 248,595
Total Direct Cost: $ 1,260,000

03/30/08 – 03/31/10 (PI: 25%)
"Stem cell mobilization in diabetes"
National Institute of Diabetes and Digestive and Kidney Diseases
R21 DK080376
Annual Direct Cost: $ 136,875
Total Direct Cost: $ 275,000

04/01/08 – 03/31/11 (Co-investigator: 10% effort) PI: (b) (6) [redacted]
"Recruiting activated endothelial progenitor cells to wounds by hyperoxia and SDF-1a"
National Institute for General Medical Sciences R01 GM081570
Annual Direct Cost: $ 250,000
Total Direct Cost: $ 750,000

04/01/08 – 10/01/09 (PI: 10% effort)
"Biomarkers profiles for carbon monoxide poisoning"
National Institute for Environmental Health Science R43 ES016720
Annual Direct Cost: $ 68,000
Total Direct Cost: $ 98,000

09/29/00 – 07/31/07 (PI: 30%)
"Specialized center of research in hyperbaric oxygen therapy"
National Center for Complementary and Alternative Therapy P50 AT00428
Annual Direct Costs: $ 999,600
Total Direct Costs: $4,998,000

03/01/94 – 07/31/01 (PI: 30 – 40%)
"CO poisoning in the context of a reperfusion injury"
National Institute for Environmental Health Science R01 ES05211
Annual Direct Cost: $ 210,000
Total Direct Cost: $ 996,500

07/01/89 – 12/31/95 (PI: 50% effort)
"CO poisoning in the context of a reperfusion injury"
National Institute for Environmental Health Science R29 ES05211
Annual Direct Cost: $ 70,000
Total Direct Cost: $ 350,000

07/01/87 – 06/30/89 (PI: 30 % effort)
"CO-induced lipid peroxidation in brain"
American Lung Association
Annual Direct Cost: $ 17,500
Total Direct Cost: $ 35,000

Publications:

Peer-reviewed journal articles:

1. Marquis, R.E., **Thom, S.R.**, Crookshank, C.A. Interactions of helium, oxygen and nitrous oxide affecting bacterial growth. Undersea Biomedical Research. 1978 5:189-198.

2. **Thom, S.R.**, and Marquis, R.E. Contrasting actions of hydrostatic pressure and helium pressure on growth of Saccharomyces cerevisiae. Underwater Physiology VII, Proceedings of the Seventh Symposium on Underwater Physiology. 1981 A.J. Bachrach and M.M. Matzen, eds. pp. 667-673.

3. **Thom, S.R.** and Marquis, R.E. Microbial growth modification by compressed gases and hydrostatic pressure. Applied and Environmental Microbiology. 1984 47:780-787.

4. **Thom, S.R.**, Lauermann, M.W., Hart, G.B. Intermittent hyperbaric oxygen therapy reduces mortality in experimental polymicrobial sepsis. Journal of Infectious Diseases. 1986 154:504-510.

5. Pfoff, D.S. and **Thom, S.R**. Preliminary report on the effect of hyperbaric oxygen on cystoid macular edema. Journal of Cataract and Refractive Surgery. 1987 13:136-140.

6. **Thom, S.R**. and Marquis, R.E. Free radical reactions and the inhibitory and lethal actions of high-

pressure gases. Undersea Biomedical Research. 1987 14:485-501.

7. Marquis, R.E. and **Thom, S.R.** Biological interactions of pressure, compressed gases and free radicals. International Meeting on Neurophysiology and Performance at High Pressure - High Pressure Nervous Syndrome 20 Years Later. (J.C. Rostain, E. Martinez, C.L. Octaren, eds.) Marseille, ARAS-SNHP 1988 pp. 65-71.

8. **Thom, S.R.** Hyperbaric oxygen therapy. *J. Intensive Care Medicine.* 1989 4:58-74.

9. **Thom, S.R**. and Keim, L. Carbon monoxide poisoning: a review. *Clinical Toxicology.* 1989 27:141-156.

8. **Thom, S.R.** Carbon monoxide-mediated brain lipid peroxidation in the rat. J. Appl. Physiol. 1990 68:997-1003.

9. **Thom, S.R.** Antagonism of CO-mediated brain lipid peroxidation by hyperbaric oxygen. Toxicol. Appl. Pharmacol. 1990 105:340-344.

10. **Thom, S.R**. and Elbuken, M.E. Oxygen-dependent antagonism of lipid peroxidation. Free Radical Biology and Medicine. 1991 10:413-426.

11. **Thom, S.R**. Inert gas enhancement of superoxide radical production. Archives of Biochemistry and Biophysics. 1992 295:391-396.

12. **Thom, S.R.** Dehydrogenase conversion to oxidase and lipid peroxidation in brain after carbon monoxide poisoning. J. Appl. Physiol. 1992 73:1584-1589.

13. **Thom, S.R**. Leukocytes in carbon monoxide-mediated brain oxidative injury. Toxicol. Appl. Pharmacol. 1993 123:234-247.

14. **Thom, S.R**. Functional inhibition of neutrophil B2 integrins by hyperbaric oxygen in carbon monoxide mediated brain injury. Toxicol. Appl. Pharmacol. 1993 123:248-256.

15. Vaporciyan, A.A., Delisser, H., Yan, H.C., Mendiguren, I., **Thom, S.R.**, Jones, M.L., Ward, P.A., and Albelda, S.M. Involvement of platelet-endothelial cell adhesion molecule-1 in neutrophil recruitment in vivo. Science. 1993 262:1580-1582.

16. **Thom, S.R**., Mendiguren, I., Van Winkle, T., Fisher, D., and Fisher, A.B. Smoke inhalation with a concurrent systemic stress results in lung alveolar injury. American Journal of Respiratory and Critical Care Medicine. 1994 149:220-226.

17. Mayevsky, A., Rogatsky, G.G., Zarchin, N., and **Thom, S.R**. Interrelations between hyperbaric oxygenation and carbon monoxide intoxication in the rat brain in vivo. Basic and Applied High Pressure Biology, (eds.) P.B. Bennett and R.E. Marquis, New York: University of Rochester Press. 1994 409-420.

18. **Thom, S.R**. Applications of Pressure Biology: Toxic and Beneficial Effects of Oxygen. Basic and Applied High Pressure Biology, (eds.) P.B. Bennett and R.E. Marquis, New York: University of Rochester Press. 1994 365-373.

19. **Thom, S.R**., Ohnishi, S.T., and Ischiropoulos, H. Nitric oxide released by platelets inhibits neutrophil B2 integrin function following acute carbon monoxide poisoning. Toxicol. Appl. Pharmacol. 1994 128:105-110.

20. Hardy, K.R. and **Thom, S.R**. Pathophysiology and treatment of carbon monoxide poisoning. Clinical Toxicology. 1994 32:613-629.

21. Ischiropoulos, H., Mendiguren, I., Fisher, D., Fisher, A.B., and **Thom SR**. Role of neutrophils and nitric oxide in lung alveolar injury from smoke inhalation. American Journal of Respiratory and Critical Care Medicine 1994 150:337-341.

22. Mayevsky, A., Meilin, S., Rogatsky, G.G., Zarchin, N., and **Thom, S.R**. Multiparametric monitoring of the awake brain exposed to carbon monoxide. J. Appl. Physiol. 1995 78:1188-1196.

23. **Thom, S.R**., Taber, R.L., Mendiguren, I.I., Clark, J.M., Hardy, K.R., and Fisher, A.B. Delayed neuropsychological sequelae following carbon monoxide poisoning and its prophylaxis by treatment with hyperbaric oxygen. Annals of Emergency Medicine 1995 25:474-480.

24. Chen, Q., Banick, P.D., and **Thom, S.R**. Functional inhibition of rat polymorphonuclear leukocyte β2 integrins by hyperbaric oxygen is associated with impaired cGMP synthesis. Journal of Pharmacology and Experimental Therapeutics. 1996 276:929-933.

25. Ischiropoulos, H., Beers, M.F., Ohnishi, S.T., Fisher, D., Garner, S.E., and **Thom, S.R**. Nitric oxide production and perivascular tyrosine nitration in brain following carbon monoxide poisoning in the rat. J. Clin. Invest. 1996 97:2260-2267.

26. Ashamalla, H.L., **Thom, S.R.,** and Goldwein, J.W. Hyperbaric oxygen therapy forradiation-induced sequelae in children. Cancer 1996 77:2407-2412.

27. Meilin, S., Rogatsky, G.G., **Thom, S.R**., Zarchin, N., Guggenheimer-Furman, E., and Mayevsky, A. Effects of carbon monoxide exposure on the brain may be mediated by nitric oxide. J. Appl. Physiol. 1996 81:1078-1083.

28. Gow, A., Duran, D., **Thom, S.R**., and Ischiropoulos, H. Carbon dioxide enhancementof peroxynitrite-mediated protein tyrosine nitration. Archives Biochemistry and Biophysics. 1996 333:42-44.

29. Ashamalla, H., Belasco, J., Wetmore, R., Ames, J., Hardy, K, **Thom, S.R**., Uri, A., and Goldwein, J. 1996. Electronic Case of the Month. OncoLink: The University of Pennsylvania Cancer Resource [Source on Worl-Wide-Web] URL:"http://oncolink.upenn.edu:80/ped_onc/cotm/apr96/cotm0494a.html"

30. **Thom, S.R**., Mendiguren, I., Hardy, K.R., Bolotin, T., Fisher, D. Nebolon, M. and Kilpatrick, L. Inhibition of human neutrophil β2 integrin-dependent adherence by hyperbaric oxygen. Am. J. Physiol. (Cell Physiology) 1997 272:C770-C777.

31. Terregino, C.A., Lubkin, C.L., and **Thom, S.R**. Impaired neutrophil adherence as an early marker of systemic inflammatory response syndrome and severe sepsis. Annals of Emergency Medicine 1997

29:400-403

32. **Thom, S.R**., Kang, M., Fisher, D., and Ischiropoulos, H. Release of glutathione from erythrocytes and other markers of oxidative stress in carbon monoxide poisoning. J. Appl. Physiol. 1997 82:1424-1432.

33. Banick, P.D., Chen, Q., Xu, Y.A., and **Thom, S.R.** Nitric oxide inhibits neutrophil β2 integrin function by inhibiting membrane-associated cyclic GMP synthesis. Journal of Cellular Physiology 1997 172:12-24.

34. Gow, A.J., **Thom, S.R**., Brass, C., and Ischiropoulos, H. Electrochemical detectors of nitric oxide in biological systems. Microchemical Journal 1997 56:146-154.

35. **Thom, S.R**., Xu, Y.A., and Ischiropoulos, H. Vascular endothelial cells generate peroxynitrite in response to carbon monoxide exposure. Chem. Res. Toxicol. 1997 10:1023-1031.

36. **Thom, S.R**. and Ischiropoulos, H. Mechanism of oxidative stress from low levels of carbon monoxide. Research Report of Health Effects Institute. 1997 80:1-19.

37. Gow, A.J., **Thom, S.R**., and Ischiropoulos, H. Nitric oxide and peroxynitrite-mediated pulmonary cell death. Am. J. Physiol. (Lung Cell. Mol. Physiol.) 1998 274:L112-L118.

38. Ischiropoulos, H., Gow, A., **Thom, S.R.,** Kooy, N.W., Royall, J.A., and Crow, J.P. Detection of reactive nitrogen species using 2,7-dichlorodihydrofluorescein and dihydrorhodamine 123. Methods in Enzymology 1998 301:627-635.

39. Schwentker, A., Evans, S.M., Partington, M., Johnson, B.L., Koch, C.J., and **Thom, S.R**. A model of wound healing in chronically radiation-damaged rat skin. Cancer Letters. 1998 127:1-8.

40. **Thom, S.R**., Ohnishi, S.T., Fisher, D., Xu, Y.A., and Ischiropoulos, H. Pulmonary vascular stress from carbon monoxide. Toxicol. Appl. Pharmacol. 1999 154:12-19.

41. **Thom, S.R**., Fisher, D., Xu, Y.A., Garner, S., and Ischiropoulos, H. Role of nitric oxide-derived oxidants in vascular injury from carbon monoxide in the rat. Am. J. Physiol. (Heart and Circ. Physiol. 45). 1999 276:H984-H992.

42. **Thom, S.R.,** Fisher, D., Xu, Y.A., Notarfrancesco, K., and Ischiropoulos, H. Adaptive responses and apoptosis in endothelial cells exposed to carbon monoxide. Proc. Natl. Acad. Sci. (USA). 2000 97:1305-1310.

43. Raub J.A., Mathieu-Nolf, M., Hampson, N.B., and **Thom, S.R.** Carbon monoxide poisoning - a public health perspective. Toxicology. 2000 145:1-14.

44. Huang, E.T., Hardy, K.R., Stubbs, J.M., Lowe, R.A., and **Thom, S.R**. Ventriculo-peritoneal shunt performance under hyperbaric conditions. Undersea & Hyperbaric Med. 2001 27:191-194.

45. Atochin, D.A., Fisher, D, Demchenko, I.T., and **Thom, S.R**. Neutrophil sequestration and the effect of hyperbaric oxygen in a rat model of temporary middle cerebral artery occlusion. Undersea & Hyperbaric

Med. 2001 27:185-190.

46. Hampson, N.B., Mathieu, D., Piantadosi, C.A., **Thom, S.R**., and Weaver, L.K. Carbon monoxide poisoning: Interpretation of randomized clinical trials and unresolved treatment issues. Undersea and Hyperbaric Medicine. 2001 28:157-164.

47. **Thom, S.R**., Fisher, D., and Manevich,Y. Roles for platelet-activating factor and nitric oxide-derived oxidants causing neutrophil adherence after CO poisoning. Am. J. Physiol (Heart and Circulatory Physiology). 2001 281:H923-H930.

48. Ufberg, J., Jaslow, D., **Thom, S.R**. Scene diagnosis of carbon monoxide poisoning by emergency medical personnel. Prehospital Emergency Care. 2001 5:407-410.

49. **Thom, S.R.** Bhopale, V., Fisher, D., Manevich, Y., Huang, P.L., and Buerk, D. Stimulation of nitric oxide synthesis in cerebral cortex due to elevated partial pressures of oxygen: an oxidative stress response. Journal of Neurobiology. 2002 51:85-100.

50. Hjelde, A., Hjelstuen, M., Haraldseth, O., Martin, J.D., **Thom, S.R**., Brubakk, A.O. Hyperbaric oxygen and neutrophil accumulation/tissue damage during permanent focal cerebral ischaemia in rats. European Journal of Applied Physiology. 2002 86:401-405.

51. Martin, J.D. and **Thom, S.R**. Leukocyte sequestration in neurological decompression sickness and prophylactic hyperbaric oxygen therapy. Aviat. Space & Environmental Med. 2002 73:565-569.

52. Thom, S.R., Mendiguren, I., and Fisher, D. Smoke inhalation-induced alveolar lung injury is inhibited by hyperbaric oxygen. Undersea & Hyperbaric Med. 2002 28:175-180.

53. Rogatsky, G.G., Meilin, S., Zarchin, N., **Thom, S.R**., and Mayevsky, A. Hyperbaric oxygen affects rat brain function after carbon monoxide exposure. Undersea & Hyperbaric Med. 2002 29:50-58.

54. Mendelman A., Zarchin, N., Meilin, S., Guggenheimer-Furman, E., **Thom, S.R**., and Mayevsky A. Blood flow and ionic responses in the awake brain due to carbon monoxide. Neurological Res. 2002 24:765-72.

55. **Thom, S.R**. and D.G. Buerk. Nitric oxide synthesis in brain is stimulated by oxygen. Advances in Experimental Medicine and Biology, 2003 510:133-137.

56. **Thom, S.R.,** Fisher, D., Zhang, J., Bhopale, V.M., Ohnishi, S.T., Kotake, Y., Ohnishi, T.,and Buerk, D.G. Stimulation of perivascular nitric oxide synthesis by oxygen. Am. J. Physiol. (Heart Circ. Physiol). 2003 284:H1230-1239.

57. **Thom, S.R**., Fisher, D., Zhang, J., Bhopale, V.M., Cameron, B., and Buerk, D.G. Neuronal nitric oxide synthase and N-methyl-D-aspartate neurons in experimental carbon monoxide poisoning. Toxicol. Appl. Pharmacol. 2004 194:280-295.

58. **Thom, S.R**. Effects of hyperoxia on neutrophil adhesion. Undersea & Hyperbaric Med. 2004 31:23-131.

59. **Thom, S.R**., Bhopale, V.M., Fisher, D., Zhang, J., and Gimotty, P. Delayed neuropathology following carbon monoxide poisoning is immune-mediated. Proc. Nat'l Acad. Sci. USA. 2004 101:13660-13665.

60. Shi, Y., Lee, C.S., Wu, J., Koch, C.J., **Thom, S.R**., Maity, A., and Bernhard, E.J. The effects of hyperbaric oxygen exposure on experimental head and neck tumor growth, oxygenation and vasculature. Head and Neck. 2005 27:362-369.

61. **Thom, S.R**., Bhopale, V.M., Velazquez, O.C., Goldstein, L.J., Thom, L.H., and Buerk, D.G. Stem cell mobilization by hyperbaric oxygen. Am. J. Physiol. (Heart Circ. Physiol). 2006 290:H1378-H1386.

62. **Thom, S.R**., Fisher, D. and Stubbs, M. Platelet function in humans is not altered by hyperbaric oxygen therapy. Undersea & Hyperbaric Med. 2006 33:81-83.

63. **Thom, S.R**., Bhopale, V.M., and Fisher, D. Hyperbaric oxygen reduces delayed immune-mediated neuropathology in experimental carbon monoxide poisoning. Toxicol. Appl. Pharmacol. 2006 213:152-159.

64. Hink, J., **Thom, S.R**., Simonsen, U., Rubin, I., and Jansen, E. Vascular reactivity and endothelial NOS activity in rat thoracic aorta during and after hyperbaric oxygen exposures. Am. J. Physiol. (Heart Circ. Physiol.). 2006 291:H1988-1998.

65. Goldstein, L.J., Gallagher, K., Baireddy, V., Bauer, S.M., Bauer, R.J., Buerk, D.G., **Thom, S.R**., Velazquez, O.C. Progenitor cell release into circulation is triggered by hyperoxia-induced increases in bone marrow nitric oxide. Stem Cells. 2006 24:2309-2318.

66. **Thom, S.R**., Bhopale, V.M., Han, S.T., Clark, J.M., and Hardy, K.R. Intravascular Intravascular neutrophil activation due to carbon monoxide poisoning. Am. J. Respir. Crit. Care Med. 2006 174:1239-1248.

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Books:

1. Neuman, T.S. and **Thom, S.R**. (eds) *Hyperbaric Oxygen Physiology and Medicine* Elsevier, Philadelphia.Saunders, Philadelphia, 2008

Major Invited Presentations (last 12 years):

National

1. **Thom, S.R.** Actin S-nitrosylation and β_2 integrin function of neutrophils - Molecular basis of hyperbaric oxygen therapy in reperfusion injuries. Univ. of Buffalo, Dept. of Physiology, 2008.

2. **Thom, S.R.** Oxidative stress modifies neutrophil adhesion functions, Harvard University/Mass. General

Hospital, Cardiovascular Research Center, 2008.

3. **Thom, S.R.** Oxidative stress is fundamental to hyperbaric oxygen therapy. Lundgren Honorary Symposium, University of Buffalo, Buffalo, N.Y, 2009.

4. **Thom, S.R.** Hypoxia inducible factor, oxidative stress and vasculogenic stem cell function. Wound Healing Symposium, Ohio State University, 2010

5. **Thom, S.R.** Physiology of oxygen in wound healing. Fall meeting – Society for Advanced Wound Care, Las Vegas NV, 2011.

6. **Thom, S.R.** Hyperbaric oxygen augments stem cell-mediated vasculogenesis. Yale University, John B. Pierce Laboratory, New Haven, CT, 2012.

7. **Thom, S.R.** Regulation of stem cell mobilization and intracellular trafficking in wounds. American College of Wound Healing and Tissue Repair, Chicago, IL, 2012

8. **Thom, S.R.** Roles for reactive oxygen and nitrogen species in stem cell biology. Association for the Advancement of Wound Care. Baltimore, MD, 2012.

9. **Thom, S.R.** Hyperbaric oxygen therapy: Removing the Mythology. Diabetic Limb Salvage, George Washington Univ. Washington, D.C., 2012.

10. **Thom, S.R.** Debate: Hyperbaric oxygen therapy for acute CO poisoning, Am. Coll. Emergency Physicians. Seattle, WA, 2013.

11. **Thom, S.R**. Stem cell mobilization and hyperbaric oxygen therapy. Diabetic Limb Salvage, George Washington Univ. Washington, D.C., 2013.

12. **Thom, S.R**. Clinical Research in Wound Healing – Negative pressure devices and hyperbaric oxygen therapy. Association of Specialty Professors Workshop on Wound Repair and Healing in Older Adults, Washington, D.C., 2014.

13. **Thom, S.R.** (1) Hyperbaric oxygen therapy mechanisms of action. (2) Microparticle-related pathophysiology. University of Pennsylvania Institute for Environmental Medicine. February, 2015

14. **Thom, S.R.** Physiology of Wound Healing/ Mechanisms of Vasculogenesis. Georgetown Conference. Washington, D.C. March 11, 2016.

14. **Thom, S.R.** (1) Stem cell mobilization by hyperbaric oxygen. (2) Vasculogenesis mechanisms in chronic wounds. Hennepin Medical Center Emergency Medicine Grand Rounds and Hyperbaric Service CME lecture. September 1, 2016.

15. **Thom, S.R.** International Workshop: New Horizons in Inflammation and Synthetic Biology. "Pro-inflammatory microparticles: A response to oxidative stress. Harvard T.H. Chan School of Public Health. November 9, 2016.

16. **Thom, S.R.** Microparticle generation by high gas pressure. University of Arkansas Physiology Grand Grounds. October 28, 2016.

17. **Thom, S.R.** Hohenberg Memorial Lecture, University of Pennsylvania. Blood-borne microvesicles and stem cells in wound repair. Philadelphia, PA June 1, 2017.

18. **Thom, S.R.** Mechanisms of hyperbaric oxygen intervention for compromised grafts and flaps. Healogics Corp. Continuing Education Series, Jacksonville, FL. July 11, 2017.

19. Thom, S.R. Microparticle production triggered by elevated carbon dioxide. Annual Meeting of the Society for Free Radical Biology and Medicine. Baltimore, MD. December 4, 2017.

20. **Thom, S.R.** Overview on hyperbaric oxygen therapy mechanisms of action. First Annual Maryland Conference on Hyperbaric Oxygen Therapy. Baltimore, MD. March 17, 2018.

21. **Thom, S.R.** Molecular mechanisms of hyperbaric oxygen therapy. Hackensack Medical Center Surgical Grand Rounds. Hackensack, NJ. May 15, 2018.

22. **Thom, S.R.** Biochemistry and physiology of hyperbaric oxygen therapy. Undersea and Hyperbaric Medical Society Scientific Meeting, Orlando, FL. June 29, 2018.

23. **Thom, S.R.** New mechanisms identified for hyperbaric oxygen therapy. Associazione Pazienti Trattati in Iperbarismo, Fidenza, Italy. March 17, 2019.

24. **Thom, S.R.** Hyperbaric oxygen therapy mechanisms of action. Second Annual Maryland Conference on Hyperbaric Oxygen Therapy. Baltimore, MD. March 30, 2019.

25. **Thom, S.R.** Molecular mechanisms of hyperbaric oxygen therapy. Dartmouth-Hitchcock Medical Center Internal Medicine Grand Rounds. Hanover, NH April 12, 2019.

26. **Thom, S.R.** Stem cell mobilization and augmented activity by hyperbaric oxygen. Annual Scientific Meeting of the Undersea and Hyperbaric Medical Society. San Juan, Puerto Rico. June 29, 2019.

International:

1. **Thom, S.R.** (1) From Germs to stem cells – fundamental mechanisms of hyperbaric oxygen therapy. (2) Clinical relevance of oxidative stress. Oxygen and infection – European Underwater Biomedical Association, Stockholm, Sweden, 2009.

2. **Thom, S.R.** Stem cell mobilization by hyperbaric oxygen. Italian Undersea and Hyperbaric Medical Society. Salsomaggiore, Italy, 2009.

3. **Thom, S.R.** Decompression-induced vascular injuries are caused by microparticles. Italian Undersea and Hyperbaric medical Society, Castiglione della Pescaia, Italy, 2010.

4. **Thom, S.R.** Pharmacology of hyperbaric oxygen therapy. Italian Society of Anesthesiology and Critical Care. Parma, Italy, 2010.

5. **Thom, S.R.** Decompression-induced microparticles initiate vascular injuries. Italian Undersea and Hyperbaric medical Society, Grosetto, Italy, 2011.

6. **Thom, S.R.** Vasculogenic stem cell mobilization and wound recruitment in diabetic patients. X Congress of the Italian Cutaneous Wound Care Society, Ancona, Italy, 2011.

7. **Thom, S.R.** Is hyperbaric oxygen useful to treat carbon monoxide poisonings? European Association of Poison Centres and Clinical Toxicologists. London, England, 2012.

8. **Thom, S.R.** Pressure and the yinyang of oxidative stress. XII biennial meeting of the International High Pressure Biology Group and the Israeli Society for Hyperbaric and Diving Medicine. Eilat, Israel, 2012.

9. **Thom, S.R.** Pressure and related changes alter microparticle production and decompression injuries. Norwegian University of Science and Technology, Trondheim, Norway. 2013

10. **Thom, S.R.** Research in Emergency Medicine – Successes and Areas of Improvement. Jeddah, Saudi Arabia. King Faisal Specialist Hospital & Research Centre Conference: "Updates in Emergency Medicine & Hyperbaric Medicine" September, 2013

11. **Thom, S.R.** Sepsis in the Emergency Department – Early diagnosis and management. Jeddah, Saudi Arabia. King Faisal Specialist Hospital & Research Centre Conference: "Updates in Emergency Medicine & Hyperbaric Medicine" September, 2013

12. **Thom, S.R.** Pathophysiology of decompression illness. Jeddah, Saudi Arabia. King Faisal Specialist Hospital & Research Centre Conference: "Updates in Emergency Medicine & Hyperbaric Medicine" September, 2013

13. **Thom, S.R.** Recent updates in CO poisoning. Jeddah, Saudi Arabia. King Faisal Specialist Hospital & Research Centre Conference: "Updates in Emergency Medicine & Hyperbaric Medicine" September, 2013.

14. **Thom, S.R**. Pro-inflammatory microparticles in diving physiology – a role in decompression stress? Pisa, Italy. Underwater and hyper-hypobaric environments: effective models of extreme physiology. January, 2014

15. **Thom, S.R**. Pathophysiology of carbon monoxide poisoning. Brisbane, Australia. Hyperbaric Technicians and Nurses Annual Scientific Meeting. August, 2014.

16. **Thom, S.R**. Stem cells and wound healing. Brisbane, Australia. Hyperbaric Technicians and Nurses Annual Scientific Meeting. August, 2014.

17. **Thom, S.R**. Stem cells mobilization by hyperbaric oxygen. Brisbane, Australia. Hyperbaric Technicians and Nurses Annual Scientific Meeting. August, 2014.

18. **Thom, S.R**. Stem cells, wound healing and hyperbaric oxygen. Wiesbaden, Germany. European Underwater and Baromedical Society1Annual Meeting. September, 2014

19. **Thom, S.R**. Role of stem cells in diabetic wound healing. Buenos Aires, Argentina. 18th International Congress on Hyperbaric Medicine. December, 2014

20. **Thom, S.R**. Circulating stem cells as a predictor of neuropathic diabetic wound healing. Melbourne, Australia. Hyperbaric Technicians and Nurses Annual Scientific Meeting. August, 2015.

21. **Thom, S.R**. Microparticle elevations and neutrophil activation in decompression sickness. Melbourne, Australia. Hyperbaric Technicians and Nurses Annual Scientific Meeting. August, 2015.

22. **Thom, S.R**. Role of oxygen in wound healing: Hyperbaric oxygen therapy and molecular and cellular regulation. 5th Congress of the World Union of Wound Healing Societies, Florence, Italy. September 26, 2016

23. **Thom, S.R.** Wound healing physiology and the role of oxygen: Stem cells and hyperbaric oxygen therapy. 5th Congress of the World Union of Wound Healing Societies, Florence, Italy. September 27, 2016.

24. **Thom, S.R.** Current perspectives in decompression sickness. Canadian Navy Diving Medical Officer Training Course. Toronto, Canada. June 9, 2017

25. **Thom, S.R.** Opioid-mediated extracellular vesicle production and NLRP3 inflammasome activation cause vascular damage. International Society on Extracellular Vesicles Annual Meeting, Barcelona, Spain. May 4, 2018.

26. **Thom, S.R.** Latest findings on pathophysiology of decompression sickness. Canadian Navy Diving Medical Officer Training Course. Toronto, Canada. June 8, 2018.

27. **Thom, S.R.** Update on CO pathophysiology and treatment. Wonju International Symposium on Hyperbaric Medicine. Wonju, Korea. October 15, 2018.

28. **Thom, S.R.** Mechanisms of action of hyperbaric oxygen therapy. Korean Society of Emergency Medicine. Incheon, Korea. October 19, 2018

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30. **Thom, S.R.** Microparticle generation and vascular injuries from high pressure exposure. 29th International Meeting -Association of Patients Treated with Hyperbaric Oxygen. Fidenza, Italy March 17, 2019.

31. **Thom, S.R.** High pressure and decompression pathophysiology. Canadian Navy Diving Medical Officer Training Course. Toronto, Canada. June 5, 2019.

32. **Thom, S.R.** Pathophysiology of CO poisoning and anti-inflammatory mechanisms of action of hyperbaric oxygen therapy. Sixth Medical Center of the PLA General Hospital, Beijing, China, June 12, 2019.

33. **Thom, S.R.** Carbon monoxide pathophysiology. International Congress of Emergency Medicine. Seoul, Korea, June 14, 2019.

34. **Thom, S.R.** Molecular mechanisms of hyperbaric oxygen therapy. International Congress of Emergency Medicine. Seoul, Korea, June 14, 2019.

35. **Thom, S.R.** Stem cell mobilization and microparticle-induced vasculopathy. Medicine Grand Rounds, University of Padua, Italy (Virtual lecture due to SARS-CoV-2) June 9, 2020.

36. **Thom, S.R.** Mechanisms of action of hyperbaric oxygen therapy. 74th National Congress of the Società Italiana di Anestesia Analgesia Rianimazione e Terapia Intensiva. (Virtual lecture due to SARS-CoV-2) October 25, 2020.

TECHNICAL PROPOSAL SUBMITTED TO THE OFFICE OF NAVAL RESEARCH

ONR N00014-21-S-B001

Title: **Neuroinflammation and glymphatic dysfunction in decompression sickness**

Institution: University of Maryland, Baltimore

Principal Investigator: Stephen R. Thom, M.D, Ph.D.

Mailing Address: (b) (6) [redacted]

(b) (6) [redacted].

Administrative:

(b) (6) [redacted]

Institutional Financial Officer: (b) (6) [redacted]; University of Maryland, Baltimore Campus, 220 Arch St. Room 02148, Baltimore, MD 21201-1531 Tel: (b) (6) [redacted]

Proposed period of performance: 1 June 2022 – 31 May 2025

Human Use:	No
Animal Use:	Yes
IACUC approval:	Yes
Recombinant DNA Research:	No

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1. TECHNICAL APPROACH & JUSTIFICATION:

Rationale: This research project is to evaluate the neuroinflammatory impact of high pressure and decompression using a murine model. The motivation is to establish the molecular basis for neurological decompression sickness (DCS). We have found that inflammatory microparticles (MPs) elevated in humans, and in animal models of DCS trigger dysfunction of the brain glial-based lymphatic (aka glymphatic) system in mice. We have identified a complex pathological interaction between brain-generated MPs and systemic inflammation that may resolve several unexplained clinical observations. We have also identified knock-out mice resistant to DCS pathology and a pharmaceutical in use in humans that protects against CNS injuries.

Approximately 30% of severe DCS cases exhibit CNS involvement (*1*). Complaints may include confusion, lethargy, difficulty with concentration or "mental cloudiness", dysphagia, visual, vestibular, and auditory disturbances. A recent population-based study demonstrated that those sustaining DCS have a 5.7-fold increased risk of developing a sleep disorder as a manifestation of CNS injury (*2*). The basis for these global changes remains unclear whereas hemi-sensory deficits and hemiplegia are typically thought to occur due to gas embolism. A review of 1070 cases of central nervous system DCS in compressed gas divers estimated that 22.7% were cerebral DCS, 66.4% spinal cord and 10.9% both (*1*).

The prevalence of some cerebral DCS manifestations is 6-fold higher in breath-hold versus compressed air divers, and breath-hold divers rarely exhibit deficits related to the spine (*3, 4*). Injuries occur in upwards of 20% of commercial breath-hold divers (*4*). Breath-hold divers' exposures are typically shallower than compressed air divers. Theoretical calculations indicate there is a risk for inert gas supersaturation because of repetitive exposures with short surface intervals (*5*). However, breath-hold divers only rarely are found to have intravascular bubbles and when found they are of low magnitude and clear rapidly from the circulation (*5, 6*).

The pathophysiology for CNS DCS remains unclear, but naturally is focused on the proposition that with decompression, insoluble gas forms bubbles either within the tissues (autochthonous bubbles) or in the vasculature (*1*). To explain the propensity for brain involvement among breath-hold divers, some have opined that hypoxia during the breath-hold induces recruitment of intrapulmonary arterial-venous shunts permitting venous nitrogen bubbles to spill over to the arterial circulation (*7*). This opinion has garnered substantial criticism (*8*). Among the arguments is a lack of evidence for intrapulmonary circulation microbubbles after dive activities and brain imaging demonstrating evidence for vasogenic edema versus an embolic insult (*8*). The stark elevation in risk among breath-hold divers leaves open the question of whether there may be an intrinsic CNS insult associated with high pressure exposures.

These observations highlight the need to identify additional factors beyond the popular view that gas bubbles are the principal mediator of DCS (*9-11*). Work funded by ONR over the past decade has led to the premise that one or more type of extracellular vesicle (EV) has a relationship to high pressure exposures and decompression, and to development of DCS. EVs have roles in many inflammatory responses (*12*). EVs include exosomes (30-120 nm diameter particles generated by the endosomal pathway), 0.1-1 μm microparticles (MPs) generated by an outward budding of plasma membrane, and ~0.5-5 μm apoptotic bodies generated during cell self-destruction (*13-16*). EVs are found in all body fluids and increase in association with virtually every human disease and injury. They are generated by most cell types, can be beneficial or exacerbate pathology, and exert effects due to content of nucleic acids, inflammatory mediators, and enzymes or organelles that generate free radicals (*12, 17-19*). Particle composition does not randomly reflect content of donor cells, and there has been considerable discussion over their potential utility as biomarkers (*20, 21*).

The number of blood-borne MPs double in mice and humans exposed to high gas pressure and rise further after decompression (*22-31*). Murine studies indicate that MPs, more so that exosomes or apoptotic bodies play a role in high pressure gas pathophysiology and possibly with gas bubble nucleation (*17, 30, 32-34*). In a field study

of breath-hold human divers we reported significant elevations of circulating MPs that bore no relationship to intravascular bubbles (*6*). In a mouse DCS model, MPs initiate a systemic inflammatory process related to neutrophil activation (*17, 30, 33, 34*). The pathway triggering MPs formation also activates the NOD-like receptor, pyrin containing 3 (NLRP3) inflammasome responsible for producing mature interleukin (IL)-1β (*35, 36*). Inflammasome assembly correlates with MP production in response to high pressure and the MPs containing high amounts of IL-1β are the primary factor causing diffuse vascular damage in the murine DCS model (*31, 37*). When these MPs are purified and injected into naïve mice, they cause the same spectrum of injuries as seen in decompressed mice (*30-32*).

We recently reported that human subjects exposed to air pressure equal to just 18 and 30 msw depth exhibit elevations of neutrophil- and platelet-derived MPs before decompression, as well as elevations of intra-particle IL-1β (*22*). These results fit the biochemical mechanism elucidated for gas pressure-induced MPs generation, and they establish a close parallel between animal studies and human responses (*36*). Open-water compressed air divers exhibit elevations of MPs and divers with DCS exhibit significantly more MPs that persist for longer periods of time than in healthy divers without DCS (*38*). Recent studies also suggest a biochemical basis for why some individuals may be prone to develop DCS. We reported a role for a plasma protein called gelsolin which influences decompression-associated MPs (*32*). Gelsolin is an abundant, highly conserved plasma protein component of innate immunity. However, levels change drastically in relation to physiological stresses (*39*). Plasma gelsolin levels fall in humans exposed to high pressure and in our murine model, and repletion with pharmaceutical grade recombinant human gelsolin can both protect against DCS development and abrogate tissue damage post-decompression in mice (*32*). Plasma gelsolin is currently in phase-II clinical trials for treating SARS-CoV-2 related pneumonia.

Pilot data in our murine model indicate that decompression stress shares similarities with several more common forms of CNS injuries. The brain glia-lymphatic (glymphatic) system facilitates brain fluid clearance and waste removal in association with advection along meningeal lymphatics (*40-46*). Neurocognitive deficits correlate with diminished glymphatic flow that leads to protein/waste accumulation (*47*). These changes occur with aging, traumatic brain injury, Alzheimer's disease, ischemic and hemorrhagic stroke (*48-50*). Glymphatic function is normally most active while sleeping, and function is perturbed by lack of sleep (*51*). The reverse is also true: Impaired glymphatic function perturbs sleep (*52-55*). We have found glymphatic dysfunction occurs in our murine DCS model. The relationship between glymphatic dysfunction and sleep disturbances poses a possible explanation for why humans sustaining DCS have a high risk of developing a sleep disorder (*2*). The goals of our proposal are to evaluate glymphatic function in the murine DCS model, explore the relationship between inflammatory MPs and glymphatics, and investigate whether gelsolin may abrogate adverse effects.

Naval relevance: DCS presents a health risk for compressed air divers and caisson/tunnel workers, and it poses a constraint to provocative diving and submarine escape. Bubbles play a role in pathophysiological responses mediating DCS, however, they are often produced without symptoms (*9-11*). Therefore, additional etiological factors have been sought to explain DCS development. MPs are elevated in association with simulated as well as *bona fide* underwater diving (*6, 23-29, 56*). Maneuvers which decrease the incidence of DCS in the animal model also diminish MPs production in human divers (*28, 29*). As MPs cause vascular damage and may also participate in gas bubble nucleation, their formation presents a feasible explanation for most aspects of DCS pathophysiology (*17, 30, 33, 34*). Hence, further research focused on MPs has direct relevance to U.S. Navy operational diving that is constrained by DCS risk. The only meaningful intervention that has come from decades of traditional DCS research is staged decompression tables to limit symptom development. This project will take a novel approach to identify the role of neuroinflammation and MPs in DCS and further explore the potential use of gelsolin as a prophylactic and therapeutic intervention.

Specific Aims:

(1) Assess glymphatics function in mice by MRI and fluorescence microscopy.

(2) Examine MPs elevations, astrocyte and microglial activation and evidence of neuroinflammation in mice.

(3) Assess role of glymphatic MPs outflow in glymphatic dysfunction and neuroinflammation.

2. STATEMENT OF WORK:

1.0 Background: Inflammatory MPs are generated by neutrophils due to an oxidative stress response when exposed to high pressure gases typical of compressed air diving (*36*). These MPs impinge on the vascular endothelium of the CNS and disturb the blood-brain barrier (BBB) (*34, 57-60*). Brain astrocytes have end-feet that maintain the BBB by surrounding CNS capillaries and their projections to synapses and axons influence neuronal signaling. Aquaporin 4 (AQP4) water channels are highly expressed in astrocyte end-feet and control the majority of glymphatic flow of CSF to the interstitial space (*40, 50*). This is a site where perivascular inflammatory mediators such as blood-borne MPs may influence brain glymphatics.

Astrocytes respond to oxidative stress by activating nuclear factor (NF)-κB which will trigger generation of a variety of EVs in brain, as well as proteins such as thrombospondin (TSP)-1 and -2 (*61*). TSP-1 is a neurogenic factor and TSP-2 plays a role in BBB repair (*62-64*). However, TSP-1 engagement by leukocytes will cause priming, chemotaxis, NLRP3 inflammasome formation/IL-1β production, and enhance endothelial attachment (*65, 66*). Pilot studies described below suggest that via NF-κB signaling, high gas pressure/decompression increases astrocyte-derived MPs that co-express glial fibrillary acidic protein (GFAP) and TSP-1. This presents an additional source for MPs that can have adverse effects. In other studies we have shown that a fraction of brain-derived MPs gain access to the bloodstream via glymphatic channels (*67*). We recently reported a pathological cycle initiated by MPs co-expressing GFAP and TSP-1 (*57*). Once liberated to the blood stream, these MPs interact with cerebral endothelial cell CD36 receptors to enhance astrocyte activation triggering biochemical alterations to astrocyte AQP4 and progressive neuroinflammation. Figure 1 illustrates the relatively complex relationship among cells and inflammatory mediators we hypothesize to be involved with CNS DCS development. Details of this figure will be explained further as pilot data are presented.

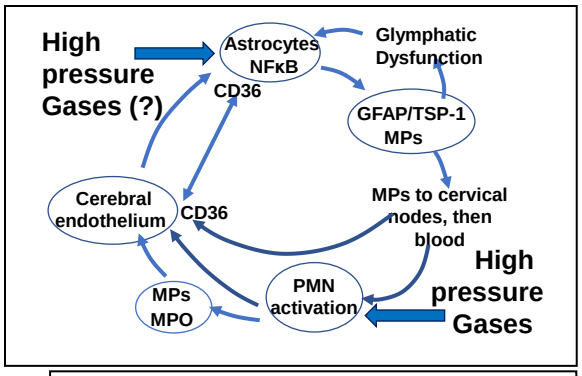


Figure 1. Mechanism schematic. MPs = microparticles MPO=myeloperoxidase.

CD36 is a class B pattern recognition receptor expressed in many tissues and found of microvascular endothelium (*68-70*). In healthy brain, CD36 is found predominantly on endothelial cells but astrocytes and microglia express CD36 over 12^{+} hours after several insults (*71, 72*). CD36 ligands include TSP-1, polyanionic lipids and lipoproteins (*71*). CD36 also binds phosphatidylserine (PS) that facilitates MPs adherence to endothelium because PS is highly expressed on the MPs surface (*73, 74*). NF-κB activation will increase CD36 expression in brain that enhances inflammatory pathways and neutrophil recruitment (*73-76*). These issues raise the prospect that either directly due to high pressure oxidative stress, or secondarily due to circulating neutrophil-derived MPs interacting with CNS endothelium, astrocytes are activated. The resulting increase in astrocyte-derived MPs appear to cause glymphatic dysfunction and exacerbate neuroinflammation. Pilot data indicate that CD36 knock-out (KO) mice are resistant to MPs-mediated adverse effects.

2.0 Scope and Objectives: This proposal is centered on examining the relationship between MPs and glymphatic dysfunction in our murine DCS model.

3.0 Requirements/Tasks: Tasks to be performed follow the sequence of specific aims.

3.1 Specific Aim 1: Assess glymphatics function in mice by MRI and fluorescence microscopy.

Preliminary data: We described our initial assessments of glymphatic function in decompressed mice in the recent ONR progress meeting. This work over the past two years was focused on establishing reliable methods. Indeed, we have now achieved these goals which include a novel here-to-fore unrecognized manner to quantitatively evaluate glymphatic function. The issues we plan to explore in this proposal include evaluating the duration of abnormalities and the molecular basis for changes due to high pressure/decompression.

Glymphatic flow is often studied by injecting contrast via the cisterna magna (*77-79*). We injected 0.5% ovalbumin (MW 45KDa) conjugated to Alexa Fluor 555 and 0.5% cadaverine (MW 0.6KDa) conjugated to Alexa Fluor 488 in artificial CSF at 2 µl/minute for 5 minutes, then incubated for 30 minutes before perfusion/fixation. Ovalbumin passes from the perivascular space to brain interstitial fluid (ISF) via clefts between astrocytes (paracellular path) versus smaller cadaverine that enters ISF via clefts and astrocytic uptake (transcellular path) (*80*). Similar to others, coronal slices (Figure 2) show cadaverine entry to be more rapid than ovalbumin. Uptake of both tracers was more rapid in mice exposed to 100 psi air pressure for 2 hours (our standard DCS model stress), seen in mice studied 60- or 90-minutes post-decompression (Figure 3). <u>This is consistent with acute inflammatory response due to high pressure/decompression.</u>

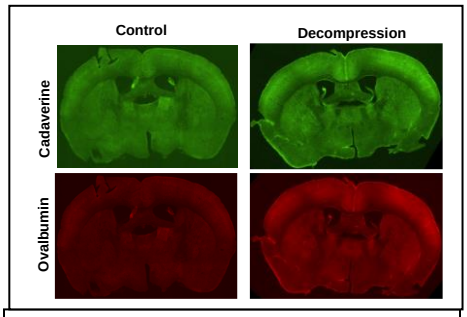


Figure 2. Coronal brain slices: Control & 30 min. post-decompression.

Glymphatic function can also be monitored by MRI with cisterna magna or IV injections of gadolinium (Gd) contrast (*81*). Unlike cisterna magna injections, the IV approach circumvents surgical procedures and alterations of intracranial pressure, but quantification of changes has not been standardized. We developed a novel, quantitative method taking advantage of the direction of flow. Glymphatic channels run adjacent to major veins, then empty into the sagittal sinus and ultimately via meningeal lymphatics to the deep cervical lymph nodes (*82*). We quantified flow as the difference in peak Gd signals at these two sites normalized by including a 60 mM Gd vial in scans. Phantom-normalized data were most robust near the Vein of Galan and relative rate of flow evaluated by comparing a 1 mm region of interest (ROI) to that at the deep cervical nodes (see Fig 4).

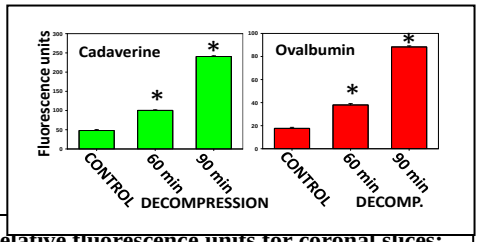


Figure 3. Relative fluorescence units for coronal slices: Control, 30 and 90 minutes post-decompression. Values are mean ± SE, n=3 all groups; * p<0.05.

Images were obtained in the University Core Facility Bruker BioSpec 70/30USR Avance III 7 Tesla horizontal bore MR scanner. The system is equipped with a BGA12S gradient system and interfaced to a Paravision 6.0 console. A 72 mm Bruker linear-volume radio frequency (RF) coil was used as a transmitter and a four-element ^{1}H RF array coil served as a receiver. Mice were anesthetized and maintained with 1.75% isoflurane. A MR compatible small-animal gating system was used to monitor respiration and maintain body temperature at 35-37°C. After initial calibration and T_2-weighted anatomic acquisitions, paramagnetic contrast (gadodiamide, Gd, 0.15 mmol (300µl) per 20 g mouse was injected IV at 50 µl/min and a series of 5 minute 3D T_1-weighted fast low angle shot acquisitions were obtained in the sagittal view (TR-13.455 ms, TE=3.82 ms, flip angle = 25^{0}, average=4, field of view = 18 x 18 x18 mm^{3},

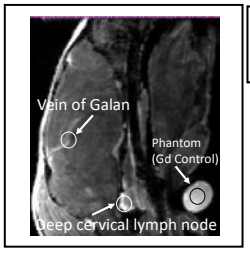


Figure 4. MRI with 1mm ROIs.

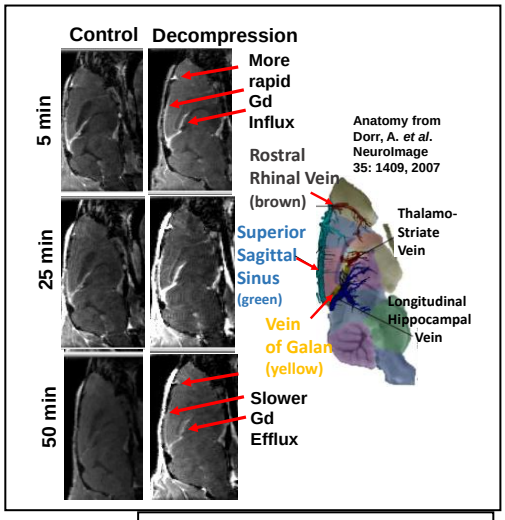


Figure 5. Brain MRI images.

imaging resolution = 0.15 x 0.15 x 0.40 mm^3, scanning time=5 min). Image processing consisted of head motion correction and voxel-by-voxel conversion to % baseline signal vs the 60 mM Gd phantom. Images in control and decompressed mice are shown in Figure 5.

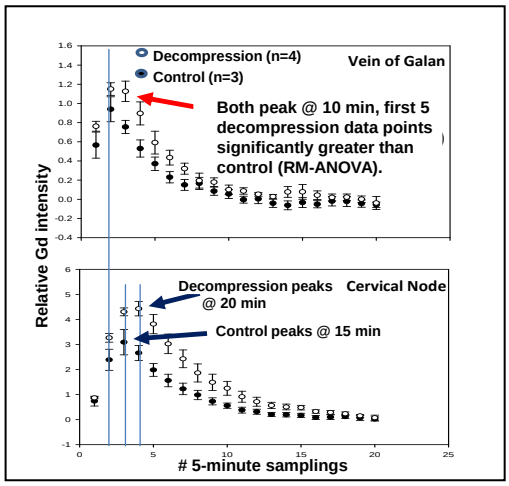


Figure 6. Normalized glymphatic flow. More rapid influx, but slower efflux seen in decompressed mice.

Quantitative data (Figure 6) demonstrated a rapid signal increase (influx) in decompressed mice after Gd infusion consistent with acute neuroinflammation, and slower efflux suggesting glymphatic dysfunction. Further, these glymphatic abnormalities are the same even when mice were studied at 1-or 2- days post-decompression. Hence, glymphatic dysfunction appears to be protracted. We plan to investigate the duration of dysfunction and identify the mechanism for the changes.

We have established the central role of MPs in several pathological processes by recapitulating inflammatory insults with injections of purified MPs from exposed mice that fail to occur using MPs from control mice (*34*, *57-60*). The same heightened Gd influx signal was seen when 60,000 blood-borne MPs isolated from a decompressed mouse were injected into a wild type mouse. These studied then advanced to using more specific sub-groups of MPs.

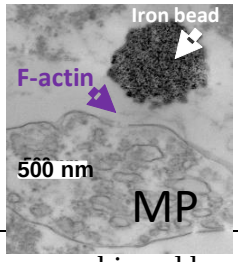


Figure 7. Electron microscope image of a F-actin positive MP extracted with a phalloidin-conjugated iron bead.

We recently demonstrating that the decompression-induced MPs carrying IL-1β and causing vascular damage expressed filamentous (F-) actin on the particle surface (*32*). We separated F-actin-positive versus -negative MPs obtained from decompressed mice. This was achieved by taking advantage of the ability of phalloidin to bind to F-actin. A suspension of MPs was incubated with phalloidin conjugated to 0.5 µm iron beads so that F-actin-positive MPs could be separated from F-actin negative MPs using a magnet. An electron micrograph of a F-actin positive MP is shown in Figure 7.

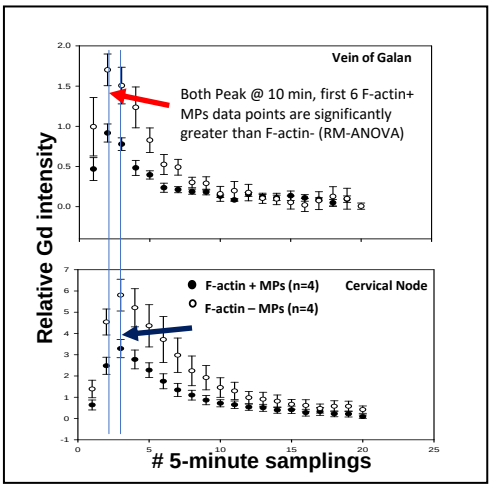


Figure 8. Glymphatic flow elevation caused by F-actin positive but not F-actin negative MPs infusions.

When separate forms of MPs were injected into naïve mice, we found that only the F-actin positive MPs caused glymphatic abnormalities (Figure 8). These data therefore indicate that blood-borne F-actin expressing MPs mediate CNS glymphatic flow changes.

With regard to the discussion above related to plasma gelsolin, one function of this protein is to depolymerize F-actin. We reported that repletion of plasma gelsolin in decompressed mice protects against vascular damage elicited by MPs due to lysis of F-actin positive MPs (*32*). In preliminary studies, we found that injection of recombinant human gelsolin abrogated glymphatic abnormalities in decompressed mice, consistent with its protective role in our published study.

Finally, above we discussed our hypothesis that CD36 receptors play a role in decompression pathophysiology. The possible roles for CD36 include functioning as a membrane receptor for circulating MPs on vascular endothelium, as a signaling molecule involved with transmissions from the vascular endothelium to astrocytes in the brain, and/or with coordinating astrocyte responses within the CNS. We have initiated a series of studies

with knock-out (KO) mice lacking the CD36 molecule. Figure 9 shows that CD36 KO mice do not show glymphatic changes when subjected to stresses imposed by 100 psi for 2 hours.

***Aim 3.1.1 Glymphatic study plans*:** We will carry on studies assessing glymphatic flow changes due to high pressure/decompression based on cisterna magna injections and Gd-MRI injection. In cohorts of mice (n=8/sampling time) we will monitor glymphatic function and also evaluate the duration of changes on a daily basis following the 100 psi air X 2 hour decompression stress until changes dissipate. We also plan to do a dose-response study evaluating changes induced by less provocative decompression. In prior work we have shown that lower pressure exposures also cause MPs elevations, although to a lesser extent than does 100 psi for 2 hours (*30*).

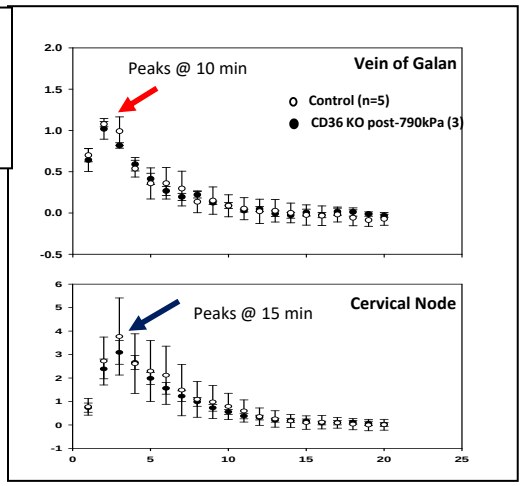


Figure 9. Glymphatic flow changes are not observed in CD36 KO mice studied at 90 minutes post decompression from 100 psi air pressure for 2 hours.

Studies will also be done to examine the effects of F-actin positive MPs more closely. Note that we do not see a slower gadolinium signal efflux in MPs-injected mice as we do when mice are exposed to the high-pressure stress (Fig 8 versus Fig 6). We hypothesize this may be related to a primary effect of high gas pressure on astrocytes in addition to effects linked to blood-borne MPs. Studies will investigate these responses by looking at effects of varying doses of F-actin positive versus F-actin negative MPs and also gelsolin administration. The gelsolin studies may prove especially important as we can discern effects when it is administered as a prophylactic agent prior to pressure, as well as a treatment post-decompression. Additional aspects of this work will be described in Specific Aim 2 & 3 (see sections 3.2 and 3.3).

Aim 3.1.2. We also plan to incorporate proton magnetic resonance spectroscopy (^{1}H-MRS) to evaluate brain metabolic changes due to high pressure/decompression. This non-invasive approach will allow correlations with glymphatic flow and MPs data looking at brain regions. We recognize the technical challenge to this approach, as some brain regions are too small to be studied discretely. However, we have published experience averaging MRS signal over a ~3 mm^3 area, and have successfully modified protocols to evaluate metabolic changes discrete to some mouse neuroanatomical structures [*e.g.* hippocampus (1.5 x 6.0 x 1.5 mm^3) and thalamus (2 x 5.5 x 1.5 mm^3)] (*83*). Despite the resolution limits, we anticipate that comparisons across mouse cohorts will allow us to determine whether regional differences and discrepancies arise that may be linked to neuroinflammatory events. Studies will be performed over a 45-minute time frame prior to Gd infusion (15-minute calibration, 30-minute acquisition).

Anticipated Results*:* We anticipate that glymphatic dysfunction due to pressure/decompression will be borne out with replicate studies. Work assessing regional glymphatic changes in combination with metabolic (^{1}H-MRS) studies will provide added insight. The spectroscopy studies will enhance understanding because we can evaluate heterogeneity in metabolic responses among brain centers that may provide better understanding of pressure and neuroinflammatory effects. The MPs injections studies are anticipated to support a primary pressure effect on brain glymphatic function that will be further strengthened with studies described below. The gelsolin studies will also expand our data base on its potential as a DCS therapeutic agent.

Future steps & potential pitfalls: We have experience with methods proposed in this specific aim, so technical issues are not anticipated. Should alternative procedures seem advantageous, we can expand studies to include cranial window placement and intracisternal or intraparenchymal contrast injection coupled with high resolution 2-photon imaging, intra-parenchymal Gd injection/MRI and whole-brain near-infrared fluorescence imaging (needed equipment and technical expertise are available in University Core Resources) (*84-86*). Finally, the

University is just finishing installation of a 9.4 T small animal MRI. Future studies can incorporate functional MRI and diffusion tensor imaging. This has translational value, as these non-invasive techniques are being pioneered for clinical glymphatic assessment (*87*). We also need to mention that while the results highlight MPs, inflammatory mediators are also carried by exosomes (some carry CD36) (*88*). Inflammatory MPs can be separated from soluble mediators and exosomes by centrifuging plasma at 21,000g (*56*). Therefore, as need arises, we can insert steps to assess whether alternative mediators play roles.

3.2 Specific Aim 2: Examine MPs elevations, astrocyte and microglial activation and evidence of neuroinflammation in mice.

