Final Report: UNC Pembroke Laser Scanning Confocal Microscopy Facility

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision, unless so designated by other documentation.

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15. SUBJECT TERMS
confocal microscopy, blast-induced TBI, brain imaging

16. SECURITY CLASSIFICATION OF:
a. REPORT UU
b. ABSTRACT UU
c. THIS PAGE UU

17. LIMITATION OF ABSTRACT UU

18. NUMBER OF PAGES

19a. NAME OF RESPONSIBLE PERSON
Ben Bahr
19b. TELEPHONE NUMBER
910-775-4383
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Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

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<th>Paper</th>
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TOTAL: 2

Number of Papers published in peer-reviewed journals:

(b) Papers published in non-peer-reviewed journals (N/A for none)

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TOTAL:
Number of Papers published in non peer-reviewed journals:

(c) Presentations

Number of Presentations: 0.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received  Paper

TOTAL:

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received  Paper

TOTAL:

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

(d) Manuscripts

Received  Paper

TOTAL:
Books

Received

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TOTAL:

| Received | Book Chapter |

TOTAL:

Patents Submitted

Bahr BA. Materials and compound combinations for cathepsin B enhancement and methods of use for treating Alzheimer’s disease, mild cognitive impairment (MCI), dementia, forms of ?-synucleinopathy, traumatic brain injury, cardiomyopathy, eye disease, and skin damage (U.S. Provisional Patent Application No. 62/262,848- filed 12/3/15; 204 claims); assigned to UNC–Pembroke

Patents Awarded

Awards

Graduate Students

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<td>Karen Farizatto</td>
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### Names of Faculty Supported

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### Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

- The number of undergraduates funded by this agreement who graduated during this period: **0.00**
- The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields: **0.00**
- The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields: **0.00**
- Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale): **0.00**
- Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering: **0.00**
- The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense: **0.00**
- The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields: **0.00**

### Names of Personnel receiving masters degrees

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### Names of personnel receiving PHDs

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**Names of other research staff**

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<td>Heather Romine</td>
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**FTE Equivalent:** 0.00

**Total Number:** 1

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**Sub Contractors (DD882)**

**Inventions (DD882)**

Materials and compound combinations for cathepsin B enhancement and methods of use for treating Alzheimer’s disease,

Patent Filed in US? (5d-1) **Y**
Patent Filed in Foreign Countries? (5d-2) **N**
Was the assignment forwarded to the contracting officer? (5e) **N**

Foreign Countries of application (5g-2):

5a: Ben A. Bahr

5f-1a: UNC Pembroke

5f-c: 1 University Dr.

Pembroke NC 28372
Scientific progress and accomplishments

1. Foreword

UNC-Pembroke received funds from the Department of Defense Research and Education Program for Historically Black Colleges and Universities and Minority-Serving Institutions (Equipment/Instrumentation) in order to acquire a Confocal Microscopy System. The system was also upgraded with Nikon’s Perfect Focus option with motorized diascopic detector, resulting in enhanced capabilities with long-term high-power resolution necessary for brain samples and labeling probes. The facility in place today now has the imaging and resolution capabilities needed to effectively analyze early signs of cellular and cytoskeletal damage, as well as associated synaptic deterioration and astrogial activation events.

2. Statement of the Problem

State-of-the-art confocal microscopy was needed to strengthen the biomedical research at UNC-Pembroke, by enhancing the University’s existing Sample Preparation and Microscopy Facility with the capability of laser scanning confocal microscopy and image capture. The previously existing microscopy facility did not have the imaging and resolution capabilities needed to effectively analyze distinct cellular and synaptic damage events during different disease states. The requested equipment included the Nikon C2+ Confocal Microscopy System. The system was needed to support the biomedical, Army-relevant neuroscience-focused research that is actively being conducted at UNC-Pembroke’s Biotechnology Research and Training Center (BRTC). The Nikon system was needed in order to provide the significantly greater image resolution and magnification needed to support Dr. Ben Bahr’s neuroscience research in the areas of traumatic brain damage, excitotoxic vulnerability, and Alzheimer’s disease. The system will provide significantly improved resolution of data images and biological samples, which is needed to understand cellular and synaptic changes associated with blast-induced neurodegeneration and in different disease states.

This Nikon system was requested since Nikon has the reputation as a world leader in advanced biological imaging, and their confocal system can work in tandem with the existing Nikon AZ100 microscope for macro images of large brain regions. We requested the C2 model since it provides both advanced imaging quality, functionality, flexibility for growth, and a reasonable cost. We also requested the Nikon product since a local sales and support team is available in North Carolina (RTP) which will provide sales, service, training, and support in a timely fashion as they did for the AZ100 microscope and software.

3. Summary of the most important results

The confocal microscopy equipment was installed by experts from Nikon, and the required training for optimal use of the system was provided to several members of the Bahr Lab as well as a few other professors from other disciplines. The confocal system and facility has supported Army-relevant neuroscience research in the Bahr Lab. Dr. Bahr’s research aims to understand the pathogenic cascade of events that initiates from excitotoxic insults (e.g. trauma, stroke, seizures) and leads to the deterioration of brain structures and functions.

