2026 Summer Research Fellowship

LABORATORY SCIENCE PROJECT DESCRIPTION

1. Project Title: ***Early neurotransmitter deficits are associated with non-cognitive risk factors in Alzheimer's model mice***
PI: ***Christine M. Crish, Associate Professor***
Location: ***Dept. of Pharmaceutical Sciences, NEOMED***

2. Abstract: A major goal of our laboratory is to understand some of the earliest pathological mechanisms that contribute to Alzheimer's brain pathology. Decades prior to the first signs of cognitive deficits, changes begin to occur in the brain that may be associated with deficits in sensory function, appetite, continence, and other fundamental processes known to predict increased risk for dementia. There is a critical need to identify and understand these early factors in order to develop early methods for detecting disease and potentially intervening.
Our lab has preliminary data showing alterations in populations of GABA and acetylcholine producing neurons in the brains of Alzheimer's (AD) model mice that precede onset of disease. Our goal is to determine which specific subpopulations of these neurons are affected in specific regions of the hippocampus and cortex at early time points in order to identify brain circuits at greatest risk for damage. These brain circuits may involve visual system dysfunction, incontinence, or other metabolic disruptions which would enable us to establish mechanistic basis for some of these early risk indicators for dementia.

3. Significance:
Dementia is diagnosed by impairment in cognitive ability but unfortunately by the time such symptoms are manifested, irreversible loss to a substantial amount of brain cells has already occurred. Thus, our research proposes to grow the overall understanding of how early changes in neural circuits that maintain sensory or homeostatic functions could signal the onset of the dementia disease process long before cognitive deficits are manifested and the brain is irreparably damaged.

4. Goals & objectives:

Goal A. Prepare tissue for immunofluorescent assays and conduct histological immunofluorescent labeling assays on brain tissue for specific neurotransmitter and AD pathology targets. Students will learn how to section fixed mouse brain sections on a microtome, store sections properly, and select region-specific sections that will be assayed. Students will learn the necessary steps on how to conduct antibody-based immunofluorescent assays including making stock laboratory solutions, calculating assay solution needs, and preparing assay incubation solutions. Students will be required to follow all assay steps to complete assays from start to finish (often a two-day process). Students will then prepare labeled tissue on slides for microscopy analysis.

Goal B. **Perform microscopy and analyze images to quantify changes in distribution of neurotransmitter-specific containing neuronal populations.**
Students will use microscopy to image immunofluorescent label of different cell types and provide quantitative analysis of expression patterns in hippocampus and/or cortex.

Goal C. Dietary manipulation of choline to improve brain neurotransmitter function
Students will help manage an ongoing study where we feed a choline-rich diet to AD mice to determine the impact of this in preventing/slowing disease-related changes to brain and early symptoms. Students will be involved in weighing and monitoring mice.

5. Research methods

Immunofluorescence and Microscopy

The student will be trained to section fixed brain tissue coronally on a freezing sliding microtome. The student will then be trained to use multicolor immunofluorescence assays to visualize expression of antibody-based labels for specific neuronal types in hippocampus and brainstem regions of mice. We will compare cell type distribution between groups of age- and sex-matched Alzheimer's model mice and healthy control mice. The student will be trained to photograph brain sections using a Zeiss AxioZoom V16 epifluorescent macroscope equipped with a digital high-resolution camera and a computer guided motorized stage and Z-axis and an Axio Imager M2 epifluorescent microscope with a digital high resolution camera and Apotome structured illumination module for tissue requiring higher magnification. Each structure of interest will be imaged at under multiple channels to capture different labels from antibody staining. Images will be z-stacked, flattened with the extended depth of focus module of the Zen microscope software and converted to tiffs or jpegs for analysis. Students will then be trained how to identify brain regions and quantify integrin label using Image Pro software and prepare publication-quality micrograph images for presentation.

6. Proposed method of data analysis

We will use SPSS for IBM Statistical Software to analyze all data. The PI will directly guide the student fellow in the use of this program in order to calculate the applicable analyses if the student has no prior experience in statistical analysis. The student will also be required to generate figures and illustrations depicting important findings using Prism and Adobe Illustrator.

7. Outcomes of research findings

This project will generate fundamental data on GABAergic and acetylcholinergic neuron expression patterns in the brain, which has not been previously investigated as an early, pre-cognitive decline biomarker. Knowledge in this area is critical because it will support future research that seeks to test novel pharmacological strategies to prevent or slow progression of dementia.

2026 SUMMER RESEARCH FELLOW MENTORSHIP/TRAINING PLAN

Training and site where research will be conducted

The student will perform the research at NEOMED in the C. Crish research lab and ancillary shared lab rooms on the fourth floor of RGE. The student accepted for this project will have an initial training phase that involves both web-based lab safety (EOHS online program). Students will have the opportunity to work directly with animals, therefore they will be required to complete relevant CITI-training modules for working with live mice. Students will receive one-on-one skills-based training with lab personnel. After these requirements are met, he or she will be directly trained by the PI (C. Crish) or senior lab staff on tissue preparation, assay conducting, microscopy, and analysis.

Resources available

The C.Crish Lab has access to all the resources necessary to train the summer fellow and enable them to carry out this work plan. The PI has active breeding colonies of AD model mice and a repository of brains collected from Alzheimer's and control mice across different disease stages/ages. The C. Crish lab owns a library of antibodies relevant to the proposed work, auxillary chemicals, laboratory supplies, and basic laboratory equipment (shakers, pipetters, incubators, etc) to conduct assays. The PI has access to all the required equipment, which is either part-owned by the PI, other colleagues in Pharmaceutical Sciences, or is core equipment of the Neurodegenerative Disease and Aging (NDA) research focus area which grants the PI free and unlimited use. The PI also owns statistical analysis software (SPSS) and image processing software (Adobe Creative Suite; Prism; Image Pro). C. Crish lab has dedicated lab bench space to accommodate lab staff and a dedicated desk/computer adjacent to the lab for use by research assistants.

Mentorship plan

The PI and student will have weekly one-on-one meetings to discuss the plan for data collection and analysis as well as to ensure that the project is moving forward at the correct pace. The PI has developed a workflow for all new lab assistants that details and tracks skills learned and their proficiency level, and this workflow will be employed for the student fellow as well.

The student will also attend the weekly C.Crish Lab research meetings to present and discuss their progress. The student fellow will work with the PI to assemble a research poster to present their data at the NEOMED OPRS summer fellowship presentation day.