Table 1: MPs in blood & node groups	Total MPs (#/µl)	% GFAP plus TSP-1	%TMEM	% Ly6G
Blood(B)-Control (n=8)	452±43	3±1	1.5±0.2	1.6±0.3
Deco/WT (n=6)	2186±134**	36±8**	13.9±2.7**	13.1±2.0**
CD36KO/Control (n=6)	604±29	6±2	1.1±0.2	1.8±0.5
Deco/36KO (n=6)	554±39	4±2	1.6±0.2	1.3±0.4
Node(N)-Control	1568±123	2.1±0.2	14.7±3.7	nd(none)
Deco/WT(n=6)	4318±282**	21.4±4.2**	20.2±4.9**	nd
CD36KO/Control (n=6)	1416±59	2.9±1.6	13.9±1.3	nd
Deco/CD36KO (n=6)	1215±33	3.9±1.1	10.2±1.8	nd

Table 1. Blood and node MPs. Cell sources of MPs were identified by cell-specific protein expression using flow cytometry. Fluorophores (6) are used in each assay with fluorescence compensation measures to assess individual proteins and presence of multiple proteins on individual MPs. We probe Ly6G (neutrophils), TMEM119 (microglia), GFAP (astrocytes), TSP-1, MBP (oligodendroglia), NPR (neurons) and podoplanin+CD31 (lymphatics). Those statistically significantly different from control are shown (p<0.05 ANOVA).**

Preliminary data: Table 1 shows MPs values in mice euthanized 90 minutes after exposure to 100 psi for 2 hours. The analysis followed published methods (*34, 57-60, 67*) with an equal number of male and female mice. The top rows in red are MPs in blood, those below are deep cervical lymph node MPs. We have published extensively on blood-borne MPs elevations related to high pressure/decompression (*34, 57-60*). Elevations of MPs in blood expressing proteins specific to neutrophils (Ly6G expression) is consistent with prior reports. Notably, in recent studies we reported that some stresses result in elevations of MPs in the deep cervical nodes that drain brain glymphatics and some of these MPs expressing CNS-specific proteins gain access to the blood (*67*). Statistically significant elevations of MPs expressing astrocyte, microglial and neutrophil-specific proteins were found in wild type (WT) C57BL/6 mice post-decompression, but this did not occur with CD36 KO mice.

Table 2	MPO	CD18
Control (8)	1.6±0.2	2.9±0.9
Deco/WT (8)	4.9±1.2**	14.4±2.1**
Deco/CD36KO (8)	1.1±0.8	3.3±1.1

Table 2. Neutrophil activation in whole blood assessed when labeled by incubation with fluorophore-conjugated antibody to Ly6G and cell activation assessed by flow cytometry as co-expression of CD18 and MPO on the cell surface following published methods (**p<0.05, ANOVA).

Neutrophil activation: We have reported that neutrophil activation with MPs production occurs in relation to pressure/decompression (*34, 57-60*). Table 2 shows that neutrophil activation does not occur in CD36 KO mice post-decompression reflected as an increase in CD18 (part of the β_2 integrin) expression and myeloperoxidase (MPO) as occurs with degranulation.

Table 3: Mouse groups: Blood & node MPs	Total MPs (#/µl)	% GFAP plus TSP-1	%TMEM	% Ly6G
IV control MPs(n=8)	619±36	3±1	1.5±0.2	1.6±0.3
Deco MPs (n=8)	2173±62**	36±2**	12.1±1.7**	16.7±2.0**
Deco MPs+ TSP IgG (3)	448±72	3±2	1.6±0.2	1.3±0.4
IV control MPs(n=8)	1767±89	2.1±0.2	14.7±3.7	nd(none)
Deco MPs (n=8)	4618±82**	25.4±1.2**	22.2±4.4**	nd
Deco MPs+ TSP IgG (3)	1915±99	2.9±1.5	9.2±1.2	nd

Effects of MPs injections: Mice were injected with 60,000 MPs isolated from blood of control mice or from mice exposed to 100 psi for 2 hours. MPs elevations in those injected with MPs from decompressed mice, but not control mice (see Table 3) were similar to those exposed to pressure in Table 1. In a recent study we reported IgG blocking antibodies to TSP-1 abrogates the adverse effects of GFAP-TSP-1 expressing MPs (*57*). When TSP-1 IgG (0.3 µg) were combined with decompression MPs prior to injection, MPs elevations were not observed. Hence, data suggest that CNS-derived MPs co-expressing GFAP/TSP-1 are responsible for MPs elevations in cervical nodes and blood. Note that this poses the possibility that brain-to-blood particle transmission exacerbates systemic as well as intra-CNS pathology.

Evidence for neuroinflammation post-decompression: A recognized astrocyte inflammatory response is NF-κB activation. NF-κB activation triggers transcription of proteins including TSP-1 and a variety of extracellular vesicles (*61*). We used immunocytochemistry to evaluate the cells expressing NF-κB in a decompressed mouse. Compared to control, cortical astrocytes prominently displayed greater GFAP and NF-κB p65 expression, and also NF-κB p65 nuclear co-localization (Figure 10).

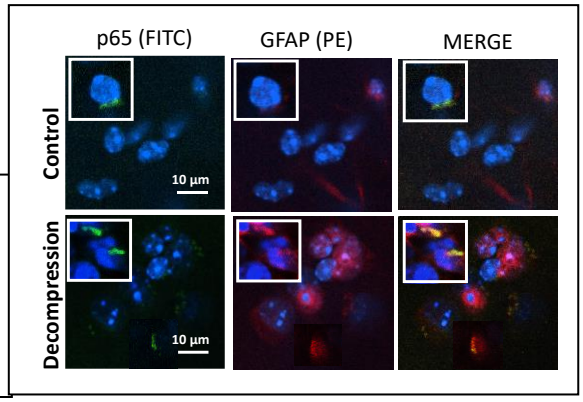


Figure 10. Representative images from cerebral cortex of a control and a decompressed mouse. Sections were stained with antibodies to GFAP (red), NF-κB p65 protein (green) and nuclei using DAPI (blue). The main images are at 60x magnification with the insert as 120x to better show p65 colocalization with nuclei.

We also prepared Western blots (Figure 11) of brain fractions enriched for endothelium and adjacent cells including astrocytes (*25, 89*) where, compared to control (lane 1), decompressed mice (lane 2) had elevations of the p65 subunit of NF-κB, serine 536 phosphorylated (Phospho-Ser^{536}) p65 NF-κB, Ly6G, MPO and CD36, and loss of AQP4 recognition using an antibody directed to its C-terminal. Note that alteration of C-terminal AQP4 recognition is reported by others in relation to glymphatic dysfunction (*90, 91*). Injections into naïve mice of MPs (lane 4) from a decompressed mouse resulted in similar protein changes but changes did not occur if MPs were first incubated with anti-TSP-1 blocking antibodies (lane 3). No changes occurred in CD36 KO decompressed mice (lane 5). Therefore, data indicate that decompression triggers extensive neuroinflammatory changes linked to CD36 and that MPs expressing TSP-1 are responsible for neuroimmune responses.

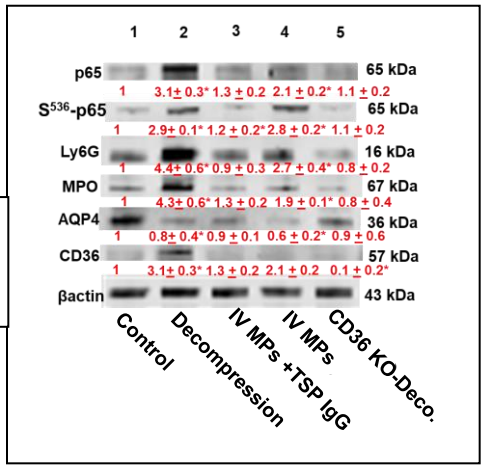
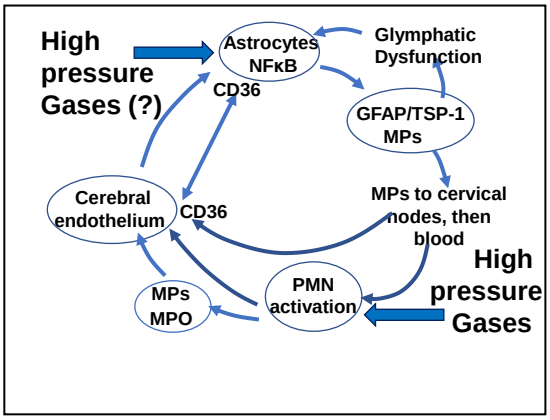


Figure 11. Western blots of brain homogenates. The numbers indicate mean ± SE band densities relative to the densities of control samples from replicate studies (n=3). *p<0.05 ANOVA

Summary: Data indicate that high pressure/decompression triggers elevations of MPs co-expressing astrocyte-specific GFAP and TSP-1 in deep cervical nodes that drain the glymphatic system and MPs gain access to blood. We previously demonstrated that gas pressure will also activate neutrophils to generate MPs, so there now is question as to whether intra-CNS MPs or those generated in blood pose the greatest risk for inflammatory changes related to pressure. High pressure/ decompression also perturbs glymphatic flow. Glymphatic flow changes do not occur if MPs production is inhibited (CD36KO mice) or if wild type mice receive TSP-1 blocking antibodies. These observations can be reconciled if the TSP-1 bearing MPs in blood interact with cerebral endothelial CD36, a process that in other forms of brain injury will exacerbate inflammation due to NF-κB mediated crosstalk with astrocytes (*57, 73-76*). In earlier studies, we reported that neutropenic mice do not exhibit MPs elevations after high pressure/decompression. Neutrophils will generate MPs in response to stimulation and these MPs carry myeloperoxidase (MPO) (*92*). There are multiple TSP-1 receptors on neutrophils in addition to CD36, but absence of activation in KO mice (shown in Table 2) may be explained because CD36 also plays a role in cytoskeletal control and Toll-like receptor signaling (*68, 93-95*). The relative excess MPO band density versus Ly6G in brain Western blots (Figure 11) is suggestive that neutrophil MPs are being sequestered in brain as well as activated neutrophils (supported by the Ly6G elevation). All data can be



reconciled by a cyclical inflammatory process depicted in Figure 1. Our data suggest that high pressure/decompression triggers astrocyte-derived MPs expressing TSP-1, and that neutrophil derived MPs impinge on brain vasculature to stimulate astrocyte NF-κB. Studies proposed in this project should discern which is the initial event but ultimately it appears that provocative exposure to high pressure/decompression establishes a feed-forward neuroinflammatory cycle involving endothelial CD36-to-astrocyte NF-κB crosstalk. Our pilot studies need further development both to discern pathophysiological relationships and to identify interventions that can abrogate these events.

Aim 3.2.1 We plan to evaluate blood and cervical lymph node MPs and blood borne neutrophil activation in mice studied in Aim 1 to evaluate the duration of neuroinflammatory events. We will use equal numbers of male and female mice. Astrocyte activation in response to some stimuli differ due to sex. For example, some stresses increases hippocampal kynurenic acid (KYNA) levels significantly, but only in males (*96*). Astrocytes are the primary source of KYNA, KYNA reduces the release of neurotransmitters such as dopamine and glutamate and can antagonize some responses (*97, 98*). If there is a high degree of variability in the female mice (*e.g.* due to changing hormonal status), studies will be repeated with female mice tracked for stage of estrus and additional studies on effects of ovarian hormones can be explored following published techniques (*99, 100*).

Aim 3.2.2 We will evaluate neuroinflammation using immunocytochemical techniques. Cell activation will be based on morphology, phospho-Ser^{536}p65 NF-kB and nuclear NF-kB (p65) colocalization in astrocytes (AQP-4 and GFAP staining), microglia (TMEM and CD68 staining), oligodendroglia (native MBP and degraded MBP) and neurons (NPR) following our published methods (*60, 101*). We will also evaluate viability by the TUNEL assay (*101, 102*). The latter addition will determine whether cell death is associated with MPs production and inflammatory changes.

These studies will be further supported by Western blot analysis as described in Figure 11. In addition to the proteins probed in pilot work, we will evaluate content of other proteins associated with NF-kB activity (*e.g.* p50, IkBα) and a closer evaluation of cytosolic p65 versus nuclear localization after cell component separation. An added aspect to this effort will include analysis of inflammatory mediators in blood-borne and cervical node MPs. Inflammatory mediators such as IL-1β damage lymphatic channels and can impair glymphatic solute clearance (*48-50, 103-105*). The effort will include assessments of particle TSP-1, MPO, IL-1β, IL-6, TNFα as we have described in prior studies, and proteins associated with NF-kB regulation [we have found inhibitor of κB kinase (IKK) -β and -γ in MPs] (*16, 56, 67, 106, 107*). Results may lead to added studies because of non-genomic actions for IKK-β (*108*).

After baseline studies, we plan to evaluate glymphatic, neuroinflammatory and blood-related changes in mice injected with gelsolin and also examine effects in neutropenic mice. The gelsolin studies will follow our published techniques using it as both a prophylactic intervention as well as treatment post-decompression (*32*). We anticipated these studies will allow assessment of how much of the post-decompression MPs elevations and cell activation arise due to TSP-1 MPs generated in brain versus the Ly6G MPs generated by neutrophils in blood.

Aim 3.2.3 We plan to characterize the biochemical changes that result in loss of Western blot AQP4 antibody recognition caused by pressure/decompression MPs, as seen in Figure 11. Others have reported neuropathological effects and glymphatic disturbances associated with changes in the C-terminal portion of AQP4 that impairs antibody recognition due to conjugation with glutamate transporter-1 (GLT-1) or other proteins (*90, 91*). We used antibody to C-terminal portion of AQP4 in pilot studies, suggesting that neuroinflammation due to injection of MPs triggers similar AQP4 biochemical changes. We plan to investigate the basis of MPs-induced AQP4 changes by first carrying out Western blots using alternative AQP4 antibodies to epitopes distant from the C-terminus, a strategy described by Wang, *et al* (*90*). We will also

immunoprecipitate AQP4 with these antibodies and probe for protein modifications by mass spectroscopy, a method we have used frequently in published reports (*109-112*).

***Anticipated Results**:* The immunohistochemical and biochemical efforts will provide information on the sequence of cell activation. Should TSP-1 IgG fail to be as effective for block MPs responses as suggested in pilot studies, work from 3.2.2 looking at alternative inflammatory mediators may point the way for identifying alternative inflammatory pathways. In future we can also investigate which alternative cell source(s) of MPs are responsible for neuropathology (*e.g*. specifically isolate GFAP, TSP-1or Ly6G MPs). This is achieved by isolating specific sub-sets of MPs based on surface protein expression for injection following our published methods (*56, 60, 67, 106, 113, 114*). Protein and lipidomic analysis of MPs can also broadly assess inflammatory mediators in future studies (*115, 116*).

***Future steps & potential pitfalls**:* We have experience with virtually all aspects of the proposed work so technical issues are not anticipated. We are cognizant, however, that there may be still more physiological stresses related to high pressure/decompression and Navy activities pertinent to diving. Should time allow, we are interested to examine the roles for some stresses in relation to inflammatory MPs and glymphatic function. Moderate hypercapnia was reported to inhibit glymphatic function (*117*). Arterial CO_2 values may rise by 3-4 mmHg during SCUBA diving because of apparatus dead space, gas density and slowed respiration related to an elevated O_2 partial pressure. These CO_2 values are comparable to those resulting from breathing ~4000 ppm CO_2, which is a level below the hyperventilation threshold (*118-120*). This issue also has pertinence to the environment within submarines where, because of engineering issues with CO_2 scrubbers, levels in submarines (also in spacecraft) may rise for varying intervals of time to 4000 to 7000 ppm (*121, 122*). We reported that due to enhanced carboxylation reactions, 1000 to 4000 ppm CO_2 triggers human and murine neutrophils to generate MPs containing IL-1β (*16*). Mice exposed to these CO_2 levels for 2 hours generate IL-1β containing MPs that cause vascular damage (*60*). Assessing TSP-1/GFAP MPs elevations due to hypercarbia +/- pressure exposures such as 100 psi for 2 hours may identify a mechanistic link for several concurrent physiological changes seen with diving activities.

Similar concerns may arise due to hypoxia and hypercarbia as occurs in breath-hold divers and may contribute to their heightened risk for CNS DCS. End tidal gas tension measurements among some divers have documented levels as low as 20-25 mmHg O_2 and concurrent CO_2 elevations close to values mentioned above (*123, 124*). Transient O_2 reductions to even just ~50-60 mmHg will trigger MPs production (*125, 126*). Assessing whether these stresses (hypoxia, hypercarbia +/- elevated inert gas pressure) have heightened adverse effects on glymphatic function and neuroinflammation are achievable within the current research plan.

3.3 Specific Aim 3: Assess role of glymphatic MPs outflow in glymphatic dysfunction and neuroinflammation. We hypothesize the CNS MPs liberated to blood after high pressure/decompression contribute to establishing a feed-forward neuroinflammatory process that causes CNS glymphatic dysfunction as shown in Figure 1. Intracerebral MPs gain access to the peripheral circulation and exacerbate neuroinflammation after several insults (*34, 57, 127, 128*). Several neuropathological models have demonstrated that obstructing glymphatic/lymphatic outflow abrogates CNS immune responses (*129-134*). We plan to use similar techniques to assess the role of CNS-generated MPs that gain access to the peripheral circulation. Note that single injections of decompression-induced MPs, as were described in pilot data and used in past published studies, can document acute responses. However, because these injected particles are typically cleared from the circulation in minutes to hours, the work cannot recapitulate all events that arise over many hours to days after decompression.

Aim 3.3.1 We will directly evaluate the role of glymphatic efflux using methods described by others in published studies (*133*). The superficial and deep cervical lymph nodes are readily identified, as we have now performed extensively (*57, 67*). Channels will be ligated, and nodes removed in parallel with sham-operated

controls. One week later after surgical recovery, mice will be subjected to 100 psi for 2 hours (and possibly other pressure profiles depending on results from Aim 1). We hypothesize that after lymphadenectomy (versus sham) decompression will not cause efflux of CNS-derived MPs to blood or neuroinflammation. Moreover, because compensatory responses to lymphatic disruption allow for continued efflux of CSF and interstitial fluid (*45*), we anticipate that pressure/decompression will not trigger the same disturbances of glymphatic function assessed with Gd-MRI and cisterna magna contrast injection studies as occur in intact, sham-operated mice.

Aim 3.2.2 An additional focus for this Aim is to determine the role of CD36 in completing the cycle of neuroinflammation. Preliminary data lead us to hypothesize that CD36 plays a key role in neuroinflammation because CD36 KO mice do not exhibit MPs elevations in response to pressure or to injections of purified MPs. The initial question we plan to address is whether CD36 is responsible for MPs attachment to cerebral endothelium, something suggested in experiments by others (*73*). Studies will assess whether endothelial CD36 facilitates MPs adherence to microvasculature using confocal imaging of IV injected fluorescent-labeled MPs. Details are as described in (*135*). Purified blood-borne MPs from control and decompressed mice will be labeled with 5-(and 6)-carboxyfluorescein diacetate, succinimidyl ester (CFSE) and injected into wild type mice without and with CD36 blocking antibodies (*76, 88*). Recipient mice will be euthanized, brains sectioned, and we anticipate that by comparing retention without versus with CD36 antibodies we can discern if the CD36 receptor is responsible for MPs adherence to the endothelium. However, a null result (no difference between groups) will tell us that the CD36 role is in cell signaling responses rather than initial particle attachment. Obviously, cell signaling may still be involved which will be addresses in a follow-up approach.

Cell signaling could involve CD36 on endothelium or on activated astrocytes. As was mentioned in Background, CD36 is normally expressed in cerebral endothelium but becomes expressed on glial cells after a brain insult (*71, 72*). We plan to address this question with conditional knock-out mice to separate CD36 roles in endothelium versus astrocytes. Mice will be obtained from Jackson Labs containing a floxed CD36 allele that can be intercrossed with GFAP-Cre or Tie2-Cre transgenic mice to generate mice with CD36-deficient astrocytes (astrocyte KO) or endothelial cells (endothelial KO), respectively. The steps necessary to establish these mice are well described and we do not anticipate technical problems, as others have done similar studies assessing the role of C-C chemokine ligand 2 in neuroinflammation (*136*). Prior to mouse use we will verify the efficiency and specificity of CD36 gene deletions by standard molecular biological methods using the University Genomics Core. Astrocyte or endothelial KOs will then be evaluated for MPs production, glymphatic flow changes, and neuroinflammation in response to decompression stresses and to injections of isolated MPs from decompressed mice.

Anticipated results*:* Technical issues are not likely to pose obstacles as others have achieved meaningful results with the mice manipulated as we propose in this Specific Aim. Work with CD36 KO mice will contribute to understanding of how inflammatory signals are transmitted into the CNS. This may have pertinence to many disorders where blood-borne MPs have a role in brain pathology (*34, 57, 58, 67, 127, 128, 137-139*).

Future steps & potential pitfalls*:* Lymphadenectomy poses a stress and can trigger functional deficits (*140*). Therefore, if complications develop in the studies planned in ***3.3.1*** we can take an alternative approach to improve clarity of mechanisms. Studies can be done using transgenic *K14-VEGFR3-Ig* mice obtained from Jackson Labs. They express a soluble VEGF-C/D trap protein that inhibits lymphangiogenesis (*141*). Lymphatic vessels are absent from the skull, thus inhibiting glymphatic drainage (*45*). We hypothesize post-decompression they will not show MPs with CNS proteins in blood or cervical nodes (nodes are still present in these mice), neuroinflammation, or glymphatic flow disturbances compared to control mice.

Statistical considerations for the entire proposal: Data will be expressed as mean $\pm$ SE. All assays will provide continuous variables. We will assess for normality; for those not satisfying this assumption, we will attempt to find appropriate transformations. If these are insufficient, non-parametric analyses will be performed.

Univariate analysis will consist of comparison of variables (*e.g.* MPs number) across each decompression pattern (Aim 1) using a t-test (or its non-parametric analog, the Wilcoxon rank sum test). Evaluating the question of a glymphatic flow changes will be done using a two-way analysis of variance. We may need to employ longitudinal analyses to assess effects of time and possible interactions between decompression severity and time. We anticipate that an N=8 per group will be adequate to arrive at valid conclusions based on power calculations using variability observed in preliminary data.

4.0 Deliverables: Progress will be reported annually to the ONR project officer and at the annual progress meeting. Peer review publications are anticipated, possibly several over the course of this project.

3. PROJECT SCHEDULE & MILESTONES:

The mice used in plans for Specific aims 1 and 2 overlap and we anticipate the goals for these two aims can be achieved within 1.5 to 2 years. Specific aim 3 goals are also likely to require a year and can be achieved by the end of this 3-year proposal.

4. MANAGEMENT APPROACH:

This is a basic science project to be conducted in the laboratory of Dr. Thom. Animal exposures and manipulations will be performed on a day-to-day basis under the direct supervision of Dr. Thom. Deepa Walia will devote full time to this project. Dr. Su Xu is a recognized expert in MRI neuroimaging and she will devote 10% effort.

Personnel:

Stephen R. Thom, M.D., Ph.D. (Principal Investigator): Dr. Thom will devote 15 % effort. He will be responsible for the overall supervision of the project, participate in the primary flow cytometry and immunohistochemical analyses, interpret all data, write all reports and compose all peer-reviewed publications.

(b) (6) [redacted], co-investigator, will devote 10 % effort to this project. She will apply her expertise with magnetic resonance imaging (MRI) and spectroscopy (MRS) to the challenge of obtaining reliable, quantifiable *in vivo* data using the murine model to evaluate the glymphatic system. She will also participate in data analysis and preparation of manuscripts with the PI.

(b) (6) [redacted], research associate, will devote 100 % effort to this project under Dr. Thom's supervision. She is highly experienced in many aspects of the project as she has been employed in the Thom lab for 6 years. She will have day-to-day responsibility for flow cytometry analysis of blood and lymph node samples for microparticles, animal surgery and coordinating glymphatic MRI studiers.

Facilities:

Equipment in the Thom Facilities: Low temperature freezer (-20°C), Spectrophotometers (Varian Cary 50 Bio UV/Vis and Coleman Lambda 1 single beam), fluorescent spectrometer (Perkin Elmer L550B) and plate reader-incubator (BioTek Synergy HT), centrifuges (Beckman TL-100 ultra, Sorvall WX Ultra 80, Sorvall Legend Micro 21R, Beckman TJ-6R, Eppendorf 541C), scintillation counter (Perkin Elmer Tri Carb 2900), Odyssey CLx infrared blot/gel/plate imager, cell counter (Coulter, ZM), Miltenyi MACSQuant three-laser bench top flow cytometer.

Microscopic imaging: The University of Maryland Core Imaging Facility has a Nikon epifluorescence microscope with computer-controlled filter wheel and video analog-to-digital conversion board in a linked IBM PC, a Biorad MRC 600 confocal microscope, a Hitachi transmission electron microscope and an ultra-microtome.

Flow Cytometry & Cell Sorting Shared Facility: The University of Maryland Core facility has four FACSCalibur Flow Cytometers, One LSR II flow Cytometer (12-parameter, 10-color, 4-laser) with digital analyzer, two FACSCiVa 10-parameter, 8-color, 3-laser high-speed digital cell sorters.

5. QUALIFICATIONS:

Dr. Thom holds both M.D and Ph.D. degrees and is actively involved with basic and applied research, as well as clinical medicine. He is board certified in Emergency Medicine as well as Undersea and Hyperbaric Medicine. In addition to playing a role in medical education he provides hands-on care to patients in the Emergency Department. His thesis leading to a PhD degree in microbial physiology focused on oxidative stress effects caused by gas and hydrostatic pressure. Thus, he has studied high pressure effects for his entire professional career. He has been publishing peer reviewed papers on hydrostatic pressure and hyperbaric gases since 1981, as is reflected in his CV (attached).

Dr. (b) (6) has over 26 years of expertise in the area of magnetic resonance imaging (MRI). She has applied advanced MR imaging techniques to animal models and clinical applications at the University of Maryland, NIH, Memorial Sloan-Kettering Cancer Center, and published 80 peer-reviewed original journal papers. She will provide her expertise in MR experiment design, optimization, and acquisition.

6. PENDING & RELATED PROPOSALS:

Current funding:

2023/06/19 – 2020/06/18
N00014-20-1-2641, Office of Naval Research
Thom, Stephen (PI)
Microparticles, inflammation and decompression sickness
This project will evaluate inflammatory microparticles in a murine model and human divers suffering from decompression sickness.
Role: PI

2019/10/25-2021/09/30
N00014-20-1-2000, Office of Naval Research
Thom, Stephen (PI)
Glymphatic function in extreme environments.
This project will evaluate brain glymphatic function in parallel mouse and human studies in response to hypoxia, hyperoxia, hypercapnia and high pressure/decompression.
Role: PI

2018/06/01-2022/05/31
R01 DK116199
(b) (6) [redacted] (co-PIs)
NOS1AP and capon associated impairment in those with diabetic foot ulcers.
The project focuses on producing further evidence to confirm the association of *NOS1AP* (nitric oxide synthase-1 adaptor protein) variation with impaired healing and discerning the functional basis of the genetic variation on capon and ultimately wound repair in those with diabetes.
Role: Co-PI

2020/06/22-2023/06/21
N00014-20-1-2593
(b) (6) [redacted] (PI)

Harnessing splenic adaptations to improve diving capabilities
The project are to evaluate adaptive responses of the spleen that may be important to improve diving capabilities by augmenting pulmonary gas exchange and oxygen delivery, lessening the risk of CO_2 toxicity, and/or alleviating the risk of decompression sickness.
Role: Co-I

2020/07/01-2022/06/30
Breath-Hold Diving: Mechanisms of Hypoxemia and Decompression Stress
The project studies breath-hold divers to verify arterial blood gases' changes at depth, detect breath-hold diving related hypoxia due to atelectasis, detect pulmonary mild interstitial edema, and test the hypothesis that an inflammatory cascade and platelet function are linked, impaired in divers and bear a relationship to cardiovascular risk.
Role: Co-I

Dr. Thom has submitted a NIH R01 proposal to evaluate inflammatory responses leading to neurological damage from carbon monoxide poisoning. He also has submitted a proposal to ONR to evaluate extracellular vesicles in human perspiration.

7. RELEVANT EXPERIENCE:

Dr. Thom has held grants that complement the focus of this application:
1. Office of Naval Research (Thom, PI): Microparticles, platelet-neutrophil aggregation and decompression sickness. N000141310613 and N000141612868

2. Office of Naval Research (Thom, PI): Pharmacological inhibition of neutrophil adherence using nitric oxide. N000141410094

3. Office of Naval Research (Thom, PI): Microparticle Production with Decompression Stress. N000141310614

4. NIH R01 DK116199 ([redacted], co-PIs) 6/01/2018 – 5/31/2022: NOS1AP and capon associated impaired healing in those with diabetic foot ulcers. This project defines variations in circulating stem cells in diabetics and migration to wound margins as the mechanism for variations in propensity to heal.

FACILITIES AND RESOURCES

Laboratory: Dr. Thom has a 1,200 square foot newly renovated laboratory space with standard facilities and fume hood equipped with monitor to sample the air surrounding the hood for any CO contamination.

Clinical: Blood samples will be collected and sent to the Thom lab for analysis. There will also be phlebotomy on volunteers to obtain blood for the ex vivo mechanistic studies described in Aim 3. IRB approval for all elements has been obtained.

Computer: Microcomputers (IBM Pentium D) are available for word processing, microscopic image analysis and statistical analysis of data at each lab technician's work space and in Dr. Thom's office. Tissue image analysis is done using Montage/MetaMorph software loaded into Pentium D processor computer with 4 GB of system memory.

Office: Dr. Thom has a 250 square foot office immediately adjacent to the laboratory.

Equipment: Major equipment includes spectrophotometers (Varian Cary 50 Bio UV/Vis, Coleman Lambda 1 single beam), fluorescent spectrometer (Perkin Elmer L550B), BioTek Synergy HT Fluorescence/Lumnescence plate reader, centrifuges (Thermo Scientific/Sorvall WX Ultra 80,

Beckman TL-100 ultra, Beckman TJ, Sorvall Legend 21R and Eppendorf 5415C preparative), scintillation counter (Perkin Elmer TriCarb 2900TR, cell counter (Beckman-Coulter Z2), plate/gel/blot imager (Odyssey CLx Infrared Imager) and table-top flow cytometer (3-laser, 6 color-Miltenyi MACSQuant Analyzer).

University of Maryland Flow Cytometry Core: A BD LSR II capable of simultaneous detection of 14 fluorochromes and 16 parameter data acquisition run on FACSDiva software. Two BD FACSCanto system sets for analysis of up to six fluorescent markers and two scatter parameters. A FACScan capable of 3 color detection. A BD FACSAria cell sorter.

University of Maryland Immunohistochemistry/Imaging Core: Three laser scanning confocal microscopes: a Zeiss LSM 5, LSM 410 and LSM 510. Core facilities also include requisite equipment, such as cryostat, for tissue preparation to accomplish imaging analysis.

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8. ATTACHMENTS:

Stephen R. Thom curriculum vitae.

(b) (6) curriculum vitae.

OMB Number: 4040-0001
Expiration Date: 12/31/2022

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE

State Application Identifier

1. TYPE OF SUBMISSION

☐ Pre-application ☒ Application ☐ Changed/Corrected Application

4. a. Federal Identifier

b. Agency Routing Identifier 342 [Chapman, Sandra]

c. Previous Grants.gov Tracking ID

2. DATE SUBMITTED

Applicant Identifier

5. APPLICANT INFORMATION

Organizational DUNS: 077758407

Legal Name: Case Western Reserve University

Department: Physiology & Biophysics

Division: School of Medicine

Street1: 10900 Euclid Ave.

Street2:

City: Cleveland

County / Parish: Cuyahoga

State: OH: Ohio

Province:

Country: USA: UNITED STATES

ZIP / Postal Code: 441060000

Person to be contacted on matters involving this application

Prefix:

First Name: Erin

Middle Name:

Last Name: Fogarty

Suffix:

Position/Title: Interim Senior Director, Grants & Contracts

Street1: 10900 Euclid Ave

Street2:

City: Cleveland

County / Parish: Cuyahoga

State: OH: Ohio

Province:

Country: USA: UNITED STATES

ZIP / Postal Code: 441060000

Phone Number: 216-368-4432

Fax Number: 216-368-0929

Email: medrespre@case.edu

6. EMPLOYER IDENTIFICATION *(EIN) or (TIN):* 34-1018992

7. TYPE OF APPLICANT: O: Private Institution of Higher Education

Other (Specify):

Small Business Organization Type ☐ Women Owned ☐ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION:

☒ New ☐ Resubmission

☐ Renewal ☐ Continuation ☐ Revision

If Revision, mark appropriate box(es).

☐ A. Increase Award ☐ B. Decrease Award ☐ C. Increase Duration ☐ D. Decrease Duration

☐ E. Other (specify):

Is this application being submitted to other agencies? Yes ☐ No ☒ What other Agencies?

9. NAME OF FEDERAL AGENCY: Office of Naval Research

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER: 12.300

TITLE: Basic and Applied Scientific Research

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT:

AQP5 and AQP1 as Targets for Prophylactic Countermeasures for Pulmonary Hyperbaric Oxygen Toxicity

12. PROPOSED PROJECT:

Start Date: 06/01/2020

Ending Date: 05/31/2021

13. CONGRESSIONAL DISTRICT OF APPLICANT

OH-011

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: | First Name: Walter | Middle Name:

Last Name: Boron | Suffix:

Position/Title: Professor and Chairman

Organization Name: Case Western Reserve University

Department: Physiology & Biophysics | Division: School of Medicine

Street1: 10900 Euclid Avenue

Street2:

City: Cleveland | County / Parish: Cuyahoga

State: OH: Ohio | Province:

Country: USA: UNITED STATES | ZIP / Postal Code: 441064970

Phone Number: 216-368-5298 | Fax Number: 216-368-5586

Email: walter.boron@case.edu

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested	136,530.00
b. Total Non-Federal Funds	0.00
c. Total Federal & Non-Federal Funds	136,530.00
d. Estimated Program Income	0.00

16. IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?

a. YES ☐ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:

DATE:

b. NO ☒ PROGRAM IS NOT COVERED BY E.O. 12372; OR

☐ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious. or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

☒ **I agree**

The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.

18. SFLLL (Disclosure of Lobbying Activities) or other Explanatory Documentation

Scan0010.pdf | Add Attachment | Delete Attachment | View Attachment

19. Authorized Representative

Prefix: | First Name: Erin | Middle Name:

Last Name: Fogarty | Suffix:

Position/Title: Interim Senior Director, Grants & Contracts

Organization: Case Western Reserve University

Department: Dean - Medicine | Division: School of Medicine

Street1: 10900 Euclid Ave

Street2:

City: Cleveland | County / Parish: Cuyahoga

State: OH: Ohio | Province:

Country: USA: UNITED STATES | ZIP / Postal Code: 441060000

Phone Number: 216-368-4432 | Fax Number: 216-368-0929

Email: medrespre@case.edu

Signature of Authorized Representative	Date Signed
Erin.Fogarty	02/12/2020

20. Pre-application | Add Attachment | Delete Attachment | View Attachment

21. Cover Letter Attachment | Add Attachment | Delete Attachment | View Attachment

Contracts & Grants

CERTIFICATION REGARDING RESTRICTIONS ON LOBBYING
(Proposals Greater than $100,000)

Proposal Title
AQP5 as a Target for Prophylactic Countermeasures for Pulmonary Hyperbaric Oxygen Toxicity

Proposal Number and/or Date February 11, 2020

Applicants should review the instructions for certification requirement under 32 CFR Part 28, "New Restrictions on Lobbying." 32 CFR Part 25, The certification shall be treated as a material representation of fact upon which reliance will be placed when the Office of Naval Research determines to award the covered transaction, grant, or cooperative agreement.

CERTIFICATION REGARDING LOBBYING ACTIVITIES

The following certification applies only to actions exceeding $100,000:

Section 1352, Title 31, U.S.C. (PL 101-121, Section 319) entitled "Limitation on use of appropriated funds to influence certain Federal contracting and financial transactions."

(1) No Federal appropriated funds have been paid or will be paid by or on behalf of the undersigned, to any person for influencing or attempting to influence an officer or employee of an agency, a Member of Congress, an officer or employee of Congress, or an employee of a Member of Congress in connection with the awarding of any Federal contract, the making of any Federal grant, the making of any Federal loan, the entering into of any cooperative agreement, and the extension, continuation, renewal, amendment, or modification of any Federal contract, grant, loan, or cooperative agreement.

(2) If any funds other than Federal appropriated funds have been paid or will be paid to any person for influencing or attempting to influence an officer or employee of any agency, a Member of Congress, an officer or employee of Congress, or an employee of a Member of Congress in connection with the Federal contract, grant, loan, or cooperative agreement, the undersigned shall complete and submit Standard Form-LLL, "Disclosure Form to Report Lobbying," in accordance with its instructions.

(3) The undersigned shall require that the language of this certification be included in the award documents for all subawards at all tiers (including subcontracts, subgrants, and contracts under grants, loans, and cooperative agreements) and that all subrecipients shall certify and disclose accordingly.

This certification is a material representation of fact upon which reliance was placed when this transaction was made or entered into. Submission of this certification is a prerequisite for making or entering into this transaction imposed by Section 1352, title 31, U.S.C. Any person who fails to file the required certification shall be subject to a civil penalty of not less than $10,000 and not more than $100,000 for each such failure.

As the duly authorized representative of the applicant, I hereby make the above certifications on behalf of the applicant.

Applicant Institution:

Case Western Reserve University

Taxpayer Identification Number (TIN): 34-1018992

Erin Foganty Senior Director, School of Medicine Office of Grants & Contracts

Printed Name, Title and Signature of Authorized Representative: (b) (6)

Date: 2/11/20

OMB Number: 4040-0010
Expiration Date: 12/31/2022

Project Abstract

The Project Abstract must not exceed one page and must contain a summary of the proposed activity suitable for dissemination to the public. It should be a self-contained description of the project and should contain a statement of objectives and methods to be employed. It should be informative to other persons working in the same or related fields and insofar as possible understandable to a technically literate lay reader. This Abstract must not include any proprietary/confidential information.

* Please click the add attachment button to complete this entry.

Add Attachment | Delete Attachment | View Attachment

AQP1_5Abstract.pdf

RESEARCH & RELATED BUDGET - Budget Period 1

OMB Number: 4040-0001
Expiration Date: 12/31/2022

ORGANIZATIONAL DUNS: 077758407 **Enter name of Organization:** Case Western Reserve University

Budget Type: ☒ Project ☐ Subaward/Consortium **Budget Period: 1** **Start Date:** 06/01/2020 **End Date:** 05/31/2021

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Walter	F	Boron		(b) (4)	0.60	0.00	0.00	(b) (4)	(b) (4)	(b) (4)

Project Role: PI/PD

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

Total Funds requested for all Senior Key Persons in the attached file 0.00

Total Senior/Key Person (b) (4)

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Technician	1.80	0.00	0.00	(b) (4)	(b) (4)	(b) (4)
1	Lab Manager	1.80	0.00	0.00	(b) (4)	(b) (4)	(b) (4)
2	**Total Number Other Personnel**					**Total Other Personnel**	(b) (4)

Total Salary, Wages and Fringe Benefits (A+B) (b) (4)

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	Funds Requested ($)

Additional Equipment: Add Attachment | Delete Attachment | View Attachment

Total funds requested for all equipment listed in the attached file	0.00
Total Equipment	0.00

D. Travel

	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	0.00
2. Foreign Travel Costs	0.00
Total Travel Cost	0.00

E. Participant/Trainee Support Costs

	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other	
Number of Participants/Trainees — **Total Participant/Trainee Support Costs**	

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	11,059.00
2.	Publication Costs	2,000.00
3.	Consultant Services	0.00
4.	ADP/Computer Services	0.00
5.	Subawards/Consortium/Contractual Costs	0.00
6.	Equipment or Facility Rental/User Fees	0.00
7.	Alterations and Renovations	0.00
8.	Animal	35,712.00
9.		
10.		
	Total Other Direct Costs	48,771.00

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	85,331.00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
Research (MTDC)	(b) (4)	(b) (4)	(b) (4)
		Total Indirect Costs	(b) (4)

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

DHHS, Uyen Tran, HHS Rep, 214-767-3261

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	136,530.00

J. Fee

Funds Requested ($)
0.00

K. Total Costs and Fee

	Funds Requested ($)
Total Costs and Fee (I + J)	136,530.00

L. Budget Justification

(Only attach one file.) AQP5_____AQP1__Budget_Justification_.p

Add Attachment | Delete Attachment | View Attachment

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		(b) (4)
Section B, Other Personnel		(b) (4)
Total Number Other Personnel	2	
Total Salary, Wages and Fringe Benefits (A+B)		(b) (4)
Section C, Equipment		0.00
Section D, Travel		0.00
1. Domestic	0.00	
2. Foreign	0.00	
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		48,771.00
1. Materials and Supplies	11,059.00	
2. Publication Costs	2,000.00	
3. Consultant Services	0.00	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	0.00	
6. Equipment or Facility Rental/User Fees	0.00	
7. Alterations and Renovations	0.00	
8. Other 1	35,712.00	
9. Other 2		
10. Other 3		
Section G, Direct Costs (A thru F)		85,331.00
Section H, Indirect Costs		(b) (4)
Section I, Total Direct and Indirect Costs (G + H)		136,530.00
Section J, Fee		0.00
Section K, Total Costs and Fee (I + J)		136,530.00

OMB Number: 4040-0001
Expiration Date: 12/31/2022

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix:
* First Name: Walter
Middle Name:
* Last Name: Boron
Suffix:
Position/Title: Professor and Chairman
Department: Physiology & Biophysics
Organization Name: Case Western Reserve University
Division: School of Medicine
* Street1: 10900 Euclid Avenue
Street2:
* City: Cleveland
County/ Parish: Cuyahoga
* State: OH: Ohio
Province:
* Country: USA: UNITED STATES
* Zip / Postal Code: 441064970
* Phone Number: 216-368-5298
Fax Number: 216-368-5586
* E-Mail: walter.boron@case.edu

Credential, e.g., agency login: walterboron

*** Project Role:** PD/PI
Other Project Role Category:

Degree Type: MD, PhD
Degree Year: 1977

***Attach Biographical Sketch**	ONR__Biosketch__WFB.pdf	Add Attachment	Delete Attachment	View Attachment
Attach Current & Pending Support		Add Attachment	Delete Attachment	View Attachment

PROFILE - Senior/Key Person 1

Prefix:
* First Name:
Middle Name:
* Last Name:
Suffix:
Position/Title:
Department:
Organization Name:
Division:
* Street1:
Street2:
* City:
County/ Parish:
* State:
Province:
* Country: USA: UNITED STATES
* Zip / Postal Code:
* Phone Number:
Fax Number:
* E-Mail:

Credential, e.g., agency login:

*** Project Role:**
Other Project Role Category:

Degree Type:
Degree Year:

Attach Biographical Sketch		Add Attachment	Delete Attachment	View Attachment
Attach Current & Pending Support		Add Attachment	Delete Attachment	View Attachment

RESEARCH & RELATED Other Project Information

OMB Number: 4040-0001
Expiration Date: 12/31/2022

1. Are Human Subjects Involved? ☐ Yes ☒ No

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ☐ Yes ☐ No

If yes, check appropriate exemption number. ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8

If no, is the IRB review Pending? ☐ Yes ☐ No

IRB Approval Date:

Human Subject Assurance Number:

2. Are Vertebrate Animals Used? ☒ Yes ☐ No

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ☐ Yes ☒ No

IACUC Approval Date: 07/18/2017

Animal Welfare Assurance Number: A3145-01

3. Is proprietary/privileged information included in the application? ☐ Yes ☒ No

4.a. Does this Project Have an Actual or Potential Impact - positive or negative - on the environment? ☐ Yes ☒ No

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ☐ Yes ☐ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place? ☐ Yes ☒ No

5.a. If yes, please explain:

6. Does this project involve activities outside of the United States or partnerships with international collaborators? ☐ Yes ☒ No

6.a. If yes, identify countries:

6.b. Optional Explanation:

7. Project Summary/Abstract AQP5___AQP1_Abstract.pdf [Add Attachment] [Delete Attachment] [View Attachment]

8. Project Narrative AQP5_AQP1__Project_Narrative_.pdf [Add Attachment] [Delete Attachment] [View Attachment]

9. Bibliography & References Cited [Add Attachment] [Delete Attachment] [View Attachment]

10. Facilities & Other Resources [Add Attachment] [Delete Attachment] [View Attachment]

11. Equipment [Add Attachment] [Delete Attachment] [View Attachment]

12. Other Attachments [Add Attachments] [Delete Attachments] [View Attachments]

Abstract

Background. Gas transport across cell membranes is essential for life but also plays central roles in disease, especially pathology of importance to the Navy. Although the dogma had been that all gases cross all membranes merely by dissolving in membrane lipids, we now know that some biological membranes have a negligible permeability to dissolved gases, whereas some membrane proteins—notably a subset of aquaporins—can conduct O_2 and CO_2. All AQPs are tetramers, and the four monomers surround a hydrophobic central pore that appears to be the main conduit for CO_2 and O_2 diffusion through these proteins.

Problem. For divers or DISSUB submariners, exposure to high O_2 can cause pulmonary oxygen toxicity (POT).

Mission relevance. POT impairs pulmonary function, reduces performance, and can lead to serious illness or even death.

Physiology. Divers and submariners can experience high alveolar O_2 partial pressure, leading to exaggerated O_2 uptake from alveolar air into alveolar type I cells (AT_ICs) and then into pulmonary capillary endothelial cells (PCECs). A high intracellular oxygen concentration leads to oxidative damage. Understanding the pathophysiology and developing prophylactic countermeasures is a high priority for Undersea Medicine.

Solution. Leverage newly discovered and characterized gas-channel physiology to identify new targets for adjuvant therapies. Aquaporin 5 (AQP5) is highly expressed in the apical (air-facing) membrane of ATICs. It has the highest-known CO_2 permeability of all AQPs, and is likely to be an excellent O_2 channel as well. AQP1 is highly expressed in PCECs. Data from our MURI team indicate that AQP1 functions as an O_2 channel in RBCs.

Hypothesis. AQP5 mediates a substantial fraction of O_2 uptake into ATIC, and AQP1 similarly contributes to O_2 uptake by PCECs. At high alveolar P_{O_2}, the genetic deletion of AQP5 or AQP1 from mice will reduce oxidative damage to ATICs and PCECs, respectively. The reduced inflammation and water permeability also will reduce pulmonary edema.

Long-term goals. Our long-term plan is to extend our AQP5 and AQP1 work to protect swine and eventually humans from acute lung and perhaps CNS injury in order to mitigate O_2 toxicity in divers through the development of drugs, identification of biomarkers, or extension of dive tables. O_2 concentration is a limiting factor in Navy diving operations and disabled-submarine scenarios.

Objective. Test the genetic proof of principle that the absence of AQP5 or AQP1 is protective in a mouse model of POT. The Boron Lab will generate and ship to Dr. Claude Piantadosi of Duke University the following: wild-type (WT) mice, "knockout (KO) mice" genetically deficient in AQP5 or AQP1, and double knockouts (dKO) mice deficient in both. The Piantadosi Lab (funded separately) will subject the mice to their O_2-toxicity protocol and then assess the mice for POT by wet/dry weights of the lungs, assays of cells and inflammatory factors collected by bronchoalveolar-lavage, and by routine pathological approaches.

Significance. If the hypothesis is true, the proposed work would establish a genetic proof of principle that reduced AQP5 or AQP1 activity would be an effective countermeasure for POT. The next step would be a more detailed of AQP5/AQP1/O_2-toxicity in mice, to pave the way for a transition to swine, and eventually to humans. This line of research could greatly improve performance and reduce pathology in divers and submariners

BUDGET JUSTIFICATION

Budget Distribution in Year 1: The direct costs requested in Year 1 can be divided into three parts: (1) $36,561 are recurring expenses for Personnel, and other Direct costs. (2) $11,059 covers General Supplies. (3) $2,000 for publication, (4) $35,712 covers the breeding and maintenance of the WT, AQP1-KO, AQP5-KO, AQP1-AQP5-Double-KO colonies, Animal maintenance supplies and expenses for shipping animals to North Carolina.

A. Personnel ($36,561)

A.1. PI: Walter F. Boron (1.2 calendar months)

The PI is responsible for the overall design of the project, supervising and advising the postdoctoral and technical personnel on a daily basis, and writing manuscripts and other reports.

A.2. Research Assistant IV: Gerald Babcock (1.8 calendar months)

Mr. Babcock will serve as lab manager. He will be responsible for submitting tissue samples for genotyping and interpreting the results for both the WT and AQP5 knockout mice. He will be responsible for coordinating the shipment of Mice to Dr. Piantadosi at Duke University School of Medicine Durham, North Carolina. Mr. Babcock's duties also include organization of the mouse colonies, ordering materials and supplies, maintenance and submission of IACUC protocols. Mr. Babcock also supervises the daily activities of Thomas Radford, our Research Assistant in charge of the Boron lab's Animal colonies. He is also the chief liaison with any of CWRU's core facilities (ARC, MPPC) used by the lab. His salary is prorated among the various projects in the lab and accounts for a 15% effort to on this project.

A.3. Research Assistant II Thomas Radford (1.8 calendar months)

Thomas is our lab technician responsible for the maintaining the Boron Lab animal colonies. His duties include Setting up breeding pairs, tracking the progress of breeding, tattooing the mice for identification, collecting tissue samples for genotyping, weaning the mice and distribution of mice after genotyping. His salary is prorated among the various projects in the lab and accounts for a 15% effort to on this project

B. *Equipment (NONE)*

C. *Travel (NONE)*

D. *Participant/Trainee Support Costs (N/A)*

E. *Other Direct Costs [aside from Equipment] ($48,771)*

E.1. Materials and Supplies ($11,059)

Genotyping fees: We estimate that we will generate ~160 WT C57BL/6, 240 AQP1, 240 AQP5-KO and 420 AQP-AQP5 double KO) through maintaining, breeding and backcrossing these colonies for the Mice experiments. We have a contract with TransnetYX to perform genotyping on WT-mice (9.45 /reaction x 160 mice = **$1,512**), AQP1-KO mice (9.45 /reaction x 240 mice = **$2,268**) AQP5-KO mice (10.36/reaction x 240 mice = **$2,508**) and AQP1/AQP5-double-KO mice (11.36/reaction x 420 mice = **$4771**)

E.2. Publication costs ($2,000)

We intend to publish one paper per year in major scientific journals. For AJP, J Physiol, or JBC, we estimate 1 x $2,000 = 2,000/year.

E.3. Consultant services (N/A)

E.4. ADP/Computer services (N/A)

E.5. Sub-awards (N/A)

E.6. Equipment or Facility Rental (N/A)

E.7. Alterations and Renovations (N/A)

E.8. Animals ($35,712)

E.8.a. Generating and maintaining mouse colonies ($24,773)

E.8.a.(1) Costs for maintaining (in-house) WT-C57BL/6 mouse colony ($3,394)

We will use mice from our own colony of WT C57BL/6. We will maintain the WT C57BL/6 colony as a source of WT mice for control experiments and mice to backcross AQP1-KO, AQP5-KO mouse lines. The cost is estimate uses the number of mice required for each aspect of the breeding (5 mice/cage). There is an estimated $1.01 Per Diem (scheduled to remain constant for 2020 but may rise $0.04/year, based on the past years) per standard cage of 5 mice. The cost for maintaining WT mice for experiments requires 40 cages of mice (~ 5 mice/cage) at ($1.01/cage x 84 days/cage, **($3,394).** Please note that these calculations estimated for selectively breeding out Males for experiments.

E.8.a.(2) Costs for breeding and backcrossing our AQP1-KO mouse colony ($6,108)

This breeding maintains isogeny between our WT-C57BL/6 line and the AQP1-KO mouse lines to supply mice to our collaborator in North Carolina. We will continue to backcross the AQP1-KO strain with our in-house strain of WT C57BL/6 to maintain isogeny in our colony. The cost is estimated using the number of cages of mice required for each Back crossing 24 cages of mice (~ 5 mice/cage) at ($1.01/cage x 84 days/cage, **($2,036)** per diem rate, but may rise $0.04/year) per diem. Breeding for experiments for year 1 will require Het x Het crosses to generate AQP1 KO breeder mice requires 24 cages of mice (~5 mice at per diem rate of ($1.01/cage x 84 days/cage **($2,036)** for year 1. Breeding AQP1-KO mice for experiments for year 1 will require AQP1-KO x AQP1-KO crosses to generate litters of AQP1-KO mice. This AQP1- KO cross requires 24 cages of mice (~5 mice at per diem rate of ($1.01/cage x 84 days/cage **($2,036)** for year 1. Please note that these calculations estimated for selectively breeding out WT Males for experiments

E.8.a.(3) Costs for breeding and backcrossing our AQP5-KO mouse colony ($6,108)

This breeding maintains isogeny between our WT-C57BL/6 line and the AQP5-KO mouse lines to supply mice to our collaborator in North Carolina. We will continue to backcross the AQP5-KO strain with our in-house strain of WT C57BL/6 to maintain isogeny in our colony. The cost is estimated using the number of cages of mice required for each Back crossing 24 cages of mice (~ 5 mice/cage) at ($1.01/cage x 84 days/cage, **($2,036)** per diem rate, but may rise $0.04/year) per diem. Breeding for experiments for year 1 will require Het x Het crosses to generate AQP5 KO breeder mice requires 24 cages of mice (~5 mice at per diem rate of ($1.01/cage x 84 days/cage **($2,036)** for year 1. Breeding KO mice for experiments for year 1 will require KO x KO crosses to generate litters of AQP5 KO mice requires 24 cages of mice (~5 mice at per diem rate of ($1.01/cage x 84 days/cage **($2,036)** for year 1. Please note that these calculations estimated for selectively breeding out WT Males for experiments

E.8.a.(4) Costs for breeding and backcrossing our AQP1/AQP5-Double-KO mouse colony ($9,163)

This breeding Generates AQP1-AQP1-Double KO Crosses for experiments by our collaborator in North Carolina. We will begin by crossing AQP1 (-/-) with AQP5 (-/-) mice to generate AQP1/AQP5 Double Heterozygous Mice. The cost is estimated using the number of cages of mice required for each crossing 32 cages of mice (~ 5 mice/cage) at ($1.01/cage x 84 days/cage, **($2,715)** per diem rate, but may rise $0.04/year) per diem. Next, we will Cross AQP1/AQP5 Double Heterozygous mice to generate AQP1-AQP5 Double KO Mice. This cross will generate very low percentage of double KO mice per Double Het cross. Breeding for AQP1-AQP5 Double-KO breeders for year 1 will require ~56 cages of mice (~5 mice at per diem rate of ($1.01/cage x 84 days/cage **($4,751)** for year 1. Breeding KO mice for experiments for year 1 will require KO x KO crosses to generate litters of AQP5 KO mice requires 24 cages of mice (~5 mice at per diem rate of ($1.01/cage x 84 days/cage **($1,697)** for year 1. Please note that these calculations estimated for selectively breeding out WT Males for experiments

E.8.b. Mouse Colony Maintenance Supplies ($1,738)

For maintaining the animal colony, supplies are requested to cover Tattoo machine, needles and dye ($469), disposable plastic ware and blades for tissue collection ($800) and reagents; alcohol and Clotasol used for tail biopsy for genotyping ($880) for animal maintenance.

E.8.c. Shipping Animals to North Carolina ($9,201)

We have budgeted for Five (**5**) shipments of mice per years to Dr. Piantadosi at Duke University School of Medicine, Durham North Carolina. Each Shipment will require ~4 Shipping containers ($30),World Carrier charges $426/container x 4 Containers ($1704), an export fee ($62.50), handling ($43.75) which is ($1,840.25./Shipment x **5** Shipments = $9,201).

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Boron, Walter Frank

eRA COMMONS USER NAME (credential, e.g., agency login): walterboron

POSITION TITLE: Professor & Chair

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
St. Louis University, St. Louis, MO	AB	05/1971	Chemistry
Washington University, St. Louis, MO	MD	05/1977	
Washington University, St. Louis, MO	PhD	05/1977	Physiology & Biophysics

A. Personal Statement

Research: We have two major areas of research, both of which bear on this grant application: (1) acid-base transporters/regulation of intracellular pH (pH_i), and (2) gas channels.

As a graduate student working on squid giant axons, I did the first experiments clearly demonstrating the active regulation of pH_i. During my early years as a graduate student and postdoctoral fellow, I also developed many of the experimental and theoretical paradigms widely used today in the acid-base field. As a postdoctoral fellow, I extended my expertise to epithelia, specifically renal tubules, and discovered the electrogenic Na/HCO_3 cotransporter (NBC). Since then, I have maintained research interests in the acid-base regulation both the brain and the kidney. Since I became an independent investigator, we were among the first to apply pH-sensitive dyes to living cells and study pH_i regulation in small vertebrate cells (e.g., kidney tubules, CNS). We also were first to clone a cDNA that encodes an NBC transporter, and went on to clone several related cDNAs that play important roles in renal acid-base transport and pH_i regulation throughout the body.

A related contribution was the development of out-of-equilibrium (OOE) CO_2/HCO_3^- solutions. One normally thinks of CO_2, HCO_3^-, and H^+ being related by the classic equilibrium $CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+$. We developed a rapid-mixing method for creating temporarily OOE states and then discovered that receptor protein tyrosine phosphatase RPTPγ is in fact a dual sensor for extracellular CO_2 and extracellular HCO_3^- in proximal tubules and most probably CNS neurons and astrocytes as well.

While studying pH_i regulation in isolated gastric glands, we discovered the first membrane with no permeability to CO_2. This led to the discovery of the first gas channel—the water channel AQP1, which is also permeable to CO_2. We now know two major families of gas channels, the AQPs and the rhesus (Rh) proteins. We showed that these channels exhibit selectivity between CO_2 and NH_3, much like ion channels exhibit selectivity for one ion (e.g., Na^+) over another (e.g., K^+). For example, AQP1, AQP4, and AQP5 are excellent CO_2 channels, but among these, only AQP1 conducts NH_3. Work by us and collaborators shows that 90 to 95% of the CO_2 permeability of red blood cells (RBCs) is due to AQP1 and the Rh complex. Ongoing research supported by a MURI championed by the ONR has provided the first evidence for channels permeable to O_2. AQP1 and the Rh complex appear to account for ~60% of the O_2 permeability of mouse RBCs. Also supported by the ONR is the development of a neutral buoyancy assay (NBA) that for the first time allows assays of movements of N_2 across cell membrane, and has led to the identification of the first N_2 channel. The NBA could be used to assess fluxes of other gases, such as the O_2 permeability of AQP5—highly relevant to the present application. Also relevant is recently completed work—now being assembled into a paper—in which we and our MURI collaborators have used Ni^{2+} and Zn^{2+} to block the central pore of a mutated AQP5, obtained the first crystal structure of AQP5 in a blocked state, and used molecular dynamics to model the diffusion of CO_2 through the unblocked central pore.