BLAST-INDUCED NEURODEGENERATION

Particularly important to the Army, the growing number of traumatic brain injury (TBI) cases associated with military service warrants Dr. Bahr’s research on blast-induced neurodegeneration. Explosives create shockwaves that cause blast-induced neurotrauma, one of the most common types of TBI linked to military service. Survivable blast-induced TBIs are often associated with reduced cognitive and behavioral functions due to a variety of factors. The confocal system has been assisting a study of the direct effects of explosive blasts on brain tissue. We removed systemic factors by utilizing rat hippocampal slices maintained in culture. The long-term slice cultures were briefly sealed air-tight in serum-free medium (SFM), lowered into a 37°C water-filled blast chamber, and 1.7-gram assemblies of cyclotrimethylene trinitramine (RDX) were detonated 15 cm outside the chamber, creating a distinct shockwave recorded at the culture plate position. Compared to control mock treatment groups of slices that received equal SFM submerge time, the blast impacts caused a dose-dependent reduction in the AMPA receptor subunit GluR1. Only a small reduction was found in slices exposed to a single RDX blast, harvested 1-2 days post-blast. However, hippocampal slices that received two consecutive RDX blasts 4 min apart exhibited a 26-50% reduction in GluR1, and it was further reduced by 60-67% after three consecutive blasts. The presynaptic markers synaptophysin and synaptotagmin were found to have similar susceptibility to multiple blast exposures as the postsynaptic protein. With confocal microscopy, the synaptic marker staining in distinct patterns around pyramidal neurons was greatly disrupted by the blast trauma. In the slice samples with clear indications of blast-induced synaptic compromise, the level of blasts used did not produce evidence of astrogial activation as measures of GFAP did not increase (a small decrease was in fact found). Actin levels were unchanged and Fluoro-Jade staining found no indication of degenerating neurons in slice cultures exposed to three RDX blasts, suggesting that synaptic alterations can occur in the absence of cellular degeneration and gliosis. Together, these results indicate that detonated RDX explosives cause distinct losses of synaptic proteins before cell death, perhaps explaining the cognitive deficits in those blast-induced TBIs with no detectable neuropathology.

PROTEIN ACCUMULATION DISEASES

Dr. Bahr’s research also aims to understand how age-related protein accumulation stress can be reduced for therapeutic purposes. Distinct protein accumulation events are suspected to lead to Alzheimer’s, Parkinson’s, Huntington’s, ALS, and other diseases. The new confocal microscopy system assisted the following study being prepared for publication:
Accumulating protein species can lead to the activation of proteasomal and lysosomal pathways. However, many studies have indicated that the two pathways exhibit stress during Alzheimer-type protein accumulation events. In particular, the Aβ42 peptide has been shown to influence proteasomes and overall proteostasis. Here, low concentration Aβ42 applied to rat hippocampal slice cultures was found to reduce proteasome activity in correspondence with increased tau phosphorylation, as well as cause a significant loss of synaptophysin, a sensitive marker of synaptic integrity. When the slice cultures were treated with the proteasome inhibitor lactacystin, the nearly complete and rapid reduction in proteasome activity was not associated with lysosomal compromise, but rather with a >50% increase in activity of the lysosomal enzyme cathepsin B (CatB).

Interestingly, the potential compensatory CatB response increased further over additional days of lactacystin treatment. To further assess this apparent inverse relationship between the proteasomal and lysosomal pathways, we tested whether enhancing the active form of CatB leads to proteasomal attenuation. Using the CatB-enhancing agent Z-Phe-Ala-diazomethylketone (PADK), an inverse effect was not found, but rather what appears to be an opposite effect, i.e. a tendency to increase proteasome activity. Surprisingly, in hippocampal slices with Aβ42-mediated proteasomal compromise, PADK indeed increased the proteasome activity to levels comparable to those found in control slices. Furthermore, PADK also reduced Aβ42-mediated tau phosphorylation, an event recently implicated as a consequence of changes in protein clearance efficiency. Such efficiency may involve cross-talk between proteasomes and lysosomes. These results suggest a distinct interaction between proteasomal and lysosomal systems, and they point to potential dual modulation against protein accumulation pathology linked to Alzheimer’s disease and other dementias. New activity probes are currently being tested to assess modulation of protein clearance pathways in living brain tissue cultures.

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The new equipment has strengthened the research training capacity of UNCP. UNCP is a minority serving institution with 16% of the students being Native American, 32% are African American, and 4% are Hispanic/Latino. Faculty are dedicated to meeting the academic needs of minority students including mentoring undergraduate students in research and advanced technologies like confocal microscopy. Dr. Bahr’s group has exposed many students to confocal techniques performed with the confocal imaging system, including undergraduates, a few Master students, as well as visiting and shadowing high school students. With the new system, UNCP students are engage in cutting edge research and are able acquire the skills and experience to prepare them for graduate programs and research careers in DOD-relevant areas. In addition, the STEM faculty of UNCP are committed to working with local K-12 students and teachers and local community college students. These students are regularly invited to tour and work in the Bahr Lab and other STEM labs on campus.


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Technology Transfer
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4. Bibliography (including previous studies that used confocal imaging)


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