The following are 4 recent papers related to AQP4 and/or gas transport:

a. Geyer RR, R Musa-Aziz, X Qin & WF Boron. Relative CO_2/NH_3 selectivities of aquaporins 0-9. *Am J Physiol Cell Physiol* 304:C985–C994, 2013. doi: 10.1152/ajpcell.00033.2013.

b. Wang C, H Hu, X Qin, B Zeise, D Xu, W-J Rappel, WF Boron & JI Schroeder. Reconstitution of CO_2 regulation of SLAC1 anion channel and function of CO_2-permeable PIP2;1 aquaporin as carbonic

anhydrase 4 interactor. *Plant Cell* 28:568–582, 2016. PMID: 26764375. PMCID: PMC4790870. doi: 10.1105/tpc.15.00637.

c. Farr GW, CH Hall, SM Farr, R Wade, JM Detzel, AG Adams, JM Buch, DL Beahm, CA Flask, K Xu, JC LaManna, PR McGuirk, WF Boron & MF Pelletier. Functionalized phenylbenzamides inhibit aquaporin-4 reducing cerebral edema and improving outcome in two models of CNS injury. *Neuroscience* 404:484–498, 2019. doi: 10.1016/j.neuroscience.2019.01.034.

d. Zhang Y, K Xu, Y Liu, B Erokwu, P Zhao, C Flask, C Ramos-Estebanez, GW Farr, GJ LaManna, WF Boron & X Yu. Increased cerebral vascularization in response to decreased water exchange across the blood-brain barrier in aquaporin-4 knockout mice. *PLoS One* 14(6):e0218415, 2019. doi: 10.1371/journal.pone.0218415.

Editorial contributions. In addition to serving on the editorial board of *Journal of Physiology* and of *American Journal of Physiology*, I was Associate Editor for 6 years of *Physiological Reviews*, and then Editor-in-Chief of *Physiol Reviews* for 6 years. Later I served for 9 years as the founding Editor-in-Chief of the journal *Physiology*. In addition, I am co-editor (with EL Boulpaep) of the textbook *Medical Physiology*, now in its third edition.

Entrepreneurship. I cofounded Aeromics Inc., which recently completed successful Phase 1 clinical trials of AER-271 for the treatment of cytotoxic cerebral edema in stroke. The parent drug AER-270 is the first aquaporin inhibitor to enter clinical trials.

Positions and Employment

1977 – 1978	Postdoctoral Fellow, Department of Physiology & Biophysics, Washington University School of Medicine, St. Louis, MO
1978 – 1980	Postdoctoral Fellow, Department of Physiology, Yale University School of Medicine, New Haven, CT
1980 – 1984	Assistant Professor, Department of Physiology, Yale University School of Medicine, New Haven, CT
1984 – 1987	Associate Professor, Department of Physiology, Yale University School of Medicine, New Haven, CT
1987 – 2007	Professor, Department of Cellular and Molecular Physiology, Yale University School of Medicine, New Haven, CT
1989 – 1998	Chair, Department of Cellular & Molecular Physiology, Yale University School of Medicine, New Haven, CT
2007 –	Myers/Scarpa Professor & Chair, Department of Physiology & Biophysics, Case Western Reserve University School of Medicine, Cleveland, OH
2013 –	Professor, Secondary Appointment, Department of Medicine, Case Western Reserve University School of Medicine, Cleveland, OH
2015 –	Adjunct Professor, Department of Physiology, Wayne State University School of Medicine, Detroit, MI
2016 –	Guest Professor, Huazhong University of Science & Technology (HUST), Wuhan, China
2016 –	Executive Director, PhD Programs, Case Western Reserve University School of Medicine, Cleveland, OH
2017 –	Professor, Secondary Appointment, Department of Biochemistry, Case Western Reserve University, School of Medicine, Cleveland, OH
2017 – 2018	Interim Chair, Department of Biochemistry, Case Western Reserve University School of Medicine, Cleveland, OH

Other Experience and Professional Memberships

1994 – 1999	Editor-in-Chief, *Physiological Reviews*
1998 – 2001	President-elect/President/Past-President, American Physiological Society
2003 – 2007	Principal Investigator, Program Project Grant DK17433
2003 – 2012	Editor-in-Chief, *Physiology*
2007 – 2017	Chairman of the Board of Directors, Aeromics, Inc.
2010 – 2017	Secretary-General, International Union of Physiological Sciences
2018 – 2020	Vice Chair, Section 3, National Academy of Medicine, Washington, DC
2018 – 2020	Councilor, Association of Chairs of Department of Physiology

Honors

1975	Grass Foundation MBL Fellow, Sigma Xi

1977	Research Service Award, National Institute of Health
1981	Searle Scholar, Searle Scholars Program
1983	Research Career Development Award, National Institute of Health
1986	Young Investigator Award, American Society of Nephrology & American Heart Association
1993	Charles W. Bohmfalk Teaching Award, Yale University School of Medicine
1993	Robert F. Pitts Lecture and Award, International Union of Physiological Sciences
1998	Carl W. Gottschalk Lecture and Award, American Physiological Society
1998	Elected, Fellow of the American Academy for the Advancement of Science
2001	Merit Award, National Institute of Health
2005	Homer Smith Award, American Society of Nephrology
2008	Sharpey-Schafer Award, The Physiological Society
2010	Palade Gold Medal (with WA Catterall and RW Tsien), Wayne State University
2011	Daggs Award, American Physiological Society
2012	British Medical Association Certificate of Honor (shared with E.L. Boulpaep & Elsevier) for Medical Physiology Updated 2nd Edition, BMA Medical Book Awards
2014	Doctor Medicinae Honoris Causa, Aarhus University, Denmark
2014	Elected, The National Academy of Medicine
2015	Elected, Fellow of the American Physiological Society

C. Contributions to Science

Discovery of and early work in the regulation of intracellular pH (pH_i) regulation: The regulation of intracellular pH (pH_i)—and the study of HCO_3^- and CO_2 transport—has been at the center of my research career since my days as a PhD student. Working on squid giant axons, Paul De Weer and I obtained the first direct evidence for the dynamic and active regulation of pH_i by showing that the fall in pH_i induced by exposing the cell to CO_2 is followed by a slower pH_i recovery due to an active-transport process. We also discovered what is now known as the ammonium prepulse technique, and used it as a tool to acid load cells. This work led to the discovery that the pH_i-regulatory process in squid axons involves HCO_3^- or a related ion. Subsequent work with John Russell was the first to show that the transport carries Cl^-. Together with the work by Roger Thomas, this research defined the first-described pH_i regulator: the Na^+-driven Cl-HCO_3 exchanger. The review by Roos and Boron put the early pH_i work in context, and has become the most-cited publication in the pH field.

a. Boron WF, De Weer P. Intracellular pH transients in squid giant axons caused by CO_2, NH_3, and metabolic inhibitors. *J Gen Physiol* 67:91–112, 1976. PubMed PMID: 1460; PubMed Central PMCID: PMC2214912.

b. Boron WF, De Weer P. Active proton transport stimulated by CO_2/HCO_3^-, blocked by cyanide. *Nature* 259:240–241, 1976. PubMed PMID: 2874.

c. Russell JM, Boron WF. Role of chloride transport in regulation of intracellular pH. *Nature* 264:73–74, 1976. PubMed PMID: 12472.

d. Roos A, Boron WF. Intracellular pH. *Physiol Rev* 61:296–434, 1981. PubMed PMID: 7012859.

Discovery of the electrogenic Na/HCO_3 cotransporter: As a postdoctoral fellow with Emile Boulpaep, we set out to test the two leading hypotheses of the day concerning the mechanism of HCO_3^- exit across the PT basolateral membrane (BLM): a HCO_3^- channel vs. Cl-HCO_3 exchange. To prepare myself to answer the question, I first explored Na-H exchange activity and made the surprising discovery of high levels of NHE (not yet cloned) activity at the BLM. We now hypothesize that this NHE1 is key to controlling the nanoenvironment near NBCe1 ($q=2$) to behave as if $q=3$. It was when trying to block this BLM NHE by removing basolateral Na^+ in the presence of HCO_3^- that I observed the striking hyperpolarization that became the signature of the electrogenic Na/HCO_3 cotransporter, later known as NBCe1. As an independent investigator, we followed up with several papers in this area. In the one with Chaillet, we became the first to calibrate an intracellular dye vs. a pH microelectrode, setting the stage for future studies with pH-sensitive dyes. In several of these, including the paper by Ganz, we explored the role of NBCs (not yet cloned) in the activation of quiescent cells. Other papers from this period established several important experimental and theoretical paradigms for studying pH_i regulation.

a. Boron WF, Boulpaep EL. Intracellular pH regulation in the renal proximal tubule of the salamander. Na-H exchange. *J Gen Physiol* 81:29–52, 1983. PubMed PMID: 6833996; PubMed Central PMCID: PMC2215563.

b. Boron WF, Boulpaep EL. Intracellular pH regulation in the renal proximal tubule of the salamander. Basolateral HCO_3^- transport. *J Gen Physiol* 81:53–94, 1983. PubMed PMID: 6833997; PubMed Central PMCID: PMC2215562.
c. Chaillet JR, Boron WF. Intracellular calibration of a pH-sensitive dye in isolated, perfused salamander proximal tubules. *J Gen Physiol* 86:765–794, 1985. PubMed PMID: 4078557; PubMed Central PMCID: PMC2228795.
d. Ganz MB, Boyarsky G, Sterzel RB, Boron WF. Arginine vasopressin enhances pH_i regulation in the presence of HCO_3^- by stimulating three acid-base transport systems. *Nature* 337:648–651, 1989. PubMed PMID: 2521920.

Cloning of the cDNA encoding the electrogenic Na/HCO_3 cotransporter and related transporters: We and others had made several unsuccessful attempts to clone NBC. Our last, with Michael Romero, was to attempt expression cloning. This, too, failed when we (as others before us) used mRNA from rabbits. However, using mRNA from salamander kidney was the key decision. Starting with a library of ~16k clones, we quickly honed in on one that encoded NBCe1. The signature that we used for cloning was the ΔVm caused by Na+ removal in the presence vs absence of CO_2/HCO_3^-. Once we had the salamander clone, we cloned the rat NBCe1 by homology … and realized that we could not express it unless we put it into a Xenopus expression vector, explaining why we and others had previously failed in expression cloning with mammalian mRNA. We quickly realized that NBCe1 is in the same family as AE1, AE2, and AE3. Our cloning of NBC clarified what it meant to be an SLC4 family member, and led to the cloning of cDNAs representing 5 other genes that encode Na^+-coupled HCO_3^- transporters. The papers by Choi, Grichtchenko, and Virkki describe 3 of these.

a. Romero MF, Hediger MA, Boulpaep EL, Boron WF. Expression cloning and characterization of a renal electrogenic Na^+/HCO_3^- cotransporter. *Nature* 387:409–413, 1997. PubMed PMID: 9163427.
b. Choi I, Aalkjaer C, Boulpaep EL, Boron WF. An electroneutral sodium/bicarbonate cotransporter NBCn1 and associated sodium channel. *Nature* 405:571–575, 2000. PubMed PMID: 10850716.
c. Grichtchenko II, Choi I, Zhong X, Bray-Ward P, Russell JM, Boron WF. Cloning, characterization, and chromosomal mapping of a human electroneutral Na^+-driven Cl-HCO_3 exchanger. *J Biol Chem* 276:8358–8363, 2001. PubMed PMID: 11133997.
d. Virkki LV, Wilson DA, Vaughan-Jones RD, Boron WF. Functional characterization of human NBC4 as an electrogenic Na^+-HCO cotransporter (NBCe2). *Am J Physiol Cell Physiol* 282:C1278–1289, 2002. PubMed PMID: 11997242.

Invention of out-of-equilibrium CO_2/HCO_3^- solutions, leading to discovery of a novel CO_2/HCO_3^- sensor: Bob Berliner, by that time retired as Dean at Yale, was kind enough to sit down with me to go over some calculations in the appendix of a paper by Nakhoul that a peculiar activation of acid extrusion in rabbit proximal tubules exposed to CO_2/HCO_3^-. Bob mused that it was too bad that we could not add the CO_2 without adding the HCO_3^- or vice versa, and in an instant the idea of OOE solutions was born. Zhao brought them to fruition in experiments on squid axons, and then Zhou used OOE solutions—which make it possible to change $[CO_2]_o$ or $[HCO_3^-]_o$ or pH_o—one at a time—to demonstrate that PT cells have on their BLMs sensors for extracellular CO_2 and HCO_3^-. Following some clever reading of the literature by Patrice Bouyer, we eventually hypothesized that the receptor protein tyrosine kinase gamma (RPTPγ), which as an extracellular domain similar to a classical carbonic anhydrase, could be the dual CO_2/HCO_3^- sensor. The papers describing that work, and another describing related work on vascular smooth muscle, are now in press.

a. Zhao J, Hogan EM, Bevensee MO, Boron WF. Out-of-equilibrium CO_2/HCO_3^- solutions and their use in characterizing a new K/HCO_3 cotransporter. *Nature* 374:636–639, 1995. PubMed PMID: 7715702.
b. Zhou Y, Zhao J, Bouyer P, Boron WF. Evidence from renal proximal tubules that HCO_3^- and solute reabsorption are acutely regulated not by pH but by basolateral HCO_3^- and CO_2. *Proc Natl Acad Sci USA* 102:3875–3880, 2005. PubMed PMID: 15728388; PubMed Central PMCID: PMC553318.
c. Zhou Y, P Bouyer & WF Boron. Role of a tyrosine kinase on the CO_2-induced stimulation of HCO_3^- reabsorption by rabbit S2 renal proximal tubule. *Am J Physiol: Renal Physiol* 291: F358–367, 2006. doi:10.1152/ajprenal.00520.2005.
d. Boedtkjer E, KB Hansen, DMB Boedtkjer, C Aalkjaer & WF Boron. Extracellular HCO_3^- is sensed by mouse cerebral arteries: Regulation in tone by receptor protein tyrosine phosphatase γ. *J Cereb Blood Flow Metab* 36:365–380, 2016. doi: 10.1177/0271678X15610787.

Discovery of gas channels: While trying to study pH_i regulation in the cells of isolated, perfused gastric glands (which we were the first to perfuse), Waisbren made the discovery of the first CO_2-impermeable membrane. After I had given a seminar about this work at Penn, Paul De Weer (who had moved there as Chair) took me aside and asked me if my hypothesis (that these CO_2-impermeable apical membranes have an unusual lipid or protein composition) was backwards: is it possible that all membranes have low intrinsic CO_2 permeability, but that some have gas channels. I reasoned that, if a CO_2 channel existed, it would be in a cell that did large amounts of CO_2 transport, and the channel would be an abundant protein that did not seem to belong in that

cell. My experience teaching respiratory physiology to medical students led me to think about erythrocytes. Peter Agre had given us the cDNA encoding AQP1, and Nakhoul tested it in pH_i assays—the first example of a gas channel. Later, we collaborated with Gerolf Gros to show that AQP1 is responsible for about half of the CO_2 permeability of RBCs. We were the first to show inhibition of CO_2 fluxes by small-molecule inhibitors, and later compared CO_2, NH_3, and H_2O permeabilities of several AQPs and Rh proteins, providing the first examples of gas selectivity by channels. We continue to explore the molecular mechanism of gas movements through channel proteins.

a. Waisbren SJ, Geibel JP, Modlin IM, Boron WF. Unusual permeability properties of gastric gland cells. *Nature* 368:332–335, 1994. PubMed PMID: 8127367.
b. Nakhoul NL, Davis BA, Romero MF, Boron WF. Effect of expressing the water channel aquaporin-1 on the CO_2 permeability of *Xenopus* oocytes. *Am J Physiol* 274:C543–C548, 1998. PubMed PMID: 9486145.
c. Endeward V, Musa-Aziz R, Cooper GJ, Chen LM, Pelletier MF, Virkki LV, Supuran CT, King LS, Boron WF, Gros G. Evidence that aquaporin 1 is a major pathway for CO_2 transport across the human erythrocyte membrane. *FASEB J* 20:1974–1981, 2006. PubMed PMID: 17012249.
d. Musa-Aziz R, Chen LM, Pelletier MF, Boron WF. Relative CO_2/NH_3 selectivities of AQP1, AQP4, AQP5, AmtB, and RhAG. *Proc Natl Acad Sci USA* 106:5406–5411, 2009. PubMed PMID: 19273840; PubMed Central PMCID: PMC2664022.

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/walter.boron.1/bibliography/40663685/public/?sort=date&direction=descending

D. Research Support

Ongoing Research Support

MURI: N00014-16-2535, Office of Naval Research
Boron, Walter F
08/01/16-7/31/21
Molecular mechanisms and pathways for gas transport across biological membranes and implications for physiology and performance.
Role: Contact PI

ONR: Pending subcontract, Office of Naval Research
Boron, Walter F
08/01/16-7/31/21
Mitigation of CNS oxygen toxicity: mitochondria-targeted antioxidant therapy and aquaporin gas channels.
Role: Collaborator

U01 GM111251-01, National Institute of General Medical Sciences (NIGMS)
Boron, Walter F
01/01/15-12/31/19 (No-cost extension)
Multi-scale modeling of gas transport through channels in living cells
Role: Contact PI

NIH: R01-DK113197, National Institute of Diabetes & Digestive & Kidney Diseases
Boron, Walter F
06/10/2017-04/30/2021
Role of RPTP-gamma in sensing and transducing acid-base disturbances in the renal proximal tubule.
Role: PI

Completed Research Support

Research grant: N00014-15-1-2060, Office of Naval Research
Boron, Walter F
02/05/15-02/04/19
Gas channels
Role: PI

Equipment grant: N00014-15-1-2920, Office of Naval Research
Boron, Walter Frank
08/15/15-08/14/16
2015 DURIP: Equipment Grant
Role: PI

Abstract

Background. Gas transport across cell membranes is essential for life but also plays central roles in disease, especially pathology of importance to the Navy. Although the dogma had been that all gases cross all membranes merely by dissolving in membrane lipids, we now know that some biological membranes have a negligible permeability to dissolved gases, whereas some membrane proteins—notably a subset of aquaporins—can conduct O_2 and CO_2. All AQPs are tetramers, and the four monomers surround a hydrophobic central pore that appears to be the main conduit for CO_2 and O_2 diffusion through these proteins.

Problem. For divers or DISSUB submariners, exposure to high O_2 can cause pulmonary oxygen toxicity (POT).

Mission relevance. POT impairs pulmonary function, reduces performance, and can lead to serious illness or even death.

Physiology. Divers and submariners can experience high alveolar O_2 partial pressure, leading to exaggerated O_2 uptake from alveolar air into alveolar type I cells (AT_ICs) and then into pulmonary capillary endothelial cells (PCECs). A high intracellular oxygen concentration leads to oxidative damage. Understanding the pathophysiology and developing prophylactic countermeasures is a high priority for Undersea Medicine.

Solution. Leverage newly discovered and characterized gas-channel physiology to identify new targets for adjuvant therapies. Aquaporin 5 (AQP5) is highly expressed in the apical (air-facing) membrane of ATICs. It has the highest-known CO_2 permeability of all AQPs, and is likely to be an excellent O_2 channel as well. AQP1 is highly expressed in PCECs. Data from our MURI team indicate that AQP1 functions as an O_2 channel in RBCs.

Hypothesis. AQP5 mediates a substantial fraction of O_2 uptake into ATIC, and AQP1 similarly contributes to O_2 uptake by PCECs. At high alveolar P_{O_2}, the genetic deletion of AQP5 or AQP1 from mice will reduce oxidative damage to ATICs and PCECs, respectively. The reduced inflammation and water permeability also will reduce pulmonary edema.

Long-term goals. Our long-term plan is to extend our AQP5 and AQP1 work to protect swine and eventually humans from acute lung and perhaps CNS injury in order to mitigate O_2 toxicity in divers through the development of drugs, identification of biomarkers, or extension of dive tables. O_2 concentration is a limiting factor in Navy diving operations and disabled-submarine scenarios.

Objective. Test the genetic proof of principle that the absence of AQP5 or AQP1 is protective in a mouse model of POT. The Boron Lab will generate and ship to Dr. Claude Piantadosi of Duke University the following: wild-type (WT) mice, "knockout (KO) mice" genetically deficient in AQP5 or AQP1, and double knockouts (dKO) mice deficient in both. The Piantadosi Lab (funded separately) will subject the mice to their O_2-toxicity protocol and then assess the mice for POT by wet/dry weights of the lungs, assays of cells and inflammatory factors collected by bronchoalveolar-lavage, and by routine pathological approaches.

Significance. If the hypothesis is true, the proposed work would establish a genetic proof of principle that reduced AQP5 or AQP1 activity would be an effective countermeasure for POT. The next step would be a more detailed of AQP5/AQP1/O_2-toxicity in mice, to pave the way for a transition to swine, and eventually to humans. This line of research could greatly improve performance and reduce pathology in divers and submariners

Title Page

PI Name: Walter F. Boron, M.D., Ph.D.

Phone: (216) 368-5298

Fax: (216) 368-5586

E-mail: walter.boron@case.edu

Institution: Case Western Reserve University

Department: Physiology & Biophysics

Division: School of Medicine

Institution Address: 10900 Euclid Avenue
Cleveland, OH 44106-4970

Current DoD contractor or grantee: Yes
ONR Program Officer: Sandra Chapman
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This proposal includes data that shall not be disclosed outside the Government and shall not be duplicated, used, or disclosed--in whole or in part--for any purpose other than to evaluate the proposal. If, however, a contract is awarded to this offeror as a result of--or in connection with-- the submission of this data, the Government shall have the right to duplicate, use, or disclose the data to the extent provided in the resulting contract. This restriction does not limit the Government's right to use information contained in this data if is obtained from another source without restriction. The data subject to this restriction are contained in (insert numbers or other identification of sheets).

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Statement of Work and Technical Approach

A. OBJECTIVES

BACKGROUND. Gas transport across cell membranes is essential for life but also plays central roles in disease, especially pathology of importance to the Navy. The dogma had been that all gases cross all membranes merely by dissolving in membrane lipids. The first crack in the dogma was our discovery[54] of the first membranes with no detectable CO_2 permeability. The second crack was our observation[4,38] that CO_2 can move through the **WATER CHANNEL AQUAPORIN 1 (AQP1)**—the first evidence for a gas channel. We and others later showed that, although several mammalian AQPs—AQP0, 1, 4, 5, 6, and 9—are highly permeable to CO_2, others are not[18,36]—the first evidence for gas selectivity by channels. Work currently in revision shows that at least AQP1 has a high permeability to O_2—the first evidence for an O_2 channel.[59] All AQPs are tetramers, and the four monomers surround a hydrophobic **CENTRAL PORE** that appears to be the main conduit for CO_2 and O_2 diffusion.

PROBLEM. In divers or DISSUB submariners, exposure to high O_2 can cause **PULMONARY OXYGEN TOXICITY (POT)**.

MISSION RELEVANCE. Pulmonary O_2 toxicity impairs pulmonary function, reduces performance, and can lead to serious illness or even death.

PHYSIOLOGY: Divers and submariners can experience a high alveolar O_2 partial pressure (P_{AO_2}), leading to exaggerated O_2 uptake from alveolar air into **ALVEOLAR TYPE I CELLS (AT_ICs)** and then into **PULMONARY CAPILLARY ENDOTHELIAL CELLS (PCECs)**. A high intracellular oxygen concentration ($[O_2]_i$) leads to oxidative damage. Understanding the pathophysiology and developing prophylactic countermeasures is a high priority for Undersea Medicine.

SOLUTION: Leverage newly discovered and characterized gas-channel physiology to identify new targets for adjuvant therapies. **AQUAPORIN 5 (AQP5)** is highly expressed in the apical (air-facing) membrane of AT_ICs. It has the highest-known CO_2 permeability of all AQPs, and is likely to be an excellent O_2 channel as well. AQP1 is highly expressed in PCECs. Data from our MURI team indicate that AQP1 functions as an O_2 channel in RBCs.[59]

HYPOTHESIS: AQP5 mediates a substantial fraction of O_2 uptake into AT_IC, and AQP1 similarly contributes to O_2 uptake by PCECs. At high P_{AO_2}, the genetic deletion of AQP5 or AQP1 from mice will reduce oxidative damage to AT_ICs and PCECs, respectively. The reduced inflammation and water permeability also will reduce pulmonary edema.

LONG-TERM GOALS: Our long-term plan (FIG 1) is to extend our AQP5 and AQP1 work to protect swine and eventually humans from acute lung and perhaps CNS injury in order to mitigate O_2 toxicity in divers through the development of drugs, identification of biomarkers, or extension of dive tables. O_2 concentration is a limiting factor in Navy diving operations and disabled-submarine scenarios.

FIG 1 TARGETING AQP5 & AQP1 FOR IMPROVED DIVER SAFETY AND PERFORMANCE

Genetic Proof of Principle in mice
↓
Genetic Proof of Principle in swine
↓
Human transition

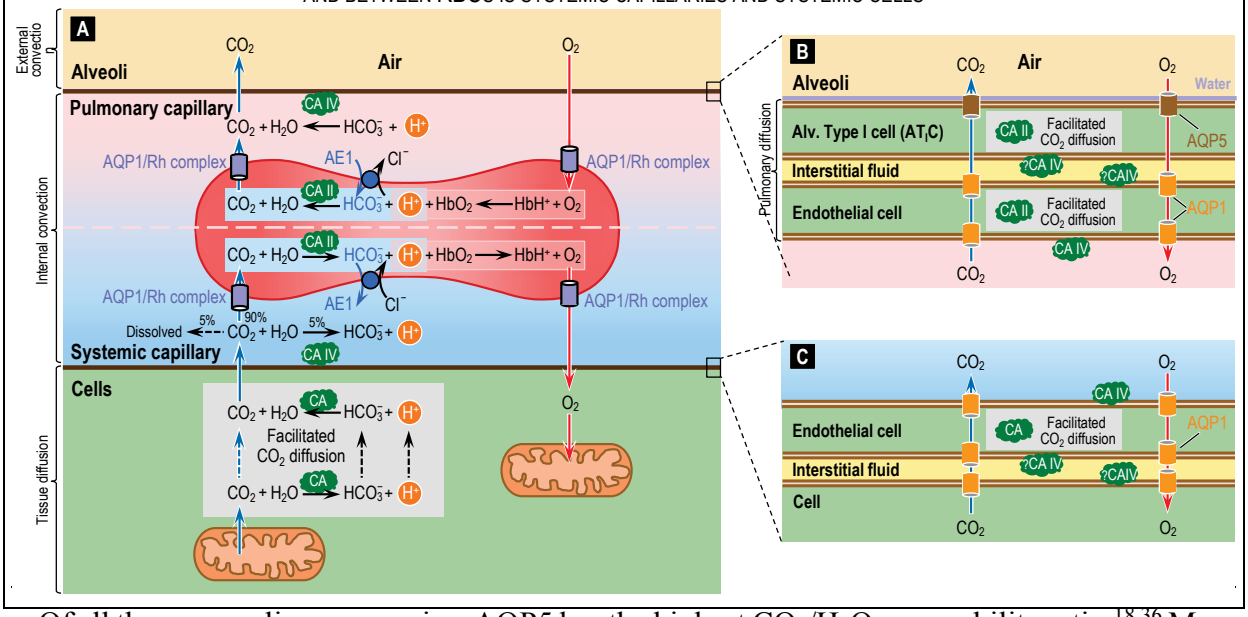
PILOT PROJECT OBJECTIVE: ***TEST THE GENETIC PROOF OF PRINCIPLE THAT THE ABSENCE OF AQP5 OR AQP1 IS PROTECTIVE IN A MOUSE MODEL OF PULMONARY OXYGEN TOXICITY.*** We propose a collaboration between two groups that are leaders in the respective expertise that they bring to the project: (A) The Boron Lab will generate and ship to Dr. Claude Piantadosi of Duke University the following: wild-type (WT) mice , "knockout (KO) mice" genetically deficient in AQP5 or AQP1, and **DOUBLE KNOCKOUTS (dKO)** mice deficient in both. And (B) the Piantadosi Lab will subject the mice to their O_2-toxicity protocol[58] and then assess the mice for POT by wet/dry weights of the lungs, bronchoalveolar-lavage (BAL), and by routine pathological approaches.

SIGNIFICANCE: If the hypothesis is true, the proposed work would establish a genetic proof of principle that reduced AQP5 or AQP1 activity would be an effective countermeasure for pulmonary oxygen toxicity. The next step would be a more detailed of AQP5/AQP1/O_2-toxicity in mice, to pave the way for a transition to swine. This line of research could greatly improve performance and reduce pathology in divers and submariners.

B. SIGNIFICANCE

Gas movement across biological membranes is one of the most fundamental processes of life. It is essential for O_2 delivery from environment to mitochondria, and CO_2 removal in the opposite direction. For nitric oxide (NO) and carbon monoxide (CO) transfer across membranes is central to biological signaling. Of special concern to the Navy is the movement of O_2 from alveolar air, across the alveolar type I cells and endothelial cells, into the pulmonary-capillary lumen and red blood cells (RBCs) (FIG 2A/TOP & B). In the systemic circulation, O_2 exits RBCs and ultimately reaches mitochondria throughout the body (FIG 2A/BOTTOM & C). Metabolically produce CO_2 moves in the opposite directions.

FIG 2 O_2 AND CO_2 TRAFFIC BETWEEN ALVEOLAR AIR AND RBCS IN PULMONARY CAPILLARY … AND BETWEEN RBCS IS SYSTEMIC CAPILLARIES AND SYSTEMIC CELLS [40]



Of all the mammalian aquaporins, AQP5 has the highest CO_2/H_2O permeability ratio.[18,36] Moreover, it is expressed at extremely high levels on the apical (i.e., air-side) membrane of the alveolar type I cells[47] across which gas exchange occurs (see brown channels in FIG 2B). AQP1 is expressed at high levels in pulmonary capillary endothelial cells. The only known roles of AQP5 and AQP1 in adults is to promote the development of **PULMONARY EDEMA**—the accumulation of fluid in the interstitium and, in more extreme cases, the alveolar air space. We propose that the physiological role of AQP5 in AT_ICs and AQP1 in PCECs is to promote O_2 and CO_2 exchange (FIG 2B).

Although the goal of this pilot grant would be to test the genetic proof of principle that AQP5 and AQP1 promote pulmonary O_2 toxicity, our work has even broader implications for the biomedical community. For Undersea Medicine, besides O_2 toxicity, a major constraint to undersea performance is **DECOMPRESSION ILLNESS (DCI)**. For submariners late in a DISSUB scenario, additional concerns are **HYPERCARBIA** and limited O_2 availability, and exposure to toxic gases. Ascent to high altitude—an issue for Marines—can lead to **ACUTE MOUNTAIN SICKNESS (AMS)** secondary to hypoxia. More generally, O_2 delivery is essential for **WOUND HEALING**. In health, optimal gas transport (O_2 to the tissues and CO_2 away) is essential for **PERFORMANCE**—both physical and cognitive. Our work on AQP5 and AQP1 could point the way to countermeasures for these other issues.

Finally, work on AQP5 and AQP1 could promote the development of **DESIGNER CHANNELS**—a mutant AQP that is permeable to one known gas, but impermeable to H_2O and other gases. As part of our MURI, we have already made progress in designing a mutant AQP1 with only minor H_2O permeability but enhanced CO_2 permeability.

Because it represents a major paradigm shift, gas channels are garnering attention well outside the DoD community, and have been the subject of several workshops, symposia, and meetings.

C. BACKGROUND

The old dogma that all gases cross all membranes merely by dissolving in the lipid phase of the membrane (for review, see ref. 1) is clearly inadequate. Our laboratory demonstrated the first gas-impermeable membranes (the apical membranes of the gastric gland)[54] and provided the first evidence for a gas (CO_2) passing through a channel (AQP1).[4,38] More recent work supported by the ONR has identified new gas-channel families (rhesus [Rh] proteins and urea transporters) and elucidated the roles of channels in CO_2 transport by plants and human RBCs.[9,10] In revision is a manuscript[59] that reports the discovery of the first O_2 channels. In addition, the ONR has supported work on the discovery of a new mechanistic class of "gas channels" (i.e., solute transporters permeable to CO_2), and the invention of the first assay for the flux across a membrane of inert gases (e.g., N_2), which could lead to the mitigation of DCI.

C.1. "BACKGROUND" GAS PERMEABILITY OF MEMBRANES

A gas channel can be effective only if the non-channel or "background" permeability of the membrane to the gas is small compared to the contribution of channels.[1,2,5,6] Some artificial lipid bilayers can have such extraordinarily high permeabilities as to mask the contribution of channels. But physiological membranes generally have low background permeabilities … how?

According to "Overton's rule"—the **SOLUBILITY HYPOTHESIS**—the **MEMBRANE PERMEABILITY TO SOLUTE X** (**$P_{M,X}$**) for a molecule diffusing through a membrane is proportional to the **SOLUBILITY OF X IN THE MEMBRANE LIPID** (**$s_{M,X}$**). Later refinements recognized that $P_{M,X}$ also depends heavily on the **DIFFUSION CONSTANT OF X IN THE MEMBRANE LIPID** (**$D_{M,X}$**)—the **SOLUBILITY-DIFFUSION HYPOTHESIS**.[12] For CO_2, the diffusion constant (D_{M,CO_2}) appears to be far more important than s_{M,CO_2}. Factors that reduce $D_{M,X}$ are increased saturation and length of the lipid hydrocarbon tail[12] and the presence of stabilizing molecules (e.g., cholesterol,[11,27] cerebrosides,[21] sphingomyelin and glycosphingolipids,[22] and unusual lipids in the unusually tough membranes of archaebacteria[33]). Integral membrane proteins can reduce the effective $s_{M,X}$ or $D_{M,X}$ by displacing or organizing membrane lipids—the **SOLUBILITY-DIFFUSION-PROTEIN HYPOTHESIS**[1]. In addition, integral membrane proteins, or soluble proteins that attach to the membrane via ionic or hydrophobic interactions, or soluble proteins that adhere to the other proteins can restrict *access* to the membrane or *egress* from the membrane—the **ACCESS-SOLUBILITY-DIFFUSION-PROTEIN-EGRESS HYPOTHESIS**.[1] In a model of the synaptic vesicle, integral and adhering proteins are so dominant that, "viewed from the outside, the lipidic surface is hardly 'visible'."[51]

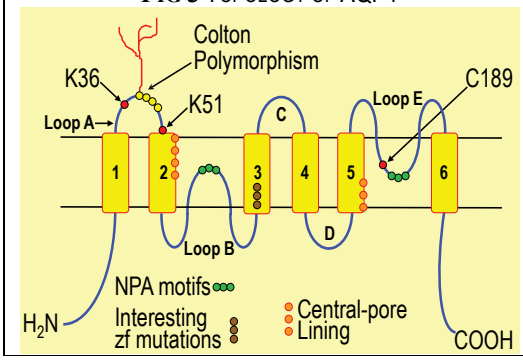
C.2. THE AQUAPORIN (AQP) FAMILY

While purifying a 32-kDa Rh protein from human RBC membranes in 1988, Agre copurified an unidentified 28-kDa protein.[8] In 1991, Preston & Agre cloned from human bone marrow the full-length cDNA that encodes the 28-kDa (269 amino acids) protein—initially called CHIP28.[42] After expressing CHIP28 in *Xenopus* oocytes and finding that it markedly increases **OSMOTIC WATER PERMEABILITY** (**P_f**),[43] Agre renamed the protein *AQP1*. Hundreds of new clones followed from animals, plants, bacteria and yeast. The AQPs are ancient and consist of two main groups, the classical AQPs (e.g., AQP1) and the aquaglyceroporins that have substantial permeability to small solutes such as glycerol.[20] Mammals have at least 13 AQP genes.

C.2.a. STRUCTURE

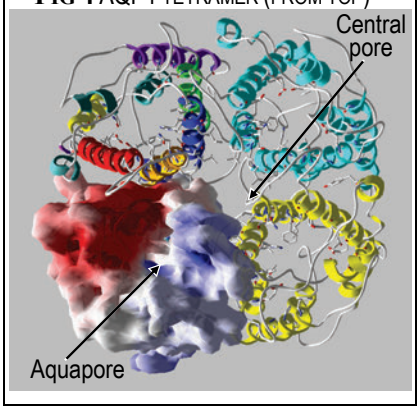
The AQP1 **monomer** evolved as a tandem dimer in which transmembrane segments (TMs) 1 through 3—including the characteristic Asp-Pro-Ala (NPA) motif on Loop B (FIG 3)—approximately repeats as TMs 4–6, including a second NPA motif on Loop E. Thus, the monomer's peptide backbone spans the membrane 6 times, with the two NPA motifs dipping into the membrane and folding over each other to form the center of the **MONOMERIC PORE**.[29] Structural studies show that AQP1

FIG 3 TOPOLOGY OF AQP1



is a *homotetramer*;[55,55] each of 4 monomers has a functional pore, and that the 4 monomers surround a hydrophobic *central pore* (FIG 4). Structures now exist for many AQPs from bacteria,[16,28,46] archaea,[32] yeast,[13] plants,[52] and mammals.[19,23–25,48,55]

FIG 4 AQP1 TETRAMER (FROM TOP)[48]



C.2.b. FUNCTION AS H_2O AND GLYCEROL CHANNELS

MERCURY. A hallmark of AQP1 is inhibition by extracellular mercurials. Agre deduced that Hg^{++} and pCMBS (organic mercurial) react with Cys-189 (see FIG 3), which faces the extracellular fluid and lies 2 residues upstream of the 2nd NPA.[44] Human AQP5 has an analogous, mercury sensitive Cys-182.

SELECTIVITY. AQP1 is permeable to H_2O but not H^+. Molecular dynamic (MD) simulations suggest that H_2O backs into the aquapore oxygen-first, flips around at the overlapping NPA loops, and then exits hydrogens-first.[50] X-ray crystallography indicates that, at its narrowest point, the aquapore is ~2.8 angstroms in diameter[81]—large enough to allow CO_2 or O_2 to pass.

Mammalian AQP3, 7 and 9 are members of the aquaglyceroporin subfamily, and have substantial permeability to solutes such as glycerol and urea as well as H_2O. Our MURI group has solved the crystal structure of AQP7 and—in a manuscript in revision[35]—has clarified how glycerol in the monomeric pore greatly slows the movement of H_2O and presumably CO_2.

C.2.c. FUNCTION AS GAS CHANNELS

C.2.c.i. Discovery

In 1998, the Boron Lab discovered the first gas channel, demonstrating that CO_2 moves through AQP1, as heterologously expressed in *Xenopus* oocytes.[4,38] Others noted that DIDS reduces P_{M,CO_2} in RBCs.[15] The Lab also showed that pCMBS (an organic mercurial targeting Cys residues) and DIDS (a amino-reactive agent targeting Lys residues) reduce the P_{M,CO_2} of AQP1[4,10]—the first examples of blockade of an identified gas channel—and showed that AQPs have gas selectivity.[18,36]

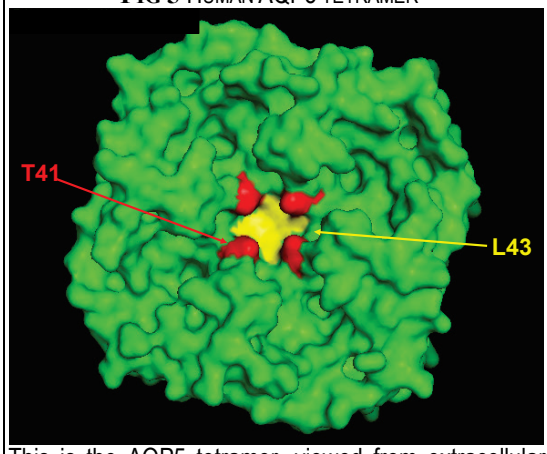
C.2.c.ii. Known physiological roles

Kaldenhoff *et al*[88] provided the first evidence that an AQP plays a critical role in a physiological process: CO_2 uptake for photosynthesis by tobacco leaves. The CO_2 permeability of AQPs has become a major target of plant research, to which the Boron Lab has contributed.[26,56] The Boron Lab and colleagues showed that AQP1 represents ~50% of P_{M,CO_2} in human RBCs.[21]

C.2.c.iii. Molecular dynamics

Molecular dynamics simulations[57] suggest that: (a) CO_2 can pass through the predominantly hydrophilic *monomeric pore* of an AQP1 monomer but prefers the hydrophobic *central pore*, and (b) the less hydrophilic O_2 could pass less easily through the aquapore, greatly preferring the central pore. A manuscript in revision confirms that about half of the CO_2 moves through AQP1 via the monomeric pore, and the other half via another pathway that is likely the central pore.[37]

FIG 5 HUMAN AQP5 TETRAMER[25]



This is the AQP5 tetramer, viewed from extracellular side. The four threonine residues at position 41 (T41)—one from each monomer—form the extracellular rim, just above the central pore (lumen colored yellow). Just below the four T41 residues, four leucine-43 (L43) residues form the extracellular end of the central pore.

C.2.c.iv. CO_2 Pathways through AQP5

POINT MUTATIONS AROUND THE EXTRACELLULAR RIM OF THE CENTRAL PORE. Forming the extracellular rim of the central pore of AQP5 (FIG 5) are four T41 residues, one from Loop A of each monomer (see FIG 3). Just below the four T41 residues are four L43 residues (see FIG 5) that mark the beginning of the central pore,

proper. We have made every possible mutation of T41.[45] None of the mutations affects P_f, and only four significantly affect P_{M,CO_2}.

(a) Replacing Thr41 with any of three bulky aromatic side chains (i.e., Phe, Tyr, Trp) significantly decreases P_{M,CO_2} in our oocyte assay.

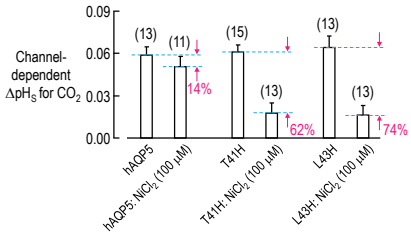
(b) Replacing Thr41 with the smallest side chain (i.e., Gly) increases P_{M,CO_2}.

Thus, we can control the CO_2 permeability of AQP5 by obstructing or widening access to the central pore.

CORK-IN-THE-BOTTLE APPROACH. We also mutated either T41 or L43 to His to create a metal-binding site (the opening of the "bottle") at/near the extracellular rim of the central pore. We predict that adding extracellular Ni^{2+} or Zn^{2+} (two "cork" options) should block CO_2 permeability through the central pore. Note: neither of these mutations, nor the addition of Ni^{2+} or Zn^{2+} to WT-AQP5 or either of the mutants, affects water permeability. Thus, our manipulations do not affect the monomeric pores.

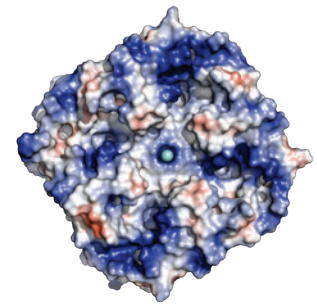
Applying Ni^{2+} (FIG 6, LEFTMOST 2 BARS) or Zn^{2+} (not shown) has little effect on the CO_2 permeability of WT AQP5. When we mutate T41 to His (T41H; FIG 6, MIDDLE 2 BARS) or mutate L43 to His (L43H; FIG 6, RIGHTMOST 2 BARS), we observe no effect on baseline CO_2 permeability (i.e., in the absence of Ni^{2+} or Zn^{2+}). However, Ni^{2+} or Zn^{2+} now markedly reduce CO_2 permeability. The experiments in this and the previous section provide the first direct evidence that a gas moves through a central pore. In fact, it is the first evidence that the central pore of a multimeric protein has any function at all—a significant advance for channel biology.

FIG 6 OOCYTE EXPRESSING AQP5: Ni^{2+} BLOCKADE OF CENTRAL PORE



CRYSTAL STRUCTURE. Ardi Vahedi-Faridi from our MURI Team has obtained the crystal structure of the AQP5-T41H mutant, with a Ni^{2+} atom plugging the central pore (Fig 7). This work establishes the proof of principle that it is possible to produce a targeted blockade of the central pore of an AQP. Moreover, preliminary MD simulations by Emad Tajkhorshid of our MURI Team suggest that this Ni^{2+} atom does indeed block the movement of CO_2 through the central pore of the T41H mutant of AQP5.

FIG 7 STRUCTURE OF HUMAN AQP5 (T41H MUTANT) WITH Ni^{2+} ATOM (CYAN) PLUGGING THE CENTRAL PORE



In summary the physiological, structural, and MD data from the MURI Team provides strong evidence that CO_2 moves through the hydrophobic central pore of human AQP5.

C.3. PULMONARY OXYGEN TOXICITY (POT)[3,7,14,41,49,53]

Pulmonary oxygen toxicity is an occupational hazard for divers breathing O_2-enriched gases at high pressure—especially for divers breathing pure O_2. POT is also an issue for hospitalized patients with critical lung disease, and patients treated in hyperbaric chambers for prolonged periods of time. Generally, inspired exposures of 0.5 ATA are considered safe, while doses above that produce lung injury as a function of dose and time.

In military and commercial diving, the current standard for the maximum predictable pulmonary oxygen exposure is **UNITS OF PULMONARY TOXICITY DOSE (UPTD)**. One UPTD equals the amount of damage caused by breathing 1 min of 100% oxygen at 1 ATA. The concept of UPTD is that a certain threshold of O_2 is required to cause lung damage, which can be measured by a decrease in **VITAL CAPACITY (VC)**. The "cumulative units of pulmonary toxicity dose" (CPTD), the "oxygen toxicity unit" (OTU) and an equation for repetitive exposures were developed to account for recovery and facilitate multi-day exposures, but these have never been validated. The main flaw in the application of the UPTD concept is that investigators often regard the change in VC as the sole indicator to determine oxidative lung damage. Difficulties with this assumption include: (a) Lung volumes exhibit intra- and interpersonal variability. (b) During O_2 breathing, absorption atelectasis

can lower VC. (c) The immersion process itself alters VC regardless of O_2 stress. (d) It is not possibly to measure VC reliably in animals.

Most studies of POT in humans, baboons, or mice, necessarily derive from dry-chamber experiments. Although, as discussed above, many human studies rely on decreases in VC to assess the degree of POT, biochemical and pathological effects on the lung are evident before VC falls. In animal studies, scavengers (such as all three forms of SOD and catalase) are known to protect lung parenchyma from the excessive production of oxidants generated by high inspired P_{O_2}. The most current information indicates that lung cell mitochondria, which produce most of the hydrogen peroxide and superoxide anion in the cell, greatly increase their oxidant production when exposed to excessive amounts of O_2. The result is direct damage to cellular macromolecules, including DNA, protein, and lipid (surfactant), the consequence of which are cell damage and cell death, especially of lung epithelial cells (human, baboon, and mouse) and endothelial cells (rats). The outcome is variable changes in lung function and gas exchange, which are also impacted by the production of nitric oxide, the autonomic nervous system, and other vasoactive mediators.

Therefore, in an experimental setting, the optimal measurements of POT are the examination of the lungs pathologically. The most useful measures are the degrees of (a) pulmonary edema, (b) lung inflammation, (c) lung DNA and protein oxidation, and (d) blinded pathological lung-injury scores. These are the measurements that we propose in the current study of genetically altered mice breathing 100% oxygen at 1 ATA continuously for 60 hours.

D. TECHNICAL APPROACH

This pilot project has only one objective: in a model of normobaric O_2 toxicity, we will determine if (a) AQP5-KO mice, (b) AQP1-KO mice, and (c) AQP5/AQP1-dKO mice experience less pulmonary oxygen toxicity than their WT counterparts.

RATIONALE. As noted above, AQP5 is the aquaporin with the highest CO_2/H_2O permeability ratio.[18,36] In rats and humans, AQP5 is localized to apical membrane (which faces air) of alveolar type I pneumocytes (see FIG 2B), but not **ALVEOLAR TYPE II PNEUMOCYTES ($AT_{II}Cs$)**.[17,31,39] Mice are similar, except that AQP5 is also present on the apical membranes of $AT_{II}Cs$.[30,34] Elsewhere in the lung, AQP5 is expressed in the non-ciliated and the basal cells of the pseudostratified columnar epithelium of the trachea, and in the most superficial cells of the stratified squamous epithelium of the upper airways.[34]

AQP1 is abundant in the capillary endothelial cells of the alveoli and of many systemic tissues.[34]

> Hypothesis #1a & b: O_2 can move through the central pores of (a) AQP5 (present in the AT_ICs) and (b) AQP1. Molecular-dynamics (MD) simulations by our MURI team, based on our new structure of AQP5/Ni^{2+}, indicates that this should be true. Previous MD simulations show that O_2 should move through the central pore of AQP1.[57] Furthermore, our work on RBCs indicates that AQP1 is an important pathway for O_2 movement across the RBC membrane.[59]

In animals with a high inspired P_{O_2}, alveolar P_{O_2} will also be high and—according to Fick's law of diffusion—produce a large flux of O_2 from the air into the cytosol of the AT_ICs (which cover 90% of the alveolar surface) and $AT_{II}Cs$. As a result, $[O_2]_i$ will rise to pathological levels in AT_ICs and—as discussed in SECTION C.3—lead to oxidative damage in these cells. This high $[O_2]_i$ will also lead to an increased flux of O_2 from the AT_ICs to the adjacent pulmonary capillary ECs, which can lead to oxidative damage in the ECs as well. The oxidative damage can secondarily lead to pulmonary edema as H_2O moves from the capillaries, through the PCECs (via AQP1) into the interstitium, and through the AT_ICs (via AQP5) into the alveolar air space (see FIG 2B).

A second consequence of the high $[O_2]_i$ in the AT_ICs and pulmonary capillary ECs is an increased diffusion of O_2 into the blood plasma, and eventually into the RBCs (see FIG 2B). Thus, the increased diffusion of O_2 from alveolar air to blood—through AT_ICs and ECs—promotes the development of O_2 toxicity in other parts of the body as well (e.g., CNS).

> Hypothesis #2a (AQP5): In mice with a genetic deletion of AQP5, the diffusion of O_2 from alveolar air into the AT_ICs and $AT_{II}Cs$ should be lower than in WT mice (see FIG 2B). As a

result, the $[O_2]_i$ should be lower, and oxidative damage should be less in these alveolar epithelial cells. The same should be true for the cells that normally express AQP5 in the other airways (see first RATIONALE paragraph, above). The AQP5-KO also may exhibit reduced pulmonary edema because of reduced inflammation and reduced H_2O permeability of the AT_ICs and AT_{II}Cs. Finally, because of the reduced O_2 flux through the AT_ICs, the AQP5-KOs should have reduced O_2 uptake into the pulmonary-capillary blood. Thus, AQP5-KO mice also should exhibit reduced oxygen toxicity elsewhere in the body, although we do not plan to test other organs in this pilot study. *Note: it would be straightforward for Dr. Dean to test the effects of this knockout in his* ***CNS OXYGEN TOXICITY (CNS-OT)*** *assays.*

Hypothesis #2b (AQP1): In mice with a genetic deletion of AQP1, the O_2 flux from AT_ICs into the PCECs (see FIG 2B) should be lower than in WT mice. $[O_2]_i$ and oxidative damage in PCECs should be less as well. The AQP1-KO also should exhibit reduced pulmonary edema, both because of reduced inflammation and because of the reduced H_2O permeability of PCECs. Finally, because of reduced O_2 diffusion through the PCECs into the pulmonary capillary blood, AQP1-KO mice also should exhibit reduced O_2 toxicity elsewhere in the body, although we do not plan to test other organs in this pilot study. *Note: it would be straightforward for Dr. Dean to test the effects of this knockout in his CNS-OT assays.*

Hypothesis #2c (AQP5/AQP1): Mice with genetic deletions of both AQP5 and AQP1 should have a combination of the favorable traits from the two single-KO strains, namely, reductions in: (a) oxidative damage to AT_ICs, AT_{II}Cs, and PCECs; (b) inflammation; (c) pulmonary edema; and (d) O_2 toxicity elsewhere in the body. *Note: again, it would be straightforward for Dr. Dean to test the effects of this dKO in his CNS-OT assays.*

APPROACH. The Boron Lab will expand and maintain colonies of (a) AQP5-KO mice, (b) AQP1-KO mice, (c) AQP5/AQP1-dKO mice, and (d) WT mice. We have backcrossed all KO strains into the WT strain for >10 generations to ensure that the strains are virtually isogenic except for the intended gene deletion(s). The Boron Lab will ship male mice at the appropriate age to Dr. Claude Piantadosi of Duke University. Because Dr. Piantadosi routinely uses mice at 6–8 weeks of age,[58] we would ship the mice at about 4 weeks of age (i.e., ~1 week after weening) so that sufficient time is available for acclimatization/quarantine at Duke. To control costs in this pilot study, we plan to limit our scope to male mice; we could extend the work to females in the future.

Dr. Piantadosi's group will subject mice to their well-established normobaric O_2-toxicity protocol:[58] a continuous exposure to 99% O_2 (or room air as a control) at sea level in chambers that control temperature, humidity and day-night cycle. (These chambers are similar to those used in Cleveland. Thus, in the future, it would be possible to perform parallel studies—e.g., blood-gas measurements—in the two venues.) The Duke group will use a standard sub-lethal harvest time (60–72 h) and compare the wet/dry weights of the lungs to assess the development of pulmonary edema. Bronchoalveolar-lavage (BAL) will yield several parameters indicative of lung injury/inflammation (e.g., inflammatory cells/ml, number of sloughed AT_ICs, albumin, LDH, TNFα, and IL-1β). They will perform these studies on 6 mice in each of 8 groups:

2 inspired gases:

- 99% O_2
- Room air

4 genotypes:

- *Aqp5–/–*
- *Aqp1–/–*
- dKO
- WT

for a total of 48 mice.

In addition, for 3–4 mice in each group, the Piantadosi Lab will fix lung tissue for routine blinded pathology and lung-injury scoring, and for lung immunochemistry (including AT_ICs distribution and number).

SIGNIFICANCE. If the hypotheses are correct, this pilot study will provide the first genetic proof of principle that AQP5 and AQP1 play an important role in gas pathology, specifically pulmonary oxygen toxicity. Next logical steps could be (1) molecular/cellular experiments to clarify the mechanism of O_2 diffusion through AQP5 and AQP1; (2) knocking central-pore mutations into mice to determine—along with oxygen-toxicity assays—whether the central pore is specifically responsible for the desirable phenotype seen in KOs; and (3) generating swine conditional-knockout embryos. Basic (6.2) research together with **Dr. Aaron Hall** would also yield insight into strategies to control the expression of AQP5 at the apical membrane of AT_ICs in mice and swine, and to control the expression of AQP1 in pulmonary capillary ECs. Collaborations with **Dr. Jay Dean** could test the predictions that interfering with pulmonary AQP5 and AQP1 could reduce the uptake O_2 into pulmonary-capillary blood and thereby mitigate CNS-OT.

The next major step would be genetic proof of principle in swine that the absence of AQP5 or AQP1 mitigates pulmonary oxygen toxicity. Finally, the transition to humans would include the development of small-molecule inhibitors of the AQP5 central pore—drugs with fast on/off rates that could be delivered as an aerosol into the inhaled air and allow the fine-tuning of the O_2 permeability of the apical membranes of AT_ICs. Other approaches could be the identification of biomarkers to identify individuals at high/low risk for pulmonary oxygen toxicity. Finally, the new knowledge gained in this research could produce insights that could lead to changes in guidelines for diving.

Project Schedule, Milestones, Deliverables

We will proceed simultaneously with generating, shipping, testing, and analyzing data from all four mouse strains as outlined in the narrative. We anticipate that the studies outlined here will require one year to complete. A key annual event will be our attendance at the Program Review for Undersea Medicine.

Our milestones will be the delivery of the mice (four strains) from the Boron Lab to the Piantadosi Lab, the generation of the planned data by the Piantadosi Lab on the four mouse strains subjected to the normobaric O_2 toxicity model, the analyses of these data, the presentation of the data at the annual Program Review for Undersea Medicine, and the publication of a scientific research paper.

The deliverables will be our annual progress reports, the report at the end of the funding period, and the aforementioned publications.

Management Approach

A. FACILITIES AVAILABLE

A.1. LABORATORY

The PI's total lab/office space is ~3000 square feet. For the proposed pilot project, we will use only a small fraction of this experimental space for genotyping studies. Adequate desk space is available for personnel working on this project to plan and track breeding of the mice, and to analyze data.

Some handling of the mice will take place in the **Mouse Physiological Phenotyping Core**, immediately adjacent to the Animal Resource Center.

A.2. EQUIPMENT

Although the Boron Lab is well equipped, the role of the Boron Group in the proposed pilot project—besides overall planning and the preparation of reports and papers—is limited to generating the necessary mice and shipping them to Duke University.

A.3. ANIMALS

Mice are housed in a state-of-the-art **Animal Resource Center**, located in the basement of our complex, immediately adjacent to the Mouse Physiological Phenotyping Core.

A.4. COMPUTERS

The personnel assigned to this project each has exclusive access to a personal computer. The Boron Lab uses a custom program for tracking the ancestry of all mice, including detailed genotyping data.

A.5. OFFICE

The Departmental office provides general grant support to the faculty.

B. PROPOSED SUBAWARDS (N/A)

The work in the Piantadosi Laboratory will be funded by a supplement directly to Duke University, not as a formal subaward.

C. PRINCIPAL INVESTIGATOR (SEE ALSO BIOSKETCH)

The PI will be **Walter F. Boron**. I have been an independent investigator for over 4 decades, and have been funded by the ONR for nearly 20 years, and have received a MURI grant, 3 DURIP grants, and 3 special ONR grants for equipment that supports the proposed research. Earlier in my career, I focused exclusively on the regulation intracellular pH (pH_i). In the mid-1990s, while studying pH_i regulation, we made the unexpected discovery of the *first gas-impermeable membranes* (the apical membranes of the chief and parietal cells of gastric glands). We concluded that these apical membranes must have special properties that not only render them resistant to autodigestion, but also gas impermeable. At a seminar at the University of Pennsylvania, Prof. Paul De Weer asked me if I had ever thought of the possibility that all membranes are gas impermeable but that the other membranes have **gas channels**. Although I initially regarded the suggestion as less than serious, the idea would not leave me. I reasoned that, if gas channels existed, they would be in a cell that did high levels of gas transport, and the channel itself would be a protein that is present at high levels but that does not otherwise seem to belong in that cell. I decided to test AQP1, which proved to be permeable to CO_2—*the first evidence for a gas channel*. In retrospect—especially in light of our new preliminary data on O_2 transport in RBCs—I think that RBCs likely have several gas channels, and that our initial focus on AQP1 was only one of several lucky guesses that we might have made. Since that time, we have been relentlessly pursuing—in parallel with our pH_i work—the subject of gas channels.

Besides our discovery of the first gas channel, we provided in our second 1998 *Am J Physiol* paper and our 2006 *FASEB J* paper *the first evidence for pharmacological blockade of a gas channel*, and in our 2009 *PNAS* paper, *the first evidence for gas selectivity of channels*. Moreover, work now in revision provides *the first evidence for O_2 channels*. Finally, based on preliminary data on AQP1, it appears that we have discovered *the first evidence of regulation of gas permeability* by a single phosphorylation. It is not for us to judge our own work … but I believe that the development of the gas-channel field will become recognized as one of the most striking paradigm shifts in the history of gas research, and a major paradigm shift in the broader field of biology.

Notable accomplishments since my last ONR grant application have been appointment in the first group of fellows of the American Physiological Society, and appointment as the incoming vice-chair of Section 3 of the National Academy of Medicine.

D. FUNDING

There is no overlap of our other grants with the present proposal. We presently have one active NIH grant and on no-cost extension (dedicated to a multi-scale modeling project of CO_2 entry into *Xenopus* oocytes).

For the grants listed below, the administrative/business contact for our institution, Case Western Reserve University, School of Medicine is: Erin Fogarty, medres@case.edu, 216-368-4432.

- U01 GM111251: "Multi-scale modeling of gas transport through channels in living cells". NIH: NIGMS. No-cost extension. Role: Contact PI. Effort: 15%, 1/1/2015 – 12/31/2019. The long-term objective is to develop a multi-scale mathematical model to study the mechanisms of gas permeation in oocytes from the atomistic to whole-cell scale, in normal and pathological states.
- N00014-16-1-2535: "Molecular mechanisms and pathways for gas transport across biological membranes and implications for physiology and performance". ONR-MURI. Annual Direct Costs: $477,048. Role: Lead PI, Effort: 20%, 8/1/2016 – 07/31/2021. The long-term objective is to understand the molecular mechanism of gas transport through the lipid phase of membranes, the role of blocking proteins in obstructing the lipid membrane, and the molecular mechanism of gas movement through channels.
- ONR subaward (pending): "Mitigation of CNS oxygen toxicity: mitochondria-targeted antioxidant therapy and aquaporin gas channels" PI: Jay Dean).
- R01 DK113197: "Role of RPTP-gamma in sensing and transducing acid-base disturbances in the renal proximal tubule". NIH: NIDDK. Annual Direct Costs: $318,909. Role: PI. Effort: 20%, 06/10/2017 – 04/30/2021. The long-term objective is to understand the mechanism by which the binding of HCO_3^- or CO_2 to the carbonic-anhydrase–like domain of RPTPγ triggers downstream signaling (i.e., dephosphorylation of tyrosine residues).

E. PLANS TO MANAGE INTERACTIONS AMONG MEMBERS OF THE RESEARCH TEAM

My office is immediately adjacent to the laboratory space, through which I walk several times a day to monitor progress, ask questions, and provide advice. The laboratory—which includes about 5 postdoctoral investigators and 1 PhD students—a lab manager, a computer specialist, and several technicians—meets weekly for a **general laboratory meeting** on Fridays at 8:30 – 10:00 am. Smaller groups meet weekly or biweekly. The **Gas Club** meets biweekly on Mondays at noon. At the smaller meeting, each member of the group summarizes his/her progress since the last meeting. Sometimes we focus the bulk of our attention on one particular subproject. Finally, we have a **Mouse Group** that meets biweekly to discuss breeding needs, but also physiological phenotyping of mice.

F. OTHER PARTIES TO WHOM PROPOSAL HAS BEEN/WILL BE SENT (N/A)

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Letters of Support

(None)

Curriculum Vitae

(See 5 following pages)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Boron, Walter Frank

eRA COMMONS USER NAME (credential, e.g., agency login): walterboron

POSITION TITLE: Professor & Chair

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
St. Louis University, St. Louis, MO	AB	05/1971	Chemistry
Washington University, St. Louis, MO	MD	05/1977	
Washington University, St. Louis, MO	PhD	05/1977	Physiology & Biophysics

A. Personal Statement

Research: We have two major areas of research, both of which bear on this grant application: (1) acid-base transporters/regulation of intracellular pH (pH_i), and (2) gas channels.

As a graduate student working on squid giant axons, I did the first experiments clearly demonstrating the active regulation of pH_i. During my early years as a graduate student and postdoctoral fellow, I also developed many of the experimental and theoretical paradigms widely used today in the acid-base field. As a postdoctoral fellow, I extended my expertise to epithelia, specifically renal tubules, and discovered the electrogenic Na/HCO_3 cotransporter (NBC). Since then, I have maintained research interests in the acid-base regulation both the brain and the kidney. Since I became an independent investigator, we were among the first to apply pH-sensitive dyes to living cells and study pH_i regulation in small vertebrate cells (e.g., kidney tubules, CNS). We also were first to clone a cDNA that encodes an NBC transporter, and went on to clone several related cDNAs that play important roles in renal acid-base transport and pH_i regulation throughout the body.

A related contribution was the development of out-of-equilibrium (OOE) CO_2/HCO_3^- solutions. One normally thinks of CO_2, HCO_3^-, and H^+ being related by the classic equilibrium $CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+$. We developed a rapid-mixing method for creating temporarily OOE states and then discovered that receptor protein tyrosine phosphatase RPTPγ is in fact a dual sensor for extracellular CO_2 and extracellular HCO_3^- in proximal tubules and most probably CNS neurons and astrocytes as well.

While studying pH_i regulation in isolated gastric glands, we discovered the first membrane with no permeability to CO_2. This led to the discovery of the first gas channel—the water channel AQP1, which is also permeable to CO_2. We now know two major families of gas channels, the AQPs and the rhesus (Rh) proteins. We showed that these channels exhibit selectivity between CO_2 and NH_3, much like ion channels exhibit selectivity for one ion (e.g., Na^+) over another (e.g., K^+). For example, AQP1, AQP4, and AQP5 are excellent CO_2 channels, but among these, only AQP1 conducts NH_3. Work by us and collaborators shows that 90 to 95% of the CO_2 permeability of red blood cells (RBCs) is due to AQP1 and the Rh complex. Ongoing research supported by a MURI championed by the ONR has provided the first evidence for channels permeable to O_2. AQP1 and the Rh complex appear to account for ~60% of the O_2 permeability of mouse RBCs. Also supported by the ONR is the development of a neutral buoyancy assay (NBA) that for the first time allows assays of movements of N_2 across cell membrane, and has led to the identification of the first N_2 channel. The NBA could be used to assess fluxes of other gases, such as the O_2 permeability of AQP5—highly relevant to the present application. Also relevant is recently completed work—now being assembled into a paper—in which we and our MURI collaborators have used Ni^{2+} and Zn^{2+} to block the central pore of a mutated AQP5, obtained the first crystal structure of AQP5 in a blocked state, and used molecular dynamics to model the diffusion of CO_2 through the unblocked central pore.

The following are 4 recent papers related to AQP4 and/or gas transport:

a. Geyer RR, R Musa-Aziz, X Qin & WF Boron. Relative CO_2/NH_3 selectivities of aquaporins 0-9. *Am J Physiol Cell Physiol* 304:C985–C994, 2013. doi: 10.1152/ajpcell.00033.2013.

b. Wang C, H Hu, X Qin, B Zeise, D Xu, W-J Rappel, WF Boron & JI Schroeder. Reconstitution of CO_2 regulation of SLAC1 anion channel and function of CO_2-permeable PIP2;1 aquaporin as carbonic

anhydrase 4 interactor. *Plant Cell* 28:568–582, 2016. PMID: 26764375. PMCID: PMC4790870. doi: 10.1105/tpc.15.00637.

c. Farr GW, CH Hall, SM Farr, R Wade, JM Detzel, AG Adams, JM Buch, DL Beahm, CA Flask, K Xu, JC LaManna, PR McGuirk, WF Boron & MF Pelletier. Functionalized phenylbenzamides inhibit aquaporin-4 reducing cerebral edema and improving outcome in two models of CNS injury. *Neuroscience* 404:484–498, 2019. doi: 10.1016/j.neuroscience.2019.01.034.

d. Zhang Y, K Xu, Y Liu, B Erokwu, P Zhao, C Flask, C Ramos-Estebanez, GW Farr, GJ LaManna, WF Boron & X Yu. Increased cerebral vascularization in response to decreased water exchange across the blood-brain barrier in aquaporin-4 knockout mice. *PLoS One* 14(6):e0218415, 2019. doi: 10.1371/journal.pone.0218415.

Editorial contributions. In addition to serving on the editorial board of *Journal of Physiology* and of *American Journal of Physiology*, I was Associate Editor for 6 years of *Physiological Reviews*, and then Editor-in-Chief of *Physiol Reviews* for 6 years. Later I served for 9 years as the founding Editor-in-Chief of the journal *Physiology*. In addition, I am co-editor (with EL Boulpaep) of the textbook *Medical Physiology*, now in its third edition.

Entrepreneurship. I cofounded Aeromics Inc., which recently completed successful Phase 1 clinical trials of AER-271 for the treatment of cytotoxic cerebral edema in stroke. The parent drug AER-270 is the first aquaporin inhibitor to enter clinical trials.

Positions and Employment

1977 – 1978	Postdoctoral Fellow, Department of Physiology & Biophysics, Washington University School of Medicine, St. Louis, MO
1978 – 1980	Postdoctoral Fellow, Department of Physiology, Yale University School of Medicine, New Haven, CT
1980 – 1984	Assistant Professor, Department of Physiology, Yale University School of Medicine, New Haven, CT
1984 – 1987	Associate Professor, Department of Physiology, Yale University School of Medicine, New Haven, CT
1987 – 2007	Professor, Department of Cellular and Molecular Physiology, Yale University School of Medicine, New Haven, CT
1989 – 1998	Chair, Department of Cellular & Molecular Physiology, Yale University School of Medicine, New Haven, CT
2007 –	Myers/Scarpa Professor & Chair, Department of Physiology & Biophysics, Case Western Reserve University School of Medicine, Cleveland, OH
2013 –	Professor, Secondary Appointment, Department of Medicine, Case Western Reserve University School of Medicine, Cleveland, OH
2015 –	Adjunct Professor, Department of Physiology, Wayne State University School of Medicine, Detroit, MI
2016 –	Guest Professor, Huazhong University of Science & Technology (HUST), Wuhan, China
2016 –	Executive Director, PhD Programs, Case Western Reserve University School of Medicine, Cleveland, OH
2017 –	Professor, Secondary Appointment, Department of Biochemistry, Case Western Reserve University, School of Medicine, Cleveland, OH
2017 – 2018	Interim Chair, Department of Biochemistry, Case Western Reserve University School of Medicine, Cleveland, OH

Other Experience and Professional Memberships

1994 – 1999	Editor-in-Chief, *Physiological Reviews*
1998 – 2001	President-elect/President/Past-President, American Physiological Society
2003 – 2007	Principal Investigator, Program Project Grant DK17433
2003 – 2012	Editor-in-Chief, *Physiology*
2007 – 2017	Chairman of the Board of Directors, Aeromics, Inc.
2010 – 2017	Secretary-General, International Union of Physiological Sciences
2018 – 2020	Vice Chair, Section 3, National Academy of Medicine, Washington, DC
2018 – 2020	Councilor, Association of Chairs of Department of Physiology

Honors

1975	Grass Foundation MBL Fellow, Sigma Xi

1977	Research Service Award, National Institute of Health
1981	Searle Scholar, Searle Scholars Program
1983	Research Career Development Award, National Institute of Health
1986	Young Investigator Award, American Society of Nephrology & American Heart Association
1993	Charles W. Bohmfalk Teaching Award, Yale University School of Medicine
1993	Robert F. Pitts Lecture and Award, International Union of Physiological Sciences
1998	Carl W. Gottschalk Lecture and Award, American Physiological Society
1998	Elected, Fellow of the American Academy for the Advancement of Science
2001	Merit Award, National Institute of Health
2005	Homer Smith Award, American Society of Nephrology
2008	Sharpey-Schafer Award, The Physiological Society
2010	Palade Gold Medal (with WA Catterall and RW Tsien), Wayne State University
2011	Daggs Award, American Physiological Society
2012	British Medical Association Certificate of Honor (shared with E.L. Boulpaep & Elsevier) for Medical Physiology Updated 2nd Edition, BMA Medical Book Awards
2014	Doctor Medicinae Honoris Causa, Aarhus University, Denmark
2014	Elected, The National Academy of Medicine
2015	Elected, Fellow of the American Physiological Society

C. Contributions to Science

Discovery of and early work in the regulation of intracellular pH (pH_i) regulation: The regulation of intracellular pH (pH_i)—and the study of HCO_3^- and CO_2 transport—has been at the center of my research career since my days as a PhD student. Working on squid giant axons, Paul De Weer and I obtained the first direct evidence for the dynamic and active regulation of pH_i by showing that the fall in pH_i induced by exposing the cell to CO_2 is followed by a slower pH_i recovery due to an active-transport process. We also discovered what is now known as the ammonium prepulse technique, and used it as a tool to acid load cells. This work led to the discovery that the pH_i-regulatory process in squid axons involves HCO_3^- or a related ion. Subsequent work with John Russell was the first to show that the transport carries Cl^-. Together with the work by Roger Thomas, this research defined the first-described pH_i regulator: the Na^+-driven Cl-HCO_3 exchanger. The review by Roos and Boron put the early pH_i work in context, and has become the most-cited publication in the pH field.

a. Boron WF, De Weer P. Intracellular pH transients in squid giant axons caused by CO_2, NH_3, and metabolic inhibitors. *J Gen Physiol* 67:91–112, 1976. PubMed PMID: 1460; PubMed Central PMCID: PMC2214912.

b. Boron WF, De Weer P. Active proton transport stimulated by CO_2/HCO_3^-, blocked by cyanide. *Nature* 259:240–241, 1976. PubMed PMID: 2874.

c. Russell JM, Boron WF. Role of chloride transport in regulation of intracellular pH. *Nature* 264:73–74, 1976. PubMed PMID: 12472.

d. Roos A, Boron WF. Intracellular pH. *Physiol Rev* 61:296–434, 1981. PubMed PMID: 7012859.

Discovery of the electrogenic Na/HCO_3 cotransporter: As a postdoctoral fellow with Emile Boulpaep, we set out to test the two leading hypotheses of the day concerning the mechanism of HCO_3^- exit across the PT basolateral membrane (BLM): a HCO_3^- channel vs. Cl-HCO_3 exchange. To prepare myself to answer the question, I first explored Na-H exchange activity and made the surprising discovery of high levels of NHE (not yet cloned) activity at the BLM. We now hypothesize that this NHE1 is key to controlling the nanoenvironment near NBCe1 ($q=2$) to behave as if $q=3$. It was when trying to block this BLM NHE by removing basolateral Na^+ in the presence of HCO_3^- that I observed the striking hyperpolarization that became the signature of the electrogenic Na/HCO_3 cotransporter, later known as NBCe1. As an independent investigator, we followed up with several papers in this area. In the one with Chaillet, we became the first to calibrate an intracellular dye vs. a pH microelectrode, setting the stage for future studies with pH-sensitive dyes. In several of these, including the paper by Ganz, we explored the role of NBCs (not yet cloned) in the activation of quiescent cells. Other papers from this period established several important experimental and theoretical paradigms for studying pH_i regulation.

a. Boron WF, Boulpaep EL. Intracellular pH regulation in the renal proximal tubule of the salamander. Na-H exchange. *J Gen Physiol* 81:29–52, 1983. PubMed PMID: 6833996; PubMed Central PMCID: PMC2215563.

b. Boron WF, Boulpaep EL. Intracellular pH regulation in the renal proximal tubule of the salamander. Basolateral HCO_3^- transport. *J Gen Physiol* 81:53–94, 1983. PubMed PMID: 6833997; PubMed Central PMCID: PMC2215562.
c. Chaillet JR, Boron WF. Intracellular calibration of a pH-sensitive dye in isolated, perfused salamander proximal tubules. *J Gen Physiol* 86:765–794, 1985. PubMed PMID: 4078557; PubMed Central PMCID: PMC2228795.
d. Ganz MB, Boyarsky G, Sterzel RB, Boron WF. Arginine vasopressin enhances pH_i regulation in the presence of HCO_3^- by stimulating three acid-base transport systems. *Nature* 337:648–651, 1989. PubMed PMID: 2521920.

Cloning of the cDNA encoding the electrogenic Na/HCO_3 cotransporter and related transporters: We and others had made several unsuccessful attempts to clone NBC. Our last, with Michael Romero, was to attempt expression cloning. This, too, failed when we (as others before us) used mRNA from rabbits. However, using mRNA from salamander kidney was the key decision. Starting with a library of ~16k clones, we quickly honed in on one that encoded NBCe1. The signature that we used for cloning was the ΔVm caused by Na+ removal in the presence vs absence of CO_2/HCO_3^-. Once we had the salamander clone, we cloned the rat NBCe1 by homology … and realized that we could not express it unless we put it into a Xenopus expression vector, explaining why we and others had previously failed in expression cloning with mammalian mRNA. We quickly realized that NBCe1 is in the same family as AE1, AE2, and AE3. Our cloning of NBC clarified what it meant to be an SLC4 family member, and led to the cloning of cDNAs representing 5 other genes that encode Na^+-coupled HCO_3^- transporters. The papers by Choi, Grichtchenko, and Virkki describe 3 of these.

a. Romero MF, Hediger MA, Boulpaep EL, Boron WF. Expression cloning and characterization of a renal electrogenic Na^+/HCO_3^- cotransporter. *Nature* 387:409–413, 1997. PubMed PMID: 9163427.
b. Choi I, Aalkjaer C, Boulpaep EL, Boron WF. An electroneutral sodium/bicarbonate cotransporter NBCn1 and associated sodium channel. *Nature* 405:571–575, 2000. PubMed PMID: 10850716.
c. Grichtchenko II, Choi I, Zhong X, Bray-Ward P, Russell JM, Boron WF. Cloning, characterization, and chromosomal mapping of a human electroneutral Na^+-driven Cl-HCO_3 exchanger. *J Biol Chem* 276:8358–8363, 2001. PubMed PMID: 11133997.
d. Virkki LV, Wilson DA, Vaughan-Jones RD, Boron WF. Functional characterization of human NBC4 as an electrogenic Na^+-HCO cotransporter (NBCe2). *Am J Physiol Cell Physiol* 282:C1278–1289, 2002. PubMed PMID: 11997242.

Invention of out-of-equilibrium CO_2/HCO_3^- solutions, leading to discovery of a novel CO_2/HCO_3^- sensor: Bob Berliner, by that time retired as Dean at Yale, was kind enough to sit down with me to go over some calculations in the appendix of a paper by Nakhoul that a peculiar activation of acid extrusion in rabbit proximal tubules exposed to CO_2/HCO_3^-. Bob mused that it was too bad that we could not add the CO_2 without adding the HCO_3^- or vice versa, and in an instant the idea of OOE solutions was born. Zhao brought them to fruition in experiments on squid axons, and then Zhou used OOE solutions—which make it possible to change $[CO_2]_o$ or $[HCO_3^-]_o$ or pH_o—one at a time—to demonstrate that PT cells have on their BLMs sensors for extracellular CO_2 and HCO_3^-. Following some clever reading of the literature by Patrice Bouyer, we eventually hypothesized that the receptor protein tyrosine kinase gamma (RPTPγ), which as an extracellular domain similar to a classical carbonic anhydrase, could be the dual CO_2/HCO_3^- sensor. The papers describing that work, and another describing related work on vascular smooth muscle, are now in press.

a. Zhao J, Hogan EM, Bevensee MO, Boron WF. Out-of-equilibrium CO_2/HCO_3^- solutions and their use in characterizing a new K/HCO_3 cotransporter. *Nature* 374:636–639, 1995. PubMed PMID: 7715702.
b. Zhou Y, Zhao J, Bouyer P, Boron WF. Evidence from renal proximal tubules that HCO_3^- and solute reabsorption are acutely regulated not by pH but by basolateral HCO_3^- and CO_2. *Proc Natl Acad Sci USA* 102:3875–3880, 2005. PubMed PMID: 15728388; PubMed Central PMCID: PMC553318.
c. Zhou Y, P Bouyer & WF Boron. Role of a tyrosine kinase on the CO_2-induced stimulation of HCO_3^- reabsorption by rabbit S2 renal proximal tubule. *Am J Physiol: Renal Physiol* 291: F358–367, 2006. doi:10.1152/ajprenal.00520.2005.
d. Boedtkjer E, KB Hansen, DMB Boedtkjer, C Aalkjaer & WF Boron. Extracellular HCO_3^- is sensed by mouse cerebral arteries: Regulation in tone by receptor protein tyrosine phosphatase γ. *J Cereb Blood Flow Metab* 36:365–380, 2016. doi: 10.1177/0271678X15610787.

Discovery of gas channels: While trying to study pH_i regulation in the cells of isolated, perfused gastric glands (which we were the first to perfuse), Waisbren made the discovery of the first CO_2-impermeable membrane. After I had given a seminar about this work at Penn, Paul De Weer (who had moved there as Chair) took me aside and asked me if my hypothesis (that these CO_2-impermeable apical membranes have an unusual lipid or protein composition) was backwards: is it possible that all membranes have low intrinsic CO_2 permeability, but that some have gas channels. I reasoned that, if a CO_2 channel existed, it would be in a cell that did large amounts of CO_2 transport, and the channel would be an abundant protein that did not seem to belong in that

cell. My experience teaching respiratory physiology to medical students led me to think about erythrocytes. Peter Agre had given us the cDNA encoding AQP1, and Nakhoul tested it in pH_i assays—the first example of a gas channel. Later, we collaborated with Gerolf Gros to show that AQP1 is responsible for about half of the CO_2 permeability of RBCs. We were the first to show inhibition of CO_2 fluxes by small-molecule inhibitors, and later compared CO_2, NH_3, and H_2O permeabilities of several AQPs and Rh proteins, providing the first examples of gas selectivity by channels. We continue to explore the molecular mechanism of gas movements through channel proteins.

a. Waisbren SJ, Geibel JP, Modlin IM, Boron WF. Unusual permeability properties of gastric gland cells. *Nature* 368:332–335, 1994. PubMed PMID: 8127367.
b. Nakhoul NL, Davis BA, Romero MF, Boron WF. Effect of expressing the water channel aquaporin-1 on the CO_2 permeability of *Xenopus* oocytes. *Am J Physiol* 274:C543–C548, 1998. PubMed PMID: 9486145.
c. Endeward V, Musa-Aziz R, Cooper GJ, Chen LM, Pelletier MF, Virkki LV, Supuran CT, King LS, Boron WF, Gros G. Evidence that aquaporin 1 is a major pathway for CO_2 transport across the human erythrocyte membrane. *FASEB J* 20:1974–1981, 2006. PubMed PMID: 17012249.
d. Musa-Aziz R, Chen LM, Pelletier MF, Boron WF. Relative CO_2/NH_3 selectivities of AQP1, AQP4, AQP5, AmtB, and RhAG. *Proc Natl Acad Sci USA* 106:5406–5411, 2009. PubMed PMID: 19273840; PubMed Central PMCID: PMC2664022.

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/walter.boron.1/bibliography/40663685/public/?sort=date&direction=descending

D. Research Support

Ongoing Research Support

MURI: N00014-16-2535, Office of Naval Research
Boron, Walter F
08/01/16-7/31/21
Molecular mechanisms and pathways for gas transport across biological membranes and implications for physiology and performance.
Role: Contact PI

ONR: Pending subcontract, Office of Naval Research
Boron, Walter F
08/01/16-7/31/21
Mitigation of CNS oxygen toxicity: mitochondria-targeted antioxidant therapy and aquaporin gas channels.
Role: Collaborator

U01 GM111251-01, National Institute of General Medical Sciences (NIGMS)
Boron, Walter F
01/01/15-12/31/19 (No-cost extension)
Multi-scale modeling of gas transport through channels in living cells
Role: Contact PI

NIH: R01-DK113197, National Institute of Diabetes & Digestive & Kidney Diseases
Boron, Walter F
06/10/2017-04/30/2021
Role of RPTP-gamma in sensing and transducing acid-base disturbances in the renal proximal tubule.
Role: PI

Completed Research Support

Research grant: N00014-15-1-2060, Office of Naval Research
Boron, Walter F
02/05/15-02/04/19
Gas channels
Role: PI

Equipment grant: N00014-15-1-2920, Office of Naval Research
Boron, Walter Frank
08/15/15-08/14/16
2015 DURIP: Equipment Grant
Role: PI

OMB Number: 4040-0001
Expiration Date: 12/31/2022

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE

State Application Identifier

1. TYPE OF SUBMISSION

☐ Pre-application ☐ Application ☒ Changed/Corrected Application

4. a. Federal Identifier N00014

b. Agency Routing Identifier 342 [Chapman,Sandra]

2. DATE SUBMITTED

Applicant Identifier

c. Previous Grants.gov Tracking ID GRANT12956827

5. APPLICANT INFORMATION

Organizational DUNS: 8043557900000

Legal Name: The Regents of the University of California; UC San Diego

Department:

Division:

Street1: UNIVERSITY OF CALIFORNIA SAN DIEGO

Street2: OFFICE OF CONTRACT & GRANT ADMIN, 0934

City: La Jolla

County / Parish:

State: CA: California

Province:

Country: USA: UNITED STATES

ZIP / Postal Code: 92093-0934

Person to be contacted on matters involving this application

Prefix: Ms.

First Name: Elizabeth

Middle Name:

Last Name: Tran-Nguyen

Suffix:

Position/Title: Contract and Grant Officer

Street1: Office of Contract and Grant Administration

Street2: 10300 North Torrey Pines Road - Level 3 West

City: La Jolla

County / Parish:

State: CA: California

Province:

Country: USA: UNITED STATES

ZIP / Postal Code: 92037-1020

Phone Number: (858)246-2383

Fax Number:

Email: ttrannguyen@ucsd.edu

6. EMPLOYER IDENTIFICATION *(EIN) or (TIN):* 95-6006-144

7. TYPE OF APPLICANT: H: Public/State Controlled Institution of Higher Education

Other (Specify):

Small Business Organization Type ☐ Women Owned ☐ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION:

☒ New ☐ Resubmission

☐ Renewal ☐ Continuation ☐ Revision

If Revision, mark appropriate box(es).

☐ A. Increase Award ☐ B. Decrease Award ☐ C. Increase Duration ☐ D. Decrease Duration

☐ E. Other (specify):

Is this application being submitted to other agencies? Yes ☐ No ☒ What other Agencies?

9. NAME OF FEDERAL AGENCY: Office of Naval Research

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER: 12.300

TITLE: Basic and Applied Scientific Research

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT:

Where is the gas? A new method to study nitrogen during diving and decompression: FY2019-000313-AS

12. PROPOSED PROJECT:

Start Date: 01/01/2020

Ending Date: 12/31/2022

13. CONGRESSIONAL DISTRICT OF APPLICANT

CA049

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: Dr. First Name: Peter Middle Name:

Last Name: Lindholm Suffix: MD, PhD

Position/Title: Professor in Residence

Organization Name: The Regents of the University of California; UCSD

Department: Emergency Medicine Division: UCSan Diego School of Medicine

Street1: 200 W. Arbor Dr. #8676

Street2: OFFICE OF CONTRACT & GRANT ADMIN, 0934

City: San Diego County / Parish:

State: CA: California Province:

Country: USA: UNITED STATES ZIP / Postal Code: 92103-1911

Phone Number: 16508230735 Fax Number:

Email: plindholm@ucsd.edu

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested	1,009,127.00
b. Total Non-Federal Funds	0.00
c. Total Federal & Non-Federal Funds	1,009,127.00
d. Estimated Program Income	0.00

16. IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?

a. YES ☐ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:

DATE:

b. NO ☒ PROGRAM IS NOT COVERED BY E.O. 12372; OR

☐ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious. or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

☒ **I agree**

The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.

18. SFLLL (Disclosure of Lobbying Activities) or other Explanatory Documentation

1242-WS00371677-GG_LobbyingForm-V1.1.pdf Add Attachment Delete Attachment View Attachment

19. Authorized Representative

Prefix: Ms. First Name: Elizabeth Middle Name:

Last Name: Tran-Nguyen Suffix:

Position/Title: Contract and Grant Officer

Organization: UC San Diego

Department: Office of Contract & Grant Adm Division: UCSan Diego School of Medicine

Street1: OFFICE OF CONTRACT & GRANT ADMIN, 0934

Street2: 9500 Gilman Drive

City: San Diego County / Parish:

State: CA: California Province:

Country: USA: UNITED STATES ZIP / Postal Code: 92093-0934

Phone Number: (858)246-2383 Fax Number:

Email: ttrannguyen@ucsd.edu

Signature of Authorized Representative	Date Signed
Tuong Vi E Tran-Nguyen	03/11/2020

20. Pre-application Add Attachment Delete Attachment View Attachment

21. Cover Letter Attachment Add Attachment Delete Attachment View Attachment

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For information on how to install Adobe Reader and configure your viewing environment please see http://www.adobe.com/go/pdf_forms_configure.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2. Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME	POSITION TITLE
Vera, David R.	Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(S)	FIELD OF STUDY
University of San Francisco	BS	1974	Biology
University of California, Davis	Ph.D.	1982	Biophysics

A. Personal Statement

During my career, I successfully translated two radiopharmaceuticals to clinical practice. The first, technetium-99m *Galactoscint* is a commercial product in Japan. It was the first receptor-targeted radiopharmaceutical to be approved by a federal regulatory agency, the Japanese FDA. The second, technetium-99m Tilmanocept (*Lymphoseek*), also a receptor-targeted radiopharmaceutical, received U.S. FDA approval in March 2013, CMS approval in October 2013, and approval by the European Medicines Agency in September 2014. In October 2014 the FDA expanded the clinical indications to include lymphatic mapping of all solid tumors. This experience, the complete translation from “bench” to “bedside”, followed by commercialization, is a rare achievement among academic investigators. Prior to Tilmanocept, the most recent FDA approval of a new technetium-99m radiopharmaceutical was in 1996. In the United States, 85 percent of Nuclear Medicine procedures use technetium-99m. These successful translations were accomplished by forming strong collaborations with my Nuclear Medicine (C.K. Hoh), and Surgery colleagues.

B. Positions and Honors

Positions and Employment

1982 - 1987 Assistant Research Biophysicist, Department of Radiology
1987 - 1993 Assistant Professor, Department of Radiology, University of California, Davis
1993 - 1997 Associate Professor, Department of Radiology, University of California, Davis
1997 - 1998 Professor, Department of Radiology, University of California, Davis
1998 - Present Professor, Department of Radiology, University of California, San Diego

Honors and Awards

1984 - 1985 New Investigator Research Award; NIDDK
1989 Berson-Yalow Award; Society of Nuclear Medicine
1996 Herbert M. Stauffer Award; Association of University Radiologists

Board Certifications

2007 American Board of Science in Nuclear Medicine

Public Service

2003 - Present Metabolic Disorders Steering Committee, Biomarker Consortium, Foundation of the NIH

C. Contributions to Science

* indicates collaborative publications with other investigators on this proposal

1. **Clinical Translation of the First Receptor-Binding Radiopharmaceutical**
   Historical Background: In 1978 William Eckelman and Richard Reba proposed (Eckelman WC & Reba RR, *J Nucl Med* 1978;19:1179-1181) a new classification for radiopharmaceuticals that localized by a “substrate-specific” mechanism. The purpose of these imaging agents, which were later named Receptor-Binding Radiopharmaceuticals (Eckelman WC, *Receptor Binding Radiotracers*, CRC Press, 1982), would be to distinguish healthy from disease tissue based on up-regulation of receptors in the diseased tissue. During the 1980’s most investigators focused on the development of receptor-binding radiotracers for brain and cardiac imaging.

Contribution to the Field: Tc-99m-galactosyl-neoglycoalbumin (TcNGA) was the subject of my PhD dissertation. In 1979, during the Second International Symposium on Radiopharmaceuticals, Kenneth Krohn, Robert Stadalnik, and I presented the first image produced by a receptor-binding radio-pharmaceutical. It was the image of a rabbit liver acquired ten minutes after injection of TcNGA, which binds to a receptor unique to hepatocytes. We proposed that a receptor-binding imaging agent should not use the tracer principle, which required that the imaging study be carried out with the smallest possible number of radioactive molecules. The goal of the tracer principle, which was considered the hallmark of a Nuclear Medicine study, was not to alter the physiologic or biochemical state of the system that is being studied. We reasoned that the true diagnostic power of a receptor-binding radiopharmaceutical lay in the bimolecular mechanism by which it localized to the target tissue. Therefore, we proposed that this class of imaging agent use injections with enough molecules that would occupy a significant fraction of the tissue receptors. The series of Tc-NGA publications from *in vitro* binding studies (ref 1), *in vivo* studies in animals (ref 2), and the clinical trials (refs 3 & 4) were essentially multiple demonstrations of this concept.

Role: My role in this Contribution was the chemical synthesis of NGA, the development and implementation of the Receptor-Binding Design model, the creation of a computer program for the kinetic analysis of the imaging data, and validation of the chemical and biologic properties. I participated in the design of the Phase 1 clinical trial, and the writing and submission of the Phase 1 application to the FDA.

a. **Vera DR**, Krohn KA, Stadalnik RC, Scheibe PO. [^{99m}Tc]galactosyl-neoglycoalbumin: *In vitro* characterization of receptor-mediated binding. *J Nucl Med* 1984;25:779-787.
b. **Vera DR**, Krohn KA, Stadalnik RC, Scheibe PO. [^{99m}Tc]galactosyl-neoglycoalbumin: *In vivo* characterization of receptor-mediated binding to hepatocytes. *Radiology* 1984;151:191-196.
c. Stadalnik RC, **Vera DR**, Woodle ES, Trudeau W, Ward RE, Krohn KA, O'Grady LF. Tc-99m-NGA functional hepatic imaging: Preliminary clinical experience. *J Nucl Med* 1985;26:1233-1242.
d. Pimstone NR, Stadalnik RC, **Vera DR**, Hutak DP, Trudeau WL. Evaluation of hepatocellular function by way of receptor-mediated uptake of a technetium-99m-labeled asialoglycoprotein analogue. *Hepatology* 1994;20:917-923.

2. **A Receptor-Binding Radiopharmaceutical Design Model for Optimization of Diagnostic Performance**

Historical Background: Early development of receptor-binding radiopharmaceuticals, especially the neuro-receptor agents, assumed that the optimal design was a chemical structure with extremely high receptor affinity and radiolabeling protocols that resulted in high specific activity (mCi per mole). The latter attribute was required to implement the tracer principle, which imposed low amounts (mole) of injected tracer.

Contribution to the Field: To test our hypothesis that a receptor-binding radiopharmaceutical should not be injected at “tracer” doses, we constructed a Design Model by which we could optimize the performance of a receptor-binding radiopharmaceutical. The model incorporated a mathematical description of the delivery (by blood flow) to the organ (or tumor) that will be imaged and localization of the radiopharmaceutical by the receptor in the organ or tumor. The latter process used a bimolecular mechanism for binding of the radiopharmaceutical to the receptor. Using engineering techniques, such as Identifiability Analysis (ref 1) and Sensitivity Analysis (ref 2), we demonstrated the requirement that the radiopharmaceutical exhibit moderate receptor affinity and the injected amount be enough to occupy a substantial fraction of the receptor within the target organ. We used another engineering technique called Parameter Plausibility to validate (ref 3) the mathematical model. Clinical confirmation of this work was achieved using Receiver Operating Characteristic analysis, another engineering technique. The work (ref 4) demonstrated that the highest diagnostic performance was achieved by the *receptor transport maximum*, which is the product of two parameters derived from the mathematical model: the *forward binding rate constant* multiplied by the square of the *receptor concentration* divided by the patient’s body weight. Moderate receptor affinity, molar dose, and kinetic sensitivity are fundamental design principles in the radiopharmaceutical sciences.

Role: My role was the development of the mathematical model, implementation the engineering methods, and creation of a computer program for the kinetic analysis of the imaging data.

a. **Vera DR**, Krohn KA, Schiebe PO, Stadalnik RC. Identifiability analysis of an in vivo receptor-binding radiopharmacokinetic system. *IEEE Trans Biomed Eng* 1985;BME-13:311-322.
b. **Vera DR**, Woodle ES, Stadalnik RC: Kinetic sensitivity of a receptor-binding radiopharmaceutical: Technetium-99m galactosyl-neoglycoalbumin. *J Nucl Med* 1989;30:1519-1530.

c. Kudo M, **Vera DR**, Trudeau WL, Stadalnik RC. Validation of in vivo receptor measurements via in vitro radioassay: Technetium-99m galactosyl-neoglycoalbumin (TcNGA) as a prototype model. *J Nucl Med* 1991;32:1177-1182.
d. Qin Z, *Hoh CK, Olson ES, Jahromi AH, Hall DJ, Barback CV, You YH, Yanagita M, Sharma K, **Vera DR**. Molecular Imaging of the glomerulus via mesangial cell uptake of radiolabeled tilmanocept. *J Nucl Med*. In Press.

3. **Clinical Translation of an FDA-Approved Sentinel Lymph Node (SLN) Imaging Agent**
Historical Background: Sentinel lymph node mapping is a technique developed by Donald Morton in the early 1990s. The sentinel lymph node, defined by Cabanas (*Cancer,* 1977), is the first lymph node to receive lymph that drains from a cancer. Prior to the FDA approval of Tilmanocept, surgeons used blue dye or a radioactive colloid to find the sentinel lymph node, which is given to the pathologist for examination. If the sentinel lymph node is free of metastases then the remaining lymph nodes will also be free of cancer cells. This information is then used to manage the patient's therapy. Both the blue dye and the radiocolloid have technical problems. The blue dye does not bind to lymph nodes. The radiocolloid, being significantly larger in size, slowly drains from the injection site (the tumor periphery) and, once in the sentinel lymph node, slowly leaks into the second (distal) lymph node within the nodal chain. The result is similar to the blue dye in that the surgeon ends up removing lymph nodes that are not sentinel to the cancer.

Contribution to the Field: Using the Receptor-Binding Design model that optimized the diagnostic performance of *Galactoscint*, we designed a receptor-binding radiopharmaceutical for sentinel lymph node mapping. Our new molecule (ref 1), ^{99m}Tc-DPTA-mannosyl-dextran, was called Lymphoseek (generic name: Tilmanocept). We used a low molecular weight dextran backbone and attached multiple units of mannose, which is the substrate for the receptor CD206. Unlike *Galactoscint*, which was designed to measure hepatocyte receptor concentration, we attached a high density of mannose units to create an extremely high affinity (K_D < 0.5 nM) to the receptor (ref 1). This property, in conjunction with a low molar dose (i.e. very high specific activity), insured that the receptor sites within the sentinel lymph node would not be saturated (i.e. all of the Tc-99m-Tilmanocept that reached the SLN would bind to the SLN). The result was rapid injection site clearance, and rapid and sustained SLN accumulation (refs 2, 3 & 4). Reference 5 extends the use of tilmanocept to imaging the primary cancer. In this paper, we demonstrated the high affinity (K_D < 0.5 nM) of tilmanocept to dendritic cells, which is the molecular basis for tumor accumulation.

Role: My role was the invention of the dextran-based technology, implementation of the Receptor-Binding Design model, and validation of the chemical and biologic properties, writing the Chemistry, Manufacturing and Controls package. I participated in the design of each of the Phase 1 clinical trials, and the writing and submission of the regulatory applications.

a. **Vera DR**, Wallace AM, *Hoh CK, Mattrey RF. A synthetic macromolecule for sentinel node detection: ^{99m}Tc-DTPA-mannosyl-dextran. *J Nucl Med* 2001; 42: 499-504.
b. Wallace AM, *Hoh CK, **Vera DR**, Darrah D, Schulteis G. A Phase I clinical study of a new radiopharmaceutical for sentinel node detection in breast cancer. *Ann Surg Oncol* 2003;10:531-538.
c. Wallace AM, *Hoh CK, Ellner SJ, Darrah DD, Schulteis G, **Vera DR**. Lymphoseek: A molecular imaging agent for melanoma sentinel lymph node mapping. *Ann Surg Oncol* 2007;14:913-921.
d. Wallace AM, Han LK, Povoski SP, Deck K, Schneebaum S, Hall NC, *Hoh CK, Limmer KK, Krontiras H, Frazier TG, Cox C, Avisar E, Faries M, King DW, Christman L, **Vera DR**. Comparative evaluation of [^{99m}Tc]tilmanocept for sentinel lymph node mapping in breast cancer patients: Results of two phase 3 trials. *Ann Surg Oncol* 2013;20:2590-2599.

4. **Multi-Modal Imaging of a Receptor-Binding Fluorescent-Radiopharmaceutical**
Historical Background: The current imaging literature abounds with descriptions of agents that can be imaged with PET or SPECT and fluorescence. Most of the agents do not provide a solution that can be realistically applied to a clinical setting. For example, Q-dots have issues with biodistribution and biologic safety. A setting that is compatible with a clinical workflow is sentinel lymph node mapping during surgery. There is a of series studies that use a cocktail of indocyanine green (ICG, a fluorescent dye) and Tc-99m-labeled *Nanocoll*, which is a colloid of human serum albumin. These studies demonstrated the ability to use SPECT/CT and fluorescence detection for sentinel lymph node mapping.

The work suffers from two disadvantages; *Nancoll* is only available in Europe, and the binding of ICG to *Nanocoll* is weak and requires purification.

Contribution to the Field: The following publications are demonstrations for a specific molecule and extend to optically-labeled receptor-binding probes in general. Reference 1 was the first demonstration that time-fluorescence curves can be acquired from the uptake of a fluorescent-labeled receptor-binding probe. This is important because at the time it was not evident that standard laboratory optical imagers were capable of the imaging sensitivity and temporal resolution needed to acquire time course data required for kinetic modeling. Reference 2 demonstrated that receptor affinity and in vivo biodistribution is not altered after that covalent attachment of multiple fluorescent dyes. Reference 3 was an analysis of the optical properties of IRDye800CW-labeled Tilmanocept, which will be used to select the chemical structure of the molecule that will be used in our "first-in-man" study. Reference 4 demonstrates the molecular mechanism (dendritic cell binding via the CD206 receptor) for fluorescence assessment of tumor margins. Reference 4 demonstrates the multi-modal imaging strategy using a dog model for prostate cancer where the dog is preoperatively imaged by PET/CT one hour after a trans-rectal injection into the prostate of Ga-68-labeled *IRDye800CW*-Tilmanocept. Two days later the dog is instrumented to a surgical robot. The pre-operative PET images are used to position the instrument arms of the robot over the sentinel lymph nodes, which after surgical exposure are visualized by the fluorescence imaging system of the robotic surgical system. Our current federal grant supports the preparation of *IRDye800CW*-Timanocept for a "First-in-Human" clinical trial. The agent has passed the required preclinical safety studies. We are currently scaling-up the synthesis and preparing the IRB/FDA documents. In reference 5, we demonstrated the ability of fluorescent-labeled tilmanocept to assess the surgical margins after resection of primary tumors.

Role: My role was the use of the dextran-based technology to attach the fluorescent label, and validation of the chemical, biologic, and optical properties of the fluorescent-labeled probe.

a. **Vera DR**, Hall DJ, *Hoh CK, Gallant P, McIntosh LM, Mattrey RF. Cy5.5-DTPA-galactosyl-dextran: A fluorescent probe for in vivo measurement of receptor biochemistry. *Nucl Med Biol* 2005;32:687-693.
b. Emerson DA, Limmer KK, Hall DJ, Han S-Y, Eckelman WC, Kane CJ, Wallace AM, **Vera DR**. A receptor-targeted fluorescent radiopharmaceutical for multi-reporter sentinel lymph node imaging. *Radiology* 2012; 265: 186-193.
c. Qin Z, Hall DJ, Liss MA, *Hoh CK, Kane CJ, Wallace AM, **Vera DR**. Optimization via specific fluorescence brightness of a receptor-targeted probe for optical imaging and positron emission tomography of sentinel lymph nodes. *J Biomed Opt* 2013; 18: 101315.
d. Anderson KM, Barback CV, Qin Z, Hall DJ, *Hoh CK, **Vera DR**, McHale MT. Molecular Imaging of endometrial sentinel lymph nodes utilizing fluorescent-labeled Tilmanocept during robotic-assisted surgery in a porcine model. PLoS ONE 2018;13(7): e0197842.

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/collections/public/1pogXyWIE-JC7yw2SO8Oawnk5/?sort=date&direction=descending

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

Endoscopy Research Award Vera (PI) July 1, 2018 – June 30, 2019
American Society for Gastrointestinal Endoscopy
"Molecular Imaging for Adenoma Detection during Gastrointestinal Endoscopy"
The goal of this project is to demonstrate tilmanocept binding to human adenoma biopsy specimens.
Role: PI

Completed Research Support

W81WXH-14-1-0578 Vera (PI) September 30, 2014 – July 29, 2018
Department of Defense, Prostate Cancer Research Program, Laboratory-Clinical Transition Program
"Translation of a Receptor-Binding PET/CT Imaging Agent for Fluorescence Imaging of Prostate Sentinel Lymph Nodes During Robotic-Assisted Surgery"
The goal of this project was to scale-up and test the pre-clinical safety of the bimodal imaging agent for prostate sentinel lymph node mapping during robotic surgery.
Role: PI

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: **Rui Carlos Pereira de Sá, Ph.D.**

eRA COMMONS USER NAME (credential, e.g., agency login): **RCPEREIRADESA**

POSITION TITLE: **Assistant Professor**

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Instituto Superior Técnico, Lisbon, Portugal	M.Sc.	09/1998	Engineering Physics
Université Libre de Bruxelles, Brussels, Belgium	D.E.A.	07/2001	Health Sciences
Université Libre de Bruxelles, Brussels, Belgium	Ph.D.	06/2008	Biomedical Sciences and Pharmacology
University of California, San Diego	Postdoc.	06/2013	Physiology, Biomedical Engineering

A. Personal Statement

I am a new investigator, establishing an independent research career in the interdisciplinary frontier between biomedical engineering and human physiology. My initial training was in Engineering Physics; my Ph.D. work focused on the influence of gravity in respiratory mechanics during sleep, using data collected before, during and after two Space Shuttle flights. Throughout my career, I have applied novel engineering approaches to respiratory physiology and pathophysiology. Since 2008 my main research tool has been functional Magnetic Resonance Imaging (MRI) of the lung, namely the development, validation and translation of a proton MR technique capable of quantifying ventilation in the human lung. Specific Ventilation Imaging uses inhaled oxygen as a T_1 contrast agent, to quantitative map specific ventilation.

In this project, I will apply my knowledge and extensive experience in imaging (MRI and multimodality) to the study of the spatial distribution of nitrogen in solution in the human body following hyperbaric breathing, using radiolabeled nitrogen and PET imaging. This project will allow me to collaborate with Dr. Lindholm, whom I've known since 1999, and briefly worked with in 2009. My main contributions to the project will be in imaging, image analysis, quality control and quantification.

Four publications relevant for this project:

- Mapping ventilation using Magnetic Resonance Imaging: **R.C. Sá**, M.V. Cronin, A.C. Henderson, S. Holverda, R.J. Theilmann, T.J. Arai, D.J. Dubowitz, S.R. Hopkins, R.B. Buxton, G.K. Prisk. Vertical distribution of specific ventilation in normal supine humans measured using oxygen-enhanced proton MRI. J Appl. Physiol. 109:1950 - 1959, 2010. (PMCID: PMC3006418)
- Validation of Specific Ventilation Imaging by comparison with Multiple Breath Washout: **R.C. Sá,** A.K. Asadi, R.J. Theilmann, S.R. Hopkins, G.K. Prisk, C. Darquenne. Validating the distribution of specific ventilation in healthy humans measured using proton MR imaging. J. Appl. Physiol., 116(8):1048-1056, 2014. (PMCID: PMC4035784)
- Persistence of ventilation defects in methacholine induced bronchoconstriction: E.T. Geier, I. Neuhart, R.J. Theilmann, G.K. Prisk, **R.C. Sá**. Spatial persistence of reduced specific ventilation following methacholine challenge in the healthy human lung. J. Appl. Physiol., 124(5):1222-1232, 2018. (PMCID: PMC6008074)

- Regional distribution of regions of low ventilation upon recovery from methacholine challenge: E.T. Geier, R.J. Theilmann, G.K. Prisk, **R.C. Sá**. Regional airflow obstruction after bronchoconstriction and subsequent bronchodilation in subjects without pulmonary disease. J. Appl. Physiol., in press, 2019. (PMCID and PMID: pending)

B. Positions and Honors

Appointments:

1998	Researcher (engineering degree thesis), Biomedical Physics Laboratory, School of Medicine, *Université Libre de Bruxelles*, Brussels, Belgium
1998 – 2003	Researcher and Ph.D. student, Biomedical Physics Laboratory, School of Medicine, *Université Libre de Bruxelles*, Brussels, Belgium
2003 – 2008	Researcher, teaching assistant and Ph.D. student, Biomedical Physics Laboratory, School of Medicine, *Université Libre de Bruxelles*, Brussels, Belgium
2008 – 2013	Post-doctoral scholar, Division of Physiology, Department of Medicine, University of California, San Diego.
2013 – 2019	Assistant Project Scientist, Division of Physiology, Department of Medicine, University of California, San Diego.
2019 –	Assistant Professor, Section of Physiology, Division of Pulmonary Critical Care and Sleep Medicine, Department of Medicine, University of California, San Diego.

Honors and Awards

Fellowships

1998	Prodex fellowship, Portuguese Ministry of Education (6 months, Master thesis)
1998 – 2002	Praxis XXI fellowship, Portuguese Ministry for Science and Technology.
2002 – 2003	Fellowship Calouste Gulbenkian Foundation (Portuguese private foundation).
2003	European Space Agency fellowship.
2009 – 2011	National Space Biomedical Research Institute postdoctoral fellowship.
2011 – 2012	National Space Biomedical Research Institute postdoctoral fellowship – competitive renewal.

Awards

1999	European Space Agency student prize awarded to “Pulse transit time for the non-invasive determination of arterial wall properties”, T. Dominique, P.F. Migeotte and **R.C. Sá** – Life odyssey symposium – 7th European conference on life science research in space.
2017	Willi Stahlhofen Award for outstanding experimental research published in the Journal of Aerosol Medicine and Pulmonary Drug Delivery in 2015-2016 - International Society for Aerosols in Medicine.

Memberships

1997 – present	Portuguese Physics society.
1999 – present	IEEE, and IEEE Engineering in Medicine and Biology Society.
2008 – present	Scientists in the World.
2008 – present	Portuguese-American Post-Graduate Society (PAPS).
2017 – present	International Society for Aerosols in Medicine (ISAM).
2017 – present	European Respiratory Society (ERS).

Reviewer

2004 – present:	IEEE Transactions on Biomedical Engineering
2009 – present:	Journal of Applied Physiology
2009 – present:	PLoS One and PLoS – Computational Biology
2010:	Journal of Asthma and Allergy
2010:	Clinics
2012:	Computer Methods and Programs in Biomedicine
2012:	European Journal of Applied Physiology
2012:	Respiratory Physiology & Neurobiology
2016 - 2017:	European Commission, Peer review committee for Marie Curie Individual Fellowships.
2017:	IEEE Journal of Biomedical and Health Informatics
2017:	Respiration

C. Contribution to Science

1. Specific Ventilation Imaging – using Magnetic Resonance Imaging to map ventilation in the human lung:

Specific ventilation Imaging uses oxygen as an inhaled contrast agent to quantitatively map the distribution of specific ventilation in the human lung, and in conjunction with measurements of regional lung density, to quantify regional ventilation. I have invented and developed this technique during my postdoctoral work, in collaboration with Dr. G. Kim Prisk[1,4]. Previously, quantification of the spatial distribution of ventilation typically required exposure to ionizing radiation and/or access to highly specialized hyperpolarized gas imaging. Specific ventilation imaging overcomes those limitations, opening a window for repeated studies, using standard, widely available clinical MRI scanners. The technique was validated against Multiple Breath Washout[2], with both techniques producing similar measures of ventilation heterogeneity. Recent progresses in the post-processing allow for the study of the persistence of the spatial distribution of ventilation following methacholinel[3], or following inhaled drug therapy (albuterol)[4].

1. **R.C.Sá**, M.V. Cronin, A.C. Henderson, S. Holverda, R.J. Theilmann, T.J. Arai, D.J. Dubowitz, S.R. Hopkins, R.B. Buxton, G.K. Prisk; Vertical distribution of specific ventilation in normal supine humans measured using oxygen-enhanced proton MRI. J. Appl. Physiol., 109:1950-1959, 2010. (PMCID: PMC3006418)
2. **R.C Sá,** A.K. Asadi, R.J. Theilmann, S.R. Hopkins, G.K. Prisk, C. Darquenne. Validating the distribution of specific ventilation in healthy humans measured using proton MR imaging. J. Appl. Physiol., 116(8):1048-1056, 2014. (PMCID: PMC4035784)
3. E.T. Geier, I. Neuhart, R.J. Theilmann, G.K. Prisk, **R.C. Sá**. Spatial persistence of reduced specific ventilation following methacholine challenge in the healthy human lung. J. Appl. Physiol., 124(5):1222-1232, 2018. (PMCID: PMC6008074)
4. E.T. Geier, R.J. Theilmann, G.K. Prisk, **R.C. Sá**. Regional airflow obstruction after bronchoconstriction and subsequent bronchodilation in subjects without pulmonary disease. J. Appl. Physiol., in press, 2019. (PMCID and PMID: pending)

2. Integrating imaging modalities – multiple MRI modalities and gamma scintigraphy:

Integrating data from different imaging modalities is one of my key contributions. A relevant example for the current application is a) ventilation-perfusion mapping[5,6] (integration of multimodal information from specific ventilation maps with quantitative lung density and lung perfusion images to produce insight on changes between these different variables[6] and to spatially quantify ventilation-perfusion in the human lung[5]); b) integrating data from gamma scintigraphy and specific ventilation imaging to study the impact of regional ventilation on particle deposition[7] and c) spatial validation of specific ventilation imaging against hyperpolarized helium[8].

5. **R.C Sá**, A. C. Henderson, T.S. Simonson, T.J. Arai, H. Wagner, R.J. Theilmann, P.D. Wagner, G.K. Prisk and S.R. Hopkins. Measurement of the distribution of ventilation-perfusion rations in the human lung with proton MRI: comparison with multiple inert gas elimination technique. J. Appl. Physiol. 123(1), 136 – 146, 2017. (PMCID: PMC5538816).
6. A.C. Henderson, **R.C. Sá**, I.A. Barash, S. Holverda, R.B. Buxton, S.R. Hopkins, G.K. Prisk. Rapid intravenous infusion of 20 ml/kg saline increases perfusion in nondependent lung in healthy supine humans. Respir. Physiol. Neurobiol. 115(3), 313-324, 2013. (PMCID: PMC3743008)
7. **R.C. Sá,** K.L. Zeman, W.D. Bennett, G.K. Prisk, C. Darquenne. Regional ventilation is the main determinant of alveolar deposition of coarse particles in the supine healthy human lung during tidal breathing. J. Aerosol Med. Pulm. Drug. Delivery, 30(5): 322 - 331, 2017 (PMCID: PMC5650708).
8. T.J. Arai, F.C. Horn, **R.C. Sá,** M.R. Rao, G.J. Collier, R.J. Theilmann, G.K. Prisk, J.M. Wild. Comparison of quantitative multiple breath specific ventilation imaging using co-localized 2D oxygen enhanced MRI and Hyperpolarized 3He MRI. J. Appl. Physiol., 125(5), 1526-1535, 2018. (PMCID: PMC6295484).

3. Signal processing for lung physiology – from times series to relevant information:

I have developed and implemented signal and image processing tools with direct application to respiratory and cardio-respiratory physiology. During my Ph.D. work, I developed a novel algorithm, based on artificial neural networks, for the accurate detection of breaths and respiratory movements acquired using respiratory inductive plethysmography, during sleep[9]. This algorithm proved to be undistinguishable from human experts performing the same task, and largely outperformed existing algorithms in sensitivity, positive predictive value, and accuracy. Another example was the use of a continuous complex wavelet-transform based method to quantify the non-stationary strength and phase delay between respiration and the corresponding oscillation in heart-rate[10] – respiratory sinus arrhythmia. This algorithm was specifically tailored to address the issue, commonly encountered during sleep, of how to quantify respiratory sinus arrhythmia when the breathing frequency is low; the algorithm was validated *in silico*[10] and proved to adequately address the issue, extending the range of

quantification of respiratory sinus arrhythmia to lower breathing frequencies and with less stringent stationarity constraints than existing methods. I have developed algorithms and software for quantitative MRI image processing, integrated into a coherent set of functions, a Matlab toolbox, for quantification and integration of different modalities of Magnetic Resonance Imaging. An example of a publicly distributed, stand-alone algorithm for deformable image registration, based on the lung outline can be found here[11].

9. **R.C. Sá** and Y. Verdandt; Automated breath detection on long duration signals using feedforward backpropagation artificial neural networks. *IEEE Transactions on Biomedical Engineering*, 49(10), 1130-1141, 2002. (PMID: 12374337)
10. L. Cnockaert, P.F. Migeotte, L. Daubigny, G. Kim Prisk, F. Grenez, and **R.C. Sá**; A method for the analysis of respiratory sinus arrhythmia using continuous wavelet transforms. *IEEE Transactions on Biomedical Engineering*, 55(5), 1640-1642, 2008. (PMID 18440911).
11. Publicly shared software for lung registration: https://github.com/UCSDPulmonaryImaging/Deforminator T.J.Arai**, R.C Sá**, A.K. Asadi. Deforminator – A Matlab toolbox for image registration of lung MRI images. Zenodo (2016) 10.5281/zenodo.45897
12. AK Asadi, **RC Sá**, TJ Arai, RJ Theilmann, SR Hopkins, R.B. Buxton, G.K. Prisk; Regional pulmonary perfusion patterns in humans are not significantly altered by inspiratory hypercapnia. J. Appl. Physiol., 127(2), 365-375, 2019
13.

4. The human lung and gravity – sleep and breathing in space and other gravity driven physiological changes:

Part of my scientific career focused on gravity and its impact on the human lung and cardio-respiratory interactions, namely the impact of low gravity exposure during space flight. I studied the influence of gravity in respiration, heart rate, and cardio-respiratory interactions during sleep, using data acquired prior, during and after two space shuttle flights; focusing on the influence of gravity on respiratory mechanics[12] and cardio-respiratory coupling. We observed a marked change in respiratory mechanics early in microgravity, with progressive return to pre-flight levels with the flight duration. Data also suggested the diaphragm is in a less efficient operating position in space, and/or the coupling between the diaphragm and rib cage respiratory compartments is less effective. These observations suggested either a muscular adaptation and/or changes in central neural control, occurring in the first week in space. During my Ph.D. I was awarded an European Space Agency prize, leading to the opportunity to study cardio-respiratory interactions during sudden gravity changes.

I collaborated with Dr. Darquenne, Dr. Prisk and Dr. Bennet (University of North Carolina – Chapel Hill) in the study of particle deposition in low gravity[13-15] using gamma scintigraphy as an imaging tool. Deposition of coarse particles in the lung periphery was found to be decreased in the lung periphery when inhalation occurs in microgravity. This study was driven by the need to set environmental standards for astronauts living and working on the moon, asteroids, mars or other low gravity environments where high levels of inhalable sized particles are present. As some of these particles present toxicological risk, knowledge of deposition site and clearance times are essential. A comparison of supine vs seated deposition in healthy humans was also performed[15]. This work was awarded the Willi Stahlhofen Award for outstanding experimental research published in the Journal of Aerosol Medicine and Pulmonary Drug Delivery in 2015-2016, by the International Society for Aerosols in Medicine.

14. **R.C. Sá**, G. Kim Prisk, M. Paiva; Microgravity alters respiratory mechanics during sleep. J. Appl. Physiol., 107: 1406–1412, 2009. (PMCID: PMC2777791)
15. G.K. Prisk, **R.C Sá**, C. Darquenne. Cardiogenic mixing increases aerosol deposition in the human lung in the absences of gravity. Acta Astronautica, 92(1): 15-20, 2013. (PMCID: PMC3747654)
16. C. Darquenne, K.L. Zeman, **R.C Sá**, T.K. Cooper, J.M. Fine, W.D. Bennett, G.K. Prisk. Removal of sedimentation decreases deposition of course particles in the lung periphery. J. Appl. Physiol., 115(4): 546-555, 2013. (PMID: 23743403)
17. **Sá RC**, Zeman KL, Bennett WD, Prisk GK, Darquenne C. Effect of Posture on Regional Deposition of Coarse Particles in the Healthy Human Lung. J. Aerosol Med. Pulm. Drug. Delivery, 28(6):423-31, 2015. (PMID: 25826480)

Complete list of published work in NCBI's: http://www.ncbi.nlm.nih.gov/sites/myncbi/rui.pereira de sa.1/bibliography/40818544/public/?sort=date&direction=descending

OMB Number: 4040-0010
Expiration Date: 12/31/2022

Project Abstract

The Project Abstract must not exceed one page and must contain a summary of the proposed activity suitable for dissemination to the public. It should be a self-contained description of the project and should contain a statement of objectives and methods to be employed. It should be informative to other persons working in the same or related fields and insofar as possible understandable to a technically literate lay reader. This Abstract must not include any proprietary/confidential information.

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1238-Abstract.pdf

RESEARCH & RELATED BUDGET - Budget Period 1

OMB Number: 4040-0001
Expiration Date: 12/31/2022

ORGANIZATIONAL DUNS: 8043557900000 **Enter name of Organization:** The Regents of the University of California; UC San Diego

Budget Type: ☒ Project ☐ Subaward/Consortium **Budget Period: 1** **Start Date:** 01/01/2020 **End Date:** 12/31/2022

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
Dr.	Peter		Lindholm	MD, PhD	(b) (4)	3.00			(b) (4)	(b) (4)	(b) (4)
Project Role:	PD/PI										
Dr.	David		Vera		(b) (4)	1.20			(b) (4)	(b) (4)	(b) (4)
Project Role:	Co-Investigator										
Dr.	Rui	Carlos	Pereira De Sa		(b) (4)	1.20			(b) (4)	(b) (4)	(b) (4)
Project Role:	Co-Investigator										

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

Total Funds requested for all Senior Key Persons in the attached file

Total Senior/Key Person (b) (4)

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	TBD - Senior Staff Scientist	10.00			(b) (4)	(b) (4)	(b) (4)
1	**Total Number Other Personnel**					**Total Other Personnel**	(b) (4)

Total Salary, Wages and Fringe Benefits (A+B) (b) (4)

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	Funds Requested ($)
Chamber Construction	5,000.00

Additional Equipment: Add Attachment | Delete Attachment | View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	5,000.00

D. Travel

	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	5,000.00
2. Foreign Travel Costs	
Total Travel Cost	5,000.00

E. Participant/Trainee Support Costs

	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other	
Number of Participants/Trainees — **Total Participant/Trainee Support Costs**	

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	55,855.00
2.	Publication Costs	1,000.00
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Communication/ NGN Expenses	509.00
9.	HS-TSC (Health Sciences Technology Services Charge)	1,756.00
10.	Animals	1,000.00
	Total Other Direct Costs	60,120.00

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	255,016.00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
MTDC	(b) (4)	(b) (4)	(b) (4)
		Total Indirect Costs	(b) (4)

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

Beth Swing
Office of Naval Research San Diego Regional Office
Phone: 619-221-5489

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	398,775.00

J. Fee

Funds Requested ($)

K. Total Costs and Fee

	Funds Requested ($)
Total Costs and Fee (I + J)	398,775.00

L. Budget Justification

(Only attach one file.) 1237-Budget_Justification.pdf

Add Attachment | Delete Attachment | View Attachment

RESEARCH & RELATED BUDGET - Budget Period 2

OMB Number: 4040-0001
Expiration Date: 12/31/2022

ORGANIZATIONAL DUNS: 8043557900000 **Enter name of Organization:** The Regents of the University of California; UC San Diego

Budget Type: ☒ Project ☐ Subaward/Consortium **Budget Period: 2** **Start Date:** 01/01/2021 **End Date:** 12/31/2021

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
Dr.	Peter		Lindholm	MD, PhD	(b) (4)	1.20			(b) (4)	(b) (4)	(b) (4)
Project Role:	PD/PI										
Dr.	Rui	Carlos	Pereira De Sa		(b) (4)	2.40			(b) (4)	(b) (4)	(b) (4)
Project Role:	Co-Investigator										

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

Total Funds requested for all Senior Key Persons in the attached file

Total Senior/Key Person (b) (4)

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	TBD - Senior Staff Scientist	12.00			(b) (4)	(b) (4)	(b) (4)
1	TBD - Research Assistant	3.60			(b) (4)	(b) (4)	(b) (4)
2	**Total Number Other Personnel**					**Total Other Personnel**	(b) (4)

Total Salary, Wages and Fringe Benefits (A+B) (b) (4)

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	Funds Requested ($)
Chamber Construction	35,000.00

Additional Equipment: Add Attachment | Delete Attachment | View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	35,000.00

D. Travel

	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	5,000.00
2. Foreign Travel Costs	
Total Travel Cost	5,000.00

E. Participant/Trainee Support Costs

	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other	
Number of Participants/Trainees — **Total Participant/Trainee Support Costs**	

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	34,465.00
2.	Publication Costs	1,000.00
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Communication/ NGN Expenses	709.00
9.	HS-TSC (Health Sciences Technology Services Charge)	2,254.00
10.	Animals	6,000.00
	Total Other Direct Costs	44,428.00

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	266,378.00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
MTDC	(b) (4)	(b) (4)	(b) (4)
MTDC	(b) (4)	(b) (4)	(b) (4)
		Total Indirect Costs	(b) (4)

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

Beth Swing
Office of Naval Research San Diego Regional Office
Phone: 619-221-5489

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	399,999.00

J. Fee

Funds Requested ($)
0.00

K. Total Costs and Fee

	Funds Requested ($)
Total Costs and Fee (I + J)	399,999.00

L. Budget Justification

(Only attach one file.) 1237-Budget_Justification.pdf

Add Attachment | Delete Attachment | View Attachment

RESEARCH & RELATED BUDGET - Budget Period 3

OMB Number: 4040-0001
Expiration Date: 12/31/2022

ORGANIZATIONAL DUNS: 8043557900000 **Enter name of Organization:** The Regents of the University of California; UC San Diego

Budget Type: ☒ Project ☐ Subaward/Consortium **Budget Period: 3** **Start Date:** 01/01/2022 **End Date:** 12/31/2022

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
Dr.	Peter		Lindholm	MD, PhD	(b) (4)	1.80			(b) (4)	(b) (4)	(b) (4)
Project Role:	PD/PI										
Dr.	Rui	Carlos	Pereira De Sa		(b) (4)	1.80			(b) (4)	(b) (4)	(b) (4)
Project Role:	Co-Investigator										

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

Total Funds requested for all Senior Key Persons in the attached file

Total Senior/Key Person (b) (4)

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	TBD - Senior Staff Scientist	4.80			(b) (4)	(b) (4)	(b) (4)
1	Chris Barback - Research Assistant	2.40			(b) (4)	(b) (4)	(b) (4)
2	**Total Number Other Personnel**					**Total Other Personnel**	(b) (4)

Total Salary, Wages and Fringe Benefits (A+B) (b) (4)

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	Funds Requested ($)

Additional Equipment: Add Attachment | Delete Attachment | View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	

D. Travel

	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	3,000.00

E. Participant/Trainee Support Costs

	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other	
Number of Participants/Trainees — **Total Participant/Trainee Support Costs**	

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	10,600.00
2.	Publication Costs	1,000.00
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Communication/ NGN Expenses	591.00
9.	HS-TSC (Health Sciences Technology Services Charge)	1,306.00
10.	Animals	2,000.00
	Total Other Direct Costs	15,497.00

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	133,135.00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
MTDC	(b) (4)	(b) (4)	(b) (4)
		Total Indirect Costs	(b) (4)

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

Beth Swing
Office of Naval Research San Diego Regional Office
Phone: 619-221-5489

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	210,353.00

J. Fee

Funds Requested ($)

K. Total Costs and Fee

	Funds Requested ($)
Total Costs and Fee (I + J)	210,353.00

L. Budget Justification

(Only attach one file.) 1237-Budget_Justification.pdf

Add Attachment | Delete Attachment | View Attachment

RESEARCH & RELATED BUDGET - Cumulative Budget

		Totals ($)
Section A, Senior/Key Person		(b) (4)
Section B, Other Personnel		(b) (4)
Total Number Other Personnel	5	
Total Salary, Wages and Fringe Benefits (A+B)		(b) (4)
Section C, Equipment		40,000.00
Section D, Travel		13,000.00
1. Domestic	13,000.00	
2. Foreign		
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		120,045.00
1. Materials and Supplies	100,920.00	
2. Publication Costs	3,000.00	
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1	1,809.00	
9. Other 2	5,316.00	
10. Other 3	9,000.00	
Section G, Direct Costs (A thru F)		654,529.00
Section H, Indirect Costs		(b) (4)
Section I, Total Direct and Indirect Costs (G + H)		1,009,127.00
Section J, Fee		0.00
Section K, Total Costs and Fee (I + J)		1,009,127.00

OMB Number: 4040-0001
Expiration Date: 12/31/2022

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: Dr.
* First Name: Peter
Middle Name:
* Last Name: Lindholm
Suffix: MD, PhD
Position/Title: Professor in Residence
Department: Emergency Medicine
Organization Name: The Regents of the University of California; UCSD
Division: UCSan Diego School of Medicine
* Street1: 200 W. Arbor Dr. #8676
Street2: OFFICE OF CONTRACT & GRANT ADMIN, 0934
* City: San Diego
County/ Parish:
* State: CA: California
Province:
* Country: USA: UNITED STATES
* Zip / Postal Code: 92103-1911
* Phone Number: 16508230735
Fax Number:
* E-Mail: plindholm@ucsd.edu

Credential, e.g., agency login:

*** Project Role:** PD/PI
Other Project Role Category:

Degree Type: MD
Degree Year: 2002

***Attach Biographical Sketch** 1234-Biosketch-Lindholm2019.p [Add Attachment] [Delete Attachment] [View Attachment]
Attach Current & Pending Support [Add Attachment] [Delete Attachment] [View Attachment]

PROFILE - Senior/Key Person 1

Prefix: Dr.
* First Name: David
Middle Name:
* Last Name: Vera
Suffix:
Position/Title: Professor
Department:
Organization Name: UC San Diego
Division:
* Street1: 9500 Gilman Drive
Street2:
* City: La Jolla
County/ Parish:
* State: CA: California
Province:
* Country: USA: UNITED STATES
* Zip / Postal Code: 92093-5004
* Phone Number: (858) 822-2574
Fax Number:
* E-Mail: dvera@ucsd.edu

Credential, e.g., agency login:

*** Project Role:** Co-Investigator
Other Project Role Category:

Degree Type: PhD
Degree Year: 1982

Attach Biographical Sketch 1235-Biosketch_Vera ONR2019.p [Add Attachment] [Delete Attachment] [View Attachment]
Attach Current & Pending Support [Add Attachment] [Delete Attachment] [View Attachment]

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Senior/Key Person 2

Prefix: Dr. * First Name: Rui Middle Name: Carlos

* Last Name: Pereira de Sá Suffix: Ph.D.

Position/Title: Assistant Professor Department:

Organization Name: UC San Diego Division:

* Street1: 9500 Gilman Drive #0852

Street2:

* City: La Jolla County/ Parish:

* State: CA: California Province:

* Country: USA: UNITED STATES * Zip / Postal Code: 92093-5004

* Phone Number: 858-822-3504 Fax Number:

* E-Mail: rcpereiradesa@ucsd.edu

Credential, e.g., agency login:

*** Project Role:** Co-Investigator **Other Project Role Category:**

Degree Type: PhD

Degree Year: 2008

Attach Biographical Sketch 1236-Bio_RuiCSa_Sept2019_Pete Add Attachment Delete Attachment View Attachment

Attach Current & Pending Support Add Attachment Delete Attachment View Attachment

RESEARCH & RELATED Other Project Information

OMB Number: 4040-0001
Expiration Date: 12/31/2022

1. Are Human Subjects Involved? ☐ Yes ☒ No

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ☐ Yes ☐ No

If yes, check appropriate exemption number. ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8

If no, is the IRB review Pending? ☐ Yes ☐ No

IRB Approval Date:

Human Subject Assurance Number:

2. Are Vertebrate Animals Used? ☒ Yes ☐ No

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ☐ Yes ☒ No

IACUC Approval Date: 11/26/2019

Animal Welfare Assurance Number: A3033-01

3. Is proprietary/privileged information included in the application? ☐ Yes ☒ No

4.a. Does this Project Have an Actual or Potential Impact - positive or negative - on the environment? ☐ Yes ☒ No

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ☐ Yes ☐ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place? ☐ Yes ☒ No

5.a. If yes, please explain:

6. Does this project involve activities outside of the United States or partnerships with international collaborators? ☐ Yes ☒ No

6.a. If yes, identify countries:

6.b. Optional Explanation:

7. Project Summary/Abstract 1239-Abstract.pdf Add Attachment Delete Attachment View Attachment

8. Project Narrative 1240-Technical_Proposal.pdf Add Attachment Delete Attachment View Attachment

9. Bibliography & References Cited Add Attachment Delete Attachment View Attachment

10. Facilities & Other Resources Add Attachment Delete Attachment View Attachment

11. Equipment Add Attachment Delete Attachment View Attachment

12. Other Attachments Add Attachments Delete Attachments View Attachments ☒

Abstract

BAA Number: N00014-20-S-B001
Technical Contact (PI): Peter Lindholm, MD, PhD
*Abstract Approved for Public Release

Where is the gas? A new method to study nitrogen during diving and decompression

A **century of study has not been able to identify the causal mechanism of decompression sickness** (DCS). We propose a new quantitative method to study Nitrogen gas (N_2) in mammals. Decompression sickness is currently hypothesized to be caused by bubble formation in tissues. However, due to the lack of direct methods to track tissue gas, **the field has used indirect methods** such as ultrasound of venous gas emboli (VGE) in the heart and mathematical modelling based on empirical testing in human divers. Current methods to predict DCS are based on statistical probability with relative risk factors such as depth and duration. It has not yet been possible to determine the causal mechanisms and explain the intra- and interindividual variability present in DCS, suggesting that **a direct method to map tissue gas distribution is a priority, as it has the potential to refine risk assessment and contribute to optimizing diving profiles.**

We will use a radioactive, positron-emitting isotope of Nitrogen (**$^{13}N_2$**) that can be breathed in a hyperbaric chamber and monitored in time and space using **positron-emission tomography (PET) combined with magnetic resonance imaging (MRI)**. With $^{13}N_2$, we can quantitatively measure the Nitrogen distribution in the body. $^{13}N_2$ has previously been produced and used in normobaric experiments with human volunteers, and a hyperbaric chamber can be constructed to inhale the gas during compression (simulated diving), widening applicability to hyperbaric breathing. We hypothesize that tissue gas bubbles will create bright spots while gas in solution will generate a signal equivalent to that tissue's saturation with Nitrogen. This new method would allow to answer the gas localization question, and address the impact of preconditioning on the amount of venous gas emboli after decompression. This approach will open a window **to study basic mechanisms and impact of interventions** related to DCS in both animals and humans.

Specific aim 1: To develop a method for inhaling $^{13}N_2$ under pressure and imaging gas distribution in a live animal. This will allow direct tracking of Nitrogen gas throughout the body, which is key to mechanistically explain decompression sickness under different conditions. We will **produce and purify the $^{13}N_2$ radioactive gas**, including compression and delivery into a pressure chamber, prior to PET and MR imaging. A chamber able to compress rodents within the PET and MRI will be developed and built. This experiment will establish the **proof of concept** that we can **track gas related to compression-decompression** in a rodent model.

Specific aim 2: Quantitative measurements of Nitrogen gas uptake and distribution during compression and excretion during and after decompression in rodents.
The dive profile (duration, pressure) will alter the tissue uptake of gas. Moreover, it has been shown that preconditioning significantly reduces VGE and reduces mortality by up to 50%. Using exercise as a preconditioning intervention, we will **test the hypothesis that preconditioning reduces the total nitrogen uptake** in rodents. We will also identify changes in **nitrogen storage, spatial distribution, and uptake and excretion temporal dynamics.**

Specific aim 3: Scaling the method to enable large animals. The method developed for rodents will be scaled to enable large animal experiments (e.g. sheep) breathing $^{13}N_2$ under pressure followed by imaging in a PET/MRI. The expected **outcome** is imaging **data** on signal intensity and gas distribution that can be **used to predict risk and optimize doses for planning human experiments**.

The proposed studies will establish a new rat model (and technical preparation for large animals and humans) that **will enable quantitative measurements of Nitrogen gas in vivo during compression-decompression** thus providing insight into tissue gas distribution and mechanisms of bubble generation during decompression. This technique will provide a valuable new tool for testing and selecting optimal interventions to prevent DCS, and optimize operational diving.

Budget Justification – University of California, San Diego (UC-San Diego)

Key Personnel at UC-San Diego

Peter Lindholm, MD, PhD - Site Principal Investigator **3.0 calendar months in Year 1**
1.20 calendar months in Year 2
1.80 calendar months in Year 3

Dr. Lindholm is a Professor in Residence, in the Department of Emergency Medicine and also holds the Gurnee endowed chair in hyperbaric and diving medicine research. He has 50+ original publications in the areas of diving medicine and radiology. As the Principal Investigator, Dr. Lindholm will lead the overall effort, recruit personnel, design and supervise experiments, analyze the results, write the reports and prepare and submit the scientific publications. The endowed chair will be used to cover additional Time year 2, equal to 1.80 months.

David Vera, PhD - Co- Investigator **1.20 calendar months in Year 1**

Professor in radiology at UCSD. He is the director of the In Vivo Imaging Shared Resource (IVISR) at UCSD, and also supervisor for the lab at Moore cancer center where the PET and MR facilities are located. He will supervise the radiochemist (TBD) and the Staff Research Associate (Mr. Barback) in developing the gas logistics and animal experiments.

Rui Carlos Pereira De Sà, PhD – Co-Investigator **1.20 calendar months in Year 1**
2.40 calendar months in Years 2
1.80 calendar months in Year 3

Dr. Sà is a physicist and assistant professor in physiology at UCSD. He will perform the mathematical and statistical programs and analysis of gas dynamics for both prediction and final analysis of gas dynamics. He will help with script writing necessary for the automation of data management and statistical analyses.

Non Key Personnel at UC-San Diego

TBD: Senior Staff Scientist **10.0 calendar months in Year 1**
12.0 calendar months in Year 2
4.80 calendar months in Year 3

A trained radiochemist with additional experience on animal experiments, he/she will be the project manager to work under supervision from Drs Vera and Lindholm. He/she will be responsible for optimizing the gas production and delivery as well as running rodent experiments during year 1-3. He/she will also assist in sheep experiments planned at subcontract Stanford year 3.

Chris Barback, Research Assistant **3.60 calendar months in Year 2**
2.40 calendar months in Year 3

The Research Assistant will assist with the direct experiments on both animals and radioactivity. Mr. Barback is experienced in small animal research, including all imaging modalities proposed (ultrasound, PET, MR). Together with Dr. Stevens, they will be the primary personnel doing the actual hands-on experiments on rats.

Other Expenses at UC-San Diego

Travel:

$13,000 ($5,000 in Year 1-2, $3,000 in Year 3) is requested for travel to ONR-NAVSEA Undersea medicine program review each year to present results of this study. In addition, domestic travel San Diego-Palo Alto will

be needed for collaboration with Stanford subcontractor. Year 3 is slightly higher due to additional travel to Stanford for experiments.

Materials & Supplies: ($100,920 TOTAL)
Some costs are based on an hourly rate. This include cost for production of radioactivity. Some consumables for experiments are needed, including animals costs, and a few additional items needed for constructing the gas delivery system that needs to enable rebreathing of gas, scavenging of gas and controlling /measuring activity (radioactivity) and concentration.

Research Equipment/Lab Supplies ($23,000 TOTAL)
Most equipment is readily available, i.e. all the major hardware such as the cyclotron, MR, PET, etc. Chamber exist on site. We will design and make an additional pressure chamber.

13N PETNET ($37,560 TOTAL)
Based on 350$ per produced batch of radioactivity and 90$ transfer/transportation cost to the lab in the basement of Moores cancer center. Currently available best estimate of costs.

PET Imaging ($13,160 TOTAL)
Based on currently available hourly rates at our facility.

Gas Analysis and Handling ($15,575 TOTAL)
Cost estimated from quote from a recent (2019) purchase of these Items, total for:
Gas chromatograph + accessories. Gas chromatograph, thermal conductivity detector (TCD), 10-port gas sampling valve, thermostatted valve oven, CTR-1 equivalent column set. SRI Instruments

Model 105S-3 scintillation detector Scintillation detector system Carowel Ramsey Instruments

Data chromatogram acquisition system Peak Simple six detector input data system jut fir HPLC, GC, USB includes sig signal inputs(0-5VDC) SRI Instruments

Animals ($9,000 TOTAL)
60 rats at 75$/each purchase and 75$handling including preconditioning, post-experiment hazard containment.

Publication Costs: ($3,000 TOTAL)
$1,000 is requested in years 1-3 for the publication costs.

Computer Supplies & Software: ($11,625 TOTAL)
- $6,125 is requested in years 1-2 for the purchase of computers for the research team
- $5,500 is requested in years 1-2 for the purchase of AMIRA software

Equipment: ($40,000 TOTAL)
Chamber Construction: $5,000 is requested in Year 1; $35,000 is requested in Year 2. An exact quote for this chamber is not possible, it will be part of the research to develop a PET/MR compatible chamber that would improve experimental quality. If not possible due to technical difficulties we can still lease a regular chamber for the proposed costs.

NGN communications costs and Health Sciences Technology Services Charge ($1,809 TOTAL)
NGN: UC San Diego Information and Technology Services (ITS) charges a flat per month fee for services to provide state-of-the-art technology infrastructure and services to the campus community. These charges are directly attributable and proportionally applied for the individual(s) included in the proposed budget on the project. These costs are not included in the campus' Facilities & Administration (F&A) rate as an indirect

cost. UC San Diego auditors have determined that it is both equitable and consistent with the OMB Circular 2 CFR 200 provisions on cost allocation that the costs be assigned to FTE on grant and contract funds. Accordingly, an allocable portion of these NGN costs are included in this budget as direct project costs.

Health Sciences Technology Services Charge (HS-TSC) ($5,316 TOTAL)
UC San Diego Health Information Services costs have been included to reflect costs associated with the increased level of security required for all personnel within UC San Diego Health, which includes UC San Diego School of Medicine, Skaggs School of Pharmacy and Pharmaceutical Sciences, and the hospitals and clinics. These charges are directly attributable and proportionally applied for the individual(s) included in the proposed budget on the project.

These costs are not included in the campus' Facilities & Administration (F&A) rate as an indirect cost. UC San Diego auditors have determined that it is both equitable and consistent with the OMB Circular 2 CFR 200 provisions on cost allocability that the costs be assigned to FTE on grant and contract funds. Accordingly, an allocable portion of these Technology Services costs are included in this proposal as direct project costs.

INDIRECT COST RATE (IDC) ($354,598 TOTAL)

- Year 1: $143,759
- Year 2: $133,621
- Year 3: $77,218

UC San Diego's indirect costs are calculated based on Modified Total Direct Costs (MTDC) as defined in 2 CFR Part 200.68 using Facilities and Administration (F&A) rates approved by the U.S. Department of Health and Human Services (DHHS).

MTDC is comprised of total direct costs less capital equipment, alterations and renovations, patient care costs, off-campus rent, tuition and fee remission, scholarships and fellowships, participant support costs, and that portion of subcontract costs in excess of $25,000. Additionally, the total amount of sub-awards to other University of California campuses are excluded. This project will be located on-campus.

Rates established by UC San Diego's F&A rate agreement dated May 23, 2018 are as follows:

- July 1, 2019, to June 30, 2020: 57.5%
- July 1, 2020, to June 30, 2021: 57.5%
- July 1, 2021, until amended: 58.0%

COLLEGES AND UNIVERSITIES RATE AGREEMENT

EIN: 1956006144A1

DATE:05/23/2018

ORGANIZATION:

University of California
San Diego Campus

FILING REF.: The preceding agreement was dated 05/12/2010

San Diego, CA

The rates approved in this agreement are for use on grants, contracts and other agreements with the Federal Government, subject to the conditions in Section III.

SECTION I: Facilities And Administrative Cost Rates

RATE TYPES: FIXED FINAL PROV. (PROVISIONAL) PRED. (PREDETERMINED)

	EFFECTIVE PERIOD				
TYPE	FROM	TO	RATE(%)	LOCATION	APPLICABLE TO
FINAL	07/01/2014	06/30/2017	55.00	On-Campus	Organized Research
PRED.	07/01/2017	06/30/2018	56.00	On-Campus	Organized Research
PRED.	07/01/2018	06/30/2019	57.00	On-Campus	Organized Research
PRED.	07/01/2019	06/30/2021	57.50	On-Campus	Organized Research
PRED.	07/01/2021	06/30/2022	58.00	On-Campus	Organized Research
FINAL	07/01/2014	06/30/2017	26.00	Off-Campus	Organized Research
PRED.	07/01/2017	06/30/2022	26.00	Off-Campus	Organized Research
FINAL	07/01/2014	06/30/2017	53.00	On-Campus	Instruction
PRED.	07/01/2017	06/30/2022	53.00	On-Campus	Instruction
FINAL	07/01/2014	06/30/2017	26.00	Off-Campus	Instruction
PRED.	07/01/2017	06/30/2022	26.00	Off-Campus	Instruction
FINAL	07/01/2014	06/30/2017	43.00	On-Campus	Other Sponsored Activities

ORGANIZATION: University of California San Diego Campus
AGREEMENT DATE: 5/23/2018

TYPE	FROM	TO	RATE(%)	LOCATION	APPLICABLE TO
PRED.	07/01/2017	06/30/2018	45.00	On-Campus	Other Sponsored Activities
PRED.	07/01/2018	06/30/2019	47.00	On-Campus	Other Sponsored Activities
PRED.	07/01/2019	06/30/2021	48.00	On-Campus	Other Sponsored Activities
PRED.	07/01/2021	06/30/2022	48.50	On-Campus	Other Sponsored Activities
FINAL	07/01/2014	06/30/2017	26.00	Off-Campus	Other Sponsored Activities
PRED.	07/01/2017	06/30/2022	23.40	Off-Campus	Other Sponsored Activities
FINAL	01/01/2014	06/30/2017	18.00	(1)	Organized Research
PRED.	07/01/2017	06/30/2022	18.00	(1)	Organized Research
FINAL	07/01/2014	06/30/2017	10.30	Off-Campus	(2) IPA
PRED.	07/01/2017	06/30/2022	10.30	Off-Campus	(2) IPA
PRED.	01/01/2017	06/30/2022	8.34	(3)	
PROV.	07/01/2022	Until Amended			Use same rates and conditions as those cited for fiscal year ending June 30, 2022.

*BASE

Modified total direct costs, consisting of all direct salaries and wages, applicable fringe benefits, materials and supplies, services, travel, and up to the first $25,000 of each subaward (regardless of the period of performance of the subawards under the award). MTDC excludes equipment, capital expenditures, charges for patient care, rental costs of off-site facilities, student tuition remission, scholarships and fellowships, participant support costs and the portion of each subaward in excess of $25,000.

(1) Nimitz Marine Facility & Marine Physical Laboratories.

(2) Intergovernmental/Personnel Act Agreements.

(3) Ship Operations Specialized Service Facility.
Base for Ship Operations Specialized Service Facility:
Modified total direct costs, consisting of all direct salaries and wages, applicable fringe benefits, materials and supplies, services, travel, and up to the first $25,000 of each subaward (regardless of the period of performance of the subawards under the award). MTDC excludes equipment, capital expenditures, charges for patient care, rental costs of off-site facilities, student tuition remission, scholarships and fellowships, participant support costs, fuel, Major Overhaul Stabilization Account (MOSA), and the portion of each subaward in excess of $25,000.

SECTION II: SPECIAL REMARKS

TREATMENT OF FRINGE BENEFITS:

The fringe benefits are specifically identified to each employee and are charged individually as direct costs. The directly claimed fringe benefits are listed below.

TREATMENT OF PAID ABSENCES
Vacation, holiday, sick leave pay and other paid absences are included in salaries and wages and are claimed on grants, contracts and other agreements as part of the normal cost for salaries and wages. Separate claims for the costs of these paid absences are not made. A separate charge is made to Federal projects for vacation benefit leave accruals.

DEFINITION OF EQUIPMENT
Equipment means tangible personal property (including information technology systems) having a useful life of more than one year and a per-unit acquisition cost which equals or exceeds $5,000.

DEFINITION OF ON-CAMPUS, OFF-CAMPUS AND SPECIAL RATES

DEFINITION OF OFF-CAMPUS RATE
The off-campus rate is applicable to those projects conducted at facilities not owned or leased by the University. However, if the project is conducted in leased space and lease costs are directly charged to the project, then the off-campus rate must be used.

PROJECTS CONDUCTED ENTIRELY ON-CAMPUS OR ENTIRELY OFF-CAMPUS:
Projects conducted entirely on-campus or entirely off-campus will be applied the on-campus or off-campus rate respectively.

PROJECTS CONDUCTED PARTIALLY OFF-CAMPUS AND PARTIALLY ON-CAMPUS:
If the project involves work at both on-campus and off-campus sites, either the on-campus or off-campus rate generally should be applied, consistent with where the majority of the work is to be performed. Salary cost is generally accepted as a measure of work performed in terms of the total project.

USE OF BOTH ON-CAMPUS AND OFF-CAMPUS RATES
The use of both on-campus and off-campus rates for a given project may be justified if both of the respective rates can clearly be identified with a significant portion of salaries and wages of the project. For purposes of this provision, significant is defined as approximately 25% or more of the total costs and a project's total salary and wage costs exceed $250,000.

OTHER SPECIAL RATES

These rates apply only to the facility or program to which they are identified. If any additional special rates become necessary the establishment of such rates should be coordinated through the cognizant negotiation agency.

The following fringe benefits are treated as direct costs:
OASDI, MEDICARE, RETIREMENT PLAN, WORKERS COMPENSATION, UNEMPLOYMENT, HEALTH/DENTAL/OPTICAL/LIFE INSURANCE, NON-INDUSTRIAL DISABILITY INSURANCE, ANNUITANT HEALTH/DENTAL INSURANCE, INCENTIVE AWARD PROGRAM, EMPLOYEE SUPPORT PROGRAM, SEVERANCE PAY AND TUITION/FEE REMISSION OF CERTAIN STUDENT EMPLOYEES.

NEXT PROPOSAL DUE DATE
A proposal based on actual costs for fiscal year ending 06/30/20, will be due no later than 12/31/20.

SECTION III: GENERAL

A. LIMITATIONS:

The rates in this Agreement are subject to any statutory or administrative limitations and apply to a given grant, contract or other agreement only to the extent that funds are available. Acceptance of the rates is subject to the following conditions: (1) Only costs incurred by the organization were included in its facilities and administrative cost pools as finally accepted: such costs are legal obligations of the organization and are allowable under the governing cost principles; (2) The same costs that have been treated as facilities and administrative costs are not claimed as direct costs; (3) Similar types of costs have been accorded consistent accounting treatment; and (4) The information provided by the organization which was used to establish the rates is not later found to be materially incomplete or inaccurate by the Federal Government. In such situations the rate(s) would be subject to renegotiation at the discretion of the Federal Government.

B. ACCOUNTING CHANGES:

This Agreement is based on the accounting system purported by the organization to be in effect during the Agreement period. Changes to the method of accounting for costs which affect the amount of reimbursement resulting from the use of this Agreement require prior approval of the authorized representative of the cognizant agency. Such changes include, but are not limited to, changes in the charging of a particular type of cost from facilities and administrative to direct. Failure to obtain approval may result in cost disallowances.

C. FIXED RATES:

If a fixed rate is in this Agreement, it is based on an estimate of the costs for the period covered by the rate. When the actual costs for this period are determined, an adjustment will be made to a rate of a future year(s) to compensate for the difference between the costs used to establish the fixed rate and actual costs.

D. USE BY OTHER FEDERAL AGENCIES:

The rates in this Agreement were approved in accordance with the authority in Title 2 of the Code of Federal Regulations, Part 200 (2 CFR 200), and should be applied to grants, contracts and other agreements covered by 2 CFR 200, subject to any limitations in A above. The organization may provide copies of the Agreement to other Federal Agencies to give them early notification of the Agreement.

E. OTHER:

If any Federal contract, grant or other agreement is reimbursing facilities and administrative costs by a means other than the approved rate(s) in this Agreement, the organization should (1) credit such costs to the affected programs, and (2) apply the approved rate(s) to the appropriate base to identify the proper amount of facilities and administrative costs allocable to these programs.

BY THE INSTITUTION:

University of California San Diego Campus

(INSTITUTION)

(b) (6) [redacted]

(SIGNATURE)

Nathan Brostrom

(NAME)

Executive Vice President, CFO

(TITLE)

(DATE)

ON BEHALF OF THE FEDERAL GOVERNMENT:

DEPARTMENT OF HEALTH AND HUMAN SERVICES

(AGENCY)

Arif M. Karim -S

Digitally signed by Arif M. Karim -S
DN: c=US, o=U.S. Government, ou=HHS, ou=PSC, ou=People, cn=Arif M. Karim -S, 0.9.2342.19200300.100.1.1=20002 12895
Date: 2018.05.24 08:49:29 -05'00'

(SIGNATURE)

Arif Karim

(NAME)

Director, Cost Allocation Services

(TITLE)

5/23/2018

(DATE) 7003

HHS REPRESENTATIVE: Janet Turner

Telephone: (415) 437-7820

UNIVERSITY OF CALIFORNIA, SAN DIEGO
FACILITIES AND ADMINISTRATIVE COST RATES
FOR THE PERIOD JULY 1, 2014
THROUGH JUNE 30, 2016

	ORGANIZED RESEARCH			INSTRUCTION			OTHER SPON. ACTIVITIES			IPAs **		NIMITZ MARINE FACIL.	
		ON-CAMPUS	OFF-CAMPUS		ON-CAMPUS	OFF-CAMPUS		ON-CAMPUS	OFF-CAMPUS				
BUILDING DEP.		5.3%			5.8%			7.5%			0.0%		0.0%
INTEREST		4.3%			2.7%			0.5%			0.0%		0.0%
EQUIPMENT		2.7%			1.1%			0.5%			0.0%		0.0%
OPERATIONS & MAINT		14.1%			9.1%			4.5%			0.0%		0.0%
LIBRARY		2.6%			8.3%			4.0%			0.0%		0.0%
GENERAL ADMIN	5.5%			3.4%			4.8%			5.8%		5.5%	
DEPT ADMIN	16.2%			13.5%			16.9%			0.0%		10.5%	
SPON PROJ ADMIN	4.0%			2.5%			4.0%			4.5%		2.0%	
STUDENT SERV ADMIN	0.3%			6.6%			0.3%			0.0%		0.0%	
ADMIN COMPONENTS	26.0%	26.0% *	26.0%	26.0%	26.0% *	26.0%	26.0%	26.0% *	26.0%	10.3%	10.3%	18.0%	18.0%
TOTAL		55.0%	26.0%		53.0%	26.0%		43.0%	26.0%		10.3%		18.0%

* Reflects provisions of Appendix III to Part 200 of Uniform Guidance—Indirect (F&A) Costs Identification and Assignment, and Rate Determination for Institutions of Higher Education (IHEs), C.8. dated September 10, 2015.
** IPAs are agreements made under the Intergovernmental Personnel Act (IPA) Mobility Program.

CONCUR: (b) (6) [redacted]
(SIGNATURE)

Executive Vice President, CFO
TITLE

5/22/2018
DATE

UNIVERSITY OF CALIFORNIA, SAN DIEGO
FACILITIES AND ADMINISTRATIVE COST RATES
FOR THE PERIOD JULY 1, 2016
THROUGH JUNE 30, 2017

	ORGANIZED RESEARCH			INSTRUCTION			OTHER SPON. ACTIVITIES			NIMITZ MPL/STS			IPAs **	
	ON-CAMPUS		OFF-CAMPUS	ON-CAMPUS		OFF-CAMPUS	ON-CAMPUS		OFF-CAMPUS	ON-CAMPUS		OFF-CAMPUS		
BUILDING DEP.		5.3%			5.8%			7.5%			0.0%			0.0%
INTEREST		4.3%			2.7%			0.5%			0.0%			0.0%
EQUIPMENT		2.7%			1.1%			0.5%			0.0%			0.0%
OPERATIONS & MAINT		14.1%			9.1%			4.5%			0.0%			0.0%
LIBRARY		2.6%			8.3%			4.0%			0.0%			0.0%
GENERAL ADMIN	5.5%			3.4%			4.8%			7.2%			5.8%	
DEPT ADMIN	16.2%			13.5%			16.9%			7.1%			0.0%	
SPON PROJ ADMIN	4.0%			2.5%			4.0%			3.7%			4.5%	
STUDENT SERV ADMIN	0.3%			6.6%			0.3%			0.0%			0.0%	
ADMIN COMPONENTS	26.0%	26.0% *	26.0%	26.0%	26.0% *	26.0%	26.0%	26.0% *	26.0%	18.0%	18.0%	18.0%	10.3%	10.3%
TOTAL		55.0%	26.0%		53.0%	26.0%		43.0%	26.0%		18.0%	18.0%		10.3%

* Reflects provisions of Appendix III to Part 200 of Uniform Guidance—Indirect (F&A) Costs Identification and Assignment, and Rate Determination for Institutions of Higher Education (IHEs), C.8. dated September 10, 2015.
** IPAs are agreements made under the Intergovernmental Personnel Act (IPA) Mobility Program.

CONCUR: (b) (6) [redacted]

(SIGNATU[redacted])

Executive Vice President, CFO
TITLE

5/29/2018
DATE

UNIVERSITY OF CALIFORNIA, SAN DIEGO
FACILITIES AND ADMINISTRATIVE COST RATES
FOR THE PERIOD JULY 1, 2017
THROUGH JUNE 30, 2018

	ORGANIZED RESEARCH			INSTRUCTION			OTHER SPON. ACTIVITIES			NIMITZ MPL/STS			IPAs **	
		ON-CAMPUS	OFF-CAMPUS		ON-CAMPUS	OFF-CAMPUS		ON-CAMPUS	OFF-CAMPUS		ON-CAMPUS	OFF-CAMPUS		
BUILDING DEP.		5.5%			5.5%			5.2%			0.0%			0.0%
INTEREST		4.5%			3.7%			4.0%			0.0%			0.0%
EQUIPMENT		3.1%			1.4%			2.0%			0.0%			0.0%
OPERATIONS & MAINT		14.3%			10.7%			6.4%			0.0%			0.0%
LIBRARY		2.6%			5.7%			4.0%			0.0%			0.0%
GENERAL ADMIN	6.8%			5.4%			9.8%			7.2%			5.8%	
DEPT ADMIN	15.2%			11.2%			8.7%			7.1%			0.0%	
SPON PROJ ADMIN	4.0%			2.7%			4.9%			3.7%			4.5%	
STUDENT SERV ADMIN	0.0%			6.7%			0.0%			0.0%			0.0%	
ADMIN COMPONENTS	26.0%	26.0% *	26.0%	26.0%	26.0% *	26.0%	23.4%	23.4%	23.4%	18.0%	18.0%	18.0%	10.3%	10.3%
TOTAL		56.0%	26.0%		53.0%	26.0%		45.0%	23.4%		18.0%	18.0%		10.3%

* Reflects provisions of Appendix III to Part 200 of Uniform Guidance—Indirect (F&A) Costs Identification and Assignment, and Rate Determination for Institutions of Higher Education (IHEs), C.8. dated September 10, 2015.
** IPAs are agreements made under the Intergovernmental Personnel Act (IPA) Mobility Program.

CONCUR: (b) (6) [redacted]
(SIGNATURE)

Executive Vice President, CFO
TITLE

5/29/2018
DATE

UNIVERSITY OF CALIFORNIA, SAN DIEGO
FACILITIES AND ADMINISTRATIVE COST RATES
FOR THE PERIOD JULY 1, 2018
THROUGH JUNE 30, 2019

	ORGANIZED RESEARCH			INSTRUCTION			OTHER SPON. ACTIVITIES			NIMITZ MPL/STS			IPAs **	
	ON-CAMPUS		OFF-CAMPUS	ON-CAMPUS		OFF-CAMPUS	ON-CAMPUS		OFF-CAMPUS	ON-CAMPUS		OFF-CAMPUS		
BUILDING DEP.		5.8%			5.5%			5.7%			0.0%			0.0%
INTEREST		4.6%			3.7%			4.4%			0.0%			0.0%
EQUIPMENT		3.5%			1.4%			2.2%			0.0%			0.0%
OPERATIONS & MAINT		14.5%			10.7%			7.0%			0.0%			0.0%
LIBRARY		2.6%			5.7%			4.3%			0.0%			0.0%
GENERAL ADMIN	6.8%			5.4%			9.8%			7.2%			5.8%	
DEPT ADMIN	15.2%			11.2%			8.7%			7.1%			0.0%	
SPON PROJ ADMIN	4.0%			2.7%			4.9%			3.7%			4.5%	
STUDENT SERV ADMIN	0.0%			6.7%			0.0%			0.0%			0.0%	
ADMIN COMPONENTS	26.0%	26.0% *	26.0%	26.0%	26.0% *	26.0%	23.4%	23.4%	23.4%	18.0%	18.0%	18.0%	10.3%	10.3%
TOTAL		57.0%	26.0%		53.0%	26.0%		47.0%	23.4%		18.0%	18.0%		10.3%

* Reflects provisions of Appendix III to Part 200 of Uniform Guidance—Indirect (F&A) Costs Identification and Assignment, and Rate Determination for Institutions of Higher Education (IHEs), C.8. dated September 10, 2015.
** IPAs are agreements made under the Intergovernmental Personnel Act (IPA) Mobility Program.

CONCUR: (b) (6) [redacted]
(SIGNATURE)

Executive Vice President, CFO
TITLE

5/24/2018
DATE

UNIVERSITY OF CALIFORNIA, SAN DIEGO
FACILITIES AND ADMINISTRATIVE COST RATES
FOR THE PERIOD JULY 1, 2019
THROUGH JUNE 30, 2021

	ORGANIZED RESEARCH			INSTRUCTION			OTHER SPON. ACTIVITIES			NIMITZ MPL/STS			IPAs **	
		ON-CAMPUS	OFF-CAMPUS		ON-CAMPUS	OFF-CAMPUS		ON-CAMPUS	OFF-CAMPUS		ON-CAMPUS	OFF-CAMPUS		
BUILDING DEP.		6.1%			5.5%			5.9%			0.0%			0.0%
INTEREST		4.8%			3.7%			4.6%			0.0%			0.0%
EQUIPMENT		3.5%			1.4%			2.3%			0.0%			0.0%
OPERATIONS & MAINT		14.5%			10.7%			7.3%			0.0%			0.0%
LIBRARY		2.6%			5.7%			4.5%			0.0%			0.0%
GENERAL ADMIN	6.8%			5.4%			9.8%			7.2%			5.8%	
DEPT ADMIN	15.2%			11.2%			8.7%			7.1%			0.0%	
SPON PROJ ADMIN	4.0%			2.7%			4.9%			3.7%			4.5%	
STUDENT SERV ADMIN	0.0%			6.7%			0.0%			0.0%			0.0%	
ADMIN COMPONENTS	26.0%	26.0% *	26.0%	26.0%	26.0% *	26.0%	23.4%	23.4%	23.4%	18.0%	18.0%	18.0%	10.3%	10.3%
TOTAL		57.5%	26.0%		53.0%	26.0%		48.0%	23.4%		18.0%	18.0%		10.3%

* Reflects provisions of Appendix III to Part 200 of Uniform Guidance—Indirect (F&A) Costs Identification and Assignment, and Rate Determination for Institutions of Higher Education (IHEs), C.8. dated September 10, 2015.
** IPAs are agreements made under the Intergovernmental Personnel Act (IPA) Mobility Program.

CONCUR: (b) (6) [redacted]
(SIGNATURE)

Executive Vice President, CFO
TITLE

5/29/2018
DATE

UNIVERSITY OF CALIFORNIA, SAN DIEGO
FACILITIES AND ADMINISTRATIVE COST RATES
FOR THE PERIOD JULY 1, 2021
THROUGH JUNE 30, 2022

	ORGANIZED RESEARCH			INSTRUCTION			OTHER SPON. ACTIVITIES			NIMITZ MPL/STS			IPAs **	
	ON-CAMPUS		OFF-CAMPUS	ON-CAMPUS		OFF-CAMPUS	ON-CAMPUS		OFF-CAMPUS	ON-CAMPUS		OFF-CAMPUS		
BUILDING DEP.		6.4%			5.5%			6.1%			0.0%			0.0%
INTEREST		5.0%			3.7%			4.7%			0.0%			0.0%
EQUIPMENT		3.5%			1.4%			2.3%			0.0%			0.0%
OPERATIONS & MAINT		14.5%			10.7%			7.4%			0.0%			0.0%
LIBRARY		2.6%			5.7%			4.6%			0.0%			0.0%
GENERAL ADMIN	6.8%			5.4%			9.8%			7.2%			5.8%	
DEPT ADMIN	15.2%			11.2%			8.7%			7.1%			0.0%	
SPON PROJ ADMIN	4.0%			2.7%			4.9%			3.7%			4.5%	
STUDENT SERV ADMIN	0.0%			6.7%			0.0%			0.0%			0.0%	
ADMIN COMPONENTS	26.0%	26.0% *	26.0%	26.0%	26.0% *	26.0%	23.4%	23.4%	23.4%	18.0%	18.0%	18.0%	10.3%	10.3%
TOTAL		58.0%	26.0%		53.0%	26.0%		48.5%	23.4%		18.0%	18.0%		10.3%

* Reflects provisions of Appendix III to Part 200 of Uniform Guidance—Indirect (F&A) Costs Identification and Assignment, and Rate Determination for Institutions of Higher Education (IHEs), C.8. dated September 10, 2015.
** IPAs are agreements made under the Intergovernmental Personnel Act (IPA) Mobility Program.

CONCUR: (b) (6) [redacted]
(SIGNATURE)

Executive Vice President, CFO
TITLE

5/29/2018
DATE

UNIVERSITY OF CALIFORNIA, SAN DIEGO
FACILITIES AND ADMINISTRATIVE COST RATES
FOR THE PERIOD JANUARY 1, 2017
THROUGH JUNE 30, 2022

	SHIP PLATFORM SSF	
BUILDING DEP.		0.10%
INTEREST		0.00%
EQUIPMENT		0.00%
OPERATIONS & MAINT		0.00%
LIBRARY		0.00%
GENERAL ADMIN	8.24%	
DEPT ADMIN	0.00%	
SPON PROJ ADMIN	0.00%	
STUDENT SERV ADMIN	0.00%	
ADMIN COMPONENTS	8.24%	8.24%
TOTAL		8.34%

CONCUR: (b) (6) [redacted]

(SIGNATURE)

Executive Vice President, CFO

TITLE

5/29/2018

DATE

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Peter Lindholm

eRA COMMONS USER NAME (credential, e.g., agency login):

POSITION TITLE: Professor in Residence, Gurnee Endowed Chair of Hyperbaric and Diving Medicine Research. Department of Emergency Medicine, University of California, San Diego

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Karolinska Institutet, Sweden	MD	09/2002	Medicine
Karolinska Institutet, Sweden	PhD	10/2002	Physiology
Hospital of Nyköping Sweden	License	08/2004	Internship
CRESE, Office of Naval Research, at Buffalo, NY	Postdoctoral	11/2005	Diving Medicine
Karolinska University Hospital, Sweden	License	11/2011	Residency in Radiology

A. Personal Statement

I moved to UCSD with my laboratory from the Karolinska Institute in July 2019 after a 2-year period at Stanford as visiting associate professor in Radiology. Prior to moving 2017 I was director of thoracic radiology at Karolinska, about 100 employees of whom 25 were radiologists. I have over 50 original publications and is internationally recognized as an expert in diving medicine, invited to conferences and courses, media etc. Education is one of my passions, both direct teaching but also to evolve and invent courses to improve the future practice of medicine through current students. I was responsible for respiratory physiology in medical school for many years and the residency program director for 4 years. I developed a weekly teaching program for residents that has been evolving since 2011, now including 125 residents in radiology from 15 hospitals in mid-Sweden. My science have focused on applied respiratory physiology with both exercise and environmental extremes (deep diving through high altitude into space). Special focus has been on gas, with hypoxia and hyperoxia in humans. I described the physiology of how glossopharyngeal breathing extends the amount of gas in the lungs beyond total lung capacity, including the first case series suggesting cerebral arterial gas embolism from glossopharyngeal insufflation. I published the first study suggesting “tracheal squeeze” as an alternative to pulmonary edema in divers bleeding after deep dives. I showed that exercise and fasting prior to diving increases the risk of loss of consciousness and drowning similar to hyperventilation. In clinical research I have developed and validated a new method to use magnetic resonance imaging to diagnose pulmonary embolism. I have the environmental physiologist’s knowledge of gas physiology and physics combined with the radiologist competence in advanced imaging procedures and radioactive materials.

B. Positions and Honors

<u>Positions and Employment</u>

1992-1993 Military service, Navy, Mine Clearance Diver, Jan 1992- Jan 1993
2002-2004 Internship ”AT-tjänst” 21 months incl. boards, Nyköping Hospital, Dec2002- Aug2004
2004-2005 Post-doctoral fellowship from the Office of Naval Research, US NAVY, and Assistant Professor, Center for Research and Education in Special Environments (CRESE), State University New York at Buffalo (SUNY), NY, USA, Oct 2004- Nov 2005

2005-2007	Senior Scientist, Centre for Environmental Physiology, Swedish Defence Research Agency (FOI), Nov 2005-Aug 2007
2007-	Associate Professor (Docent) in Physiology, Karolinska Institutet
2007-2011	Physician for the Swedish sports diving federation
2008-2009	Hyperbaric oxygen&diving physician, on-call and chamber service only, Karolinska Univ. Hosp.
2009	Visiting Scholar, Department of Radiology, University California San Diego, Sept-Dec 2009
2011-2017	Staff radiologist part-time, Karolinska University Hospital
2011-2015	Residency program director, Karolinska University Hospital
2013-2019	Associate Professor in Radiology, Karolinska Institutet
2015-2016	Head of section; Residents May 2015- June 2016, Karolinska University Hospital
2015-2016	Head of section; Thoracic October 2015- June 2016, Karolinska University Hospital
2016-2017	Director of Thoracic Radiology June 2016-Jan 2017, Karolinska University Hospital
2017-2019	Senior Consultant part-time (Överläkare), Karolinska University Hospital
2017-2018	Visiting associate professor, Dept Radiology, Stanford School of Medicine, CA

Other Experience and Professional Memberships

1999-	Member, Undersea Hyperbaric Medical Society
2002-	Member, Swedish medical association
2004-	Member, Swedish society for sports medicine
2008-	Member, European society of radiology
2015-	Member, Radiological Society of North America

Honors

1997	Awarded one semester as exchange student at Imperial College London, Pathology and Microbiology /Medical School.
2005	Mauro Ficini Young Investigator Award "Studi e ricerca sull'apnea" December 2, BLUE 2005 Conference, including prize €1000, PISA, ITALY
2009	Junior research group leader grant 100 000 SEK Karolinska Institutet
2010	President's Award: Best poster, Investigation of a Halcyon RB80 Semi-closed Rebreather in Connection with a Diving Accident. The Undersea and Hyperbaric Medical Society Annual Meeting
2012	Teaching distinction: The residents "Rose" (ST-läkarnas ros) 2012, published in the Swedish medical journal "läkartidningen".
2015	SHINE, Winning contribution in category Physical activity in chronic disease: Oxelerate AB and Peter Lindholm 25000 SEK

Patents

PCT: WO-2012-128704, A DEVICE AND METHOD FOR SUPPLYING AND DOSING GAS TO A BREATHING PERSON, 27 September 2012. EU, USA and Canada. Released/aborted 2018.
4 equal owners: Peter Lindholm, Oskar Frånberg, Fredrik Frånberg and Åke Larsson.

C. Contributions to Science (50+ original publications, 29 as first or last author)

My research started in the physiology of breath-hold diving, where I came up with an experimental method to convincingly show that humans can conserve oxygen during breath-hold diving, similar to deep-diving mammals (#1). We also discovered that breath-hold diving post-exercise (#5) or fasting (#15) increase the risk of involuntary loss of consciousness and that this can explain drownings. Studying the limits of hypoxia in human diving subjects (#7, 17), led to a change in current "text-book physiology" from 30mmHg to 20 mmHg. I came up with the idea of tracheal or bronchial strain for the minor bleedings that divers sometimes suffer in distinction to pulmonary edema that is another issue in diving. Today tracheal squeeze is a common term among the diving community, which originates from two of my papers (#4) and (#18). I have continued to study immersion pulmonary edema that show similarities with high altitude pulmonary edema (HAPE) on high resolution computed tomography (#44).

 # 1 Lindholm P, Sundblad P and Linnarsson D. Oxygen-conserving effects of apnea in exercising men.
J Appl Physiol 87: 2122-2127, 1999, PMID: 10601158

 # 4 Lindholm P. and S. Nyren Studies on inspiratory and expiratory glossopharyngeal breathing in breath-hold divers employing magnetic resonance imaging and spirometry. Eur J Appl Physiol 94(5-6): 646-51. 2005 PMID: 15942772
 # 5 Lindholm P, Gennser M. Aggravated hypoxia during breath-holds after prolonged exercise Eur J Appl Physiol 93: 701-707, 2005 PMID: 15778900
 # 7 Lindholm P. Lundgren CEG Alveolar gas composition before and after maximal breath-holds in competitive divers UHM 2006 Nov-Dec;33(6):463-7 PMID: 17274316
 # 15 Lindholm P, Conniff M, Gennser M, Pendergast D, Lundgren CEG Effects of fasting and carbohydrate consumption on voluntary resting apnea duration Eur J Appl Physiol. 2007 Jul;100(4):417-25 PMID: 17406888
 # 17 Lindholm P Loss of motor control and/or loss of consciousness during breath-hold competitions Int J Sports Med. 2007 Apr;28(4):295-9. PMID: 17024640
Lindholm P, Ekborn A, Oberg D, Gennser M. Pulmonary edema and haemoptysis after breath-hold diving at residual volume. J Appl Physiol. 2008 Apr;104(4):912-7. PMID: 18202166
 # 44 Lindholm P, Swenson ER, Martinez-Jimenez S, Guo HH. From ocean deep to mountain high: Similar CT findings in immersion and high altitude pulmonary edema. Am J Respir Crit Care Med. 2018 Oct 15;198(8):1088-1089. doi: 10.1164/rccm.201803-0581IM. PMID: 30044644

Glossopharyngeal breathing, a technique used by post-polio and patients with spinal cord injuries to improve breathing by using the glossopharynx as a piston pump to hyperinsufflate the lungs with air. This technique was gaining popularity in breath-hold diving since it increased pulmonary gas volume above total lung capacity. We showed the physiology in healthy humans hyperinsufflating their lungs more than 30% over TLC and the effects and risks it entailed with increased chest flexibility and larger oxygen stores but also when overdone syncope from diminished blood flow, and in extreme cases arterial gas embolism with neurological symptoms. We also tested it as a training method for increased lung volume (and buoyancy) in elite swimmers and in patients.

 # 14 Nygren-Bonnier M., Gullstrand L., Klefbeck B., **Lindholm P**. Effects of Glossopharyngeal Pistoning for Lung Insufflation in Elite Swimmers. Med Sci Sports Exerc. 2007 May;39(5):836-41. PMID: 17468583
 # 16 Loring SH, O'donnell CR, Butler JP, **Lindholm P**, Jacobson F, Ferrigno M. Respiratory mechanics during glossopharyngeal breathing in competitive breath-hold divers. J Appl Physiol. 2007 Mar;102(3):841-6 PMID: 17110514
 # 23 Lindholm P, Norris CM Jr, Braver JM, Jacobson F, Ferrigno M. A fluoroscopic and laryngoscopic study of glossopharyngeal insufflation and exsufflation. Respir Physiol Neurobiol. 2009 Jun 30;167(2):189-94. PMID: 19383557
 # 20 Nygren-Bonnier M, Wahman K, **Lindholm P**, Markström A, Westgren N, Klefbeck B. Glossopharyngeal pistoning for lung insufflation in patients with cervical spinal cord injury. Spinal Cord. 2009 May;47(5):418-22. PMID: 19002147
 # 29 Schiffer TA, **Lindholm P**. Transient ischemic attacks from arterial gas embolism induced by glossopharyngeal insufflation and a possible method to identify individuals at risk. Eur J Appl Physiol. 2013 Mar;113(3):803-10 PMID: 22983570

In pulmonary embolism we have developed and are improving a robust, non-gated, non-contrast MRI-protocol that work clinically and showed that residents can learn to interpret these in a short amount of time. We have preliminary validation and an ongoing clinical trial in Sweden.

 # 36 Nyrén S, Nordgren Rogberg A, Vargas Paris R, Bengtsson B, Westerlund E, **Lindholm P**. Detection of pulmonary embolism using repeated MRI acquisitions without respiratory gating: a preliminary study. Acta Radiol. Acta Radiol. 2017 Mar;58(3):272-278. Epub 2016 Jul 19. PMID: 27273375
 # 41 Nordgren-Rogberg A. Nyrén S, Westerlund E, **Lindholm P**.
How to train radiology residents to diagnose pulmonary embolism using a dedicated MRI protocol. Acta Radiologica Open, 2017 Sep 27;6(9) PMID: 28989798
 # 46 Nordgren Rogberg A, Gopalan D, Westerlund E, **Lindholm P**. Do radiologists detect chronic thromboembolic disease on computed tomography?. Acta Radiol. 2019 Epub March 21.

 # 49 Medson K, Vargas-Paris R, Nordgren-Rogberg A, Sigbergsdottir A, Nyrén S, **Lindholm P**. Primary diagnosis of pulmonary embolism with unenhanced MRI for patients not eligible for CTPA: Clinical Outcome. European Journal of Radiology Open. Accepted Aug 2019

The citation for My Bibliography is provided here: https://profiles.ucsd.edu/karl.lindholm

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

Endowed chair in hyperbaric and diving medicine research at UCSD. Started July 26, 2019.
No current grant support in the US, and most Swedish grants finished 2018 or 2019.

Swedish Heart and Lung foundation (PI: Lindholm) 01/01/2013-12/31/2020
Magnetic resonance imaging for Pulmonary Embolism
The goal of this project is to assess a freebreathing, non-gated, non-contrast method using MRI to diagnose pulmonary embolism. First paper published in 2016, two PhD students projected to finish 2020.

Innovation fund from Stockholm City Council (PI: Lindholm) 01/01/2018-12/31/2019
Vi can solve the shortage of radiologists with competence in mammography
Goal: To develop a training system for mammography as well as a licensing tool. Simulation of clinical work using labeled/annotated images. To implement deliberate practice and simulation with an enriched data set. We are using Swedish registry to extract and develop AI-training database for e.g. mammography and also enriched datasets for teaching and simulation in radiology. Currently 4 million images.

Fraenckel fund for aviation research (PI: Lindholm) 01/01/2019-12/31/2019
Automated detection of venous gas emboli in high altitude decompression.
Goal: to use modern computers and current software technology with deep learning algorithms (“artificial intelligence) to reprocess old doppler data on vascular bubbles in decompression to generate an automatic method (the old method is heavily dependent on skilled human operators).

Abstract

BAA Number: N00014-20-S-B001
Technical Contact (PI): Peter Lindholm, MD, PhD
*Abstract Approved for Public Release

Where is the gas? A new method to study nitrogen during diving and decompression

A **century of study has not been able to identify the causal mechanism of decompression sickness** (DCS). We propose a new quantitative method to study Nitrogen gas (N_2) in mammals. Decompression sickness is currently hypothesized to be caused by bubble formation in tissues. However, due to the lack of direct methods to track tissue gas, **the field has used indirect methods** such as ultrasound of venous gas emboli (VGE) in the heart and mathematical modelling based on empirical testing in human divers. Current methods to predict DCS are based on statistical probability with relative risk factors such as depth and duration. It has not yet been possible to determine the causal mechanisms and explain the intra- and interindividual variability present in DCS, suggesting that **a direct method to map tissue gas distribution is a priority, as it has the potential to refine risk assessment and contribute to optimizing diving profiles.**

We will use a radioactive, positron-emitting isotope of Nitrogen (**$^{13}N_2$**) that can be breathed in a hyperbaric chamber and monitored in time and space using **positron-emission tomography (PET) combined with magnetic resonance imaging (MRI)**. With $^{13}N_2$, we can quantitatively measure the Nitrogen distribution in the body. $^{13}N_2$ has previously been produced and used in normobaric experiments with human volunteers, and a hyperbaric chamber can be constructed to inhale the gas during compression (simulated diving), widening applicability to hyperbaric breathing. We hypothesize that tissue gas bubbles will create bright spots while gas in solution will generate a signal equivalent to that tissue's saturation with Nitrogen. This new method would allow to answer the gas localization question, and address the impact of preconditioning on the amount of venous gas emboli after decompression. This approach will open a window **to study basic mechanisms and impact of interventions** related to DCS in both animals and humans.

Specific aim 1: To develop a method for inhaling $^{13}N_2$ under pressure and imaging gas distribution in a live animal. This will allow direct tracking of Nitrogen gas throughout the body, which is key to mechanistically explain decompression sickness under different conditions. We will **produce and purify the $^{13}N_2$ radioactive gas**, including compression and delivery into a pressure chamber, prior to PET and MR imaging. A chamber able to compress rodents within the PET and MRI will be developed and built. This experiment will establish the **proof of concept** that we can **track gas related to compression-decompression** in a rodent model.

Specific aim 2: Quantitative measurements of Nitrogen gas uptake and distribution during compression and excretion during and after decompression in rodents.
The dive profile (duration, pressure) will alter the tissue uptake of gas. Moreover, it has been shown that preconditioning significantly reduces VGE and reduces mortality by up to 50%. Using exercise as a preconditioning intervention, we will **test the hypothesis that preconditioning reduces the total nitrogen uptake** in rodents. We will also identify changes in **nitrogen storage, spatial distribution, and uptake and excretion temporal dynamics.**

Specific aim 3: Scaling the method to enable large animals. The method developed for rodents will be scaled to enable large animal experiments (e.g. sheep) breathing $^{13}N_2$ under pressure followed by imaging in a PET/MRI. The expected **outcome** is imaging **data** on signal intensity and gas distribution that can be **used to predict risk and optimize doses for planning human experiments**.

The proposed studies will establish a new rat model (and technical preparation for large animals and humans) that **will enable quantitative measurements of Nitrogen gas in vivo during compression-decompression** thus providing insight into tissue gas distribution and mechanisms of bubble generation during decompression. This technique will provide a valuable new tool for testing and selecting optimal interventions to prevent DCS, and optimize operational diving.

UNIVERSITY OF CALIFORNIA, SAN DIEGO UCSD

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Sennite Meche
Contract and Grant Officer
TELEPHONE (858) 822-6554
E-MAIL: smeche@ucsd.edu

OFFICE OF CONTRACT AND GRANT ADMINISTRATION
University of California, San Diego
9500 Gilman Drive #0934
La Jolla, CA 92093-0934

OVERNIGHT MAIL DELIVERY ADDRESS:
10300 North Torrey Pines Road, Third Floor West
La Jolla, CA 92037

Date: October 15, 2019

Office of Naval Research
875 N Randolph Street,
Arlington, VA 22217

Re: Proposal in response to Funding Opportunity: N00014-19-S-B001

Dear Sir or Madam,

On behalf of The Regents of the University of California; University of California, San Diego (UCSD); the following proposal is presented for your consideration:

Title: Where is the gas? A new method to study nitrogen during diving and decompression
Principal Investigator(s): Dr. Peter Lindholm
Total Amount Requested: $**1,197,597**
Project Period: January 01, 2020 – December 31, 2022

UCSD is an educational, non-profit, research institution whose main principles are based on open and accessible dissemination of research for the public benefit.

UCSD reserves the right to negotiate any resulting agreement so that the terms align with UCSD policies, including the freedom to publish and no citizenship restrictions.

Additional technical information may be obtained from the Principal Investigator named above. Inquiries concerning the contractual or budgetary aspects should be directed to the undersigned. I confirm, on behalf of The Regents of the University of California; University of California, San Diego, that this proposal is endorsed, and has been submitted by the Office of Contract and Grant Administration at UC San Diego.

Sincerely,

(b) (6)

Sennite Meche
Contract and Grant Officer

TECHNICAL PROPOSAL COVER PAGE

BAA Number: N00014-20-S-B001

Title of Proposal: Where is the gas? A new method to study nitrogen during diving and decompression

Submitted To: Office of Naval Research

Offerors:
Prime: The Regents of the University of California; University of California San Diego

Technical Contact (PI):
Peter Lindholm, MD, PhD
Professor in Residence
Gurnee Endowed Chair of Hyperbaric
and Diving Medicine Research
Department of Emergency Medicine
University of California San Diego
200 W. Arbor Dr. #8676
San Diego, CA 92103
plindholm@health.ucsd.edu
+16508230735

Administrative/Business Contact:
Elizabeth Tran-Nguyen
Contract and Grant Officer
Office of Contract & Grant Administration
University of California San Diego
10300 North Torrey Pines Road
Level 3 West
La Jolla, California 92037
ttrannguyen@ucsd.edu | ocga.ucsd.edu
(858)246-2383

Proposed Period of Performance:
01/01/2020 - 12/31/2022

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TECHNICAL APPROACH

RESEARCH PLAN

List of abbreviations:

ATA:	atmospheres absolute pressure
DCS:	decompression sickness
MRI:	magnetic resonance imaging
$^{13}N_2$:	gaseous radioactive isotope of nitrogen, emitting positrons, tracer for PET
PET:	positron emission tomography
SNR:	signal to noise ratio
VGE:	venous gas emboli

1.1 Background

Decompression sickness (DCS) is a major operational concern for diving operations and submarine escape. DCS **has been studied extensively with established methods**, including empirical testing in humans subjected to various depth/time profiles that remain the "gold standard" (Vann et al. 2011, Mahon 2014). Tables have been developed and continue to evolve to support diving operations; however, we **still do not know exactly how to predict or counteract DCS** (Mahon 2014).

During hyperbaric air breathing conditions (diving), nitrogen is taken up in the body with a resulting supersaturation of gas relative to sea level pressure of 1 ATA (1 atmosphere absolute). This supersaturation may create bubbles upon decompression (Tikuisis 2003). These bubbles are most likely both in tissues and in the blood but direct imaging of tissue bubbles has to date been beyond the reach of existing methods. In blood, **venous gas emboli (VGE)** can be heard or seen directly with ultrasound in the right ventricle of the heart (Spencer 1969, Karlsson 2009, Blogg 2014) VGE **is the most commonly used proxy of direct testing for DCS**. Although ultrasound-measured VGE has a high negative predictive value for DCS, the positive predictive value of ultrasound-measured VGE is poor. When significant bubbles (grade Kisman Masurel III-IV (Nishi 2003) are found on ultrasound, the risk for DCS is about 10% (Mahon 2014).

Mathematical models of gas uptake have been proposed for **over 100 years** (Boycott 1908, Tikuisis 2003). In modeling, different "tissue compartments" are used with various half-times based on fitting these mathematical models to empirical datasets on "hits", cases of DCS from human (and animal) testing. These simulated "tissue compartments approach" has showed that a model with slow and fast tissues better predicts DCS than a model with only a single "tissue." The concept that blood is a fast compartment, muscle intermediate, and fat is a slow compartment is a pedagogical model that does not correspond exactly with these mathematical models of tissues. **The difficulty of directly measuring gas uptake and tissue bubbles makes all these models empirical and approximate** (Mahon 2014, Tikuisis 2003), and without validation, unable of actually predicting true nitrogen gas tension and change in the body.
Nitrogen gas (N_2) can be manipulated to include a radioactive, positron-emitting isotope ($^{13}N_2$). This process was tested in human subjects by Weathersby et al. (1986) using a volume detector to study the signal from the knee during normobaric rebreathing of this gas (i.e.,

without diving). Since then only a few human experiments have been done for ventilation-perfusion studies. Those trials were not done by inhalation, rather by injection of water containing $^{13}N_2$ with subsequent images collected on where the gas was excreted in the lung (Rhoades 1989 1&2, Wellman 2010).

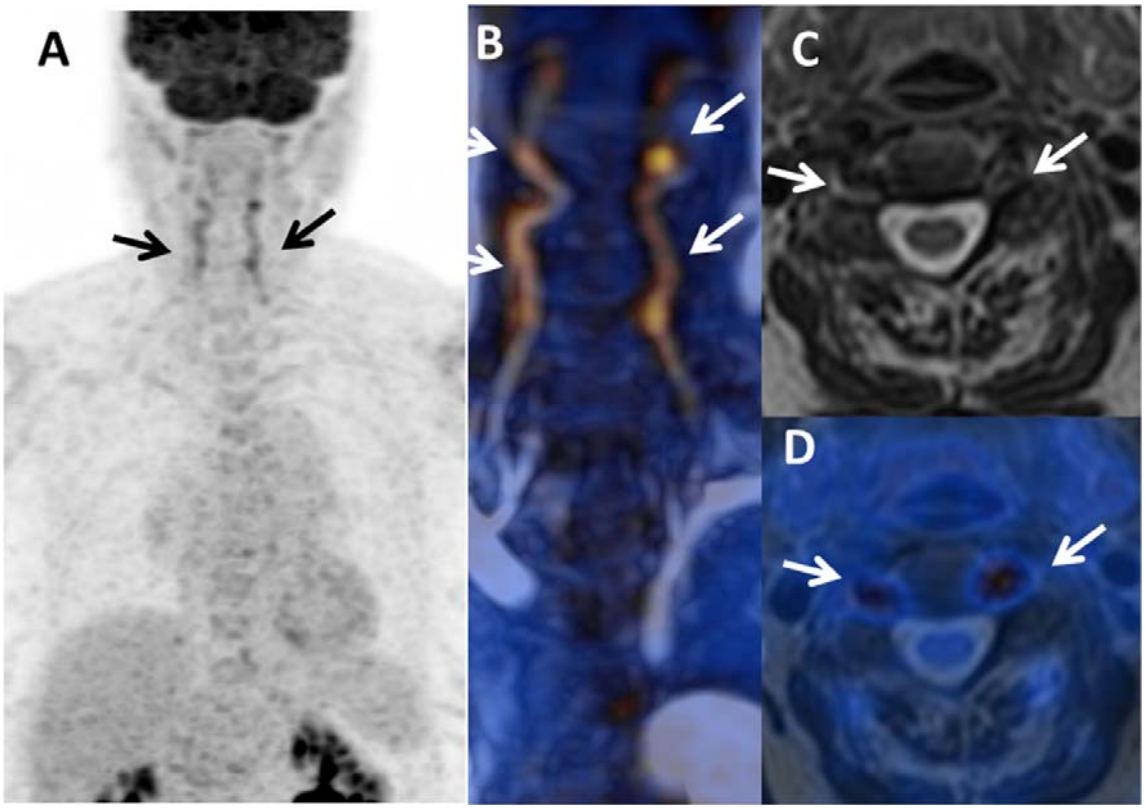
Development in the field of medicine has created the ability to image positrons at unprecedented spatial and temporal resolutions using **positron-emission tomography combined with magnetic resonance imaging (PET/MRI)** (Mannheim 2018). PET systems are clinically used primarily with a radiolabeled sugar fluorodeoxyglucose (FDG) to measure metabolism and detect malignant tumors. But the positron released from a radiolabeled sugar molecule or from a nitrogen isotope will be detected by the same technology, thus enabling use of available PET-scanners to image gas.

PET/MRI enables accurate 3-dimensional tracing of radiolabeled activity (Mannheim 2018) in the animal or human body. **It could be possible to follow $^{13}N_2$ gas from inhalation to blood and peripheral tissues, and accurately image and measure the spatial distribution and temporal dynamics of the nitrogen in the body.** The half-life (9.96 minutes) of the $^{13}N_2$ suggests that a 2-hour experiment is feasible and 2-3 repeated experiments in a human are feasible within acceptable radiation limits for a healthy volunteer (our calculation based on Weathersby et al 1986). It is thus possible to breathe $^{13}N_2$ during simulated diving in a hyperbaric chamber, then exit the chamber and image the body in a PET to determine where the gas was distributed during the "dive". Furthermore, **consecutive scanning would show how gas is excreted over time** from supersaturation to normobaria. A stationary gas bubble in a tissue would generate a stronger signal that can be mapped to the correspondent anatomical location.

Tissue bubbles could be identified as follows: a gas distributed in a tissue such as muscle generates a specific signal intensity for such a tissue. If gas bubbles are generated in the tissue "hot spots" of higher PET signal are created where these bubbles are located. If VGE are created and lodged in endothelial crevices (Tikuisis 2003) there will be areas of higher signal intensity along blood vessels.

Stanford University has recently developed machine learning algorithms to de-noise the signal, effectively enabling higher signal-to-noise than previously attained (Gong 2018). Such algorithms could be used for PET and will soon be academically and/or commercially available (personal communication with Professor J. Pauly, Stanford).

Below is an image from a PET/MRI study of vasculitis (Laurent et al. 2019) where the inflammatory cells have an increased uptake of **radioactive glucose** (18F-FDG). The vessels are clearly seen due to higher uptake (orange) while the surrounding tissues have less signal (blue). **We envision that gas and hot-spots could be differentiated similarly to panel B**.



PET/MRI used for the initial diagnosis of giant cell arteritis (GCA) in a female with temporal headaches and acute-phase reactants without any vascular signs or arthralgia. PET/MRI showed an inflammatory pattern with clear uptake (>liver uptake, grade 3) in vertebral arteries ((A) Maximum intensity projection, and (B) fusion MR angiography/PET; arrows) associated with arterial wall thickening on: (C) MR axial T2-weighted image and (D) T2-weighted/PET fusion. (Image from Laurent et al. 2019).

Where is the gas? experiments with preconditioning by exercise (Wisløff 2001) or drugs (Wisløff 2004) increasing nitric oxide have shown that the amount of VGE can be reduced after decompression in rats. The effect was strong enough to eliminate a mortality of 50% during the first hour post decompression (Wisløff 2001). Following the observed reduction in VGE's, **a question that remains to be answered is: where did the gas go?** was it never taken up during the compression phase or was it excreted differently in the preconditioned versus non-preconditioned animals?
A similar result has been observed in humans: using exercise as preconditioning, subjects showed fewer or no VGE in the right ventricle of the heart post-diving the day after exercise, as compared to the control group that had not exercised the day before diving (Dujic et al. 2004). The postulated mechanism depends on the Nitric Oxide system, with more NO post exercise affecting endothelium and thus bubble formation (Wisløff et al 2003, 2004). But it is not clear if off-gassing was constant and the lack of bubbles was simply due to a decrease in nucleation.

The individual methods and technologies with **PET/MRI, $^{13}N_2$, hyperbaric chambers** and rebreathers have all been used separately for human experiments but **never been tested in concert, neither in animals nor humans.** We believe these techniques can be combined to create a new gold standard for quantitatively measuring gas physiology in decompression research. Therefore, **$^{13}N_2$ has the potential to improve detection and tracking of gas over time compared to ultrasound imaging of VGE**. This technique could enable a significant and major improvement in understanding the role of N_2 in DCS.

1.2 Specific Aims

Specific aim 1: To develop a method for inhaling $^{13}N_2$ under pressure and imaging gas distribution in a live animal. This will allow direct tracking of Nitrogen gas throughout the body, which is key to mechanistically understand decompression sickness under different conditions. We will produce and purify the $^{13}N_2$ radioactive gas, including compression and delivery into a hyperbaric chamber, prior to PET and MR imaging. A hyperbaric chamber able to accommodate rodents within the PET and MRI will be developed and built. This experiment will be proof of concept that we can track gas related to compression-decompression in a rodent model.

Specific aim 2: Quantitative measurements of Nitrogen gas uptake and distribution during compression and excretion during and after decompression in rodents.
The dive profile (duration, pressure) will alter the tissue uptake of gas. Moreover, it has been shown that preconditioning significantly reduces VGE and reduces mortality by up to 50%. Using exercise as a preconditioning intervention, we will test the hypothesis that preconditioning reduces the total nitrogen uptake in rodents. We will also identify changes in nitrogen storage, spatial distribution, and uptake and excretion temporal dynamics.

Specific aim 3: Scaling the method for large animals. The method will be scaled to enable large animal experiments (e.g. sheep) breathing $^{13}N_2$ under pressure and then imaging in a PET/MRI. The expected outcome is imaging data on signal intensity and gas distribution that can be used to predict and optimize doses for planning large animal and/or human experiments.

1.3 Approach

Specific aim 1: To develop a method for inhaling $^{13}N_2$ during pressure and imaging gas distribution in a live animal.

Nitrogen-13-labeled nitrogen gas ($^{13}N_2$) will be produced using the method developed by Suzuki & Kwata (1977). Parks & Krohn (1978) routinely produced 800 mCi of purified $^{13}N_2$ using the Suzuki & Kwata method, which irradiates a water target with protons using the $^{18}O(p,\alpha)^{13}N$ nuclear reaction. We will receive irradiated water [the target solution (TS)] from a cyclotron (Siemens Medical System model *Eclipse RDS 111*) operated by the PETNET Corp. This facility is located 10 minutes (one half-life of ^{13}N) away from the UCSD Radiochemistry facility (Letter of support PETNET). We will use the Parks and Krohn (1978) method for gas collection over water in a recording spirometer. We will assay the radiochemical and radionuclidic purity using gas chromatography.

We anticipate adequate activity for one and possibly two diving/imaging experiments (1 for 45 minutes of compression, 2 when only 30min compression is used). The microPET scanner (GE Healthcare *Vista DR*) requires approximately 0.200 mCi injections for images of adequate quality. Table 1 lists the timings for the experiments listed in this proposal. The half-life of ^{13}N is 9.96 minutes.

Table 1. Time Course		
Radioactivity (mCi)	Time (min)	Process
800		End of Irradiation

400	10	Transfer from target to shipping vial at PETNET
100	20	Courier delivery to UCSD
25	20	Purification & trapping of gas within the spirometer
3.125	30	Inhalation by rat #1
1.56	10	Transfer to PET scanner Start PET imaging
0.195	30	Start PET imaging of rat #2

A Haskel type booster pump (a pneumatic driven gas booster) will be used compress the $^{13}N_2$ into the hyperbaric chamber.

Normobaric *Animal experiment:*
We will set up and test the technique on a few (target 4) rats in normobaric conditions. The animals will breathe air (2 animals) or pure oxygen for 1 hour to off-gas nitrogen (2 animals). Then they will be sedated (with isoflurane) and placed upon the micro-PET (Vista DR from GE Healthcare) gantry bed. They will be advanced into the imager until their hips rest in the center of the field of view (to avoid signal from radioactivity in the breathing gas passing through mouth and lungs).
Next, ventilation will switch to air including $^{13}N_2$ for 30 minutes and then back to regular air with the exhaled air collected separately. Activity will be monitored on the inspiratory and expiratory side (inhaled and exhaled gas separately) as well as in 3D imaged regions by the PET. All gas (isoflurane and radioactive nitrogen) will be collected. Then the bed will be advanced into the MRI to generate a anatomical map of the rat, for overlay with the PET signal. Upon completion of the scans, each rat will be euthanized, and kept until all activity has been allowed to decay away. During the excretion of gas, imaging will alter between brain and hip areas. The primary imaging data targets will be the brain and the pelvic area (with hips, large muscles etc.) which are also areas that do not move in a sedated animal (as opposed to lungs and heart).

We expect this part to show where the radioactive gas goes in the rat, and that the signal can be detected appropriately by the PET. We also expect this step to produce calibration procedures, finetuning signal to noise etc. for imaging. We will also confirm the safety in handling of the sedated animals with radioactivity involved. Oxygen prebreathing will use the "oxygen window" effect (Behnke 1967) to increase the partial pressure vacancy in tissues for $^{13}N_2$, thus increasing $^{13}N_2$ uptake during normobaric conditions and a resulting difference in uptake and excretion between rodents in normobaric conditions.

The tissue distribution of nitrogen uptake and off-gassing will be examined to generate a map of intensity over time for specific tissues such as neurons, white matter, bone, cerebrospinal fluid, subcutaneous fat, etc. These studies will serve as a baseline for subsequent studies under pressure. A comparison based on differences in gas uptake between control and prebreathing would be a scientific finding on its own, given the importance of pre-breathing oxygen for high altitude flight and extravehicular activity in space (Foster 2013, Conkin 2017).

PET MRI compatible chamber for rodents:
We will develop a chamber system for rats made of an acrylic tube, a substance that is transparent to the gamma radiation that results from the positron-electron annihilation, thus not impacting detection, and made to fit within a PET/MRI. MRI compatible chambers are

available for animal studies (https://www.reimerssystems.com/mri-compatible-chambers), but we would have to make or order a custom design that enable additional shielding (lead blocks on the parts protruding from the PET gantry) for radioprotection of human operators. This chamber will have throughputs for isoflurane and ventilation purposes, also enabling collecting any residual gas for safe handling until radioactivity has decayed.

We expect this part will lead to a fully developed hyperbaric chamber that can be positioned inside a PET-MRI system with full imaging capabilities for sedated rats during compression and decompression while breathing either the radioactive gas, or off gassing while breathing room air (with collection of exhaled gas that partly contain anesthetics and in part radioactivity).

Specific aim 2: Quantitative measurements of Nitrogen gas uptake and distribution during compression and excretion during and after decompression in rodents.

We will probe the sensitivity of the technique applied in normobaric conditions in aim 1 to a hyperbaric condition and an exercise preconditioning protocol. We will create scenarios with different amounts of gas in tissues, blood gas bubbles versus gas in solution in a live animal. This can be achieved by different durations at pressure, a longer compression will result in more gas uptake (Boycott 1908, Tikuisis 2003).
Differences in gas in solution versus venous gas emboli can be created by preconditioning with exercise (Wisløff and Brubakk (2001)) or drugs (Wisloff 2003) increasing nitric oxide (L-NAME, Nitroglycerin etc.). We aim to use exercise since drugs cause various side effects and may not be used in future human experiments.
Compressing rats to 700 kPa (200 feet of seawater) for 45 minutes has been shown to create vast amounts of VGE and has a 50% lethality within 60 minutes after decompression without preconditioning (Wisloff 2001). We will add a control group with 30 minutes compression to reduce gas load and enable comparisons of tissue gas uptake depending on duration at pressure and VGE depending on preconditioning.

Rats (target 40) will be split into four equal groups:
control (no preconditioning exercise) and 30 minutes compression
control (no preconditioning exercise) and 45 minutes compression
exercise preconditioning and 30 minutes compression
exercise preconditioning and 45 minutes compression

Exercise groups:
The exercise cohort will be placed onto a motorized, rat sized treadmill for 1.5 hours of exercise 18-20 hrs. prior to compression in the chamber. This exercise protocol follows Wisloff et al 2001. After this exercise period the rats will be moved back to their regular cage until the next day ("dive").

Compression/Decompression, all animals.
Rats (300-350 grams) will first be anesthetized (isoflurane). They will be shaved and naired over the heart (to enable ultrasound, a probe will be attached to the chest). $^{13}N_2$ will be introduced as the breathing gas at the start of compression and ended upon conclusion of the decompression. Continuous imaging (about 2 hours) will be obtained of primarily the head and the hips (ultrasound probe will limit imaging of the chest area). Upon completion of the scan, the rat will be positioned (without ultrasound probe) into the MRI for generation of an

anatomical map corresponding to PET images of the head and hips. Surviving rats will be euthanized while still under anesthesia.

We expect this experiment to highlight the differences in amount and spatial distribution of gas is in the preconditioned animals' vs those that did not underwent exercise preconditioning, potentially explaining why exercise preconditioning massively reduces VGE and mortality. We hypothesize 3 possible routes:

1) Gas is excreted faster when bubbles occur, leaving a higher residual radiation in rats subjected to preconditioning with exercise.
2) Gas is excreted slower when endothelial bubbles trap gas coming from the tissues in bubbles instead of being transported in solution and excreted in the lungs.
3) That less gas is dissolved in tissue during compression, implying that the lungs or endothelium changes gas diffusivity for nitrogen after exercise, reducing gas taken up by the body.

Basically, we will determine: **1) where is the gas in the animals that survive and 2) if the temporal dynamics of bubbles during decompression is altered by exercise preconditioning**.

Specific aim 3: Scaling the method for large animals.

Adapting methods to enable experiments in sheep, in preparation for future human experiments.

Resolution in a PET machine scales in 3-dimentional voxels that are independent of the size of the animal. This suggests that larger animals (or human) will yield better anatomical and tissue resolution, as the larger size of structures will enable better spatial association of anatomical locations and the sites of the gas accumulation. MRI resolution on the order of ~1 mm scale (isotropic) suggest that in a sheep or human subject, separation can be made within the brain or spinal cord from different structures.

Creare (personal communication, Dr. Knaus) is developing an MRI-Compatible Hyper/Hypobaric chamber for human research under a Navy SBIR program (N68335-17-C-0328). Creare is currently designing and building the first prototype chamber, which will be certified under the ASEM PVHO (Pressure Vessels for Human Occupancy) standards for human use. The chamber could potentially be available for human use in the 2021 timeframe. The specifications are for a pressure rating of 4 atmospheres absolute (100 fsw) with a chamber fitted for an MRI scanner with a 70cm bore (inner diameter). The PET/MRI systems currently used for humans (General Electric SIGNA™) have a 60 cm bore suggesting that human experiments with a chamber from Creare may need a customized build (diameter) of the acrylic tube. There will be possible collaborations with Creare, but it would be preferable to purchase a human rated chamber for the sheep experiments. The alternative (as sheep are not proposed to be imaged during compression in this phase) is to use a regular transportable steel animal chamber.

Images will be examined for signal quality, signal to noise (SNR) ratio and for allowable temporal resolution with standard image reconstruction. Machine-learning algorithms will be employed to improve SNR at the reconstruction level and allow a better temporal resolution.

The expected technical outcomes of this study is the development and testing of procedures to generate gas for a large animal studies, including delivery of gas during compression and imaging in a PET/MRI system made for human imaging.

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1.5 Future Naval Relevance

The proposed experiments **will add a quantitative method** to study basic gas physics and gas physiology that is relevant for understanding decompression in humans in both **operational diving and submarine escape**. In particular, it enables measurement of the spatial distribution of gas in three dimensions and the temporal dynamics of gas loading and off-loading. It would be **the first study to map tissue gas directly *in vivo***. The method could be used to test both countermeasures and individual susceptibility. We will be able to study the pathophysiology and anatomy of gas distribution by actually seeing and measuring (signal intensity) the distribution of gas uptake in the body.

This approach will combine proven technology developed in the last decade (**Positron Emission Tomography and Magnetic Resonance Imaging**) with an **experimental gas isotope of nitrogen ($^{13}N_2$)** developed and tested 30-50 years ago, leading to a new technique with immense potential for decompression research. We will use this gas in combination with a proven intervention that showed that the amount of venous gas bubbles generated during decompression could be modulated by exercise preconditioning. This step will serve as a proof of concept for this new method and to **enable future experimental studies** to measure the behavior **of nitrogen gas in the human body**.

Development of this technique enables a multitude of studies related to gas physics and physiology in relation to compression and decompression, and could start a new era of research in decompression and diving **contributing to a solution of decompression sickness risk management**.

1.6 Project Schedule and Milestones

TIMELINE:
Year 1:

1) Radiochemistry work to produce, purify and establish administration of gas.
2) 'Dive' a few rats to setup model with exercise, bubbles and ultrasound of VGE.
3) Gas modeling, calculations, image quality tests, data collection and analysis
4) Administer gas in vivo in 4 rats, at atmospheric pressure to calibrate method, test in PET.
5) Construct a PET-compatible hyperbaric chamber for sedated rats.
6) Design a gas compression and gas evacuation system for sedated rats and radioactive gas.

Year 2:

1) Finish construction and test of PET-compatible hyperbaric chamber for rats.
2) 'Diving' 40 rats with $^{13}N_2$. With or without prior preconditioning exercise, different durations. Imaging with PET and MRI. Data analysis, optimization, dose modulation.
3) Data analysis, manuscript preparation on rat experiment.
4) Construct/adapt a gas rebreathing system to work with sheep in a transportable hyperbaric chamber.

Year 3:

1) Transport equipment built in previous years to a large (Human size) PET-MR system. To enable $^{13}N_2$ gas purification on site, in preparation for future large animal research.
2) Testing rebreathing apparatus of $^{13}N_2$ inside a human /large animal sized PET-MRI.
3) Analysis of data and optimization of $^{13}N_2$ dose, analyze and produce images.
4) Additional rat experiments at UCSD with different diving profiles.
5) Data analysis, manuscript preparation.

1.7 Reports

- Annual Technical and Financial Progress Reports
- Final Report within 60 days of the completion of the project

MANAGEMENT APPROACH

Describe the overall management approach and provide rationale for participation of key team members. Describe the planned relationships with any subawardees or collaborators. This is a single PI award; if there are subawardees or collaborators, explain how the proposed team fits the single PI structure. If appropriate, briefly describe anticipated schedule.

Peter Lindholm is the Principal Investigator on the project and will bear the main responsibility for the successful execution of the proposed research. As outlined in the timeline in the research plan, The PI and co-I's are well-versed in training undergraduate, graduate, and postgraduate students and will be responsible for the activity performed in his/her research group.
One year of effort is needed for a radiochemist to set up and refine the logistics around gas production. This time is needed to define the path to human experiments. The co-I Dr. Vera worked with $^{13}N_2$ in the 1980's and a gas production target exist at the PETNET cyclotrone facility in San Diego. During the first year we will also reproduce published animal experiments (Wisloff & Brubakk 2001) but without $^{13}N_2$ to set up pressurization techniques, rat exercise and ultrasound VGE detection. Year 2 and 3 will be experimentation with both diving and radioactive gas in rats. For the first part we can use our small animal chamber while designing and building a PET-compatible chamber for a sedated rodent in a radioactive environment. Then we will start scaling up for larger animals to get closer to imaging a human. For year 3 we plan to use live sheep, pending further support, that will most likely be imaged at the Stanford PET/MR system under the direction of Prof. Butts-Pauly. Pending support we will transport a "sheep"-sized chamber to the Stanford PET-MRI for those experiments, possibly constructing a dedicated chamber with radioactive shielding.

CURRENT AND PENDING SUPPORT

LINDHOLM, P.

ACTIVE
None

PENDING
None

PRINCIPAL INVESTIGATOR QUALIFICATIONS

Peter Lindholm, MD, PhD, Principal Investigator is a full Professor at UCSD, (in residence). He is also the Gurnee endowed chair of hyperbaric and diving medicine research at Dept. of Emergency Medicine. Dr Lindholm has 25 years of research experience in the field of diving medicine/ diving physiology. He is also a radiologist with experience (licensed in Sweden) in imaging and radiation.

David Vera, PhD, Co-Investigator and Professor in Radiology at UCSD. He will assist in supervising the radiochemist (Dr. Stevens) and the Staff Research Associate (Mr. Barback) in developing the gas logistics and is also supervisor for the lab at Moore cancer center where the PET and MR facilities are located.

Rui Sà, PhD, Co-Investigator and Assistant Professor in Physiology at UCSD. He will perform the mathematical and statistical programs and analysis of gas dynamics for both prediction and final analysis of gas dynamics. He will help with script writing necessary for the automation of data management and statistical analyses.

Michael Lang, PhD, (In-kind not paid) Adjunct Assistant Professor at UCSD. He will be assisting with the development of compression chambers.

TBD, radiochemist: Senior staff researcher and a trained radiochemist. He will develop and optimize the gas production and delivery as well as running rodent experiments during year 1-2.

Christopher Barback, Staff Research Associate at UCSD. He will assist in running experiments on rodents in the radiochemistry lab. He has training in running the different imaging modalities in Prof Vera´s lab.

RESPONSIBILITY

Offerors must provide the following information to ONR in order to assist in ONR's evaluation of the offeror's responsibility:
Describe how you have adequate resources or the ability to obtain such resources as required to complete the activities proposed.

Dr Lindholm has access to more than one mobile animal chamber, with some cost-sharing or transportation cost since it cannot be stored at the PET-facility when not in use.
Dr Vera has a research lab facility where radiochemistry could be optimized. This lab has all the advanced or expensive equipment readily available and a cyclotrone nearby. This include PET, MR, and cardiac ultrasound (for rodents) and routines for ordering and handling animals via the UCSD animal facility. The specific procedures for $^{13}N_2$ will need to be set up and optimized.

Describe how you have the ability to comply with the grant conditions, taking into account all existing and currently prospective commitments of the applicant, nongovernmental and governmental.

Dr Lindholm has an academic position dedicated to research in part covered by an endowed chair. This will cover time for this project in addition to any portion of FTE taken from the grant. Thus, the portion of salary from the grant will less than the actual time invested. Co-Investigators will allocate the time covered by the grant for the proposal. This proposal has the support from the chairs of both departments Emergency Medicine (Dr. Chan) and Radiology (Dr. Norbash).

Describe your performance history; specifically, your record in managing Federal awards and the extent to which any previously awarded amounts will be expended prior to future awards.

In Sweden, Dr Lindholm has held multiple grants from both government and private funding. Co-I Dr Vera both has current federal grants (as PI:s) for other unrelated projects.

Describe your record of integrity and business ethics. Describe qualifications and eligibility to receive an award under applicable laws and regulations.

Dr Lindholm has a position as full professor in Residence at UCSD. He has all qualifications and eligibilities required. He has always acted with the utmost integrity.

Describe your organization, experience, accounting, and operational controls and technical skills, or the ability to obtain them (including as appropriate such elements as property control systems, quality assurance measures, and safety programs applicable to the efforts to be performed).

The PI Dr Lindholm has been a faculty member at UC San Diego since 7/26/2019. He has the qualifications and resources necessary to lead the project and the ability to comply with the grant conditions. UC San Diego has in place a comprehensive program ensuring all its employees obey the highest ethics and record of integrity. University policies establish standards of ethical behavior for all members of the university community and prescribe procedures for due process and discipline for deviation from those standards. Faculty at UC San Diego must periodically obtaining certification on a variety of topics, including cybersecurity awareness, confidentiality of student records and privacy rights, sexual violence and sexual harassment prevention, and intellectual property compliance and conflicts of interest. The prime offeror, UC San Diego, as well as the subaward offerors, Stanford University, have numerous annual audits of all regulatory and accounting systems by federal agencies. UC San Diego has approved property control systems, an Office of Research Compliance and Integrity, and an Environmental, Health and Safety department. Finally, the two institutions all have top-ranked engineering schools, and annually attract a large number of exceptional graduate students who want to pursue an advanced degree in science or engineering. This, together with the scientific reputation of the PI team, will enable the enlistment in the project of a highly-qualified cadre of national and international students capable of performing the proposed research tasks.

FACILITIES AND EQUIPMENT

No Federal Government-furnished facilities or equipment are proposed for use.

UNIVERSITY OF CALIFORNIA SAN DIEGO (UCSD)

Overview:
The University of California San Diego (UCSD) Campus was established in 1959 and has become in the ensuing 60 years one of the nation's leading research universities. The UCSD School of Medicine has more than 900 physicians and scientists on the faculty, and the highly regarded medical teaching program accepts about 125 new students per year. Additionally, over 670 residents and fellows receive postdoctoral training in research laboratories and the patient care setting. The facilities, equipment and other resources available to Project PI (Dr Lindholm), Co-investigators (Dr. Vera and Dr Sà) and their research teams, provide a first-class scientific environment that is strongly supportive of the proposed research, and include everything needed to undertake and complete the proposed project successfully.

DEPARTMENT OF EMERGENCY MEDICINE

The department has an animal chamber for research that is transportable and can house multiple small animals enabling the proposed rat experiments. Made and certified by Pacific Coast Welding & Machine Inc. Its rated 90 Psi (7ATA). There is also a human multi-place chamber at Hillcrest Hospital rated for 6ATA that can be used to develop and test the rebreather systems to use with radioactive gas.

MOORES CANCER CENTER

The UCSD Moores Cancer Center (MCC) is one of 44 NCI designated Comprehensive Cancer Centers in the United States and is the only Comprehensive Cancer Center in the San Diego region.

Moores Cancer Center Shared Resource Facilities

The Moores Cancer Center maintains shared resource facilities for cancer related research. The proposed project will mainly utilize the In Vivo Imaging Shared Resource (IVISR), animal facilities, and the molecular imaging program.

In Vivo Imaging Shared Resource (IVISR)

David Vera, PhD, is the director of the In Vivo Imaging Shared Resource (IVISR). The IVISR provides optical, CT, ultrasound, and high-resolution planar gamma imaging, magnetic resonance imaging, and PET imaging of mice and rats. Services include CT, PET, optical imaging (time-resolved and continuous wave), gamma camera imaging, ultra-sound/optoacoustic imaging, high resolution tissue imaging, image processing/kinetic modeling, protocol development, and radiochemistry.

Equipment includes: a GE *eXplore Vista DR* mciroPET, a GE *eXplore Locus* microCT, a GE *eXplore Optix*, a Fluoptics *FluoBeam800*, Biospace Gamma Camera, a 4.7 T MRsolutions *4000MRS* MRI, and a Visual Sonics *Vevo LAZR 2100* ultra-sound/optoacoustic imager. The resource is in the basement of the Moores Cancer Center building. The IVISR is an IACUC-approved Level-2 satellite vivarium, which can house mice and rats up to 72 hours.

Radiochemistry, Molecular Imaging Center, UCSD Moores Cancer Center

The UCSD Production Facility currently has a portfolio of eight IND radiopharmaceuticals that support the human research activities in the Departments of Neurosciences, Psychiatry, and Radiology, as well as the UCSD Moores Cancer Center.

The Molecular Imaging Program at UCSD is housed in the UCSD Moores Cancer Center. The program includes a radiochemistry facility supports a high-resolution gamma camera, and a microPET scanner in the In Vivo Imaging Shared Resource of the Cancer Center. The Radiochemistry facility houses a 75mm Von Gahlen hotcell and a Von Gahlen dual mini cell for radiolabeling with positron-emitting radio-isotopes, a HPLC with gamma, absorbance, fluorescence, and refraction detection, a radiochromatographic strip scanner, two automated synthesizers (G.E. *FastLab 2* and G.E. *TracerLab FN-FX*), and an Beckman *Gamma 8000* auto-well gamma counter.

ANIMAL FACILITIES

Institutional

The University of California, San Diego is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International (NIH Assurance Number; A3033-1, USDA Animal Research Facility Registration Number: 93-R-0437, AAALAC Institutional Number: 00050), and holds an approved NIH Assurance and USDA License. There are currently approximately 102,000 square feet of animal facilities at 26 locations. Support includes quarantine rooms, sterile operating rooms; post-surgical recovery rooms, radiology and diagnostic laboratory services. Veterinary care is provided on a 24-hour basis, including weekends and holidays, by a staff comprised of four veterinarians and three animal health technicians. Research support services include training classes, seminars and wet labs offered throughout the year.

Animal Facilities to be used in these studies:

1. *In Vivo Imaging Shared Resource (IVISR) at the Moores UCSD Cancer Center, La Jolla*
2. *Vivarium, Moores UCSD Cancer Center, La Jolla*
3. *Research Animal Facility, UCSD Medical Center, Hillcrest*

The *In Vivo Imaging Shared Resource (IVISR)* resides in the basement of the Moores UCSD Cancer Center; The 330-square foot facility has been designated a satellite vivarium by the UCSD IACUC and can house rats or mice for up to 48 hours. Animals can be transferred

from all UCSD and AAALAC-approved non-UCSD vivaria. Rabbits are housed in the *Research Animal Facility*, which is a 15-minute drive from the *IVISR*. The *vivarium* in the Moores UCSD Cancer Center contains capacity of ~8,800 mice cages including standard housing and barrier facilities. Both vivaria have biohazard cabinet-equipped procedure rooms and biohazard suites.

The IVISR is served by an intranet of eight Personal Computers running Windows. Four of these computers directly acquire imaging data from one of the four rodent imagers: CT, PET, gamma camera, and optical scanner. All of the computers are linked to a Dell server, which is connected to a tape backup subsystem.

RESEARCH LABORATORIES

Molecular Imaging Program

The UCSD Molecular Imaging Program, directed by Dr David Vera, has a laboratory is located on the fourth floor of the Moores Cancer Center, occupying 300 square feet and an elevator ride to the *In Vivo Imaging Shared Resource* in the basement.

Laboratory Equipment

General Equipment

particle size analyzer
UV/VIS spectrophotometer
top loading balance
microcentrifuge
analytical balance
clean bench
vacuum pumps (3)
strip chart recorder (4)
refrigerators (4)
shell freezer
pH Stat
freezer (2)
high-speed tabletop centrifuge
refrigerated centrifuge
recirculating water baths (2)
pH meters (3)
shaker bath
ultralow freezer (2)
tabletop ultracentrifuge
water purification system
vacuum oven
fluorescence spectrometer with integrating sphere

Chemical Synthesis and Radiolabeling

fume hoods (4)
dose calibrator (3)
high-vacuum pump trap system
FTIR Spectrometer
Hot cell (75 mm)
NMR (500 MHz)
rotary evaporator
lyophilizer (3)
ultrafiltration/dialysis systems (4)
gas chromatograph with autosampler
HPLC/mass spectrometer

Chromatography and Electrophoresis

Multi-laser light scattering detector, HPLC
Gradient HPLC (2)
UV/Vis detector, HPLC (4)
FPLC pump (3)
fraction collector (2)
radioactivity detector, HPLC (2)
fluorescence detector, HPLC
HV electrophoresis power supply (3)
chromatographic cold box
TLC imaging scanner
refractive index detector, HPLC

Nuclear Counting

Autowell gamma counter (2)
Autowell liquid scintillation counter
high voltage supply (2)
Flow gamma detectors (2)
TLC imaging scanner
Bioscan multichannel detector systems (2)

NaI(Tl) detectors (2)
PMT/pre amp (2)
timing SCA analog ratemeter (2)
spectroscopy amplifier SCA (2)
amplifier (2)
scaler, 4 channel digital

Computing Hardware & Software
Dual CPU server (3)
PC clone computers (10)
AMIRA image processing software (6 licenses)
HP laser printers (3)

Imaging, Small Animal
MRI Scanner (MRsolutions, 4.7T)
PET Scanner (G.E. *eXplore Vista DR*)
CT Scanner (G.E. *eXplore Locus*)
Optical Scanner (G.E. *eXplore Optix MX*)
Ultrasound (Visual Sonics, Vevo 770)
Gamma camera (Biospace Mesures, *δ-IMAGER*)
Histological Imaging: BioRad *FX-Pro-plus* & Vibratome *Model 8250*
Heating recirculator with pads
Vaporizer (3)
Dose calibrator (3 ea, Capintec CRC15)
Decay cabinet (511 kev)
L-blocks (511 kev)

RESEARCH PROPOSAL SUBMITTED TO THE OFFICE OF NAVAL RESEARCH

PROPOSAL TITLE: Assessment of Kv2.2 channels as therapeutic targets for oxygen toxicity in rats following HBO exposure

LABORATORY: Naval Medical Research Center
Undersea Medicine Department
503 Robert Grant Avenue
Silver Spring, MD 20910-7500

PRINCIPAL INVESTIGATOR: Aaron Hall, Ph.D.

CO-INVESTIGATOR(S):

USE OF HUMAN SUBJECTS (Y/N): N

USE OF ANIMALS (Y/N): Y rats

RECOMBINANT DNA RESEARCH (Y/N): N

PERSONNEL/ENVIRONMENTAL HAZARDS (Y/N): Y

PROPOSED START DATE: 1 JULY 2022

PROPOSED BUDGET ($):

	DIRECT	INDIRECT	TOTAL
FY22	102,317	(b) (4)	(b) (4)
TOTAL	102,317	(b) (4)	(b) (4)

I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports and agreed-upon products to the Office of Naval Research.

Principal Investigator Date
Phone: 301-319-7189
Fax: 301-319-7378
Email: Aaron.a.hall6.civ@mail.mil

Approval Authority Date
Phone: 301-319-7402
Fax: 301-319-7410
Email: stephen.t.ahlers.civ@mail.mil

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C. Statement of Objectives:

Enriched oxygen breathing gases are used extensively in closed circuit under water breathing apparatus (CC-UBA) systems to increase stealth and reduce the amount of dive time dedicated to decompression. Prolonged exposure to hyperbaric oxygen (HBO) with a pO2 greater than 0.4ATA (140kPa) increases the prevalence of oxygen toxicity (1), which effects several tissue beds including: skeletal muscle, lung, and the CNS. These effects present as symptoms such as fatigue and tracheobronchitis (2). Manifestation of these symptoms can have a deleterious impact on diver safety, comfort, and mission effectiveness.

Voltage-gated potassium channels regulate cell function globally, with profound effects on cellular and system level homeostasis. The primary function of these channels is to both regulate cell membrane potential and to repolarize the cell membrane following an action potential (3). In a human study at NSMRL, the potassium chanel Kv2.2 was identified as functionally enriched in the blood after a 6.5hr, 2.0ATA HBO exposure. Kv2.2 has not been previously studied under hyperoxic conditions. Kv2.2 is an oxygen sensitive voltage gated potassium channel (4).

The **objective of this study** is to determine whether Kv2.2 function contributes to the manefestation of oxygen toxicity in rats and is thus a target for countermeasure development.

D. Statement of Hypotheses and Specific Research Aims:

We hypothesize that pharmacological/biological manipulation of Kv2.2 activity will modify oxygen toxicity symptoms associated with HBO exposure. Our specific research aims are:

1.) Determine effect of Kv2.2 antagonists on pulmonary oxygen toxicity in rats following a 6.5hr 2.0ATA HBO exposure.
2.) Determine the effect of Kv2.2 agonists on pulmonary oxygen toxicity in rats following a a 6.5hr 2.0ATA HBO exposure

E. Statement of the Approach:

NMRC has a well-established rodent HBO model which will be used for this study. Rats will have a baseline assessment of muscle and pulmonary function, be randomized to receive intervention, and then be compressed to 2.0 ATA on oxygen for 6.5 hours. Rats will then have functional assessment of muscle and pulmonary function and be euthanized for blood and tissue collection. This 2.0 ATA oxygen model was selected to mirror the conditions of the human study.

F. Statement of Significance:

One of the major limiting factors for Navy Divers utilizing rebreather diving rigs (MK 16 or MK 25) is limited bottom time due to the manifestation of oxygen toxicity symptoms. Increasing the capacity to utilize higher pO_2 during diving missions will reduce decompression obligations and improve mission capability by directly shifting a greater proportion of dive time away from decompression towards mission execution. Currently it is recognized that diver physiology is a limiting factor in diving operations. The current study seeks to identify new targets to enhance tolerance to HBO while simultaneously preserving diver safety and comfort.

G. Key Word/Phrase Listing:

Oxygen toxicity, hyperbaric oxygen, Voltage Gated Potassium Channels, Rodents.

H. Body of Proposal:

1. The Idea

Human studies conducted at NSMRL indicated that the Kv2.2 channel was upregulated in the serum of individuals exposed to a 2.0ATA hyperbaric oxygen exposure for 6.5 hours which induced symptoms of pulmonary oxygen toxicity. Kv2.2 has not been studied under conditions of hyperbaric hyperoxia. We propose to examine whether blockade or stimulation of the Kv2.2 changes the onset of pulmonary injury associated with HBO exposure.

2. Introduction and Background

Prolonged hyperbaric oxygen exposure produces symptoms in the lungs, central nervous system, and skeletal muscle. A study conducted at NSMRL exposed human subjects to a 2.0ATA hyperbaric oxygen exposure for 6.5hrs. Blood samples collected pre-dive and 1hr post-dive were analyzed for changes in protein content which demonstrated functional enrichment of 3 proteins, APOD, KCNB2 (Kv2.2), and VP13C (Figure 1). KV2.2 is an oxygen sensitive potassium channel subunit which assists with repolarization of membrane potential after action potentials in a variety of tissues including vascular endothelium, skeletal muscle, and brain. Due to this oxygen sensitivity, we propose to examine the role Kv2.2 channels play in the development of symptoms associated with HBO toxicity.

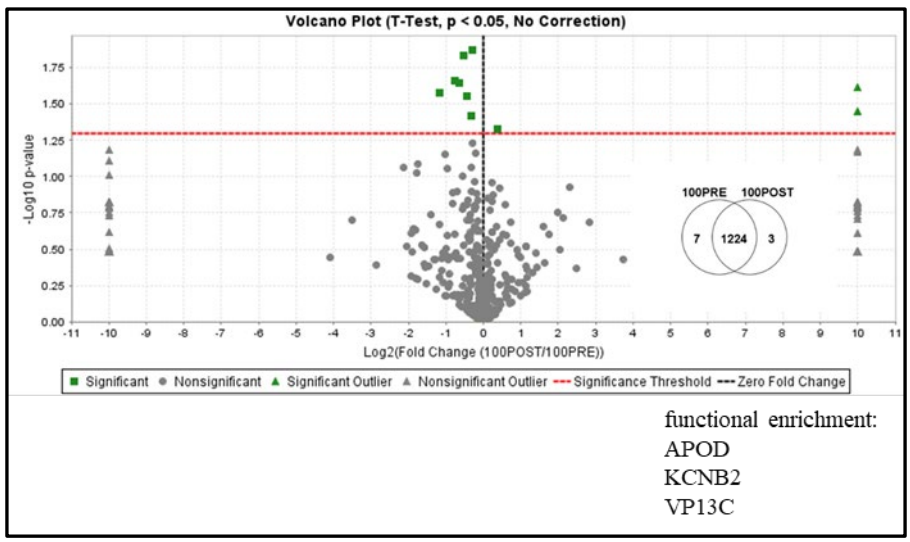

Figure 1: Volcano Plot showing functional changes in serum proteins following a 6.5hr 2.0ATA HBO exposure in human subjects

Kv2.2 channels belong to the Shab subfamily of potassium channel subunits and form both homodimers and heteromeric channels with potassium channel subunits from other subfamilies such as Kv8.2 (5). These channels regulate repolarization rates following action potentials and are involved in sleep/arousal (6), smooth muscle function (7), and pulmonary vascular function (8). In vivo, these channels are activated by nitric oxide (9) and have an oxygen sensitivity (4).

3. Technical Approach

To evaluate Kv2.2 channels as therapeutic targets for oxygen toxicity we will execute 2 aims of study. In aim 1, we will use the tarantula based toxin GxTx1E to antagonize the Kv2.2 channel *in vivo* prior to exposure to a 6.5hr 2.0ATA HBO exposure. In aim 2, we will indirectly activate the Kv2.2 channel using the guanylyl cyclase agonist Riociguat then antagonize Kv2.2 function using GxTx1E to determine the relative contribution of Kv2.2 to any effects on oxygen

toxicity observed. Due to gender differences observed with oxygen exposure and Kv2.2 expression/function both male and female groups will be studied.

Aim 1: Rats (n=48) will be randomized into 4 groups (n=12/group) consist of an equal number of male and female rats: Control Air, Control HBO, GxTx1E Air, GxTx1E HBO. Following standardized acclimatization, rats will have baseline functional assessments of peripheral muscle strength and pulmonary function. Rats will then receive toxin (or control) injection and be placed in a hyperbaric chamber. The rat will be pressurized to 2.0ATA, have their breathing gas switched to 100% oxygen, and be held for 6.5 hours. Following the 6.5hour exposure the rats will be returned to surface and have post-dive assessment of muscle and pulmonary function.

Aim 1: Rats (n=48) will be randomized into 4 groups (n=12/group) consist of an equal number of male and female rats: Control Air, Control HBO, Riociguat HBO, Riociguat+GxTx1E HBO. Following standardized acclimatization, rats will have baseline functional assessments of peripheral muscle strength and pulmonary function. Rats will then receive toxin (or control) injection and be placed in a hyperbaric chamber. The rats will be pressurized to 2.0ATA, have their breathing gas switched to 100% oxygen, then be held for 6.5 hours. Following the 6.5 hour exposure the rats will be returned to surface and have post-dive assessments of muscle and pulmonary function.

The following functional parameters will be measured in all rats over the course of the HBO exposure study:

1.) Peripheral muscular strength and fatigue resistance – This endpoint will determine how afferent signaling affects local muscular fatigue in the absence of central motor input by assessing time required to reach 50% of maximal tetanic force production in rodent hind limb muscle via electrical stimulation (120hz) of the motor nerve (central fatigue) or stimulation (120hz) of the motor end plate (peripheral fatigue). Somatosensory evoked potentials (SEP) will be recorded using a stimulator (Stimulator S 88, Grass, Quincy, MA) and EEG amplifier (Wide band A. C. pre-amplifier, 7P3c and Polygraph D.C. driver amplifier. Data will be sampled at a rate of 1 kHz on PC and exported for off-line analysis.

2.) Pulmonary function- This endpoint will determine how afferent signaling effects pulmonary function in un-anesthetized rats using whole body plethysmography. The specific endpoint used will be enhanced pause (Penh), which is a dimensionless unit that correlates with pulmonary resistance, in addition to minute ventilation, tidal volume, and respiratory rate. A hypercapnic challenge protocol will be utilized to evaluate ventilation responsiveness. This measurement will be measured using a whole-body plethysmograph (Buxco Electronics Ltd., NY) connected to a standalone PC running Fine-Point software and exported for off-line analysis.

Oxygen toxicity parameters 1 and 2 will be measured pre-dive and post-dive. After the functional assessment, the rats will be euthanized and histological examination of the gastrocnemius muscle and the lungs will be conducted.

4. Transition Plan

Once the described experiments are completed, the results will be submitted for publication in a peer reviewed journal. The findings from this research study will be shared with Dr. Sandra Chapman at ONR in the form of regular research reports. Final results will also be shared with Dr. David Fothergill to support the ongoing manned diving experiments utilizing the HBO model used in this study. Additionally, if one of the treatment groups demonstrates improved outcomes following HBO exposure, a follow on study will be submitted to ONR to transition these findings to a higher order animal model.

I. Facilities available:

The diving experiments outlined in this application will be performed at NMRC where all the necessary equipment is available and operational for use. These include:

A. Hyperbaric Labs Capability Summary

General: The hyperbaric laboratories in Building 511 presently contain 7 hyperbaric chambers ranging in size from 0.5 to 30 cubic feet floodable volume. The chambers support Research Programs in Decompression Sickness and Oxygen Toxicity. Capabilities are available for single or multiple small animals as well as single or multiple large animals.

Depth Hull #	Floodable Volume	Pressure	Depth (fsw)	Depth (ATA)	Gas Capabilities
H-176-1 (H2)	5 CF	500 psig	1120	35	Air & Mixed Gas (HeO_2)
H-209	5 CF	500 psig	1120	35	Air & Mixed Gas (HeO_2)
H-192	20 CF	325 psig	730	23.0	Air Chamber , w/ O_2 or Air closed circuit overboard dump
H184 (H1)	5 CF	325 psig	730	23.0	Air, O_2, & N_2
H-140	5 CF	115 psig	258	8.8	O_2 & N_2
MLAC	450 CF	550 psig	1230	38	Air Chamber , w/ O_2, Air, or Mixed Gas closed circuit overboard dump
SSAC – (4 chambers)	.025 CF	35 psig	78	3.3	Air, O_2, N_2, Mixed Gas
SRC	.065 CF	35 psig	78	3.3	O_2 & N_2

Breathing Gas: Various configurations allow for many combinations of gas delivery. Some chambers require subjects to breathe directly from the chamber atmosphere, while others are designed with a closed circuit overboard dump breathing system. These systems allow switching of the breathing gas on the fly during diving operations independent of the chambers atmosphere.

Gas Analysis: Sample points throughout the systems monitor and control Oxygen and Carbon Dioxide in both the chamber atmosphere and the auxiliary systems.

Infusion: Venous and arterial infusion lines (for swine subjects) are available on surface or at depth.

Vital Signs / Life Support Parameters: Capabilities for swine include the ability to monitor pulse, respiration, blood pressure, EEG, EKG and rectal temp; monitoring O_2 and CO_2 of chamber atmosphere and exhaled breathing gases. Capabilities for rodents include EEG, EKG and O_2 and CO_2 of chamber atmosphere as well as exhaled breathing gases.

Environmental Control: Chamber temperature and humidity are monitored. Most chambers have provisions for limited control of these variables.

Exercise: Three of the 5 cu ft. chambers hold rotating exercise cages for multi-rat studies. Additional rotating cages are available on the bench (1 ATA). A treadmill is available for large animals.

B. General Laboratory Space:

In addition to the hyperbaric chamber facility, there is a fully equipped laboratory which contains a water bath, luminometer, low- and high- speed centrifuges; FACS-STAR 5 flow cytometer, phosphoimager and densitometer, ELISA washer/plate reader, gas chromatograph/mass spectrometer. Animals will be housed at the AAALAC certified facilities

described above under the care and supervision of the Institution's Laboratory of Animal Medicine and Science Division.

C. Animals:

Animals will be housed at the above facilities under the care and supervision of the Institution's Laboratory of Animal Medicine and Science Division.

J. Annual Budget ($):

SUMMARY

Item / Category	FY 2022
Personnel	82,391
Equipment and Maintenance	0
Expendable Supplies	6,427
Animal Purchase and Per Diem	9,499
Travel	2,500
Contracts/Consultants	0
Other Direct Costs	1500
Total Direct	102,317
Indirect	(b) (4)
TOTAL BUDGET	135,000
Budget Estimate:	135,000

DETAILS FOR FY22

Personnel	Title	% Effort	Cost
Aaron Hall, PhD	Research Physiologist	10	$(b) (4)
Richard Mahon, MD	Senior Scientist	5	$(b) (4)
Katherine Buddo, PhD	Post-Doctoral Fellow	10	$(b) (4)
William Hickman	Chamber Technician	5	$(b) (4)
Nicholas Talbott	Chamber Technician	10	$(b) (4)
Virginia Livingston	Animal Technician	10	(b) (4)

Krystal Bockting	Animal Technician	10	$(b) (4)
Alan Metelko, PhD	Statistician	5	$(b) (4)
Total personnel cost			$82,391

Equipment and Maintenance	**Cost**
Chamber maintenance	0
Chamber Specific Equipment/Fabrication	0
Large Equipment Purchase (>3K)	0
Total Equipment and Maintenance Cost	0

Expendable Supplies	**Cost**
Consumable lab supplies	$5,000
Oxygen	$1,427
Total Expendable Supplies Cost	$6,427

Animal Purchase and Per Diem	**Cost**
96 rats	$8,395
Animal per diem	$1,104
Total Animal Purchase and Per Diem Cost	$9,499

Travel	**Cost**
Program Review	$2,500
Total Travel Cost	$2,500

Contracts/Consultants	**Cost**
Publication Costs	$1,500
Fees (Miscellaneous)	
Total Contracts/Consultants Cost	0

Total Direct Cost	$102,317
Indirect Cost	(b) (4)

TOTAL FY22 COST	$135,000

K. Full Curricula Vitae

Please see attached.

L. Current and pending support of each investigator:

Aaron Hall:

1.) The impact of carbon dioxide on simulated DISSUB survival and decompression sickness at 5ATA air in a 70kg swine model. NAVSEA FY21-23 (delayed). Total Funding: $786,692, Hall 0.2 FTE
2.) T&E of Submarine Rescue Systems Decompression Plan. DHA 6.7, FY22-26. Total Funding $1,504,000, Hall 0.25 FTE
3.) Breath Test for Pulmonary Oxygen Toxicity, USAMMDA, FY22-23. Total Funding $2,902,000, Hall 0.5 FTE

M. Other agencies to which this proposal has been or will be submitted: none

N. Special information regarding certain types of experiments:

The present proposal involves the use of experimental animals. All research involving animals will be performed at the WRAIR/NMRC facility that has AAALAC accreditation and all regulations and laws will be fulfilled. Documentation of Institutional Animal Care and Use Committee (IACUC) approval for the animal use protocol will be forwarded under separate cover.

O. Appendices

Aaron Hall, PhD	POSITION TITLE Research Physiologist Undersea Medicine Department Naval Medical Research Center

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Florida, Gainesville, FL	BS	2002	Microbiology/cell science
University of South Florida, Tampa, FL	MS	2005	Medical Sciences
University of South Florida, Tampa, FL	PhD	2009	Medical Sciences

Please refer to the application instructions in order to complete sections A, B, and C of the Biographical Sketch.

PUBLICATIONS

1. Ajmo, CT Jr, Vernon, DOL, Collier, L, Hall, AA, Garbuzova-Davis, S, Willing, AW, Pennypacker, KR: The spleen contributes to stroke induced neurodegeneration. J. Neurosci. Res. 86:2227-34, 2008, PMID: 18381759.
2. Hall, A., Guyer, A., Leonardo, C., Ajmo, C. Jr., Collier, L., Willing, A., Pennypacker, K.: Human Umbilical Cord Blood Cells Directly Suppress Ischemic Oligodendrocyte Cell Death. J. Neurosci. Res. 87(2): 333-341 2009. PMID: 18924174.
3. Herrera, Y, Katnik, C, Rodriguez, J, Hall, A, Willing, AE, Pennypacker, KR, Cuevas, J: Sigma-1 receptor modulation of ASIC1a channels and ASIC1a-induced Ca2+ influx in rat cortical neurons. J. Pharm Exp. Ther. PMID: 18723775.
4. Hall, A.A., Herrera, Y., Ajmo C.T., Cuevas, J., Pennypacker K.R.: Sigma receptors suppress multiple aspects of microglial activation. Glia 57(7): 744-754, 2009. PMID: 19031439.
5. Ajmo Jr., C.T., Collier, L.A., Leonardo, C.C., Hall, A.A., Green, S.M., Womble, T.A., Cuevas, J., Willing, A.E., Pennypacker, K.R.: Blockage of Adrenorecptors Inhibits the Splenic Response to Stroke. Exp. Neurology. 2009 Apr 14 [Epub ahead of press] PMID: 19371742.
6. Leonardo, C.C., Hall, A.A., Collier, L.A., Gottschall, P.E., Pennypacker, K.R.: Inhibition of gelatinase activity reduces neural injury in an ex vivo model of hypoxia-ischemia. Neuroscience. 2009 Jun 2;160(4):755-66. [Epub 2009 Mar 9]. PMID: 19272421.
7. Hall AA, Leonardo CC, Collier LA, Rowe DD, Willing AE, Pennypacker KR.: Delayed treatments for stroke influence neuronal death in rat organotypic slice cultures subjected to oxygen glucose deprivation. Neuroscience. Neuroscience. 2009 Dec 1;164(2):470-7. Epub 2009 Aug 29
8. Leonardo CC, Hall AA, Collier LA, Ajmo CT Jr, Willing AE, Pennypacker KR.: Human umbilical cord blood cell therapy blocks the morphological change and recruitment of CD11b-expressing, isolectin-binding proinflammatory cells after middle cerebral artery occlusion. J Neurosci Res. 2010 May 1;88(6):1213-22
9. Hall AA, Pennypacker KR.: Implications of Immune System in Stroke for Novel Therapeutic Approaches. Translational Stroke Research. Jan 2010

10. Leonardo CC, Hall AA, Collier LA, Green SM, Willing AE, Pennypacker KR.: Administration of a Sigma Receptor Agonist Delays MCAO-Induced Neurodegeneration and White Matter Injury. Transl. Stroke Res. Jun;1(2):135-145.
11. Cord blood administration induces oligodendrocyte survival through alterations in gene expression. Rowe DD, Leonardo CC, **Hall AA**, Shahaduzzaman MD, Collier LA, Willing AE, Pennypacker KR. Brain Res. 2010 Dec 17;1366:172-88. Epub 2010 Sep 29.
12. The spleen contributes to stroke induced neurodegeneration through interferon gamma signaling. Seifert HA, Leonardo CC, **Hall AA**, Rowe DD, Collier LA, Benkovic SA, Willing AE, Pennypacker KR. Metab Brain Dis. 2012 Jun;27(2):131-41. Epub 2012 Feb 22.
13. Assessment of the effects of acute and repeated exposure to blast overpressure in rodents: toward a greater understanding of blast and the potential ramifications for injury in humans exposed to blast. Ahlers ST, Vasserman-Stokes E, Shaughness MC, **Hall AA**, Shear DA, Chavko M, McCarron RM, Stone JR. Front Neurol. 2012;3:32. Epub 2012 Mar 5.

ABSTRACTS

1. Hall, A, Cruz, Y, Katnik, C, Cuevas, J, Pennypacker, KR: The sigma receptor agonist 1,3-DI-O-TOLYLGUANIDINE, suppresses activation of primary microglia and the subsequent release of TNF-a and nitric oxide. SEPS Meeting, 2005.
2. Hall, A, Cruz, Y, Katnik, C, Cuevas, J, Pennypacker, KR: Sigma receptor agonists suppress activation of primary microglia and the subsequent release of TNF-a and nitric oxide. Exp. Neurol. 198:570, 2006.
3. Hall, AA, Cruz, Y, Katnik, C, Cuevas, J, Pennypacker, KR: The sigma receptor agonist 1, 3-di-o-tolylguanidine suppresses the intracellular calcium signaling necessary for the activation of microglia. Soc. Neurosci. Abst. 711.5, 2006.
4. Ajmo, CT, Collier, LA, Hall, AA, Cuevas, J, Willing, AE, Pennypacker, KR: Splenic response to stroke is not dependent on neurotransmission via the splenic nerve. Soc. Neurosci. Abst. 2007.
5. Hall, AA, Herrera, Y, Ajmo, CT, Cuevas, J, Willing, AE, Pennypacker, KR: Sigma receptor mediated suppression of microglial activation is a calcium dependent process. Soc. Neurosci. Abst. 2007.
6. Herrera, Y, Katnik, C, Hall, AA, Pennypacker, K, Cuevas, J:. Modulation of acid-sensing ion channels by sigma receptors. Soc. Neurosci. Abst. 468.10/J25, 2007.
7. Peer-Reviewed
8. Hall, A.A., Leonardo, C.C., Collier, L.A., Cuevas, J., Willing, A.E., Pennypacker, KR: Human Umbilical Cord Blood Treatment Inhibits Nitric Oxide Production and Neuronal Death in Organotypic Slice Cultures Subjected to Oxygen Glucose Deprivation. Stroke 40(4):e170, 2009.

EXPERIENCE/EMPLOYMENT

2000 - 2004	Laboratory Technician, Biochemistry & Molecular Biology, University of Florida, Gainesville, FL
2009	Postdoctoral Research Fellow, University of South Florida, Tampa, FL
2009-2011	Research Associate II, Henry M Jackson Foundation, Civilian contractor, Naval Medical Research Center, Silver Spring, MD
2011-present	Research Physiologist, Naval Medical Research Center, Silver Spring, MD

References:

1. U. S. Navy Diving Manual, Revision 7, Published by direction of Commander, Naval Sea Systems Command
2. Shykoff BE, Pulmonary effects of submerged oxygen breathing in resting divers:repeated exposures to 140kPa Undersea and Hyperbaric Medical Society DEC 2007; 35(2): 131-143
3. Blaine JT, Ribera AB. Kv2 channels form delayed-rectifier potassium channels in situ. J Neurosci. 2001;21(5):1473-1480. doi:10.1523/JNEUROSCI.21-05-01473.2001

4. Jason X.-J. Yuan, Oxygen-sensitive K+ channel(s): where and what? American Journal of Physiology-Lung Cellular and Molecular Physiology 2001 281:6, L1345-L1349

5. Gayet-Primo J, Yaeger DB, Khanjian RA, Puthussery T. Heteromeric $K_V2/K_V8.2$ Channels Mediate Delayed Rectifier Potassium Currents in Primate Photoreceptors. J Neurosci. 2018 Apr 4;38(14):3414-3427. doi: 10.1523/JNEUROSCI.2440-17.2018. Epub 2018 Feb 26. PMID: 29483285; PMCID: PMC5895036.

6. Hermanstyne, Tracey O et al. "Kv2.2: a novel molecular target to study the role of basal forebrain GABAergic neurons in the sleep-wake cycle." *Sleep* vol. 36,12 1839-48. 1 Dec. 2013, doi:10.5665/sleep.3212

7. Felicitas Schmalz, Jacqueline Kinsella, Sang Don Koh, Fivos Vogalis, Anne Schneider, Elaine R. M. Flynn, James L. Kenyon, and Burton Horowitz. Molecular identification of a component of delayed rectifier current in gastrointestinal smooth muscles. American Journal of Physiology-Gastrointestinal and Liver Physiology 1998 274:5, G901-G911

8. Xu C, Lu Y, Tang G, Wang R. Expression of voltage-dependent K(+) channel genes in mesenteric artery smooth muscle cells. Am J Physiol. 1999 Nov;277(5):G1055-63. doi: 10.1152/ajpgi.1999.277.5.G1055. PMID: 10564112.

9. Steinert JR, Robinson SW, Tong H, Haustein MD, Kopp-Scheinpflug C, Forsythe ID. Nitric oxide is an activity-dependent regulator of target neuron intrinsic excitability. Neuron. 2011;71(2):291-305. doi:10.1016/j.neuron.2011.05.037

OMB Number: 4040-0001
Expiration Date: 10/31/2019

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE

State Application Identifier

1. TYPE OF SUBMISSION

☐ Pre-application ☒ Application ☐ Changed/Corrected Application

4. a. Federal Identifier N00014

b. Agency Routing Identifier 342 [Chapman, Sandra]

c. Previous Grants.gov Tracking ID

2. DATE SUBMITTED

Applicant Identifier

5. APPLICANT INFORMATION

Organizational DUNS: 0696872420000

Legal Name: University of South Florida

Department: Sponsored Research

Division: Research and Innovation

Street1: 4019 East Fowler Avenue Suite 100

Street2:

City: Tampa

County / Parish: Hillsborough

State: FL: Florida

Province:

Country: USA: UNITED STATES

ZIP / Postal Code: 336172008

Person to be contacted on matters involving this application

Prefix:

First Name: Brenda

Middle Name:

Last Name: Simmons

Suffix:

Position/Title: Sponsored Research Administrator

Street1: 4019 East Fowler Avenue Suite 100

Street2:

City: Tampa

County / Parish: Hillsborough

State: FL: Florida

Province:

Country: USA: UNITED STATES

ZIP / Postal Code: 336172008

Phone Number: 813-974-8778

Fax Number: 813-974-4962

Email: bsimmons@usf.edu

6. EMPLOYER IDENTIFICATION *(EIN) or (TIN):* 59-3102112

7. TYPE OF APPLICANT: H: Public/State Controlled Institution of Higher Education

Other (Specify):

Small Business Organization Type ☐ Women Owned ☐ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION:

☒ New ☐ Resubmission

☐ Renewal ☐ Continuation ☐ Revision

If Revision, mark appropriate box(es).

☐ A. Increase Award ☐ B. Decrease Award ☐ C. Increase Duration ☐ D. Decrease Duration

☐ E. Other (specify):

Is this application being submitted to other agencies? Yes ☐ No ☒ What other Agencies?

9. NAME OF FEDERAL AGENCY: Office of Naval Research

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER: 12.300

TITLE: Basic and Applied Scientific Research

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT:

Electrodermal activity during hyberbaric oxygen exposure: rat study

12. PROPOSED PROJECT:

Start Date: 02/01/2019

Ending Date: 01/31/2020

13. CONGRESSIONAL DISTRICT OF APPLICANT

FL-014

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: | First Name: Jay | Middle Name:

Last Name: Dean | Suffix:

Position/Title: Professor

Organization Name: University of South Florida

Department: Molecular Pharm and Physiology | Division: College of Medicine

Street1: 12901 Bruce B Downs Blvd MDC8

Street2:

City: Tampa | County / Parish: Hillsborough

State: FL: Florida | Province:

Country: USA: UNITED STATES | ZIP / Postal Code: 336129445

Phone Number: 813-974-1547 | Fax Number:

Email: jdean@health.usf.edu

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested	24,977.00
b. Total Non-Federal Funds	0.00
c. Total Federal & Non-Federal Funds	24,977.00
d. Estimated Program Income	0.00

16. IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?

a. YES ☐ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:

DATE:

b. NO ☒ PROGRAM IS NOT COVERED BY E.O. 12372; OR

☐ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious. or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

☒ **I agree**

**The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

18. SFLLL (Disclosure of Lobbying Activities) or other Explanatory Documentation

Add Attachment | Delete Attachment | View Attachment

19. Authorized Representative

Prefix: | First Name: Keith | Middle Name:

Last Name: Anderson | Suffix:

Position/Title: Director

Organization: University of South Florida

Department: Sponsored Research | Division: Research and Innovation

Street1: 4019 East Fowler Avenue Suite 100

Street2:

City: Tampa | County / Parish: Hillsborough

State: FL: Florida | Province:

Country: USA: UNITED STATES | ZIP / Postal Code: 336172008

Phone Number: 813-974-2897 | Fax Number: 813-974-4962

Email: rsch-awards@usf.edu

Signature of Authorized Representative	Date Signed
Raegen Einsmann	02/08/2019

20. Pre-application Add Attachment | Delete Attachment | View Attachment

21. Cover Letter Attachment Add Attachment | Delete Attachment | View Attachment

OMB Number: 4040-0010
Expiration Date: 12/31/2019

Project Abstract

The Project Abstract must not exceed one page and must contain a summary of the proposed activity suitable for dissemination to the public. It should be a self-contained description of the project and should contain a statement of objectives and methods to be employed. It should be informative to other persons working in the same or related fields and insofar as possible understandable to a technically literate lay reader. This Abstract must not include any proprietary/confidential information.

* Please click the add attachment button to complete this entry.

Add Attachment | Delete Attachment | View Attachment

1234-ABSTRACT_Dean EDA CNS-OT study.p

RESEARCH & RELATED BUDGET - Budget Period 1

OMB Number: 4040-0001
Expiration Date: 10/31/2019

ORGANIZATIONAL DUNS: 0696872420000 **Enter name of Organization:** University of South Florida

Budget Type: ☒ Project ☐ Subaward/Consortium **Budget Period: 1** **Start Date:** 02/01/2019 **End Date:** 01/31/2020

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Jay		Dean		(b) (4)	0.24			(b) (4)	(b) (4)	(b) (4)

Project Role: PD/PI

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

Total Funds requested for all Senior Key Persons in the attached file

Total Senior/Key Person (b) (4)

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Lab Manager - (b) (4)	1.08			(b) (4)	(b) (4)	(b) (4)
1	**Total Number Other Personnel**					**Total Other Personnel**	(b) (4)

Total Salary, Wages and Fringe Benefits (A+B) (b) (4)

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	Funds Requested ($)

Additional Equipment: Add Attachment Delete Attachment View Attachment

Total funds requested for all equipment listed in the attached file

Total Equipment

D. Travel

		Funds Requested ($)
1.	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	
2.	Foreign Travel Costs	
	Total Travel Cost	

E. Participant/Trainee Support Costs

		Funds Requested ($)
1.	Tuition/Fees/Health Insurance	
2.	Stipends	
3.	Travel	
4.	Subsistence	
5.	Other	
	Number of Participants/Trainees — **Total Participant/Trainee Support Costs**	

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	820.00
2.	Publication Costs	
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Telemetry (refurbish and batteries)	2,444.00
9.	Animal Costs	1,000.00
10.	GSR Amp for AD Instruments	1,170.00
	Total Other Direct Costs	5,434.00

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	16,707.00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
MTDC	(b) (4)	(b) (4)	(b) (4)
		Total Indirect Costs	(b) (4)

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

DHHS, Shon Turner, 214-767-3261

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	24,977.00

J. Fee

Funds Requested ($)

K. Total Costs and Fee

	Funds Requested ($)
Total Costs and Fee (I + J)	24,977.00

L. Budget Justification

(Only attach one file.) 1238-BUDGET JUSTIFICATION_Dean EDA CN$ Add Attachment Delete Attachment View Attachment

RESEARCH & RELATED BUDGET - Cumulative Budget

		Totals ($)
Section A, Senior/Key Person		(b) (4)
Section B, Other Personnel		(b) (4)
Total Number Other Personnel	1	
Total Salary, Wages and Fringe Benefits (A+B)		11,273.00
Section C, Equipment		
Section D, Travel		
1. Domestic		
2. Foreign		
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		5,434.00
1. Materials and Supplies	820.00	
2. Publication Costs		
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1	2,444.00	
9. Other 2	1,000.00	
10. Other 3	1,170.00	
Section G, Direct Costs (A thru F)		16,707.00
Section H, Indirect Costs		(b) (4)
Section I, Total Direct and Indirect Costs (G + H)		24,977.00
Section J, Fee		
Section K, Total Costs and Fee (I + J)		24,977.00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: * First Name: Jay Middle Name:
* Last Name: Dean Suffix:
Position/Title: Professor Department: Molecular Pharm and Physiology
Organization Name: University of South Florida Division: College of Medicine
* Street1: 12901 Bruce B Downs Blvd MDC8
Street2:
* City: Tampa County/ Parish: Hillsborough
* State: FL: Florida Province:
* Country: USA: UNITED STATES * Zip / Postal Code: 336129445
* Phone Number: 813-974-1547 Fax Number:
* E-Mail: jdean@health.usf.edu

Credential, e.g., agency login:

*** Project Role:** PD/PI **Other Project Role Category:**

Degree Type: PhD
Degree Year: 1986

***Attach Biographical Sketch** 1237-Biosketch_Dean, Jay.pdf [Add Attachment] [Delete Attachment] [View Attachment]
Attach Current & Pending Support [Add Attachment] [Delete Attachment] [View Attachment]

PROFILE - Senior/Key Person 1

Prefix: * First Name: Middle Name:
* Last Name: Suffix:
Position/Title: Department:
Organization Name: Division:
* Street1:
Street2:
* City: County/ Parish:
* State: Province:
* Country: USA: UNITED STATES * Zip / Postal Code:
* Phone Number: Fax Number:
* E-Mail:

Credential, e.g., agency login:

*** Project Role:** **Other Project Role Category:**

Degree Type:
Degree Year:

Attach Biographical Sketch [Add Attachment] [Delete Attachment] [View Attachment]
Attach Current & Pending Support [Add Attachment] [Delete Attachment] [View Attachment]

RESEARCH & RELATED Other Project Information

OMB Number: 4040-0001
Expiration Date: 10/31/2019

1. Are Human Subjects Involved? ☐ Yes ☒ No

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ☐ Yes ☐ No

If yes, check appropriate exemption number. ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8

If no, is the IRB review Pending? ☐ Yes ☐ No

IRB Approval Date:

Human Subject Assurance Number:

2. Are Vertebrate Animals Used? ☒ Yes ☐ No

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ☒ Yes ☐ No

IACUC Approval Date:

Animal Welfare Assurance Number: A4100-01

3. Is proprietary/privileged information included in the application? ☐ Yes ☒ No

4.a. Does this Project Have an Actual or Potential Impact - positive or negative - on the environment? ☐ Yes ☒ No

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ☐ Yes ☐ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place? ☐ Yes ☒ No

5.a. If yes, please explain:

6. Does this project involve activities outside of the United States or partnerships with international collaborators? ☐ Yes ☒ No

6.a. If yes, identify countries:

6.b. Optional Explanation:

7. Project Summary/Abstract 1235-Project Abstract.pdf [Add Attachment] [Delete Attachment] [View Attachment]

8. Project Narrative 1236-Technical Proposal.pdf [Add Attachment] [Delete Attachment] [View Attachment]

9. Bibliography & References Cited [Add Attachment] [Delete Attachment] [View Attachment]

10. Facilities & Other Resources [Add Attachment] [Delete Attachment] [View Attachment]

11. Equipment [Add Attachment] [Delete Attachment] [View Attachment]

12. Other Attachments [Add Attachments] [Delete Attachments] [View Attachments]

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED PERSONAL DATA
Project Director/Principal Investigator and Co-Project Director(s)/Co-Principal Investigator(s)

The Federal Government has a continuing commitment to monitor the operation of its review and award processes to identify and address any inequities based on gender, race, ethnicity, or disability of its proposed PDs/PIs and co-PDs/PIs. To gather information needed for this important task, the applicant should submit the requested information for each identified PD/PI and co-PDs/PIs with each proposal. Submission of the requested information is voluntary and is not a precondition of award. However, information not submitted will seriously undermine the statistical validity, and therefore the usefulness, of information received from others. Any individual not wishing to submit some or all the information should check the box provided for this purpose. Upon receipt of the application, this form will be separated from the application. This form will not be duplicated, and it will not be a part of the review process. Data will be confidential.

Project Director/Principal Investigator

Prefix:

*** First Name:** Jay

Middle Name:

*** Last Name:** Dean

Suffix:

Gender: Male

Race (check all that apply):

- [ ] American Indian or Alaska Native
- [ ] Asian
- [ ] Black or African American
- [ ] Native Hawaiian or Other Pacific Islander
- [ ] White
- [x] Do Not Wish to Provide

Ethnicity: Non-Hispanic or Latino

Disability Status (check all that apply):

- [ ] Hearing
- [ ] Visual
- [ ] Mobility/Orthopedic Impairment
- [ ] Other
- [ ] None
- [x] Do Not Wish to Provide

Citizenship: US Citizen

Abstract

(b) (4)
[redacted]). Aim 1 will be done at USF in the laboratory of co-I Dr. J. B. Dean using unanesthetized, instrumented adult male Sprague-Dawley rats. The goal of this aim is to evaluate the utility of measuring electrodermal activity (EDA) across the skin as a physiological marker of an impending grand mal tonic-clonic seizure, which the hallmark symptom of central nervous system oxygen toxicity (CNS-OT) while breathing hyperbaric oxygen (HBO_2). We hypothesize that increased EDA can be detected prior to onset of seizures while breathing HBO_2 and thus used to predict an impending episode of CNS-OT. Increased EDA has been observed prior to onset of epileptic seizures at room pressure in rodents. To accomplish this aim, rats are anesthetized and implanted with a radio telemetric module for measuring various physiological parameters. At least one week later, under anesthesia, two EDA electrodes are attached to the rodent's back using either surface electrode attachment or subdermal attachment. 24 hours later, the animal is placed in the animal chamber inside the hyperbaric chamber and the two EDA wire leads are connected to the animal. The EDA lead wires are restrained by a tether harness. The animal is dived to 5 ATA HBO_2 until onset of first and second bouts of seizures. The importance of this study is to determine if electrodermal activity, which can be easily monitored in animals and humans, is a useful physiological marker of an impending CNS-OT "hit"; that is, does EDA increase prior to seizure onset, and can it be used as signal for taking corrective action by lowering inspired oxygen and thus averting CNS-OT seizures.

Budget justification:

Key personnel—($11,273/yr 1)

Jay B. Dean (PhD; 2% effort) (b) (4) [redacted] Dr. Dean is director of the USF Hyperbaric Biomedical Research Laboratory and an expert in hyperbaric physiology and neurophysiology. Dr. Dean will take responsibility for all the proposed animal experiments in Aim 1, which measures EDA before and during onset of CNS-OT seizures in rats.

(b) (4) [redacted] (BS; 9% effort) is the lab manager for the USF Hyperbaric Biomedical Research Laboratory. (b) (4) [redacted] is involved in all animal experiments including surgery to implant the radio telemetry module and in assisting the Co-I in conducting and analyzing the experiments.

Materials and supplies—($820/yr 1) Funds are requested to purchase surgical supplies, anesthetics and analgesics, and gases that are used for implanting radio-telemetry modules and EDA leads under sterile conditions. Gases are also used for administering HBO to induce CNS-OT.

Other: telemetry ($2,444/yr 1), animal costs ($1,000/yr 1), and GSR Amp for ADInstruments data acquisition system ($1,170/yr 1)—Funds are requested to have Data Sciences International (DSI) refurbish four F-40 radio-telemetry modules that we currently have in the lab. Funds are also requested for 12 animals, which includes purchase price plus shipping and per diem costs. Finally, a GSR amplifier that interfaces with a preexisting ADInstruments data acquisition system is requested to measure EDA in rats.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME Jay B. Dean, PhD	POSITION TITLE Professor of Molecular Pharmacology & Physiology
eRA COMMONS USER NAME (credential, e.g., agency login)	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Central Michigan University, Mt. Pleasant, MI	B.S.	8/79	Biology, Chemistry
Michigan Technological Univ., Houghton, MI	M.S.	5/81	Biol. Sci. (Respiratory Control)
The Ohio State University, Columbus, OH	Ph.D.	12/86	Physiology (Control of body temperature)
Univ. of North Carolina, Chapel Hill, NC	Post-Doc.	12/86-11/91	Neurophysiology (Resp.Control)

A. Personal Statement

My lab studies the effects of O_2, CO_2 and barometric pressure on the function of the mammalian CNS, with an emphasis on the cardio-respiratory and gastro-esophageal control systems. Ongoing research focuses on understanding how hyperoxia and hypercapnia interact to generate reactive O_2 and N_2 species that, in turn, activate neuronal activity in brain stem neurons. This research has relevance to understanding normal and abnormal function in cardio-respiratory control during periods of disordered breathing, environmental stresses, and in understanding neurological problems arising from the use of hyperoxia in clinical medicine and combat operations. We are particularly interested in the following: 1) neurophysiology of CNS oxygen toxicity (seizures) and its mitigation by ketone metabolic therapy; 2) understanding how CO_2 and H-ions are "sensed" by chemoreceptor neurons in the caudal medulla oblongata; and 3) understanding how gastro-esophageal CO_2 ventilation supplements pulmonary ventilation during acute and chronic respiratory acidosis, digestion and other conditions that stimulate gastric acid secretion.

B. Positions and Honors

Positions and Employment

1986-88	NINCDS Postdoctoral Fellow, Dept. of Physiology, Curriculum in Neurobiology, UNC-CH (D.E. Millhorn)
1988-90	NRSA Postdoctoral Fellow, Dept. of Physiology, UNC-CH (D.E. Millhorn)
1990-91	Res. Assist. Prof./Parker B. Francis Fellow in Pulmonary Res., Dept. of Physiology, UNC-CH (D.E. Millhorn)
12/91-97	Assistant Professor, Dept. of Physiology & Biophysics, Wright State University, Dayton, OH
1997-02	Associate Professor, Dept. of Physiology & Biophysics, Wright State University, Dayton, OH
7/02-6/03	Professor, Dept. of Physiology & Biophysics, Wright State University, Dayton, OH
7/03-10/04	Acting-Chairman, Dept. of Anatomy & Physiology, Wright State Univ., Dayton, OH
11/04-3/05	Vice Chair of Administration, Dept. of Anatomy & Physiology, Wright State Univ., Dayton, OH
3/05-6/06	Vice Chair of Administration, Dept. of Neuroscience, Cell Biology and Physiology, WSU, Dayton, OH

7/1/06-pres. Professor, Dept. of Molecular Pharmacology & Physiology, Coll. of Med., Univ. of South Florida, Tampa, FL

7/1/10-10/30/11 Acting Chair, Molecular Pharmacology & Physiology, Coll. of Med., Univ. of South Florida, Tampa, FL

Other Experience and Professional Memberships

1992-94 NSF, Special Emphasis Panel for Research Planning Grants and Career Advancement Awards for Women

1998 NIH, Special Emphasis Panel

2000 NIH, Respiratory and Applied Physiology Study Section, Temporary Member

2002 NIH, Respiratory Physiology Study Section, Ad-hoc Member

3/2003 NIH grant review panel for SCOR grants, Sleep Apnea

10/03-6/07 Regular member, NIH Respiratory Integrative Biology and Translational Research Study Section (RIBT)

1999-00 Co-organizer & Lecturer, International Symp., Central Chemoreception 2000, Ruhr Univ., Bochum, Germany

2001-02 Co-chair & Lec., Featured Topic, EB2002, Role of gap junctions in CO_2-chemoreception & respiratory control

2000-01 Guest Co-Ed., Special Issue, Respir. Physiol., Central Chemoreception 2000 (vol. 129:1-278, 2001)

2003-04 Chair & Lecturer, Featured Topic, EB 2004, Oxygen Sensing by Neural Tissues

4/10-6/10 Co-Ed., Highlighted Topic, J. Appl. Physiol., Central CO_2 Chemoreception & Cardio-respiratory Control

1999-pres. Editorial Board, Journal of Applied Physiology

2008-2010 Associate Editor, Journal of Applied Physiology (Hyperbaric Physiology, Respiratory Control)

2002- 2012 Editorial Board, Undersea and Hyperbaric Medicine

1987- pres. Member, Society for Neuroscience

1997- pres. Member, American Physiological Society

1997- pres. Member, Undersea and Hyperbaric Medical Society

2000- pres. Member, Society for Free Radical Biology and Medicine

2004- pres. Member, Aerospace Medical Association

Honors

7/03-6/06 Brage Golding Distinguished Professor of Research, Wright State Univ., Dayton, OH

Selected peer-reviewed publications pertinent to this application (from a total of >60)

Ciarlone, G.E., Hinojo, C.M., Stavitzski, N.M., and Dean, J.B. (2019) CNS function and dysfunction during exposure to hyperbaric oxygen in operational and clinical settings. Invited review for Redox Biology, Special Issue entitled, "Redox Biology in Physiology and Disease" (in peer review; manuscript no. REDOX_2019_115)

Ciarlone, G.E., and Dean, J.B. (2019) Acute hyperoxia increases CO_2-chemosensitivity in rat caudal solitary complex neurons. American Journal of Physiology, Cell Physiology (in revision; manuscript C-00368-2018R1)

Ari, C., Koutnik, A. P., DeBlasi, J., Landon, C. S., Rogers, C. Q., Vallas, J., Bharwani, S., Puchowicz, M., Bederman, I., Diamond, D.M., Kindy, M.S., Dean, J.B., and D'Agostino, D. P. e. (2019). Delaying latency to hyperbaric oxygen-induced CNS oxygen toxicity seizures by combinations of exogenous ketone supplements. Physiological Reports. doi: 10.14814/phy2.13961

Morris K.F., Nuding S.C., Segers L.S., Iceman K.E., O'Connor R., Dean J.B., Ott M.M., Alencar P.A., Shuman D., Horton K.K., Taylor-Clark T.E., Bolser D.C., and Lindsey B.G. (2017) Carotid chemoreceptors tune breathing via multipath routing: Reticular chain and loop operations supported by parallel spike train correlations. J Neurophysiol 119(2):700-722. doi: 10.1152/jn.00630.2017

Ciarlone G. E. and Dean J. B. (2016a) Normobaric hyperoxia stimulates superoxide and nitric oxide production in the caudal solitary complex of rat brain slices. Am J Physiol Cell Physiol 311: C1014–C1026,

Ciarlone G. E. and Dean J. B. (2016b) Acute hypercapnic hyperoxia stimulates reactive species production in the caudal solitary complex of rat brain slices but does not induce oxidative stress. Am J Physiol Cell Physiol 311: C1027–C1039, 2016.

Gianni D'Angelo, Raffaele Pilla, Jay B. Dean, Salvatore Rampone (2016) Toward a soft computing-based correlation between oxygen toxicity seizures and hyperoxic hyperpnea. Soft Computing (METHODOLOGIES AND APPLICATION), DOI 10.1007/s00500-017-2512-z

Matott, M.P., Ciarlone, G.E., Putnam, R.W., and Dean, J.B. (2014) Normobaric hyperoxia (95% O_2) stimulates CO_2-sensitive and CO_2-insensitive neurons in the caudal solitary complex of rat medullary tissue slices maintained in 40% O_2. Neuroscience 270: 98-122

Held, H.E., Pilla, R., Ciarlone, G.E., Landon, C.S., Dean, J.B. (April 2014) Female rats are more susceptible to central nervous system oxygen toxicity than male rats. Physiological Reports 2 (e00282) DOI: 10.14814/phy2.282; http://physreports.physiology.org/content/2/4/e00282.short

Pilla, R., Held, H.E., Landon, C.S., and Dean, J.B. (2013) High doses of pseudoephedrine hydrochloride accelerate onset of CNS oxygen toxicity seizures in unanesthetized rats. Neuroscience 246: 391-396.

D'Agostino, D.P., Pilla, R., Held, H.E., Landon, C.S., Puchowicz, M., Brunengraber, H., Ari, C., Arnold, P. and Dean, J.B. (2013) Therapeutic ketosis with ketone ester delays central nervous system oxygen toxicity seizures in rats. Am. J. Physiol. Reg., Integr. and Comp. Physiol. 304(10): R829-R836

Pilla, R., Landon, C.S., and Dean, J.B. (2013) A potential early physiological marker for CNS oxygen toxicity: hyperoxic hyperpnea precedes seizure in unanesthetized rats breathing hyperbaric oxygen. J. Appl. Physiol. 114: 1009-1020.

D'Agostino, D. P., McNally, H.A., and Dean, J.B. (2012) Development and testing of hyperbaric atomic force microscopy (AFM) and fluorescence microscopy for biological applications, J. Microscopy 246(2): 129-142

Dean J.B. (2011) Theory of gastric CO_2 ventilation and its control during respiratory acidosis: Implications for central chemosensitivity, pH regulation, and diseases causing chronic CO_2 retention, Respir. Physiol. Neurobiol. 175(2): 189-209; with Commentary by Forster H.V. (2011) Gastric acid secretion contributes to elimination of CO_2 during hypercapnia, Respir. Physiol. Neurobiol. 175: 189-209

Dean, J.B. Putnam, R.W. (2010) The caudal solitary complex is a site of central CO_2 chemoreception and integration of multiple systems that regulate expired CO_2, Resp. Physiol. & Neurobiol. 173(3): 274-287.

Garcia III, A.J., Putnam, R.W. and Dean, J.B. (2010) Paper 1 of 2: Hyperbaric hyperoxia and normobaric reoxygenation increase excitability and activate oxygen-induced potentiation (OxIP) in CA1 hippocampal neurons. J. Appl. Physiol. 109: 804-819

Garcia III, A.J., Putnam, R.W. and Dean, J.B. (2010) Paper 2 of 2: Hyperoxic stimulation of synchronous orthodromic activity and induction of neural plasticity does not require changes in excitatory synaptic transmission. J. Appl. Physiol. 109: 820-829

Dean JB. (2010) Hypercapnia causes cellular oxidation and nitrosation in addition to acidosis: implications for CO_2 chemoreceptor function and dysfunction, J. Appl. Physiol. 108(6):1786-1795

Nichols, N.L., Wilkinson, K.A., Powell, F.A., Dean, J.B. and Putnam, R.W. (2009) Chronic hypoxia suppresses the CO_2 response of solitary complex (SC) neurons from rats. Respir. Physiol. & Neurobiol., 168:272-280

D'Agostino, D.P., Olson, J.E., and Dean, J.B. (2009) Acute hyperoxia increases lipid peroxidation and induces plasma membrane b lebbing in human U87 glioblastoma cells. Neuroscience 159: 1011-1022

Dean, J.B. and D'Agostino, D. P. (2008) Chapter 10. Pressure effects on human physiology. In: Physiology and Medicine of Hyperbaric Oxygen Therapy (edited by Tom S. Neuman and Stephen R. Thom), Elsevier Inc., Philadelphia, pp. 189-202

D'Agostino, D.P., Colomb, D.G., and Dean, J.B. (2009) Effects of hyperbaric gases on membrane nanostructure and function in neurons. J. Appl. Physiol. 106: 996-1003

D'Agostino, D.P., Putnam, R.W., and Dean, J.B. (2007) Superoxide ($\cdot O_2^-$) production in CA1 neurons of rat hippocampal slices exposed to graded levels of oxygen. *J. Neurophysiol.* 98: 1030 – 1041.

Mulkey, D.K., Henderson III, R.A., Putnam, R.W., and Dean, J.B. (2003) Pressure ($\leq$4 ATA) increases membrane conductance and firing rate in the rat solitary complex, *Journal of Applied Physiology*, 95(3): 922-930

Mulkey, D.K., Henderson III, R.A., Putnam, R.W., and Dean, J.B. (2003) Hyperbaric oxygen and chemical oxidants stimulate CO_2/H^+-sensitive neurons in rat brain stem slices, *Journal of Applied Physiology*, 95 (3): 910-921

Dean, J.B., Mulkey, D.K., Garcia III, A.J., Putnam, R.W., and Henderson III, R.A. (2003) Neuronal sensitivity to hyperoxia, hypercapnia, and inert gases at hyperbaric pressures, *Journal of Applied Physiology* 95(3): 883-909

Dean, J.B., Mulkey, D.K., Henderson III, R.A., Potter, S.J., and Putnam, R.W. (2004) Hyperoxia, Reactive O_2 Species, and Hyperventilation: O_2-Sensitivity of Brain Stem Neurons, *Journal of Applied Physiology*, 96: 784-791.

Mulkey, D.K., Henderson III, R.A., Putnam, R.W., and Dean, J.B. (2004) Oxidative stress decreases intracellular pH, Na^+/H^+ exchange and increases excitability of solitary complex neurons from rat brain slices, *American Journal of Physiology*, *Cell Physiology*, 286: C940-C951.

Mulkey, D.K., Henderson III, R.A., Ritucci, N.A., Putnam, R.W., and Dean, J.B. (2004) Chemical oxidants acidify solitary complex (SC) neurons in rat. In: *Undersea and Hyperbaric Medicine*, 31(1): 107-111

Ritucci, N.A., Dean, J.B., and Putnam, R.W. (2005) Somatic versus dendritic responses to hypercapnia in chemosensitive locus coeruleus neurons from neonatal rats. *Am. J. Physiol. Cell Physiol.*, 289: C1094-C1104

Dean, J.B. and Mulkey, D.K. (2000) Continuous intracellular recording from mammalian neurons exposed to hyperbaric helium, oxygen or air, *Journal of Applied Physiology* 89:807-822.

Mulkey, D.K., Henderson III, R.A., Olson, J.E., Putnam, R.W., and Dean, J.B. (2001) Oxygen measurements in brainstem slices exposed to normobaric and hyperbaric oxygen, *Journal of Applied Physiology*, 90: 1887-1899

D. Research Support

Ongoing Research Support

ONR N00014-16-1-2537 Dean (PI) 6/1/2016 – 5/31/2019
(no cost extension to be requested: -8/31/2019)

Office of Naval Research, Undersea Medicine Program

Cellular mechanisms of CNS oxygen toxicity during CO_2 retention and ketone metabolic therapy

The goal of this research project is to further understand how conditions that accelerate and delay onset of CNS oxygen toxicity (CNS-OT) seizures (Sz) affect each of the following phenomena that are implicated in CNS-OT in rodents: i) increased production of reactive O_2 and N_2 species \ in the brain stem; ii) electrical signaling by medullary neurons; and iii) respiration during the period preceding Sz; i.e., hyperoxic hyperventilation. iv) An additional goal is to determine the effects of chronic CO_2 retention on cardiorespiratory and gastric physiology.

ONR N00014-18-1-2701 D'Agostino (PI) 08/01/2018 – 7/31/2021

Office of Naval Research

Optimizing ketone metabolic therapy and identifying biomarkers for mitigation and prediction of CNS oxygen toxicity: animal studies

The major goal of this project is to define the efficacy and anticonvulsant mechanism of ketones using in vivo and in vitro models

JBD's Role: Co-I

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Volume 1: Technical Proposal

BAA Number: ONR BAA Number: N00014-19-S-B001

Title of Proposal: Aim 1, Animal studies (USF Tampa): Feasibility of electrodermal activity for detecting seizures elicited by central nervous system oxygen toxicity

Prime Offeror: Office of Naval Research, Undersea Medicine Program

Technical Contact: P.I., Jay B. Dean, Ph.D., Professor
Dept. of Molecular Pharmacology & Physiology
Hyperbaric Biomedical Research Laboratory
Morsani College of Medicine, MDC 8
12901 Bruce B. Downs Blvd.
Tampa, FL 33612
(813) 974-1547, office phone
(b) (6) [redacted] cell phone
(813) 905-9965, office fax
jdean@health.usf.edu

Administrative/business contact:
Brenda Simmons
Sponsored Research Administrator
University of South Florida
4019 East Fowler Avenue, Suite 100
Tampa, FL 33617-2008
813-974-8778
bsimmons@usf.edu

Proposed period of performance:
February 2019-January 2020

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• TECHNICAL APPROACH AND JUSTIFICATION[1]

1. Introduction

HBO_2 & CNS oxygen toxicity. Hyperbaric oxygen (HBO_2) is breathed during hyperbaric oxygen therapy (HBOT) for treating problematic wounds and resolving disorders caused by inert gas bubbles and emboli [1]. HBO_2 is also used for certain specialized undersea activities covered under recreational, commercial, and military diving operations [2, 3], and pressurized disabled submarine (DISSUB) emergencies that require escape or rescue [4-6]. In these contexts, the greatest benefit of using O_2-enriched gas mixtures or pure O_2 is the avoidance of maladies that involve inert gases [7-9]. Breathing a hyperoxic gas prior to decompression, a strategy known as oxygen prebreathing or isobaric denitrogenation, can be used to reduce or omit decompression obligations and avert the risk for decompression sickness (DCS) not only in divers completing prolonged dive profiles, but also in pressurized DISSUB personnel who are exposed to hyperbaric air inside a compromised submarine at a maximum rescuable depth equivalent of 5 ATA ambient pressure [4-6]. Importantly, high dose O_2 can also be used in conjunction with closed-circuit underwater breathing apparatus (e.g., MK-25 and LAR V Dräger rebreathers) in military clandestine operations to maintain stealth (no exhaled gas bubbles) during shallow water transit [3, 9, 10].

What limits exposure to HBO_2 in the foregoing situations is the risk of developing central nervous system oxygen toxicity (CNS-OT) following a latent period of safe oxygen breathing. CNS-OT presents as various non-convulsive signs and symptoms, many of which appear to be of brainstem origin involving cranial nerve nuclei, autonomic nervous system centers, and cardiorespiratory centers, which ultimately spread to higher cortical centers and terminate as grand mal tonic-clonic seizures [11]. The initial safe latent period makes the use of HBO_2 practical in hyperbaric and undersea medicine; however, the latent period is highly variable between individuals and within the same individual on different days, making it difficult to predict onset of lethal indications of CNS-OT. Consequently, currently accepted guidelines for safe HBO_2 exposure are highly conservative; for example, current guidelines for safe exposure at 50 feet of sea water (fsw) breathing pure oxygen is 10 minutes [12].

The purpose of this research project is to test the hypothesis that skin electrical resistance, which is known as electrodermal activity (EDA), increases during the pre-seizure period of CNS-OT. The utility of this approach is that increased EDA, if it precedes seizures sufficiently early, could be used to take corrective action and avert seizures; i.e., reduce the level of inspired partial pressure of oxygen (P_IO_2). Early changes in animal physiology that precede seizures during exposure to HBO_2 are known as **physiological markers** or **physio-markers** of an impending seizure [11]. In the proposed study, the animal's physiology will be measured using radio telemetry and EDA electrodes attached to the back of the animal while breathing HBO_2 in a

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[1] ***Abbreviations used in grant proposal:*** **AFM,** atomic force microscopy; **ATA,** atmospheres absolute pressure; **CBF,** cerebral blood flow; **CNS,** central nervous system; **CNS-OT,** central nervous system oxygen toxicity; **DCS,** decompression sickness; **DISSUB,** disabled submarine; **EDA,** electrodermal activity; **EEG,** electroencephalogram; **F_IO_2,** fractional concentration of inspired oxygen; **fsw,** feet of sea water; **HBO_2,** hyperbaric oxygen; **HBOT,** hyperbaric oxygen therapy; **$NormO_2HBHe$,** normoxic hyperbaric helium (heliox); **He,** helium; **LSz,** latency time to seizure; **LSz1 & LSz2,** latency time to 1st and 2nd bouts of seizures; **O_2,** oxygen; **P_B,** barometric pressure; **PO_2,** partial pressure of oxygen; **P_IO_2,** partial pressure of inspired oxygen; **t_0,** time zero for calculation of LSz; **USN,** United States Navy

pressure chamber. Onset of CNS-OT will be determined by the appearance of neurological and behavioral (motor) seizures [13].

Logistics and personnel. (b) (4) [redacted] Aim 1 (the present study) will be done at USF in the laboratory of Dr. J.B. Dean using unanesthetized, instrumented adult male Sprague-Dawley rats. Experiments for Aim 1 will be done by the co-I. (J.B. Dean, 2% FTE) assisted by (b) (4) [redacted] (Lab Manager, 9% FTE).

2. Specific Aim

Aim 1. *Determine the effects of HBO_2 on electrodermal activity (EDA) in Sprague-Dawley rats*. Changes in EDA will be correlated with other physiological and behavioral parameters including cortical electroencephalogram activity, behavioral motor seizures, heart rate and pattern, respiration, body temperature, and animal activity.

3. Background

HBO_2 & physio-markers that precede seizures. Potential physio-markers under investigation for predicting an impending seizure during exposure to HBO_2 include the following: hyperoxic hyperpnea, which is also known as hyperoxic hyperventilation [14, 15]; heart rate and pattern [16, 17]; and hyperoxic hypothermia [18-20]. Several other possible predictors exist [3, 10, 21], most of which appear to be of brainstem origin [11]. The proposed study seeks to determine if increased EDA is another physio-marker of an impending CNS-OT seizure.

Electrodermal activity (EDA). EDA is a measure of the electrical activity of sympathetic (autonomic) nerves that innervate the skin [22-24]. Previously, it has been shown that EDA increases prior to onset of epileptic seizures in humans [22]. **The proposed study will test the hypothesis** that increased sympathetic neural activity precedes seizure genesis during HBO_2 exposure in a commonly used small animal model of CNS-OT; i.e., the adult male Sprague-Dawley rat [15, 25-28]. Good EDA signals can be measured from the back of a semi restrained (tethered), unanesthetized rat [24].

The pathophysiology of CNS-OT: role of increased autonomic neural activity during early exposure to HBO_2. Research, much of it supported by the ONR Undersea Medicine Program, has shown that exposure to HBO_2 initially results in a transient parasympathetic response comprised of decreased heart rate, cardiac output, blood pressure, and sympathetic tone [29]. Initially, cerebral blood flow (CBF) is decreased by vasoconstriction and delays a significant increase in brain tissue P_{O_2} [30-35]. Likewise, minute ventilation decreases initially due to hyperoxic inactivation of peripheral chemoreceptors [14, 15]. With extended exposure to HBO_2, this cardiopulmonary response is overtaken by increased sympathetic outflow resulting in hyperventilation, and hypertension [15, 29, 36]. We plan to determine if part of the increased sympathetic nervous system response includes an increase in EDA. In parallel with the sympathetic response, a buildup in nitric oxide causes escape from O_2-induced cerebral vasoconstriction and CBF increases causing a surge in neural tissue P_{O_2} and reactive O_2 and N_2 species production and, shortly thereafter, seizures [32, 37, 38].

Knowledge Gaps. This is the first study to determine if increased EDA precedes onset of CNS-OT in mammals. Changes in sympathetic EDA will be correlated with other physiological parameters including respiration, heart rate, core body temperature and the latency time to onset of neurological and behavioral seizures (LSz). If changes in EDA activity correlate with the LSz, then attempts will be made to determine if monitoring relative level of EDA can be used to avert CNS-OT seizures in our proposed animal model.

4. Specific Experiments

Aim 1. Determine the effects of HBO_2 on EDA in unanesthetized rats.

Hypothesis 1. Skin electrical resistance, which is known as EDA and a function of sympathetic neural activity, increases during the early pre-seizure period of CNS-OT and forewarns of an impending seizure.

Rationale 1. We know that the early pre-seizure period of exposure to HBO_2 is a period of increased autonomic nervous system activity that initially begins as parasympathetic activation that then switches over to increased sympathetic neural activity [11]. We also know that increased EDA precedes onset of epileptic seizures under normobaric (room pressure) normoxic conditions [22]. Furthermore, CNS-OT seizures are thought to resemble the seizures in idiopathic epilepsy [11]. Thus, we postulate that increased EDA activity precedes onset of HBO_2-induced convulsions.

Technical Approach 1.

Overview. Each rat will be implanted with a telemetric unit to measure cortical electroencephalograph (EEG) activity. Brain oxygen toxicity will be measured by onset of neurological seizures (increased EEG activity) and behavioral (visible tonic-clonic motor) seizures. Latencies to first and second bouts of seizures will be determined (LSz1 & LSz2). The first bout of seizures is usually small and short duration [13]. Typically, seizures stop immediately or within 1-2 minutes once P_{IO_2} is reduced. EDA will be measured by quantifying skin conductance changes due to changes in sympathetic nervous activity measured by either skin or sub-dermal electrodes. Initially, a small pilot experiment will determine the best method to measure EDA in rats during exposure to HBO_2 (5 ATA O_2); both skin electrodes and sub-dermal wire electrodes will be tested. Once this is established, each animal will be exposed to 5 ATA HBO_2 until the onset of the 2nd bout of seizures (LSz2). The animal will be decompressed on air and returned to the vivarium after it exhibits normal behavior. Five to seven days later, the same animal will be dived on normoxic heliox using 4%O_2 + 96% helium (He) to produce 0.2 ATA O_2 + 4.8 ATA He at total ambient pressure of 5 ATA; i.e., normoxic hyperbaric helium (NormO_2HBHe). An equivalent period of exposure to NormO_2HBHe serves as a control for the effects of pressure per se to confirm that any observed changes in EDA during HBO_2 are due to increased P_{O_2} and not due to hyperbaric pressure per se. The order of HBO_2 and NormO_2HBHe dives are not randomized because we need to know the duration of time spent in HBO_2 (LSz1 & LSz2) so that an equivalent duration of exposure to NormO_2HBHe is tested.

All experiments are conducted in Sprague-Dawley rats (male, 200-350g). The outbred/stock rat was chosen because it is the representative animal model this lab has used in its previous studies of the neurophysiology of CNS-OT [13, 15, 26-28, 39-44]. Males are used

because of the prevalence of males in U.S. Special Operations Forces and because female rats are more sensitive to HBO_2 and oxygen toxicity [27, 45], an added complexity to the study that we plan to address later in a future study.

General Approach: hyperbaric radio telemetry & EDA electrodes. All experiments will use radio telemetry (1st survival surgery, invasive) followed at least 1 week later by attachment of two EDA electrodes using either surface electrode attachment or sub-dermal attachment (2nd survival surgery, minimally invasive). 24 hours after the 2nd survival surgery, the animal will be placed in the animal chamber inside the hyperbaric chamber. Two leads will be attached to the EDA electrodes and the wires restrained by a tether harness and the animal dived to 5 ATA HBO_2 until onset of 1st and 2nd bouts of seizures. Five to 7 days later, the animal will be dived for an equivalent period using $NormO_2HBHe$ as the control for the effects of pressure per se.

Radio telemetric module implantation & EDA electrodes attachment. Each rat is transferred to the USF Hyperbaric Biomedical Research Lab and prepared for survival surgery using aseptic methods. The rat is anesthetized with isoflurane and implanted with a DSI radio telemetry module; the F-40 telemetry unit with 1 pair of biopotential leads, or alternatively the 4ET with 4 pairs of biopotential leads. The telemetry unit is implanted in the abdomen as described in the BUMED and USF Animal Use Protocols. F-40 wire leads are implanted through the skull to measure cortical EEG activity and core body temperature, whereas the 4ET module measures EEG activity, respiratory muscle electromyogram activity, electrocardiogram, and core body temperature. After recovery from surgery, rats are returned to the vivarium that same day.

At least 1 week following telemetric module implantation, each rat be anesthetized using isoflurane and the fur removed from the middle back (circular area about 2.5 cm in diameter) using a depilatory agent (Shiseido) and a pair of surface electrodes (3mm diameter) will be attached to the skin using a quick-hardening dental resin (Nissin Dental Products, Inc., MEND-REX). Alternatively, after removal of fur, a pair of Ag-Cl wires will be inserted beneath the surface of the skin (~2 mm sub-dermal) and attached using the quick-hardening dental resin. Animals will be fitted with a protective rodent jacket to prevent accidental removal of the EDA electrodes and returned to the vivarium upon recovery.

Dive protocols. The animal will be brought back to the lab and placed in the animal chamber (still wearing the protective rodent jacket). The telemetry module will be turned on via a magnet, EDA leads attached to the skin/sub-dermal electrodes. In the latter case, the point of lead attachment is secured using either liquid tape or heat shrink tubing. The animal chamber is sealed, and EDA leads are tethered and passed through the wall of the animal chamber and secured to the exterior of the animal chamber. Once sealed, the animal chamber is continuously ventilated with either air, pure oxygen (HBO_2), or 4%O_2 in helium ($NormO_2HBHe$). The animal chamber sits atop the radio telemetry receiver. The animal chamber and receiver sit inside a hyperbaric chamber that is pressurized with air or 100% N_2 in parallel with the animal chamber. Baseline telemetry recordings are made under control conditions (room air, 1 ATA) for 45-60 minutes. The two chambers are then pressurized simultaneously at a rate of 0.5 atmospheres/minute to 5 ATA. **For HBO_2 dives,** upon reaching 5 ATA, the animal chamber is switched over from air (0.2 $O_2 \times 5$

ATA = 1 ATA O_2 and 4 ATA N_2)[2] to 100% O_2 (5 ATA O_2). The [O_2] in the animal chamber is measured continuously (CWE Instruments, Gemini Respiratory Gas Analyzer) and changes quickly (< 10 sec). Time zero (t_0) for measuring LSz1 & LSz2 is marked once the fractional concentration of oxygen (F_IO_2) equals 1.0 in the animal chamber. The animal remains on HBO_2 until 2nd bout of seizures at which time is switched to air and decompressed at a rate of ~0.5 ATA/minute to avoid DCS. **For NormO$_2$HBHe dives**, upon reaching 5 ATA, the animal chamber is switched over from air (1 ATA O_2 and 4 ATA N_2) to NormO$_2$HBHe (0.2 ATA O_2 and 4.8 ATA He) and t_0 marked when F_IO_2 = 0.2 ATA O_2.[3] Total time for any given experimental session is ~1.5-3.5 hours/run. All dives are monitored in real time using a CCD video camera and recorded digitally. The animal is visualized continuously for telltale signs of CNS-OT, including tonic-clonic seizures of the upper limbs, neck and head (Table 1) coincident with increased cortical EEG activity. If the 4-ET radio transmitter is used, then additional physiological parameters monitored in real time include breathing, heart rate, and body temperature.

Measuring LSz1 & LSz2. The LSz1 and LSz2 will be determined using our modified Racine Scale of seizure behaviors [13]. The behavioral phenotype of CNS-OT in juvenile and adult Sprague-Dawley rats for seizures ranges in severity from Stage 1 through Stage 6 (Table 1). Intensity of convulsions increases with increasing seizure state number. Normal rat behaviors range from self-grooming, chewing bedding, sleeping, etc. Upon onset of cortical seizures, the rat is categorized with one of the behavioral stages listed below.

Stage	Behavior/ Phenotype
1	Freezing, staring, hard blinking and horizontal head movement
2	Hunched back, gnawing, vertical head jerks
3	Rearing with forelimbs lifted
4	Wet dog shakes
5	Rearing and forelimbs lifted with clonus
6	Generalized with forelimb and hindlimb clonus, rearing and falling

Table 1. Modified Racine Scale for behavioral seizures in Sprague-Dawley rats.

The risk for CNS-OT is measured as the LSz; specifically, first bout of seizures and second bout of seizures or LSz1 and LSz2, respectively. Two bouts of seizures are measured because the first bout is usually underwhelming in terms of intensity and duration (Stage 1), lasting only a few seconds and is then followed by normal behaviors for next several minutes to tens of minutes before the second, usually greater episode of seizures (States 2-6). If, however, the first bout of seizures is severe and long-lasting as in Stage 6 then the "dive" will be terminated at that point without waiting for the second episode of seizures.

Analysis 1. Based on our previous telemetric studies [15, 26, 28], we know that we can get useful data in 85% of the experiments. Failures (at 15% rate) may occur that require early removal of a rat from the study due to one of the following problems: death during surgery, electrode failure

[2] The brief exposure to 4 ATA N_2 is not thought to cause inert gas narcosis since much higher levels of hyperbaria are required to induce nitrogen narcosis in rats [46, 47].

[3] The brief exposure time (~4 min) to hyperoxia (>0.2 to 1.0 ATA O_2) during compression on air to maximum chamber pressure (5 ATA) is not expected to effect EDA since the dose of O_2 is sub-lethal; i.e., will not produce seizures.

post-surgery/during experimental sessions, radio-transmitter failure (transmitter battery failure), and unexpected illness/death during the study. Thus, a correction factor of 1.15 is used in animal numbers calculation to allow for 15% of animals that do not survive the experiment to completion for one of the above reasons. For these experiments, power analysis indicated a power = 0.8-0.9 for n=10 rats per group. This is based on our previous experiments using these procedures and the telemetric data generated. Using these assumptions and facts, we estimate the maximum number of animals needed for the experiments in Aim 1 as follows: 10 rats × 1.15 = 11.5 ≅ 12 rats.

GraphPad Prism version 7.04 software will be used for all statistical analyses. All values will be reported as means ± SE. As in our previous study of physio-markers during HBO_2 [15], data will not be normalized to the control values in air or 1 ATA O_2; however, because of individual variability in the latency time to seizure (LSz1 & LSz2) between animals, physiological data points will be aligned or zeroed to the onset of behavioral seizures (motor movements of the upper limbs and head; Table 1) during neurological seizure (increased EEG activity). Physiological data will be analyzed in reverse chronological order until the sample size begins decreasing as animals with the shortest-duration LSz values drop out of the pooled data. Data are analyzed by averaging the values derived from the raw data every 10 sec and a linear regression done at times 1, 2, 4, 8, and 16 mins (or similar intervals) preceding seizures 1 and 2. All statistical comparisons were considered significant at $P < 0.05$.

Anticipated Results 1. We predict that EDA will increase several minutes prior to onset of 1st seizures. During the interictal period that precedes the second seizure, we predict that EDA will remain elevated and exhibit a second surge in activity prior to the 2nd seizures. We do not expect $HBHeNormO_2$ (pressure per se) to have a significant effect on EDA. We also predict that EDA will increase in parallel with minute respiration and potentially heart rate prior to seizures.

Problems/Alternative Approaches 1. No significant problems are anticipated since we routinely conduct hyperbaric radio telemetric experiments in rats. Making EDA measurements will be new to our lab; however, the methods are already established in the literature [24] and should not be any more difficult than make a standard electrocardiogram recording.

5. General Procedures for Aim 1.

Hyperbaric Radio-Telemetry & EDA. The radio-telemetry system consists of an implantable F-40 or 4ET radio-transmitter able to amplify and broadcast signals via a receiver (DSI PhysioTel, model RPC-2) connected to an acquisition interface unit (ACQ 7700 Ponemah) via electrical penetrations in the wall of the hyperbaric chamber. The acquisition interface unit is connected to a computer for real time data collection and storage. The same acquisition unit also records chamber pressure and temperature, which are measured, respectively, by a thermocouple and pressure gauge directly connected to the acquisition system via BNC cables. Each animal will be continuously monitored via a video camera (AXIS 221 Network Camera) and the video of each experiment will be recorded as well. Raw data will be collected using DSI Ponemah software (P3 Ponemah Physiology Platform, version 4.90). ADE activity will be recorded using an ADInstruments Powerlab with GSR Amp. Statistical analysis will be performed using GraphPad PRISM®, version 7.04.

The hyperbaric system consists of two main elements: 1) a plexiglass chamber (~3 liter capacity, Diamond Box, Buxco, Electronics Inc., model PLY3114), that will house the rat during

the experiment, and 2) a hyperbaric chamber (Reimers System Inc. - 7.8 ATA MWP), that contains the plexiglass chamber and functions as the pressure vessel. Both chambers are connected to an air compressor (oil-less rotary scroll compressor – model DK6086, Powerex). The outer chamber will be pressurized using air or nitrogen (capacity ~205 liters) to minimize the risk of an electrical-induced fire.

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Future Naval Relevance

Naval unique Special Warfare/Special Operations diving maneuvers and submarine operations are limited by health risks associated with breathing various gas mixtures at normobaric and hyperbaric pressures. Included in the list of health risks is CNS-OT. Submariners must be able to survive a possible CNS-OT "hit" while breathing HBO_2 in a pressurized DISSUB emergency as they perform isobaric denitrogenation (oxygen prebreathe), to reduce the risk for DCS, in preparation of dropout decompression during a buoyant ascent. Navy divers are affected by HBO_2 because the risk of developing CNS-OT greatly limits bottom time during operations. For example, the current, highly conservative limit for breathing HBO_2 at 50 feet of sea water (fsw) is 10 minutes [9]. Research during World War II, however, showed that when divers were pushed to the onset of acute toxic symptoms of CNS-OT, including non-convulsive indications (e.g., vertigo, nausea, vomiting, dizziness) and seizures, that half of the divers could tolerate longer exposure to HBO_2 at 50 fsw. Many divers withstood at least twice as much oxygen, and some individuals tolerated exposures to HBO_2 upwards of ten times [11, 46]. The USN's goal is to expand the envelope of clandestine diving operations; that is, longer and safer dives. Currently, the only approved strategy for prevention of CNS-OT is reduction of depth (chamber pressure), and thus, P_{IO_2}, and/or the time of exposure (oxygen concentration product). One strategy currently under study, however, to extend bottom time on O_2 is to monitor the early physiological changes in a diver that precedes seizures; i.e., so-called physio-markers [11, 15]. In the present study, we plan to test whether electrodermal activity (EDA) of the sympathetic nervous system is a useful physio-marker. The proposed EDA study in rodents will be done in parallel with a study in humans at Duke University and in collaboration with University of Connecticut. This study also complements ongoing animal studies in our lab looking at other physio-markers of interest to the

USN including hyperoxic hyperventilation, heart rate and pattern, and hyperoxic hypothermia during HBO_2.

Project Schedule & Milestones

Table 2.				
Year (12 months):	Early 2019-Early 2020			
Months:	1-4		5-8	9-12
Setup EDA system & test EDA electrodes (surface vs. subdermal); JBD, (b) (4)				
Implant radio transmitters & dive animals on HBO_2 & $NormO_2HBHe$; JBD, (b) (4)				
Analyze physiology data; JBD, (b) (4) [redacted]				
Write manuscript; JBD with (b) (4) [redacted]				

Legend to Table 2.

JBD, Dr. Jay B. Dean (P.I. USF study); (b) (4) [redacted]

Reports & Data Deliverables

Technical Progress Reports

- written report submitted as requested by ONR Program Officier; and
- participation in the annual NAVSEA/ONR Undersea Medicine (UM) Program Review and any planned workshops.

Presentation Materials

- Viewgraphs included with each annual progress report;
- Data slide updates provided semi-annually/annually for ONR-UM Program Officer

Published manuscripts resulting from ONR-funded research.

Management Approach

1. Use of personnel. (b) (4) [redacted]. We will have weekly lab meetings in which we discuss the upcoming schedule of experiments projected for the next several weeks. Care will be taken to ensure that both team members know their role in the project. Ms. Landon will be involved in radio-telemetry surgery and assist the P.I. in animal experiments and with analyzing data from the proposed experiments.

2. Government research interfaces. We plan to communicate the findings of our studies as they progress with members of the undersea medical scientific community (b) (4) [redacted] & ONR headquarters (S. Chapman & colleagues). This is so we can receive input from the USN directly if we find it necessary to revise our protocol to better meet the needs of the U.S. Fleet or improve the study based on data and input supplied by colleagues. In addition, we

will communicate regularly with other ONR performers in academia who work in related areas of undersea medicine; for example, (b) (4) [redacted]

Facilities & Other Resources (including Government Furnished Equipment/Hardware/ Software):

(b) (4) [redacted]

(b) (4)

[redacted]

(b) (4) [redacted]

<u>Normobaric Research Stations.</u> 1) This cell culture/brain slice electrophysiology and imaging station includes the following items*:* electrophysiology amplifier (Axoclamp 2B patch clamp amplifier); 2 Burleigh microdrives; Nikon FN1 fixed-stage microscope equipped with: DIC optics and IR video microscopy, fluorescence attachment and Roper CoolSnap camera; brain slice recording chamber; vibration isolation table; window discriminators & rate meters; 6 channel timer/ stimulator with SIU; 2 oscilloscopes; 2 channel DC amplifier/filter; Digidata 1322A/pCLAMP data acquisition system with computer; water bath; temperature controller; and fiber optic illuminator. **2)** The other cell culture/brain slice electrophysiology and imaging station includes: Nikon TE2000 inverted microscope, which is mated to a fluorescence microscopy attachment. Important pieces of equipment for fluorescence microscopy include: Roper Cool Snap Digital Camera; Xenon light source; light guide; Lambda 10-3 shutter controller and interface; Nikon Elements Software for data collection and analysis.

<u>Gas Tank Farm</u>: A 528 ft^2 cement patio, enclosed by chain-link & barbed-wire fencing and overhanging roof, houses the following items used to compress/decompress the pressure chambers: oil-less scroll air compressor (model SES; MWP 115 PSIG) and a vertical air handling tank (400 gallons, 93" tall x 36" dia.; MWP 137 PSIG); 1-HP oil-less rotary vane vacuum pump (30 gallons tank) is plumbed to two chambers (b) (4) [redacted]) for producing altitudes over 20,000 feet; and space for up to 60 K-type gas bottles; and gas-mixing station for blending customized gas mixtures. A dual cylinder horizontal roller is used to mix gases cylinders that may have separated out. Compression and vacuum lines penetrate the back wall and terminate on the interior wall of MDL1026 with a manifold control panel so that helium, nitrogen or air can be directed to anyone one of up to six different work stations. In addition, various cylinders of O_2, CO_2 & N_2/He are plumbed from the tank farm to each work station for pressurizing sample cylinders used to produce HBO_2 media.

Histology: A full service research histology laboratory is available for histological and cytological preparations, tissue array construction, immunostaining and in situ hybridization in the Dept. of Molecular Pharmacology and Physiology (M. Barter). A Dako automated immunostainer, a Chemicon ATA 100 tissue arrayer and 2 cryostats as well as the equipment items necessary for paraffin processing, sectioning and staining are available.

Biostatistics Shared Resource: The Biostatistics Shared Resource staffs two doctoral-level biostatisticians available for consultation on all statistical aspects of research studies including: study design, database design, sample-size analysis, data analysis and interpretation, statistical/software instruction and manuscript writing. Available software tools include: SAS, SPSS, S-Plus, R, and nQuery.

Computer: Each research station maintains 1-2 computers for online data collection and offline data analysis. In addition, each office (3 total: P.I., lab manager & trainees) maintains a computer for word processing and each member of the lab owns a lap top computer.

Other: BioTek ELX800 Spectrophotometer (BioTek Instrument) for biochemical assays; Kerr Tissue Recording System for cell or tissue electrophysiology recordings if needed. General lab equipment includes: deionized water still; water baths, 2 Mettler analytical balances; pH meters; sonicators and refrigerators and freezers, including a -80°F freezer. Also available is additional equipment used for brain slicing and fabrication of microelectrodes and micropipettes: 3 micropipette pullers; Nikon Labophot & Optiphot microscopes; Nikon patch pipette polisher; Zeiss microscope with phase contrast optics; 3 Pelco Vibratomes for making tissue slices.

Animal: The USF Division of Comparative Medicine (DCM) serves as the advocate for animals involved in research at USF, and provides a fully accredited, centralized service of pathogen-free animal procurement, husbandry, health surveillance, and quality control. DCM is fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, International (AAALAC #000434). Its program and facilities for animal care and use are managed in accordance with the National Research Council Guide for the Care and Use of Laboratory Animals, the PHS Policy on Humane Care and Use of Laboratory Animals (PHS assurance #A4100-01), and the Animal Welfare Regulations. DCM has an assurance of compliance on file with OLAW and NIH, and is a registered research facility with the United States Department of Agriculture (USDA registration #58-R-0015). Animals are purchased as needed through Huntingdon Laboratories (formerly Harlan Laboratories).

Cell Culture: Available if needed. P.I. has just received cell culture incubator and hood from the former NEDU electrophysiology laboratory. In addition, we have access to departmental shared cell culture facility 327 ft^2 and two specialized cell culture incubators (Galaxy 48R, New Brunswick Instruments) that are capable of maintaining cell cultures under a wide range of oxygen concentrations (1% to 95%). is arranging for HBRL to acquire a new cell culture facility in addition to the shared facility currently available.

Office: The Department of Molecular Pharmacology and Physiology provides staff support for this project, including the following: (b) (4) [redacted]

[redacted] (b) (4) [redacted]

Other Aspects of the Research Environment/Facilities: The College of Engineering supports an instrument shop, which the PI has access to at the cost of $50/hr labor plus materials. The PI also has access (fee basis) to an assortment of machinists/model makers and electricians in the Tampa Bay area who do work for hire with USF faculty. (b) (4) [redacted] A full-time PhD degree program is available with various research tracks including Neuroscience and Physiology.

Current and Pending Project and Proposal Submissions

Jay B. Dean, Ph.D.

Project 1, JBD (PI)—the proposed study 3/2019 - 2/2020

1) Title of Proposal and Summary: Animal studies, Aim 1 (USF Tampa): Feasibility of measuring electrodermal activity for detecting seizures elicited by central nervous system oxygen toxicity. The goal of this project is to determine if electrodermal activity (skin electrical resistance) changes significantly in a small animal model before onset of seizures while breathing hyperbaric oxygen (HBO_2). If so, then increased electrodermal activity is a possible physiological marker of an impending seizure otherwise known as CNS oxygen toxicity, CNS-OT.

2) Source and amount of funding:

Total annual direct costs: $16,707

Grant No.: this proposal

3) Percentage effort devoted to project—2%

4) Identity of prime Offeror—ONR Undersea Medicine

5) Technical contact—P.I.: Jay B. Dean, Ph.D.; 813-974-1547/813-905-9965; jdean@health.usf.edu

6) Administrative/business contact—Sandra Chapman, Ph.D.; Undersea Medicine Program Officer; Warfighter Performance Department (Code 34); Office of Naval Research; 875 N Randolph St.; Arlington VA 22203; (703) 588-2429; Sandra.chapman@navy.mil

7) Period of performance—2/2019 through 1/2020

8) The proposed project and all other projects or activities requiring a portion of time of the Principal Investigator and other senior personnel must be included, even if they receive no salary support from the project(s)

P.I., J.B. Dean—2%

(b) (4) [redacted] —9%

9) The total award amount for the entire award period covered (including indirect costs, IDC) must be shown as well as the number of person-months or labor hours per year to be devoted to the project, regardless of source of support

Total direct costs + Total IDC = $16,707 + $8,270 = $24,977

10) State how projects are related to the proposed effort and indicate degree of overlap.—this is the proposed study

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Project 2, JBD (PI) 6/1/2016—5/31/2019 (NCE August 2019)

1) Title of Proposal and Summary: Cellular mechanisms of CNS oxygen toxicity during CO_2 retention and ketone metabolic therapy. The goal of this research project is to further understand how conditions that accelerate and delay onset of CNS oxygen toxicity (CNS-OT) seizures affect each of the following phenomena that are implicated in CNS-OT in rodents: i) increased production of reactive O_2 and N_2 species \ in the brain stem; ii) electrical signaling by medullary neurons; and iii) respiration during the period

preceding Sz; i.e., hyperoxic hyperventilation. iv) An additional goal is to determine the effects of chronic CO_2 retention on cardiorespiratory and gastric physiology.

2) Source and amount of funding:
Total annual direct costs: $219,933
Grant No.: ONR N00014-16-1-2537

3) Percentage effort devoted to project: 30%

4) Identity of prime offeror: ONR Undersea Medicine
5) Technical contact—P.I.: Jay B. Dean, Ph.D.; 813-974-1547/813-905-9965; jbdean813@gmail.com
6) Administrative/business contact—Sandra Chapman, Ph.D.; Undersea Medicine Program Officer; Warfighter Performance Department (Code 34); Office of Naval Research; 875 N Randolph St.; Arlington VA 22203; (703) 588-2429; Sandra.chapman@navy.mil
7) Period of performance—6/1/2016 through 5/31/2019 plus requesting NCE through August 2019
8) The proposed project and all other projects or activities requiring a portion of time of the Principal Investigator and other senior personnel must be included, even if they receive no salary support from the project(s)
P.I., J.B. Dean—30%
(b) (4) [redacted] —60%
9) The total award amount for the entire award period covered (including indirect costs, IDC) must be shown as well as the number of person-months or labor hours per year to be devoted to the project, regardless of source of support
Total direct costs + Total IDC = $977,569
10) State how projects are related to the proposed effort and indicate degree of overlap.—there is no scientific overlap

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Project 3, JBD (co-I) 2018-2021
1) Title of Proposal and Summary: Optimizing ketone metabolic therapy and identifying biomarkers for mitigation and prediction of CNS oxygen toxicity: animal studies. The major goal of this project is to define the efficacy and anticonvulsant mechanism of ketones using in vivo and in vitro models
2) Source and amount of funding:
Total annual direct costs: $228,559
Grant No.: ONR N00014-18-1-2701
3) Percentage effort devoted to project: 10%
4) Identity of prime offeror: ONR Undersea Medicine
5) Technical contact—P.I.: (b) (4) [redacted]
6) Administrative/business contact—Sandra Chapman, Ph.D.; Undersea Medicine Program Officer; Warfighter Performance Department (Code 34); Office of Naval Research; 875 N Randolph St.; Arlington VA 22203; (703) 588-2429; Sandra.chapman@navy.mil
7) Period of performance—8/1/2018 through 7/31/2021
8) The proposed project and all other projects or activities requiring a portion of time of the Principal Investigator and other senior personnel must be included, even if they receive no salary support from the project(s)
P.I., J.B. Dean—10%
(b) (4) [redacted] —20%
9) The total award amount for the entire award period covered (including indirect costs, IDC) must be shown as well as the number of person-months or labor hours per year to be devoted to the project, regardless of source of support
Total direct costs + Total IDC = $1,021,278

10) State how projects are related to the proposed effort and indicate degree of overlap.—there is no scientific overlap

(b) (4) [redacted]
No extramural funding to report

Qualifications: See attachments

Jay Dean, PI, 2% effort: Dr. Dean is the Director of the USF Hyperbaric Biomedical Research Laboratory. He takes overall responsibility for the performance of the study. He will conduct, in collaboration with his laboratory personnel, all the proposed experiments, assist in the analysis of the data, direct writing of papers, and deliver data presentations at national/international meetings and symposia. Dr. Dean's technical expertise is studying the electrophysiology and oxidative neurobiology of mammalian neurons in reduced animal and whole animal models (rat). Dr. Dean has employed a variety of technical approaches including hyperbaric electrophysiology (in vitro), hyperbaric microscopy (in vitro), hyperbaric radio-telemetry and plethysmography (in vivo) and other instrumented approaches in rats to study the effects of normobaric gases and hyperbaric gases at cellular and organismal levels.

(b) (4) [redacted]**, Lab Manager, 9%:** (b) (4) [redacted] will provide technical support to the laboratory of Dr. Dean, including radio-telemetry surgery, animal maintenance, solution preparation, ordering of supplies, budget maintenance, data analysis and figure preparation, including computer graphics work. She is greatly experienced with all aspects of laboratory management.

Biographical Sketches follow on next pages…

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME Jay B. Dean, PhD	POSITION TITLE Professor of Molecular Pharmacology & Physiology
eRA COMMONS USER NAME (credential, e.g., agency login)	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Central Michigan University, Mt. Pleasant, MI	B.S.	8/79	Biology, Chemistry
Michigan Technological Univ., Houghton, MI	M.S.	5/81	Biol. Sci. (Respiratory Control)
The Ohio State University, Columbus, OH	Ph.D.	12/86	Physiology (Control of body temperature)
Univ. of North Carolina, Chapel Hill, NC	Post-Doc.	12/86-11/91	Neurophysiology (Resp.Control)

A. Personal Statement

My lab studies the effects of O_2, CO_2 and barometric pressure on the function of the mammalian CNS, with an emphasis on the cardio-respiratory and gastro-esophageal control systems. Ongoing research focuses on understanding how hyperoxia and hypercapnia interact to generate reactive O_2 and N_2 species that, in turn, activate neuronal activity in brain stem neurons. This research has relevance to understanding normal and abnormal function in cardio-respiratory control during periods of disordered breathing, environmental stresses, and in understanding neurological problems arising from the use of hyperoxia in clinical medicine and combat operations. We are particularly interested in the following: 1) neurophysiology of CNS oxygen toxicity (seizures) and its mitigation by ketone metabolic therapy; 2) understanding how CO_2 and H-ions are "sensed" by chemoreceptor neurons in the caudal medulla oblongata; and 3) understanding how gastro-esophageal CO_2 ventilation supplements pulmonary ventilation during acute and chronic respiratory acidosis, digestion and other conditions that stimulate gastric acid secretion.

B. Positions and Honors

Positions and Employment

1986-88	NINCDS Postdoctoral Fellow, Dept. of Physiology, Curriculum in Neurobiology, UNC-CH (D.E. Millhorn)
1988-90	NRSA Postdoctoral Fellow, Dept. of Physiology, UNC-CH (D.E. Millhorn)
1990-91	Res. Assist. Prof./Parker B. Francis Fellow in Pulmonary Res., Dept. of Physiology, UNC-CH (D.E. Millhorn)
12/91-97	Assistant Professor, Dept. of Physiology & Biophysics, Wright State University, Dayton, OH
1997-02	Associate Professor, Dept. of Physiology & Biophysics, Wright State University, Dayton, OH
7/02-6/03	Professor, Dept. of Physiology & Biophysics, Wright State University, Dayton, OH
7/03-10/04	Acting-Chairman, Dept. of Anatomy & Physiology, Wright State Univ., Dayton, OH
11/04-3/05	Vice Chair of Administration, Dept. of Anatomy & Physiology, Wright State Univ., Dayton, OH

3/05-6/06 Vice Chair of Administration, Dept. of Neuroscience, Cell Biology and Physiology, WSU, Dayton, OH
7/1/06-pres. Professor, Dept. of Molecular Pharmacology & Physiology, Coll. of Med., Univ. of South Florida, Tampa, FL
7/1/10-10/30/11 Acting Chair, Molecular Pharmacology & Physiology, Coll. of Med., Univ. of South Florida, Tampa, FL

Other Experience and Professional Memberships

1992-94 NSF, Special Emphasis Panel for Research Planning Grants and Career Advancement Awards for Women
1998 NIH, Special Emphasis Panel
2000 NIH, Respiratory and Applied Physiology Study Section, Temporary Member
2002 NIH, Respiratory Physiology Study Section, Ad-hoc Member
3/2003 NIH grant review panel for SCOR grants, Sleep Apnea
10/03-6/07 Regular member, NIH Respiratory Integrative Biology and Translational Research Study Section (RIBT)
1999-00 Co-organizer & Lecturer, International Symp., Central Chemoreception 2000, Ruhr Univ., Bochum, Germany
2001-02 Co-chair & Lec., Featured Topic, EB2002, Role of gap junctions in CO_2-chemoreception & respiratory control
2000-01 Guest Co-Ed., Special Issue, Respir. Physiol., Central Chemoreception 2000 (vol. 129:1-278, 2001)
2003-04 Chair & Lecturer, Featured Topic, EB 2004, Oxygen Sensing by Neural Tissues
4/10-6/10 Co-Ed., Highlighted Topic, J. Appl. Physiol., Central CO_2 Chemoreception & Cardio-respiratory Control
1999-pres. Editorial Board, Journal of Applied Physiology
2008-2010 Associate Editor, Journal of Applied Physiology (Hyperbaric Physiology, Respiratory Control)
2002- 2013 Editorial Board, Undersea and Hyperbaric Medicine
1987- 2011 Member, Society for Neuroscience
1997- pres. Member, American Physiological Society
1997- 2009 Member, Undersea and Hyperbaric Medical Society
2000- 2013 Member, Society for Free Radical Biology and Medicine
2004- pres. Member, Aerospace Medical Association

Honors

7/03-6/06 Brage Golding Distinguished Professor of Research, Wright State Univ., Dayton, OH

Selected peer-reviewed publications pertinent to this application (from a total of >60)

Ciarlone, G.E., and Dean, J.B. (2019) Acute hyperoxia increases CO_2-chemosensitivity in rat caudal solitary complex neurons. American Journal of Physiology, Cell Physiology (in revision; manuscript C-00368-2018R1)

Ciarlone, G.E., Hinojo, C.M., Stavitzski, N.M., and Dean, J.B. (2019) CNS function and dysfunction during exposure to hyperbaric oxygen in operational and clinical settings. Invited review for Redox Biology, Special Issue entitled, “Redox Biology in Physiology and Disease” (accepted pending minor revisions; manuscript no. REDOX_2019_115)

Ari, C., Koutnik, A. P., DeBlasi, J., Landon, C. S., Rogers, C. Q., Vallas, J., Bharwani, S., Puchowicz, M., Bederman, I., Diamond, D.M., Kindy, M.S., Dean, J.B., and D'Agostino, D. P. e. (2019). Delaying latency to hyperbaric oxygen-induced CNS oxygen toxicity seizures by combinations of exogenous ketone supplements. Physiological Reports. doi: 10.14814/phy2.13961

Morris K.F., Nuding S.C., Segers L.S., Iceman K.E., O’Connor R., Dean J.B., Ott M.M., Alencar P.A., Shuman D., Horton K.K., Taylor-Clark T.E., Bolser D.C., and Lindsey B.G. (2017) Carotid

chemoreceptors tune breathing via multipath routing: Reticular chain and loop operations supported by parallel spike train correlations. J Neurophysiol 119(2):700-722. doi: 10.1152/jn.00630.2017

Ciarlone G. E. and Dean J. B. (2016a) Normobaric hyperoxia stimulates superoxide and nitric oxide production in the caudal solitary complex of rat brain slices. Am J Physiol Cell Physiol 311: C1014–C1026,

Ciarlone G. E. and Dean J. B. (2016b) Acute hypercapnic hyperoxia stimulates reactive species production in the caudal solitary complex of rat brain slices but does not induce oxidative stress. Am J Physiol Cell Physiol 311: C1027–C1039, 2016.

Gianni D'Angelo, Raffaele Pilla, Jay B. Dean, Salvatore Rampone (2016) Toward a soft computing-based correlation between oxygen toxicity seizures and hyperoxic hyperpnea. Soft Computing (METHODOLOGIES AND APPLICATION), DOI 10.1007/s00500-017-2512-z

Matott, M.P., Ciarlone, G.E., Putnam, R.W., and Dean, J.B. (2014) Normobaric hyperoxia (95% O_2) stimulates CO_2-sensitive and CO_2-insensitive neurons in the caudal solitary complex of rat medullary tissue slices maintained in 40% O_2. Neuroscience 270: 98-122

Held, H.E., Pilla, R., Ciarlone, G.E., Landon, C.S., Dean, J.B. (April 2014) Female rats are more susceptible to central nervous system oxygen toxicity than male rats. Physiological Reports 2 (e00282) DOI: 10.14814/phy2.282; http://physreports.physiology.org/content/2/4/e00282.short

Pilla, R., Held, H.E., Landon, C.S., and Dean, J.B. (2013) High doses of pseudoephedrine hydrochloride accelerate onset of CNS oxygen toxicity seizures in unanesthetized rats. Neuroscience 246: 391-396.

D'Agostino, D.P., Pilla, R., Held, H.E., Landon, C.S., Puchowicz, M., Brunengraber, H., Ari, C., Arnold, P. and Dean, J.B. (2013) Therapeutic ketosis with ketone ester delays central nervous system oxygen toxicity seizures in rats. Am. J. Physiol. Reg., Integr. and Comp. Physiol. 304(10): R829-R836

Pilla, R., Landon, C.S., and Dean, J.B. (2013) A potential early physiological marker for CNS oxygen toxicity: hyperoxic hyperpnea precedes seizure in unanesthetized rats breathing hyperbaric oxygen. J. Appl. Physiol. 114: 1009-1020.

D'Agostino, D. P., McNally, H.A., and Dean, J.B. (2012) Development and testing of hyperbaric atomic force microscopy (AFM) and fluorescence microscopy for biological applications, J. Microscopy 246(2): 129-142

Dean J.B. (2011) Theory of gastric CO_2 ventilation and its control during respiratory acidosis: Implications for central chemosensitivity, pH regulation, and diseases causing chronic CO_2 retention, Respir. Physiol. Neurobiol. 175(2): 189-209; with Commentary by Forster H.V. (2011) Gastric acid secretion contributes to elimination of CO_2 during hypercapnia, Respir. Physiol. Neurobiol. 175: 189-209

Dean, J.B. Putnam, R.W. (2010) The caudal solitary complex is a site of central CO_2 chemoreception and integration of multiple systems that regulate expired CO_2, Resp. Physiol. & Neurobiol. 173(3): 274-287.

Garcia III, A.J., Putnam, R.W. and Dean, J.B. (2010) Paper 1 of 2: Hyperbaric hyperoxia and normobaric reoxygenation increase excitability and activate oxygen-induced potentiation (OxIP) in CA1 hippocampal neurons. J. Appl. Physiol. 109: 804-819

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Dean JB. (2010) Hypercapnia causes cellular oxidation and nitrosation in addition to acidosis: implications for CO_2 chemoreceptor function and dysfunction, J. Appl. Physiol. 108(6):1786-1795

Nichols, N.L., Wilkinson, K.A., Powell, F.A., Dean, J.B. and Putnam, R.W. (2009) Chronic hypoxia suppresses the CO_2 response of solitary complex (SC) neurons from rats. Respir. Physiol. & Neurobiol., 168:272-280

D'Agostino, D.P., Olson, J.E., and Dean, J.B. (2009) Acute hyperoxia increases lipid peroxidation and induces plasma membrane b lebbing in human U87 glioblastoma cells. Neuroscience 159: 1011-1022

Dean, J.B. and D'Agostino, D. P. (2008) Chapter 10. Pressure effects on human physiology. In: Physiology and Medicine of Hyperbaric Oxygen Therapy (edited by Tom S. Neuman and Stephen R. Thom), Elsevier Inc., Philadelphia, pp. 189-202

D'Agostino, D.P., Colomb, D.G., and Dean, J.B. (2009) Effects of hyperbaric gases on membrane nanostructure and function in neurons. J. Appl. Physiol. 106: 996-1003

D'Agostino, D.P., Putnam, R.W., and Dean, J.B. (2007) Superoxide ($\cdot O_2^-$) production in CA1 neurons of rat hippocampal slices exposed to graded levels of oxygen. *J. Neurophysiol.* 98: 1030 – 1041.

Mulkey, D.K., Henderson III, R.A., Putnam, R.W., and Dean, J.B. (2003) Pressure ($\leq$4 ATA) increases membrane conductance and firing rate in the rat solitary complex, *Journal of Applied Physiology*, 95(3): 922-930

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D. Research Support

Ongoing Research Support

ONR N00014-16-1-2537 Dean (PI) 6/1/2016 – 5/31/2019
(no cost extension to be requested: -8/31/2019)

Office of Naval Research, Undersea Medicine Program

Cellular mechanisms of CNS oxygen toxicity during CO_2 retention and ketone metabolic therapy

The goal of this research project is to further understand how conditions that accelerate and delay onset of CNS oxygen toxicity (CNS-OT) seizures (Sz) affect each of the following phenomena that are implicated in CNS-OT in rodents: i) increased production of reactive O_2 and N_2 species \ in the brain stem; ii) electrical signaling by medullary neurons; and iii) respiration during the period preceding Sz; i.e., hyperoxic hyperventilation. iv) An additional goal is to determine the effects of chronic CO_2 retention on cardiorespiratory and gastric physiology.

(b) (4) [redacted]

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
(b) (4) [redacted]	Laboratory Manager/ Senior Biological Scientist
eRA COMMONS USER NAME N/A	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
(b) (4) [redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]

A. Positions

(b) (4) [redacted]

Honors

(b) (4) [redacted]

B. Publications

(b) (4) [redacted]

(b) (4) [redacted]

VOLUME 2: Cost Proposal

Part 1: Itemized Budget

Next Page

Proposal Budget Worksheet

								NIH Salary Cap	Fringe Options	Fringe Rate	Health Insurance	Fiscal Year 2018	Life Insurance
Principal Investigator:	Jay Dean							(b) (4)	Family	(b) (4) %	(b) (4)	Annual	(b) (4)
Sponsoring Agency:	University of Conneticut							COL Escalation	Spouse	(b) (4) %	$(b) (4)	Annual	Type Appt
Project Title:								0%	Individual	(b) (4) %	(b) (4)	Annual	12
	Electrodermal activity during hyberbaric oxygen exposure rat study							Tuition / Hour	No Health	(b) (4) %	(b) (4)	Annual	9
Start Date:	2/1/19	End Date:	1/31/20					(b) (4)	Temporary	(b) (4) %	(b) (4)	Annual	3
Grant Years:	1							Escalation	Post Doc	(b) (4) %	(b) (4)	Annual	9&3
								(b) %	Student	(b) (4) %	(b) (4)	Annual	6

	ROLE ON PROJECT	TYPE APPT (3,9,12)	EFFORT ON PROJ	PERSON MONTHS Cal	Acad	Smmr	BASE SALARY	TYPE OF FRINGE	Year 1	Year 2	Year 3	Year 4	Year 5	ALL YRS
A. Key Personnel														
Jay Dean	PI	12	2.00%	0.24	0	0	$(b) (4)	Family	(b) (4)	$0	$0	$0	$0	(b) (4)
			0.00%	0	0	0			$0	$0	$0	$0	$0	$0
			0.00%	0	0	0			$0	$0	$0	$0	$0	$0
			0.00%	0	0	0			$0	$0	$0	$0	$0	$0
			0.00%	0	0	0			$0	$0	$0	$0	$0	$0
			0.00%	0	0	0			$0	$0	$0	$0	$0	$0
			0.00%	0	0	0			$0	$0	$0	$0	$0	$0
			0.00%	0	0	0			$0	$0	$0	$0	$0	$0
			0.00%	0	0	0			$0	$0	$0	$0	$0	$0
								Key Personnel Total	$(b) (4)	$0	$0	$0	$0	$(b) (4)
B. Other Personnel														
Carol Landon	Lab Manager	12	9.00%	1.08	0	0	$(b) (4)	Spouse	$(b) (4)	$0	$0	$0	$0	(b) (4)
			0.00%	0	0	0			$0	$0	$0	$0	$0	$0
			0.00%	0	0	0			$0	$0	$0	$0	$0	$0
			0.00%	0	0	0			$0	$0	$0	$0	$0	$0
			0.00%	0	0	0			$0	$0	$0	$0	$0	$0
			0.00%	0	0	0			$0	$0	$0	$0	$0	$0
			0.00%	0	0	0			$0	$0	$0	$0	$0	$0
			0.00%	0	0	0			$0	$0	$0	$0	$0	$0
			0.00%	0	0	0			$0	$0	$0	$0	$0	$0
			0.00%	0	0	0			$0	$0	$0	$0	$0	$0
			0.00%	0	0	0			$0	$0	$0	$0	$0	$0
								Other Personnel Total	(b) (4)	$0	$0	$0	$0	$(b) (4)
Total Personnel									$11,273	$0	$0	$0	$0	$11,273
							# of Students	0						
C. Equipment			(no F&A if Federal)						$0	$0	$0	$0	$0	$0
D. Travel														
	1. Domestic								$0	$0	$0	$0	$0	$0
	2. International								$0	$0	$0	$0	$0	$0
E. Participant/Trainee Costs									$0	$0	$0	$0	$0	$0
F. Other Direct Costs														
	1. Materials and Supplies								$820	$820	$0	$0	$0	$1,640
	2. Publication Costs								$0	$0	$0	$0	$0	$0
	3. Consultant Costs								$0	$0	$0	$0	$0	$0
	4. ADP/Computer Services								$0	$0	$0	$0	$0	$0
	6. Equipment/Facility Rental								$0	$0	$0	$0	$0	$0
	7. Alterations/Renovations								$0	$0	$0	$0	$0	$0
	8. Other		Telemetry (refurbish and batteries)						$2,444	$2,444	$0	$0	$0	$4,888
	9. Other		Animal Costs						$1,000	$1,000	$0	$0	$0	$2,000
	10. Other		GSR Amp for AD Instruments						$1,170	$1,170	$0	$0	$0	$2,340
	5. Subaward/Consortium													
	Subaward 1 <25K						0		$0	$0	$0	$0	$0	$0
	Subaward 1 >25K		(no F&A if Federal)						$0	$0	$0	$0	$0	$0
	Subaward 2 <25K						0		$0	$0	$0	$0	$0	$0
	Subaward 2 >25K		(no F&A if Federal)						$0	$0	$0	$0	$0	$0
	Subaward 2 <25K						0		$0	$0	$0	$0	$0	$0
	Subaward 2 >25K		(no F&A if Federal)						$0	$0	$0	$0	$0	$0
		Subaward Total							$0	$0	$0	$0	$0	$0
Total Other Direct Costs									$5,434	$5,434	$0	$0	$0	$10,868
Total Direct Costs									$16,707	$5,434	$0	$0	$0	$22,141

*No tuition required for MCDM students

*Please note that for federal grants consortium F&A is not included in the direct cost limitations

	Year 1	Year 2	Year 3	Year 4	Year 5	ALL YRS
Total Direct Costs	$16,707	$5,434	$0	$0	$0	$22,141
DIRECT w/o subs F&A	$16,707	$5,434	$0	$0	$0	$22,141
BASE ● MTDC ○ TDC	$16,707	$5,434	$0	$0	$0	$22,141
F&A COSTS	$8,270	$2,690	$0	$0	$0	$10,960
TOTAL COSTS	$24,977	$8,124	$0	$0	$0	$33,101
F&A RATE	(b) (4)					
Target (direct costs)	$25,000	$0	$0	$0	$0	$25,000

Direct Labor:
Budget Justification.

1) Personnel salaries, wages and fringe benefits (% effort).

Please see the attached computer-generated budget pages at the end of this section. A breakdown of the salaries per investigator per year is also provided.

Budget justification:

Key personnel—($11,273/yr 1) Jay B. Dean (PhD; 2% effort) is a Co-I on Dr. Chon's project and the lead investigator of the subcontract at USF Tampa for Aim 1 (animal experiments). Dr. Dean is director of the USF Hyperbaric Biomedical Research Laboratory and an expert in hyperbaric physiology and neurophysiology. Dr. Dean will take responsibility for all the proposed animal experiments in Aim 1, which measures EDA before and during onset of CNS-OT seizures in rats. (b) (4) [redacted] (BS; 9% effort) is the lab manager for the USF Hyperbaric Biomedical Research Laboratory. (b) (4) [redacted] involved in all animal experiments including surgery to implant the radio telemetry module and in assisting the Co-I in conducting and analyzing the experiments.

Materials and supplies—($820/yr 1) Funds are requested to purchase surgical supplies, anesthetics and analgesics, and gases that are used for implanting radio-telemetry modules and EDA leads under sterile conditions. Gases are also used for administering HBO to induce CNS-OT.

Other: telemetry ($2,444/yr 1), animal costs ($1,000/yr 1), and GSR Amp for ADInstruments data acquisition system ($1,170/yr 1)—Funds are requested to have Data Sciences International (DSI) refurbish four F-40 radio-telemetry modules that we currently have in the lab. Funds are also requested for 12 animals, which includes purchase price plus shipping and per diem costs. Finally, a GSR amplifier that interfaces with a preexisting ADInstruments data acquisition system is requested to measure EDA in rats.

5) Indirect Costs

University of South Florida has a modified indirect rate of ((b) (4) [redacted]% × total direct costs). Please see attached budget page.

6) Total cost

$24,977

Chapter One

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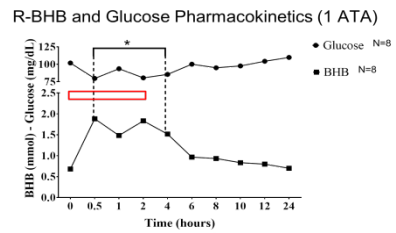
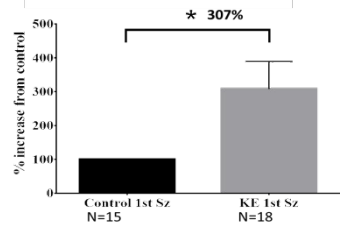
Optimizing Ketone Metabolic Therapy and Identifying Biomarkers for Mitigation and Predicting CNS Oxygen Toxicity: Animal Studies

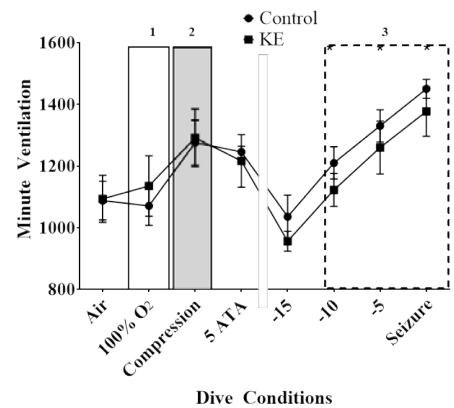
Dominic P. D'Agostino, PhD (PI)
Jay B. Dean (Co-I); Angela M. Poff (Co-I)

Summary: Ketogenic metabolic therapy (KMT) is an effective mitigation strategy to delay Central Nervous System Oxygen Toxicity (CNS-OT) seizures in rats, and has a proven safety in humans. The current study tests the most promising ketogenic agents (KAs) and lower dose on CNS-OT, cognition, behavior and motor performance. The diverse neuropharmacological and signaling mechanisms of KMT remain under investigation, but the current study supports a KMT-induced increase in antioxidant capacity.

Naval Applications: Naval Special Warfare and Special Operations diving and submarine operations are limited by the risk CNS-OT. KMT is a promising mitigation strategy for CNS-OT, enhanced safety and performance resilience.







Aims: Our 1st aim was to understand the dose-dependence of KE adminstration for delaying CNS-OT without impairing cognitive or motor performance. 2nd aim was to determine the effects of HBO ± KE on presumptive physiological markers (e.g. body temperature, heart rate, respiratory rate) that can be monitored in real time to predict an impending Sz. 3rd aim was to study the anti-convulsant mechanisms of therapeutic ketosis by measuring redox potential of blood from animals pre-and post- Sz with and without KE treatment. As designed, the proposed studies answer crucial questions needed for advancing the science and future human application of KMT for CNS-OT and warfighter performance resilience.

Issues: No issues

FY22 Accomplishments, Discoveries, & Inventions

- KMT delays Sz_1 by 307% overall
- Cognition and performance remain unchanged; anxiolytic effect
- Hyperoxic hyperventilation & bradycardia precedes seizures
- Antioxidant capacity is increased with KMT
- 4 Manuscripts and 2 Book Chapters
  - Stavitzski NM, et.al. *AJP Reg.* 2021 PMID: 34132115
  - Brunner et.al (2021). *Nutrients*,13(11),4082. PMID: 34836344
  - Prins PJ.(2021) *Frontiers in Physiology*, 12, 780755(2021).
  - Poff, A.M et.al.(2021) *Frontiers in Nutrition*, 8, 783659.
  - 2 book chapters on KMT in Ketogenic Diet and Metabolic Therapies (2nd Edition) Oxford University Press

FY23 Goals

- Test the safety and efficacy of new Glycerol-AcAc Triester

PI Contact Email: ddagosti@usf.edu

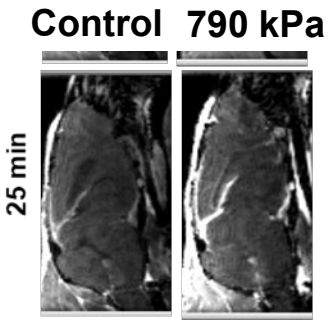
Glymphatic function in extreme environments

UNIVERSITY of MARYLAND SCHOOL OF MEDICINE

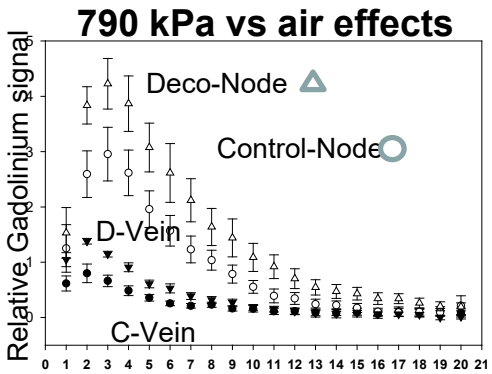
Stephen R. Thom, MD, PhD

Background: The brain glial lymphatic, or 'glymphatic' pathway and meningeal lymphatics are the anatomical routes for cerebrospinal and interstitial brain fluid flow. They play a critical role in CNS health with involvement in sleep/wake cycle, cognition, and memory. We hypothesize that diving-related high pressure/decompression have adverse effects on glymphatic function.

Naval & Scientific Benefits: Glymphatic function has an intimate association with neuroinflammation. Dysfunction may cause diver and submariner impaired sleep/wake pattern, impaired cognition and development of CNS decompression sickness.



Gadolinium MRI of glymphatic flow



Glymphatic flow is increased with pressure/decompression

Objectives: Aim 1: Develop techniques & assess glymphatic function in murine models.

Aim 2: Measure inflammatory microparticle (MPs) in deep cervical nodes that drain brain glymphatics and in blood.

Aim 3: Document neuroinflammatory responses.

Issues: Results document a complex series of events when pressure exposures exceed 'safe' standards including microglial activation and inflammatory MPs formation.

FY22 Accomplishments, Discoveries, & Inventions

- Pressure/decompression activates astrocytes & microglia.
- Significant glymphatic flow enhancement occurs with decompression and is abrogated by eradication of MPs.
- Documented the role of CNS derived inflammatory microparticles with brain ↔blood inflammatory progression.

FY23 Goals

- New start - **Neuroinflammation and glymphatic dysfunction in decompression sickness**

Principal Investigator Contact Email:
(b) (6) [redacted]

GLYMPHATIC FUNCTION IN EXTREME ENVIRONMENTS

Stephen R. Thom

University of Maryland School of Medicine
(b) (6)

Background

The brain glial lymphatic, or 'glymphatic' pathway and meningeal lymphatics are the anatomical routes for cerebrospinal and interstitial brain fluid flow and thus play a critical role in central nervous system (CNS) health. This process is driven by astrocyte aquaporin (AQP)-4 function. It is typically increased with acute neuroinflammation and decreased following chronic inflammatory events. We hypothesize that diving-related stresses perturb glymphatic function.

Objectives

Aim 1: Glymphatic function will be assessed in murine models. Aim 2: Inflammatory MPs changes in deep cervical nodes that drain brain glymphatics and their presence in peripheral blood will be evaluated by flow cytometry. Aim 3: Neuroinflammatory responses will be evaluated using immunohistochemical techniques.

Methods

Glymphatic function was evaluated by contrast MRI in a 7 Tesla instrument and by confocal microscopy following intra-cisternal injections of fluorescence-labeled ovalbumin (MW 45KDa to assess paracellular transport) and cadaverine (MW 0.6KDa – assess astrocyte mediated transcellular transport). MPs in cervical nodes and blood were evaluated by flow cytometry. Neuroinflammation was assessed by Western blots of vasculature-enriched brain fractions following colloidal silica injections and immunohistochemistry.

Results

1. Astrocyte and microglial activation post-decompression. Following 2 hour exposure to 25 psi (273 kPa) or 100 psi (790 kPa), but not after just 1 hour exposure to 25 psi (within US Navy safe decompression limit), mouse brain sections exhibit glial cell activation based on staining for astrocyte-specific glial fibrillary acidic protein (GFAP) co-localization with p65 phosphorylated (activated) nuclear factor (NF)-κB; and increases of microglial-specific ionized calcium-binding adaptor protein-1 (IBA-1) [Figure 1]. Western blots are consistent with this and also show loss of astrocyte aquaporin-4 [Figure 2].

Fig.1. Histochemistry evidence of CNS leukocyte activation with pressure. Upper panels show GFAP (astrocyte, in green) and phosphor-NF-kB (red). Lower panels show IBA-1 (microglial protein) and nuclear staining (DAPI).

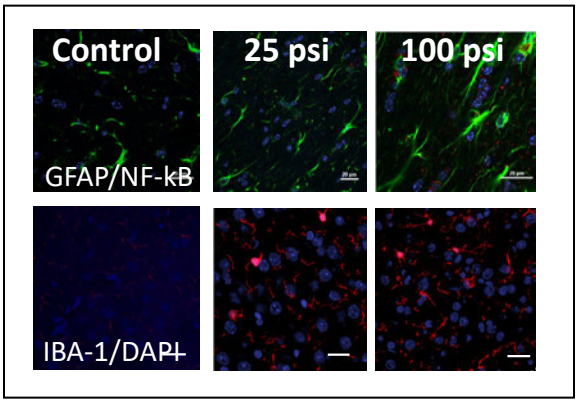
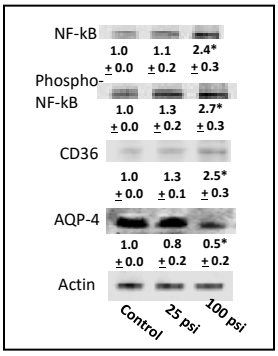


Fig. 2 shows brain protein changes quantified by Western blots. Numbers show band densities vs actin loading relative to control and mea ± SE (n=4-6). Significant elevations of NF-kB, phosphor-NF-kB and CD36, with reduction of AQP-4 occur with 100 psi exposure.



Glymphatic flow is enhanced after provocative decompression-MRI. Glymphatic channels run adjacent to veins and MRI/ IV gadolinium

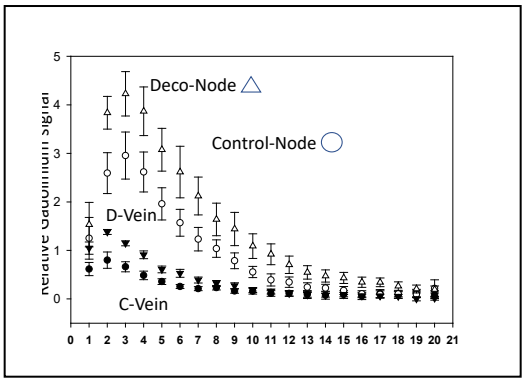
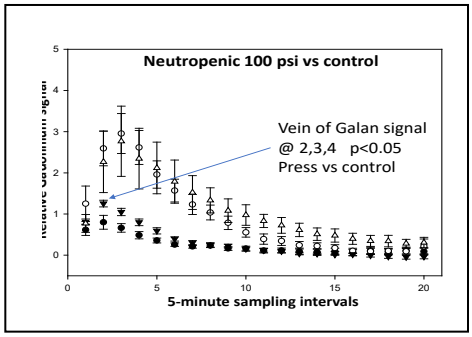


Fig. 3 Glymphatic flow by gadolinium MRI.

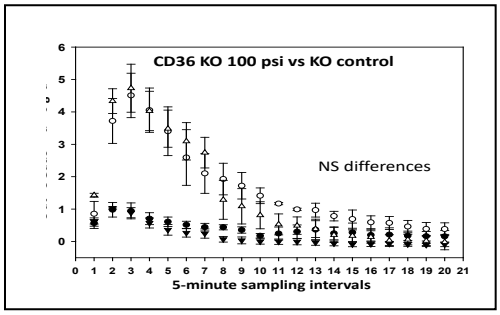
enhancement was pronounced at the Vein of Galan. An assessment of total lymphatic flow was made by also quantifying gadolinium intensity at deep cervical nodes. Figure 3 shows mean ± SE (n=4-6) T1 ratios from control mice and mice after decompression from 100 psi air pressure for 2 hours. Notably, flow changes were virtually the same after 25 psi X 2 hour, but no different from control following 25 psi X 1 hour exposures (data not shown).

Prior studies in this murine model have found that neutropenia abrogates vascular damage and changes in blood-borne inflammatory microparticles. As shown in Figure 4, neutropenic mice still exhibit



elevations of perivenous glymphatic flow after decompression, although total lymphatic flow (node signal) does not differ from control.

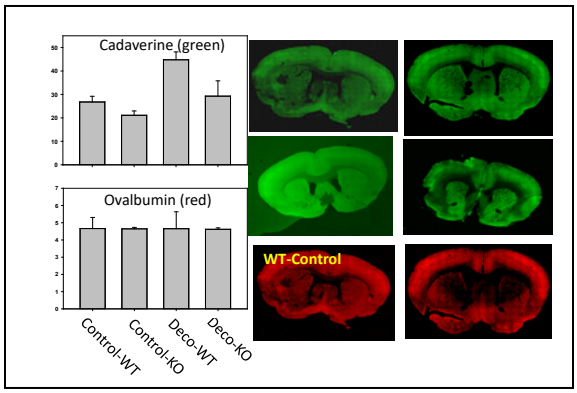
MRI studies were also done with mice lacking CD36, a membrane-bound pattern recognition receptor that has been implicated in neuroinflammatory events. Figure 5 shows that



glymphatic flow in CD36 KO mice does not differ between air-exposed (control) mice and those exposed to 100psi for 2 hours.

3. Glymphatic flow enhancement – Cisterna magna injections: We emphasize studies by MRI as there are fewer possible artifacts, but most glymphatic flow studies have been performed by monitoring contrast injected directly into the CNS. We find that exposure to 100 psi X 2 hours results in enhanced transcellular flow of cadaverine, but not paracellular transport of ovalbumin. Notably, changes are not observed in CD36 KO mice (Figure 6),

4. Microparticle elevations in cervical nodes



and blood parallel glymphatic flow changes. Pressure-exposed wild type (WT) but not CD36 KO mice exhibited elevations of MPs. Cell sources of MPs were identified by cell-specific protein expression. Table 1 shows results after 2 hour exposures to pressure for those expressing GFAP (astrocytes) + thrombospondin (TSP), TMEM119 (microglia) NPR (neurons) and MBP (oligodendroglia).

Exposure	MPs/ul	%GFAP	%TMEM	%NPR	%MBP
Cervical node MPs (n=4-14) +TSP					
Control	1526±97	4.1±1.0	14.8±2.4	14.0±1.6	25.0±2.1
273 kPa	1533±113	4.0±0.4	19.0±1.8	16.0±2.2	26.0±4.6
446 kPa	2362±201*	6.1±1.5*	21.0±3.6*	31.1±6.3	38.9±3.6
790 kPa	4084±260*	14.5±2.0*	25.1±6.6*	26.9±1.8*	39.3±3.1*
Blood MPs (n=4-14)					
Control	567±21	4.9±1.0	15.4±1.9	22.0±1.7	25.8±2.3
273 kPa	1522±181*	5.4±1.6	15.4±1.2	16.5±2.2	25.5±6.3
446 kPa	1965±137*	9.1±3.3*	18.1±3.6	33.0±3.2	34.3±4.2
790 kPa	2594±82*	20.4±4.5*	24.3±3.2*	30.9±2.6*	37.0±2.7*

Conclusions: Exposures to high pressure trigger neuroinflammation manifested by MPs elevations and enhanced glymphatic flow.

Notes: