NEOMED

Office of Research and Sponsored Programs' Student Research Fellowship Program

2024 Project Catalog

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PROGRAM GOALS, PARTICIPATION POLICY AND PROGRAM REQUIREMENTS

GOALS

The fellowship projects provide summer experiences for NEOMED's medical and pharmacy students, in a variety of disciplines. The Summer Research Fellowship Program is a mentored research program, designed to provide intensive training in research procedures and principles on projects in basic and clinical disciplines; to enhance students' research horizons; and develop scientific presentation and writing skills. These projects are funded by the Office of Research and Sponsored Programs (ORSP).

PARTICIPATION

Phase 1, M1, M2, P1, P2, and graduate students *in good standing* may participate in the ORSP's Summer Research Fellowship Program.

M3, M4, P3 and P4 students who have completed their clerkships and have no conflict with their electives, may participate in the ORSP's Summer Research Fellowship Program.

A M4 and P4 student must have written documentation of the time permitted to complete the summer project.

If the project is to cross-over into any elective time, the student must obtain written approval of the elective director indicating the time frame that will be allotted to the fellowship project.

The project investigator will have to approve the plan.

Special requests will be considered if it is arranged and approved in advance.

PROGRAM REQUIREMENTS

Prior research experience is not required for research projects. However, research experience may be a factor for selection for a specific project and will be up to the discretion of the individual project investigator.

Students are required to complete all applicable training prior to beginning their research projects. Required training will be determined by the project investigator.

All students are required to complete the online Collaborative Institute Training Initiative (CITI) Human Subjects Research – Social-Behavioral-Educational Module Certificate. (See page 3 for details.) If you have completed this training during the past three years you do not have to repeat it. You can provide a certificate of completion if you are selected for a fellowship.

Fellowship Stipends and Commitment
Through the Office of Research and Sponsored Programs

1. All students agree to fulfill a commitment with the project investigator for completion of a research fellowship. Each project investigator is volunteering their time and expertise to train the fellow. It is the student's responsibility to be prompt, available for the project for the contracted time and attend to all requirements of the fellowship.

2. The total stipend for the research fellowship will be $3,000. Student research fellows are contracted employees and will be paid in two equal installments: the first payment will be issued at the midpoint of the fellowship and the second payment will be issued upon successful completion of the fellowship.

NOTE: When a student and the principal investigator of a specific student research fellowship opportunity have - through mutual discussion - determined a match exists and the student "commits" to work on that specific project with the principal investigator, that "commitment" is firm. The decision by a student to revoke their prior commitment in order to accept another fellowship opportunity is considered a professionalism issue and may result in a professionalism concern note (PCN) being filed in the student record.

Collaborative Institute Training Initiative (CITI) at the University of Miami
Human Subjects Research – Social-Behavioral-Educational Module Certificate

1. All students who are selected for a summer research fellowship will be required to take the computerized on-line researcher course at:

   https://www.citiprogram.org

   If you have taken this training within the past year you do not need to repeat it. Please provide a copy of the completion certificate to Nona Hose in the Office of Research and Sponsored Programs, Office G-235 if you are selected for a fellowship.

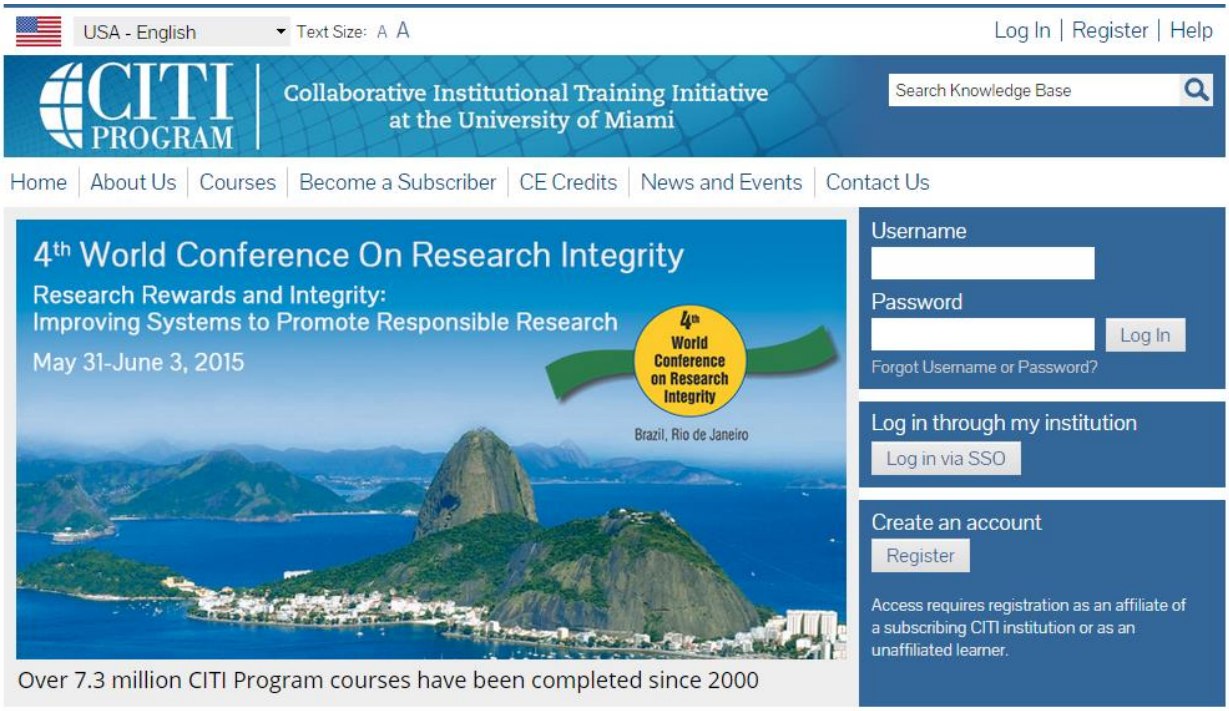
2. Description of course from the CITI Program:

   "Basic HSR modules are suitable for all persons involved in research studies involving human subjects, or who have responsibilities for setting policies and procedures with respect to such research, including IRBs. These modules are typically assembled into a basic course, which is the learner's first exposure to the content. Refresher modules, which can be assembled into refresher courses presented to learners at intervals defined by the institution, are designed to provide continuing education in human subject research issues. The standalone courses are intended for institutional/signatory officials, IRB administration (administrators, directors, coordinators, and other support staff), and IRB chairs.

   HSR module topics include: basics of IRB regulations and the review process, assessing risk to subjects, avoiding group harms, conflicts of interest, cultural competence, FDA-regulated research, genetic research, HIPAA-regulated research, informed consent, international research, Internet research, IRB member responsibilities, IRB chair responsibilities, records-based research, research in schools, research with protected populations, research with vulnerable subjects, the role of the community member, unanticipated problems and reporting, and students in research."

3. A certificate of completion will be awarded. Send this certificate to Nona Hose, Executive Administrative Assistant, Office of Research and Sponsored Programs, NEOMED

4. You will not be permitted to participate in any research without this certification.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CITI PROGRAM
Collaborative Institutional Training Initiative at the University of Miami
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4th World Conference On Research Integrity
Research Rewards and Integrity:
Improving Systems to Promote Responsible Research
May 31-June 3, 2015
4th World Conference on Research Integrity
Brazil, Rio de Janeiro
Over 7.3 million CITI Program courses have been completed since 2000
Username
Password
Log In
Forgot Username or Password?
Log in through my institution
Log in via SSO
Create an account
Register
Access requires registration as an affiliate of a subscribing CITI institution or as an unaffiliated learner.

Student Research Symposium

Friday, November 22, 2024

All ORSP sponsored fellows are **required** to participate in the Student Research Symposium.

Details as to preparation, deadline, etc., for poster presentations will be provided at a later date.

NEOMED
Office of Research & Sponsored Programs

Student Research Fellowship Program

APPLICATION MATERIALS

APPLICATION AND HIRING PROCEDURES

PLEASE NOTE: The application and interview process begins as soon as the project catalog is distributed. Please submit your applications as soon as possible.
The deadline to apply is Friday, March 1, 2024.

1. Students who are required to complete a summer course remediation are strongly discouraged from participating in any student research fellowship program that overlaps with the remediation exam study period. Please contact Craig Theissen, Director of Academic Support at **ctheissen@neomed.edu** or (330) 325-6758 for additional information.

2. Application/Interview process:
   a. Complete the application form online.
   b. Submit a *curriculum vitae* along with your application online.

3. Hiring Process:

   Students and project investigators should approach the fellowships as job opportunities. Students are asked to submit an application and curriculum vitae to the project investigator(s) of their choice. The project investigators will then contact the student(s) in which they are interested and set up an interview. After interviews are conducted, the project investigator will make his/her selection and offer the position to the student of his/her choice. Once a student has accepted the offer of a fellowship, the project investigator will notify Nona Hose in the Office of Research and Sponsored Programs.

   The project investigators will be asked to fill out a NEOMED Training Checklist form indicating any safety training that will be required (lab safety, animal care and use, etc.). This checklist will be provided to the student and to NEOMED's Safety Office.

   All onboarding paperwork and applicable safety training must be done before the student can begin working on a project.

   You may contact Nona if you have any questions or need additional information.
   Nona Hose, Executive Administrative Assistant
   Office of Research & Sponsored Programs, Room G-235
   Phone: 330-325-6499
   E-Mail: nhose@neomed.edu

CONTACT LIST FOR PROJECT APPLICATIONS

Applications may be submitted electronically at the link below:

Project Investigator contact information:

Anatomy and Neurobiology department's main office is located in E-116

Lisa Cooper, Ph.D.
Email: lcooper@neomed.edu

Alexander Galazyuk, Ph.D.
Email: agalaz@neomed.edu

Tobin Hieronymus, Ph.D.
Email: thieronymus@neomed.edu

Julia Huyck, Ph.D.
Email: jhuyck@kent.edu

Bradley Winters, Ph.D.
Email: bwinters@neomed.edu

Jesse Young, Ph.D.
Email: jwyoung@neomed.du

Family & Community Medicine department's main office is located in G-115

Michael Appleman, M.A.Ed.
Email: mappleman@neomed.edu

Kristin Baughman, Ph.D.
Email: kbaughma@neomed.edu

Stacey Gardner-Buckshaw, Ph.D., M.P.A.
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Hannah Haynie, M.S.
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Amy Lee, M.D., M.P.H., M.B.A.
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Feng Dong, Ph.D.
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Yanqiao Zhang, Ph.D.
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Pharmaceutical Sciences department's main office is located in RGE-400

Christine Dengler-Crish, Ph.D.
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Sheila Fleming, Ph.D.
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Takhar Kasumov, Ph.D.
Email: tkasumov@neomed.edu

Xinwen Wang, Ph.D.
Email: xwang2@neomed.edu

Psychiatry department's main office is located in B-226

Natalie Bonfine, PhD and Stacey Barrenger, Ph.D.
Email: nbonfine@neomed.edu
sbarrenger@neomed.edu

NEOMED

Office of Research & Sponsored Program's Student Research Fellowship Program

Project Descriptions

Submit your application to Dr. Lisa Cooper

Title: Mechanosensitivity in the bone and cartilage cells of mammals
PI: Lisa Cooper, Department of Anatomy and Neurobiology
LOCATION: NEOMED, C-156

1) **Abstract**: Within mammals, cells of cartilage and bone are mechanosensitive and respond to loads both within their living matrix as well as when loaded in a petri dish (in vitro). However, little is known of differences in the responsiveness of these cells in animals that live in extreme habitats. To increase our understanding of the variation in bone and cartilage cell sensitivity to loads across mammals, this study aims to establish a fundamental understanding of cell mechanosensitivity in terrestrial (mouse), aerial (bat) and marine (whale) mammals. This study uses species specific-primers and standard PCR techniques to compare dynamics of gene expression in cells harvested from bone and loaded in vitro. We hypothesize that the cells of bats will be either just as mechanosensitive or more mechanosensitive compared to the mouse, but those of the whale will be much less responsive to loads compared to mice and bats. Results will establish a new, foundational dataset. We therefore expect our results will add a critical understanding to the physiology of connective tissues across a taxonomically broad survey of mammals. Students interested in participating in this research should demonstrate fluency in PCR and cell culture.

2) **Significance of the Research:** The mammalian skeleton dynamically responds to loads but little is understood of how mechanosensitivity to loads differs between mammals that occupy different habitats. We expect that life in an aerial habitat has altered skeletal function, and indeed the wing bones of bats flex and bend with wing beats. Deformation in their skeleton is elastic, and such changes in shape would easily break the bones of most mammals. In comparison, the flipper bones of whales are stiff like terrestrial mammals, but only experience compressive loads and are therefore free from stresses associated with their exceptional mass or shear loading. Although the field of skeletal biology has an understanding of the mechanosensitivity of cells living in the skeleton of mice, we have yet to develop a critical understanding of the cellular physiology and function in mammals that occupy aerial and aquatic habitats. This study will increase our understanding of the diversity of mammalian bone and cartilage cell mechanosensation and allow for unique insights into the cellular consequences of life in an extreme habitat. By comparing gene expression profiles, our data will elucidate the loads at which bone cells begin to respond to their loading environment. Fresh tissues of bats and whales are exceptionally rare. The PI has a colony of bats and conducts field work in the Arctic and has collected cells of whales that are currently ready for analysis. Our contribution will be significant because the data will assist in developing a nuanced understanding of the cellular evolution underlying mammalian radiations into fluid habitats. It is also likely that our results will vertically advance our understanding of cartilage and bone cell plasticity within the mammalian skeleton associated with locomotion in novel habitats.

4) **Goals and Objectives of the Research:** By comparing gene expression levels, our data will elucidate whether the bone and cartilage cells of bats and whales are uniquely mechanosensitive compared to mice.

5) **Research Methods Learned by the Summer Fellow:** Applicants should have experience and fluency in PCR and cell culture. First, the researcher will be trained to participate in every phase of project research, including specimen preparation and analyses. Opportunities for students to gain experience with unusual model organisms are rare, and the skills gained through involvement with this project should substantially broaden the researcher's skill sets. The

student will learn to conduct PCR experience and assist in cell culture experiments, harvest tissues, and analyze data.

6) **Research Methods and Data Analysis** – The cells of whale, mouse and bat cartilage and bone are available for gene expression assays using standard relative PCR and species-specific primers. For cell culture, fresh and frozen cells that are terminally differentiated will be plated onto collagen coated dishes and incubated at physiological temperatures. Cells will be transferred to chamber slides and regular glass slides, and after an acclimatization period, will be loaded within an incubator for short cycles daily, over a period of days and then used for morphological comparisons as well as for gene expression assays. This protocol is already established in the PIs lab.

7) **Expected Outcomes** – Three possible outcomes are anticipated for this study. Our null hypothesis is that compared to mice, gene expression profiles will be similar between bats, whales and mice. If the bat cells display differences in gene expression, this is likely associated with the exceptionally flexible bone matrix and greater loads in the cartilage associated with flight performance. If the whale cells display differences in gene expression, this is probably associated with life in a compressive loading environment. Our findings, regardless of outcome, will lay the foundation for future work quantifying mechanosensitivity across the skeleton between these taxa. We expect this work to be the first of several publications.

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

Research will be conducted in C-156 using cells harvested from Cooper's colony of bats and samples collected from the field. Protocols are already established, and all necessary laboratory equipment and disposables are already in use as this is an ongoing project.

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

Submit your application to Dr. Alexander Galazyuk

Submit your application to Dr. Alex Galazyuk
Project Title: Neural mechanisms underlying age-related hearing loss
PI Name: Alex Galazyuk
Location: NEOMED

Abstract: Age-related hearing loss remains one of the most common chronic conditions of aging. It begins from the gradual loss or impairment of the inner and outer hair cells in the cochlea. This loss leads to the development of deficits in the central auditory system which eventually cause difficulties in processing temporally complex sounds such as speech, especially in noisy environments. Typically, individuals experience a notable decline in their hearing abilities after the age of 65, whereas cochlea degradation begins much earlier in life. Within the field, there exists a consensus that central plasticity, often referred to as central gain enhancement, serves as a compensatory mechanism to counterbalance the loss of input from the cochlea to the central auditory system due to aging. The postsynaptic mechanism underlying this compensation is largely unknown. It has been hypothesized that alterations in the balance between excitation and inhibition may play the key role. The goal of this project is to elucidate the postsynaptic mechanisms that contribute to the central gain and to identify pharmacological therapy to improve hearing performance in aged individuals.

Significance: Age-related hearing loss remains one of the most common chronic conditions of aging. Older listeners experience difficulties understanding speech, particularly in noisy environments. While audibility partially accounts for these functional deficits, elderly listeners with normal hearing and intact cognitive function still have poorer speech recognition ability in noise compared to young listeners. Central auditory processing has been shown to play a key role in compensation for the loss of cochlea function with age. This compensation has been hypothesized to rely on altered balance between excitation and inhibition and often referred as central gain enhancement. At present, little is known about postsynaptic mechanism(s) underlying central gain alteration. Deep knowledge about changes in both the excitatory and inhibitory components of this mechanism can help us to better understand the cellular basis for aging. The proposed study will improve our knowledge of the central mechanisms responsible for auditory aging and provide a foundation for the development of treatment strategies.

Goals and Objectives: The student will identify changes in sound-evoked responses of inferior colliculus neurons in different age groups of unanesthetized mice.

Methods: The student will learn how to record single neuron electrical activity of neurons in the inferior colliculus in response to different sound frequency and intensity. The student will learn to use electrophysiological setup to stimulate mice with sounds and record action potentials of auditory neurons in response to these stimuli. The student will also have an opportunity to observe other techniques in the lab, including fabrication of glass recording microelectrodes and generation of automated sound stimulation protocol.

Data Analysis: Data collected by the student will be analyzed using custom-made software. For each recorded neuron its so-called frequency response area based on more than 3,000 sounds with different combinations of sound frequency and intensity will be constructed and analyzed.

Anticipated Findings: The anticipated findings from this project will identify the differences in sound processing with age.

Student Fellow Training/Mentoring Plan

The student will have the opportunity to meet individually with the PI regularly. In addition to working directly with the PI, the student's training will be continuously monitored by a postdoctoral fellow. Research will be conducted at NEOMED. All resources necessary for the described experiments are available. We work as part of the NEOMED Hearing Research Group, and students in the lab will have the opportunity to interact with the other group members.

Submit your application to Dr. Tobin Hieronymus

Title: **Digital Muscular and Fascial Anatomy Using Contrast-Enhanced MicroCT**
PI: Tobin Hieronymus, Associate Professor of Anatomy & Neurobiology
Research Location: NEOMED

Abstract: Smooth muscle plays a critical role in the function of many organ systems, but our ability to understand the effects of smooth muscle contraction are limited by our current inability to directly record smooth muscle activity *in-vivo*. Unlike skeletal muscle, smooth muscle does not depend on cell-membrane depolarization to coordinate contraction, so standard techniques such as electromyography (EMG) will not work. Current research in Hieronymus lab is focused on two major aims: (1) applying established methods of measuring brain activity (calcium indicator fluorometry) to the novel setting of peripheral smooth muscle tissues to directly record activity, and (2) developing selective, localized, and reversible interventions to manipulate smooth muscle contraction for functional studies. This summer research project will make use of recent advances in microCT imaging to characterize the architecture of smooth muscle tissue in bird skin (our lab's experimental model system for smooth muscle fluorometry and manipulation), with particular attention to its relationship to the superficial and deep fascia of the human-comparable musculoskeletal elements of the forelimb.

Significance: In addition to the smooth muscle lining the GI tract, airways, and vasculature, avian skin contains bundles of smooth muscle responsible for moving the feathers, similar to the arrector pili muscles in human skin. Because they are abundant and comparatively large, these muscles form a readily accessible experimental model for studies of smooth muscle physiology. My lab is developing a contact fluorometry instrument to record smooth muscle activity *in vivo* and investigating means to locally and reversibly knock down smooth muscle function—both of these aims feed into the broader goal of testing hypotheses of smooth muscle function *in vivo* during normal behavior. Current electrophysiology-based methods do not allow for direct measurement of smooth muscle contraction, and instead rely on indirect measures of correlated skeletal muscle contraction, which are only present in some systems. The ability to directly measure and reversibly manipulate smooth muscle function *in vivo* has implications across urogenital, gastrointestinal, and cardiopulmonary physiology.

Goals & Objectives: The goal of this study is to provide the first tomographic characterization of smooth muscle bundles in avian skin. The student's research experience will focus on assessing interconnections between dermal smooth muscle bundles, superficial fascia, and underlying deep fascia and skeletal muscle of the forelimb. The specific question to be addressed is whether the unusual connections of dermal smooth muscle to underlying bone in the avian forelimb are mediated by deep fascia and associated muscular tissue.

Research Methods: The student will apply Diffusable Iodine Contrast Enhancement (DICE) and Selectively Perfused Iodine Contrast Enhancement (SPICE), both recently developed techniques for imaging normally radiolucent tissues with X-ray computed tomography (CT). These techniques employ the radiodensity of iodine as a contrast agent for CT imaging and are thus comparable to the use of injectable iodinated contrast media for angiography. Paraffin histology of focal samples will be used to confirm tissue relationships in areas of interest.

Data Analysis: Analysis for this project will include segmentation using Avizo CT analysis software to generate computer models of the musculature and surrounding tissues.

Contribution to Overall Research Effort: Completion of this project will augment continuing experiments with avian feather muscles. This work will also produce a stand-alone set of anatomical results suitable for student-led publication.

Student Fellow Mentoring Plan: Student fellows will take part in weekly lab meetings with all lab members to identify and address lab-wide issues and tasks. Student fellows will also be expected to attend the weekly journal club for the Musculoskeletal Research Focus Area, providing exposure to a broad range of research topics as well as a chance to interact with researchers at different career stages—typical attendance in summer includes 2-4 summer fellows, 2-3 technicians, 1-4 graduate students, 3 postdocs, and 3-4 faculty PIs. Timely completion and reporting of the student fellows' projects will be ensured by weekly one-on-one meetings with the PI, not only to organize work but also to work through main tasks side by side (*e.g.,* worked examples of analysis, drafting, editing and presentation) both during the summer and through the following year as needed. Materials and equipment for the proposed research are currently available in the PIs lab.

Submit your application to Dr. Julia Huyck

1. Project title, Principal Investigator name, title and location
Project: Processes underlying immature auditory perception during adolescence
Principal Investigator: Julia Jones Huyck Ph.D., Associate Professor & Program Coordinator, Speech Pathology and Audiology, Kent State University, and Voluntary Adjunct Assistant Professor, Department of Anatomy and Neurobiology, NEOMED.
Location: Speech Pathology & Audiology Program, Kent State University (1325 Theatre Drive, Kent, OH)

2. Abstract of project
Hearing and listening are critical to how adolescents communicate, learn new information, and engage with technology and culture; however, performance on auditory perceptual tasks takes a long time to become mature. Because few studies of auditory perception have centered on typically developing adolescents, little is known about the mechanisms underlying this immaturity. This project will evaluate the extent to which auditory stimulus encoding and various cognitive processes contribute to immature auditory perception during adolescence, using a combination of perceptual testing, neuropsychological and language testing, eye-tracking, and auditory evoked potentials (electrophysiology).

3. Significance of the project
Despite growing evidence that older children and adolescents have immature auditory perception (Buss et al., 1999; Hartley et al., 2000; Johnson, 2000; Wightman and Kistler, 2005; Bishop and Dawes, 2008; Lutfi et al., 2010; Wightman et al., 2010; Banai et al., 2011; Ross et al., 2011; Buss et al., 2017; Huyck and Wright, 2017; Huyck, 2018; Huyck and Rosen, 2018), most developmental studies only evaluate children up to 9 to 12 years of age and do not span the entire age range from early adolescence to adulthood. Thus, little is known about the processes underlying the prolonged maturation of hearing and listening abilities. The scarcity of information about auditory perception by typically developing adolescents provides little basis of comparison for adolescents with communication disorders. Thousands of children and adolescents in the United States have normal hearing thresholds but report difficulty on some listening tasks, either due to bottom-up perceptual deficits, language disorders, top-down cognitive issues, or some combination thereof (Loo et al., 2013; Bellis and Bellis, 2015; Moore, 2015). The lack of knowledge of the processes underlying the prolonged maturation of hearing and listening abilities in typical listeners can lead to difficulties in the diagnosis of auditory processing disorders (Loo et al., 2013; Ludwig et al., 2014; Moore, 2015). This project will yield experimental protocols that will be applicable for the development of diagnostic tests regarding auditory processing in adolescents and young adults and provide insights for the development of rehabilitation strategies to treat disorders affecting auditory processing in this population.

4. Goals and objectives
The goal is to evaluate the extent to which auditory stimulus encoding and various cognitive processes contribute to immature auditory perception during adolescence.

Learning objectives:
- The fellow will become familiar with the medical research environment by actively participating in lab meetings and departmental journal clubs.
- The fellow will learn to collect data on auditory learning from adolescents and adults using custom computer programs.
- The fellow will learn to administer neuropsychological tests to assess cognitive skills.
- The fellow will learn to collect auditory evoked potentials and eye-tracking data.

5. Research methods to be used *Human Subjects*: All procedures are approved by the Institutional Review Board at Kent State University (IRB #15-355 & #20-299). 10- to 23-year-olds will be recruited from northeast Ohio through flyers and letters sent home from local schools. Participants will have normal

hearing as confirmed by a pure tone audiogram. They will be excluded if they (or their parents) report that they have a history of hearing loss, language impairments, learning disabilities, attention deficit/hyperactivity disorder, traumatic brain injury, or other major neurological problems. Participants will be compensated for their time with gift cards. Study procedures pose minimal risk.

Auditory perception, sensory encoding and temporal processing will be measured using combination of perceptual testing, pupillometry and blink-rate to index cognitive processes engaged during active listening, auditory evoked potentials to index temporal and spectral encoding during unattended stimuli and standardized neuropsychological and language tests.

6. Proposed method of data analysis
Most data will be collected via custom computer programs. Some listening tasks and standardized tests will require manual scoring. Data will be analyzed using hierarchical regression and linear mixed models.

7. Fellow's contribution to overall research
The bulk of the data collection from our juvenile participants will occur over the summer. Assistance with data collection from teens is critical to the success of the project. Data collection is time-intensive because each participant must come in four times for about 2 hours each time. In addition, two fellows, students, or lab employees must be present at each session.

Summer Research Fellow Training/Mentoring Plan
Research will be conducted in Dr. Huyck's laboratory, which is part of the Speech Pathology and Audiology Program at Kent State and the Hearing Research Group (HRG) in the Department of Anatomy and Neurobiology at NEOMED. Dr. Huyck's lab emphasizes professionalism, enthusiasm, and scientific rigor. The fellow will receive training from Dr. Huyck and her collaborators/students in data collection and/or analysis. Lab members meet weekly to develop new projects, address technical concerns, and discuss results and related research. The fellow will meet individually with Dr. Huyck on a weekly basis. If there are regular meetings of the Hearing Research Group (HRG) in the summer of 2024, the student will attend those meetings as well.

For more information on this project please contact:
Julia Jones Huyck, Ph.D.; jhuyck@kent.edu; 330-672-0249
Associate Professor, Speech Pathology and Audiology Program, Kent State University
Voluntary Adjunct Assistant Professor, Department of Anatomy and Neurobiology, NEOMED

Submit your application to Dr. Bradley Winters

Project Title: Dendritic arbor analysis of patch-clamped lateral superior olive neuron types
PI Name: Bradley Winters (bwinters@neomed.edu)
Location: NEOMED Rootstown Campus

Abstract: The superior olivary complex (SOC) in the brainstem of mammals integrates information from the two ears enabling sound localization. This ability underlies selective auditory attention and is disrupted by hearing loss and in children with central auditory processing disorder (CAPD). Principal neurons of the lateral superior olive (LSO PNs) are critical for these functions. The classical view of the LSO is a homogeneous block of cells that extracts ongoing interaural level differences (ILDs), however, LSO is increasingly implicated in encoding interaural time differences (ITDs) for broadband transients and amplitude modulations. Cellular properties are fundamental to how neurons extract and encode information. ILD/ITD processing places disparate demands on neuronal properties and there is cellular diversity in the LSO that is not well-understood. It is also critical to understand how different types of information may be organized in higher processing centers of the inferior colliculus (IC).

We found that LSO PNs consist of inhibitory and excitatory cell types with different projection patterns, intrinsic membrane properties, and morphology. We will further probe the functional implications of our preliminary findings on the intrinsic membrane properties of LSO PN types by examining the synaptic drive onto these cells with the goal of finding input-output relationships that support different sound localization coding strategies. Preliminary studies show that inhibitory LSO PNs have lower activation threshold, however, cell-type specific synaptic drive could accentuate or offset these differences. We also found that excitatory LSO PNs have more complicated dendritic arbors suggesting they may integrate more synaptic inputs which could favor ILD coding. Since LSO PN dendrites mainly receive excitatory inputs, this finding suggests the hypothesis that excitatory LSO PNs receive more excitatory inputs than inhibitory LSO PNs. To test this, we will examine the number, strength, balance, short-term dynamics, and channel kinetics of synaptic inputs *ex vivo* using whole-cell patch-clamp. The recorded neurons will be filled with a marker and the brain slices fixed and mounted so that their dendritic arbors can be analyzed.

Significance: Our overarching hypothesis is that LSO PN cellular diversity supports both ILD and ITD coding and neurotransmitter system, intrinsic excitability, and projection pattern provide means to organize differentially extracted information in the IC. This project will yield foundational insights into the cellular organization of the SOC which may be disrupted by hearing loss and contribute to poorly understood disease states such as CAPD.

Goals and Objectives: The student will help us reconstruct the dendritic arbor of LSO PNs that were filled with a marker during electrophysiological recordings. This will allow us to correlate differences in synaptic drive between LSO PN types with cell morphology.

Methods: The student will image cells using a light microscope and then digitally reconstruct them using NeuroLucida software.

Data analysis: Data collected by the student will be analyzed using the NeuroLucida system and Microsoft Excel. This will produce a table of neuronal properties such as total dendritic length, number of branch points, number of primary dendrites, etc., that will be compared with previously recorded synaptic properties.

Anticipated Findings: The anticipated findings are critical to understanding the relationship between cell morphology and synaptic drive which in turn helps determine how LSO PNs integrate timing vs. level information.

Student Fellow Training/Mentoring Plan: The student will meet regularly with the PI in addition to working with other members of the lab, postdoc and other students. The student will be encouraged to observe and potentially participate in other lab activities/experiments to get a better understanding of how research labs operate. The Winters lab is part of the close-knit NEOMED Hearing Research Group which the student will have the opportunity to interact with.

Submit your application to Dr. Jesse Young

I. PROJECT TITLE, PRINICIPLE INVESTIGATOR AND LOCATION

Project Title: An Empirical Investigation of Functional Variation in Trabecular Bone Morphology

Principle Investigator: Jesse W. Young, Professor, Dept of Anatomy and Neurobiology

Location: Comparative Biomechanics Laboratory, D103; Department of Anatomy and Neurobiology, NEOMED

II. ABSTRACT

Bone researchers have long assumed that trabecular bone form might be structurally optimized to resist the common loading regimes. However, few empirical data exist to directly associate variation in trabecular bone morphology with functional variation in load resistance. In this research project, we will use 3D printing to create physical models that precisely vary aspects of trabecular number, thickness, and spacing. Models will be loaded using a universal material testing machine, allowing us to quantitatively diagnose how variation in trabecular form impacts the capacity for load resistance.

III. SIGNIFICANCE OF THE RESEARCH

Trabecular bone, also known as cancellous or spongy bone, is the generic name given to the network of bony struts that lie in the internal aspect of most long bones, particularly in the epiphyseal and metaphyseal regions. Because trabecular bone is 1) proximate to limb joint surfaces (and therefore may experience pronounced loading during locomotion) and 2) metabolically very active throughout life, bone researchers have long assumed that trabecular bone form might be structurally optimized to resist the common loading regimes. Various measures of trabecular bone volume, shape, and orientation have been cited in the literature as reliable indicators of activity levels and the magnitude and direction of loading history. However, most previous studies are correlational or based on computer models. Few empirical data exist to directly associate variation in trabecular morphology with functional variation in load resistance.

IV. GOALS AND OBJECTIVES

The goal of this research will be to use 3D printing to construct a set of physical models representing a range of variation in trabecular number, thickness, and shape. By precisely varying the number, thickness, and spacing of these struts, we will mimic variation in trabecular morphology in a quantitative, controlled manner. These models will then be loaded in using a universal testing machine, allowing us to systematically test for associations between specific aspects of trabecular shape and variation in overall strength (i.e., capacity for load resistance prior to structural failure). Ultimately, these results will help inform which specific aspects of trabecular bone morphology are the most informative in trying to diagnose function.

V. INVESTIGATIVE METHODS

Volumetric models will be created in the free, open source modelling program Blender (www.blender.org). Each model will consist of two flat plates connected by a network of linear struts arranged in a lattice pattern. Models will be created along a gradient of variation in trabecular number, thickness, and spacing. Each computer model will then be ported and 3D printed using a resin printer. Models will be loaded in axial compression (i.e., pushing the two flat plates of the models towards each other) using an Instron EletroPuls E3000 universal testing machine, allowing us to precisely measure the ultimate strength of each model in resisting compression (i.e., the amount of force, in Newtons, required to fracture the model).

VI. DATA ANALYSES

Quantitative data on the material strength of each model will be analyzed using full-factorial three-way analyses of variance (ANOVAs) in the statistical program R. Three-way ANOVA will allow us to statistically test for the independent and synergistic effects of trabecular spacing, number, and thickness on overall strength.

VII. CONTRIBUTION TO OVERALL PROJECT

Analyses of possible functional variation in trabecular bone morphology will contribute to Young Lab research efforts over the past decade to understand the mechanistic underpinnings of musculoskeletal anatomy as it relates to locomotor performance in mammals (e.g., Young 2005, 2009; Young et al. 2010; Russo and Young 2011; Young et al. 2014; Boyer et al. 2019; Foster et al. 2019; Smith et al. 2020; Young et al. 2020; Mossor et al. 2022; Magrini et al. 2023). Potential research products will include 1) a student presentation at the annual NEOMED student research conference, 2) student authorship on a research presentation at the annual conference of the American Association of Biological Anthropologists, and 3) student authorship on an eventual manuscript summarizing research findings.

Summer Research Fellow Training/Mentoring Plan

Over my 15 years at NEOMED, I have mentored seven postdoctoral research fellows, two graduate students, and 41 pre-doctoral trainees (i.e., medical students, undergraduate students, and high school students). I am committed to fostering a positive, rewarding research experience for all students in my laboratory. In the current context, this goal will be achieved through the mentoring program described below.

First, the fellow will be trained to participate in every phase data analysis, interpretation, and dissemination. This involvement will promote mastery of several skills necessary to accomplish holistic biomechanical research, such as the analysis of quantitative data and the use of common software packages (e.g., MATLAB and R). Opportunities for medical students to gain experience with *in vivo* biomechanical research are rare, and the skills gained through involvement with this project should substantially broaden the fellow's expertise. Additionally, I will mentor the fellow in a structured literature review, providing the student with the necessary theoretical and empirical background to understand the impetus for our research and the chosen methodology for addressing the research questions. Where merited, the fellow will be given authorship on any presentations and publications stemming from this project, even after the student is no longer actively working in the laboratory.

The student will be given the opportunity to participate in weekly brown bag seminars and journals clubs sponsored by the NEOMED Musculoskeletal Biology Research Focus Area. Additionally, the fellow will participate in all Young Laboratory meetings.

All research will take place in the NEOMED Comparative Biomechanics Research Lab (D-103), collaboratively run by Drs. Young, German, and Grider-Potter. The Comparative Biomechanics Lab has all of the equipment and computer resources needed to carry out this research.

Submit your application to Dr. Kristin Baughman

Project Title: The Inclusion of Policy Content and Recommendations within Gerontology and Geriatric Medicine Journals: A Bibliometric Analysis

PI: Kristin Baughman, PhD, Associate Professor of Family & Community Medicine, at NEOMED, Office G127.

Background: Including policy content in scientific articles is an important step in the implementation of new practices and the evaluation of current practices. This study will examine the inclusion of policy content and recommendations within gerontology and geriatric medicine journals. **Methods:** A bibliometric analysis will be conducted examining author guidelines, solicitation of policy articles, and other related journal characteristics. The study will allow us to determine which journals are most likely to include policy as a crucial component of the research process.

Significance: Discovering new treatments and preventive strategies through scientific research is not sufficient to improve health outcomes. The new evidence must be implemented within the healthcare system and policies/guidelines updated to support the new evidence-based discoveries. It can take years before a new practice is implemented. Yet, few scientific journals include articles that address the implementation of best practices and the related changes to policies and guidelines.

The challenges of implementation are particularly challenging within the field of Gerontology. Older adults with multiple, complex chronic conditions are often excluded from clinical trials (Carpenter et al. 2022) so that policies and guidelines are not updated to incorporate new treatments for older adults. Sometimes treatments which are effective for most adults are found to be of limited use for older adults and policies must be changed to discourage the use of treatments that may do more harm than good in older adults.

Several academic organizations state the importance of policy within their mission statements. For example, the American Geriatrics Society's vision "to maintain our health, safety, and independence as we age" is grounded in influencing programs and policies within the healthcare system (https://www.americangeriatrics.org/where-we-stand) and the Gerontological Society of America stresses the importance of translating research evidence into policy (https://www.geron.org/Advocacy/GSA-Policy-Initiatives). Yet, few scientific articles address policy content or recommendations.

Goals and objectives: The objective of this research is to examine the extent to which policy content is addressed within gerontology and geriatrics journals. We will examine author guidelines and the types of articles that are solicited by each journal to determine if the journal is publishing or encouraging the publication of policy content or recommendation papers.

Research methods: We will conduct a bibliometric analysis of peer-reviewed journals within the field of gerontology and geriatric medicine. Variables will include mention of policy content in author guidelines, types of articles accepted for publication, publisher, impact factor, frequency of publication, as well as other relevant factors.

Proposed method of data analysis: Descriptive statistics will be used to describe the journals most likely to solicit articles with policy content. The statistical package, SAS, will be used to analyze the data.

Summer Fellow contributions: The summer fellow will contribute to the literature review, assist in collecting data from journal websites, and perform the descriptive analysis. The student will also draft an abstract to present at the NEOMED Student Research Symposium and prepare either a poster or oral presentation for the symposium.

Training/Mentoring Plan

Plan for training/mentoring: Kris Baughman, PhD (associate professor of Family & Community Medicine at NEOMED) will be the primary contact and mentor for this project. In addition, the student will have the opportunity to work with Ruth Ludwick, PhD RN (professor emeritus from Kent State University's College of Nursing). Dr. Baughman will meet with the student to develop a work plan which will include the following: 1) review of the literature on implanting policies within the healthcare system and how to develop a research question and hypothesis, 2) training in collecting data from journal websites, 3) data management, 4) and how to analyze data using quantitative software. Follow-up meetings will be held at least weekly to discuss project progress, and to provide the student with any necessary guidance or information. The student will also be included in research team meetings.

Description of resources available: Workspace within the Department of Family & Community Medicine is available if needed but the student is also welcome to work from home. Statistical software will also be provided and meetings will occur in person or by zoom.

Site where the research will be conducted: The research will be conducted either at NEOMED or remotely.

NOTE: This summer fellowship is funded by the NEOMED Student Training in Aging-Related Research (NEOSTAR) program and offers the same stipend as the research office. **We encourage students interested in Geriatrics to apply and offer additional shadowing experiences with Geriatricians throughout the summer.**

Submit your application to Dr. Stacey Gardner-Buckshaw

Title: Adolescent Substance Use Disorder in Northeast Ohio – Exploratory Study

Principal Investigator: Stacey Gardner-Buckshaw, Ph.D., MPA, Associate Professor and Director of Community Engagement, Department of Family and Community Medicine, NEOMED **Location:** Department of Family and Community Medicine, G-151, and a collaborating hospital (such as Akron Children's or Metro – planning meetings scheduled for late January 2024).

Abstract and Significance: Primary care providers (PCPs, including primary care physicians, physician assistants, and nurse practitioners) are frequently the first line care for many patients with substance use disorders (SUD). The same is also true for pediatricians, however most SUD research is conducted in adults even though many patients with SUD report engaging with alcohol and other drugs in adolescents. The IOM has recommended health professions education and training programs should provide educational opportunities in SUD treatment in primary care. This study will explore the feasibility of treating pediatric patients with SUD in a primary care setting, and to assist PCPs in the successful incorporation of MAT into all levels of medical education and primary care practice,

Goals and Objectives: The long-term goal of the project is to identify how to recognize risk for SUD in adolescents, design effective interventions, and treat adolescent SUD in a primary care setting. The short-term objective to be achieved this summer is to identify a target adolescent population to target for the study using data from health system electronic medical records and/or community youth risk behavior survey data. This data is required to secure grant funding for the project from federal sources. To help accomplish this, the summer research fellow will conduct a study to inform project development. The student will use existing data to inform study design, engaging stakeholders from Northeast Ohio. Then, will compile and report the information to NEOMED program directors and other stakeholders responsible for the SUD treatment expansion and/or follow-up training program. The fellow may also design an independent, related research project using secondary data under faulty direction that will be disseminated at NEOMED Scholarship Day.

Significance of Anticipated Findings: Results of this study will inform best practices in MAT and other SUD recovery interventions and training for PCPs, and will likely inform a forthcoming grant-funded project proposal.

Investigative Methods: The student will help design and implement an exploratory study to look for themes among program stakeholders regarding what content is needed (priorities) for PCPs implementing adolescent SUD intervention in practice, as well as content to be presented in trainings (accessibility).

Proposed Method of Data Analysis: This project requires simple descriptive statistics, as well as understanding of the various qualitative and quantitative methods.

Student Role: Under direction, the student will take a leadership role in all parts of the research process including but not limited to: institutional review, informed consent, protocol development, data collection and analyses, and dissemination. The student will meet at least bi-weekly with collaborating NEOMED faculty as needed.

Mentoring Plan:

1. Student meets with Dr. Gardner-Buckshaw and community preceptor bi-weekly.
2. Educational topics we will cover, in the context of the project:
    A. Establishing a research question
    B. Conducting a gap analysis/literature review

C. Writing a grant proposal
D. Protection of human subjects
E. Data collection/management
F. Posters and presentations

3. The student will work with Dr. Gardner-Buckshaw to submit a regional or national presentation of their work.

Resources Available: the student will receive space in the DFCM, with access to computers and a telephone (as required) for research-related activities. The student will also receive research and statistical support as needed. Funds from a SAMHSA grant, or DFCM's student support budget, may be used for student travel to meetings/interviews/presentations, or for poster printing for dissemination.

Submit your application to Ms. Hannah Haynie

Title: Changes in Prescriber Attitudes About Medication-Assisted Treatment Training from Before and After the COVID-19 Pandemic

Principal Investigator: Hannah Haynie, M.S., SOAR Clinic Manager, Department of Family and Community Medicine, NEOMED
Co-Investigator: Dr. Stacey Gardner-Buckshaw, Ph.D., Associate Professor, Department of Family and Community Medicine, NEOMED
Location: Department of Family and Community Medicine, G-123; research will be conducted at NEOMED.

Abstract and Significance: Primary care providers (PCPs, including primary care physicians, physician assistants, and nurse practitioners) are frequently the first line care for many patients with opioid use disorders. The IOM has recommended health professions education and training programs should provide educational opportunities in pain assessment and treatment in primary care. To address this critical training need, NEOMED and Metro Health Services collaborated to offer Medication-Assisted Treatment (MAT) training designed specifically for PCPs. The recent opioid crisis has created an increased need for addiction treatment.

To assist PCPs in the successful incorporation of MAT into all levels of medical education and primary care practice, NEOMED and Metro Health have been offering training sessions since November 2017. The 12-hours of training includes 4 hours of self-directed online MAT training offered by SAMHSA and AAAP, 4 hours in-person MAT and 4 hours supplemental training designed for PCPs. The supplemental training teaches clinicians the significance of opiate use disorders and identification; MAT implementation best practices, and how to converse with patients about MAT; misconceptions about MAT; means to address stigmas associated with MAT; and motivational interviewing. This plan goes beyond the required 8 hours of required MAT for the X waiver and is designed to ensure that PCPs achieve a level of comfort in delivering this type of treatment. Even with recent changes in legislation, training has continued to ensure provider competency and comfortability when providing MAT services.

A manuscript describing the initial study was accepted for publication by the *Journal of the American Board of Family Medicine* in November 2022.

Goals and Objectives: The goal of this study is to build upon what we learned over the program years and assess how participation in the NEOMED/Metro Health MAT training for PCPs influenced implementation of MAT into primary care practice. Given that no trainings were held in 2020, we also wish to measure differences in PCP attitudes and intent to implement buprenorphine treatment into primary care practice before the COVID-19 pandemic compared to providers trained in 2021 or later (after the initial COVID-19 surge). To accomplish this, the summer research fellow will compile evaluation data- collected in the past and present, for the development of a manuscript describing the program and its outcomes. The fellow may also design an independent, related research project using secondary data under faulty direction that will be disseminated at NEOMED Scholarship Day.

Significance of Anticipated Findings: This study holds the promise of contributing to evidence-based interventions, ultimately working towards mitigating the profound consequences of the dual challenges posed by the opioid epidemic and the ongoing COVID-19 pandemic.

Investigative Methods: The student will compile training pre-test and post-test evaluation data and analyze the results using quantitative methods.

Proposed Method of Data Analysis: This project requires simple descriptive statistics, frequencies, crosstabs and comparative means tests.
Student Role: Under direction, the student will take a leadership role in all parts of the research process including but not limited to: institutional review, informed consent, questionnaire development, survey interviewing, data collection and analyses, and dissemination. The student will meet at least bi-weekly with collaborating NEOMED faculty and staff, as needed.

Mentoring Plan:
1. The student will meet with the research team bi-weekly.
2. Educational topics we will cover, in the context of the project:
    A. Establishing a research question
    B. Conducting a gap analysis/literature review
    C. Protection of human subjects
    D. Data collection/management
    E. Posters and presentations
3. The student will work with the research team in the DFCM to submit a regional or national presentation of their work.

Resources Available: The student will receive space in the DFCM, with access to computers and a telephone (as required) for research-related activities. The student will also receive research and statistical support, as needed. Funds from a SAMHSA grant, or DFCM's student support budget, may be used for student travel to meetings/interviews/presentations, or for poster printing for dissemination.

Submit your application to Dr. Amy Lee

Title: Investigating Clinical Services Structures for Christine's Hope
Principal Investigator: Amy Lee, MD, MPH, Professor and MPH Program Director
Location: NEOMED

Abstract: Christine's Hope is a non-profit based in Cuyahoga County. Its mission statement is to "create meaningful engagements and a sense of belonging, dignity, and respect for Citizens who are differently-abled. As a nonprofit, all proceeds are used to create a bright future for adults with cognitive delays." The Consortium of Eastern Ohio Medical University has worked with its founder, Bess Vrettos, on projects to enhance programming. Ms. Vrettos has stated that they would like to offer clinical telehealth services to their clients. This project will take steps to help Ms. Vrettos decide whether it might be possible to achieve FQHC, FQHC look-alike, free clinic, or community health center status is in her organization's best interest. Students will interview key informants and research the structures of these entities and present their findings.

Significance of the overall research: In 2016, more than 61 million Americans had a disability. Lagu et al. published a study that suggested that physicians have negative attitudes about people with disabilities and have concerns about resources and accommodations to treat these patients. Some physicians do not accept them into their practice. Because people with disabilities may not have accommodations in clinical practices, organizations such as Christine's Hope help to bridge the gap by understanding the needs of these patients. This project will provide recommendations on how Christine's Hope can improve the clinical experience of people with disabilities.

Goals and objectives: This project will be for the summer fellow will be to investigate the best options to provide clinical services through Christine's Hope.

- SMART Objective 1: By August 31, 2024, provide pros and cons of different clinical structure options for providing clinical services to people with disabilities through Christine's Hope.
- SMART Objective 2: By August 31, 2024, provide at least 3 recommendations of the directions that Christine's Hope should take to initiate clinical services.

Research methods that will be used/learned: The fellow will do a web search and interview key informants, which will include Christine Hope's board of directors and administrators of FQHC and community health centers.

Proposed methods of data analysis: The student will use do a content theme analysis from the key informant interviews using Quirkos and compile the pro and cons of the different structures in a table format.

How the anticipated findings from the summer research fellow contribute to the success of the overall research being investigated: The products will be used by administrators of Christine's Hope to consider as their next steps toward providing clinical services.

Appendix
Plan for training/mentoring the summer research fellow—individual, group, lab meetings, journal clubs, seminars, etc. The student will do the following: Attend regular meetings with the faculty advisor (remote or in person).

Demonstrate project management techniques, such as creating agendas and meeting summaries and adhering to a project timeline.

Take the Writing for the Sciences course offered by Coursera (free version) to improve their writing skills.

Submit an abstract for the NEOMED student research forum in the fall.

Visit Christine's Hope at least once during the experience to tour the facility and learn about the experience of people with disabilities.

Interview key informants.

Provide stated outcomes.

Description of resources available. The student will have a desk in the Department of Family and Community Medicine. Students will be given guidance by their faculty team and community contacts on who should be contacted and other community sources, as guided by the principal investigator.
Site where the research will be conducted. The research will be conducted remotely and at Christine's Hope.

Sources

- Lagu T, et al. I am not the doctor for you: physicians' attitudes about caring for people with disabilities. Health Affairs. Vol. 41, No. 10: Disability & Health. https://doi.org/10.1377/hlthaff.2022.00475
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- COPE Health Solutions. FQHC and FQHC look-alike programs: key differences and criteria for designation. https://copehealthsolutions.com/cblog/fqhc-and-fqhc-look-alike-programs-key-differences-and-criteria-for-designation/ Accessed January 11, 2024.

Submit your application to Dr. Feng Dong

Project title: Role of CXCR4 in aortic stenosis
Principal Investigator: Feng Dong, Associate Professor, RGE 234

Abstract of project:
Previously, we found blunted stromal cell-derived factor-1 (SDF-1): CXCR4 axis in diabetes, and our preliminary results show an increase in chronic cardiac myocyte CXCR4 expression in diabetic murine hearts. Moreover, CXCR4 activation in diabetes produces a profound negative inotropic effect (which may seem counterintuitive, but we think it is a key adaptation in the diabetic heart). Furthermore, our preliminary results demonstrate a significantly increased mortality rate of diabetic (high fat, high sugar [HFHS]) mice null for CXCR4 in cardiac myocytes compared to HFHS diabetic wild-type mice. Recently, with our CXCR4 endothelial cell-specific knockout mice, we found that the deletion of CXCR4 in endothelial cells leads to aortic stenosis. This proposal leverages novel models of loss of CXCR4 function in endothelial cells to investigate the role of CXCR4 in aortic stenosis and define the mechanisms of how CXCR4 knockout could affect cardiac function.

The significance of the overall research:
Upon completing these studies, we will have determined the importance of the SDF-1: CXCR4 axis in aortic stenosis. Novel physiology and treatment strategies will be developed based on a detailed understanding of the mechanisms involved in aortic stenosis.

The goals and objectives for the summer research project what aspect of the overall research will be the focus of the student's summer research experience? What is the specific research question being addressed by the summer research project?

The goal of our proposed studies is to define the molecular mechanisms and physiology associated with the development of aortic stenosis. The focus of the student's summer research experience will be the scientific research procedures and principles on aortic stenosis.
The specific research question addressed by the summer research project is: Determine the role of CXCR4 in cardiac function using our EC CXCR4 null mouse models.

The research methods that will be used/learned by the student:
The experiments will expose students to various cellular, molecular, and biochemical techniques such as the culture of cells, western blot, and q-PCR. The students will also be exposed to microscopic techniques and animal surgeries such as confocal microscopy and echocardiography.

The proposed methods of data analysis:
Comparisons between 2 groups will be made with a 2-tailed Student t-test. Comparisons among multiple groups will be made with 2-way ANOVA followed by the Tukey post
hoc analysis.

A statement of how the anticipated findings from the summer research fellow contribute to the success of the overall research being investigated? The summer research project is a part of an ongoing project in the lab. Our preliminary results show that the deletion of CXCR4 in endothelial cells leads to aortic stenosis. Anticipated findings from the summer research will answer an important question: How could the CXCR4 knockout affect aortic stenosis and cardiac function?

Student Fellow Training/Mentoring Plan
Plan for training/mentoring the summer research fellow – individual, group, lab meetings, journal clubs, seminars, etc.

After proper training, the variety of the experiments (cell biology, molecular biology, and microscopy) ensures that each student will have unique and specific tasks relating to the overall completion of the project. The students will be taught troubleshooting methods and encouraged to design alternative strategies and hypotheses based on their findings. Students will present their results and project updates in formal lab meetings and informally to the PI. The meetings will discuss the relevant literature, improving critical thinking, and oral presentation skills. The students will present their research at NEOMED (Cardiovascular group, IMS Department).

Description of resources available. lab space for students within the open laboratory of the department (4000 sq. ft.). In addition, PI has access to all core facilities, which include an animal surgery room equipped with ventilators, surgical instruments, and echocardiography systems, as well as a station for processing and embedding tissue in paraffin; fully functional tissue culture facilities, dark rooms, FACS, RT-PCR, gel imaging and software for analyses. The laboratory is located in a modern complex that houses the Department of Integrative Medical Sciences and Pharmaceutical Science.

Site where the research will be conducted.
Most work will be done in RGE 200, and some will be done in room RGE 217, 218.

Submit your application to Dr. Jessica Ferrell

Title: TGR5 & Alcohol-Associated Liver Disease
PI: Dr. Jessica Ferrell, PhD; jfrancl@neomed.edu; x6468; Assistant Professor
Location: NEOMED, Department of Integrative Medical Sciences, Laboratory F-205A

Abstract: Bile acids are the natural ligand for Takeda G protein-coupled receptor 1 (TGR5), an anti-diabetic and anti-inflammatory receptor expressed in the liver, intestine, and brain. It is also a potential therapeutic target for obesity, metabolic dysfunction-associated steatotic liver disease (MASLD) and alcohol-associated liver disease (AALD). *Tgr5*-/- mice have significantly increased expression of fibroblast growth factor 21 (FGF21) upon administration of alcohol via unknown mechanisms. FGF21 is a growth factor involved in suppression of carbohydrate consumption, including ethanol and sugar. FGF21 was shown to be induced after alcohol consumption in rodents and primates/humans, and administration of FGF21 significantly reduces alcohol consumption. However, it is unknown whether the increased Fgf21 in *Tgr5*-/- mice affects alcohol consumption or nutrient preference. The aim of this study is to determine the role of TGR5 in FGF21 signaling, and to determine if *Tgr5*-/- mice have altered preference for ethanol consumption.

Significance: Our data indicates that in *Tgr5*-/- mice, alcohol induces changes in fibroblast growth factor 21, a hormone involved in mediating preference for carbohydrate and ethanol consumption. *Tgr5*-/- mice also have changes in leptin signaling, involved in the control of food intake and satiety, in the white adipose, liver, and brain, though the mechanisms by which this occurs are unknown. Studying how TGR5 is involved in ethanol and nutrient preference could lead to novel therapeutic targets for metabolic syndrome or AALD.

Goals and Objectives: The proposed research will further uncover the role of FGF21 and TGR5 in alcohol consumption and nutrient preference. The goals for the summer research student are to learn scientific technique and experimental design, data analysis and interpretation, and to demonstrate professional presentation of scientific results.

Research Methods: Wild type mice will be subjected to 2-bottle-choice study (10% ethanol or water control) coupled with BAR-501 activation of Tgr5, and tissue will be collected for: qPCR to determine changes in gene expression, Western blotting to determine changes in protein expression, lipid and bile acid analyses, and tissue histology.

Data Analysis: Appropriate statistical tests (Student's *t*-test, one-way ANOVA, etc.) using GraphPad Prism Software will be performed to determine statistical significance ($p<0.05$).

Contribution of Findings: It is expected that the findings obtained from this project will lead to better understanding of the role of TGR5 signaling under normal and pathophysiological conditions and will be an instrumental base in further studying the role and FGF21 liver injury, nutrient preference, and alcohol consumption during the pathogenesis of AALD.

Student Fellow Training/Mentoring Plan: The student will complete safety and lab training modules prior to the start date. The training plan for the student encompasses individual and group mentorship from Dr. Ferrell (mentor), senior lab technicians, and Ph.D. students who will be available to help instruct in the techniques necessary to complete this research. The student will become familiar with the research topic by reading primary and review journal articles. Basic lab techniques will be introduced through one-on-one instruction and will progress to independent work when appropriate. In addition to lab work, the student will be expected to keep records of the experiments and will learn to interpret the data collected. These results will be discussed with the mentor as necessary and during weekly lab meetings. Additionally, lab members participate in biweekly

Diabetes, Obesity, and Metabolism Research Focus meetings, which include data and journal article presentations by graduate students, post-docs and staff. The student will attend these meetings and will have the opportunity to present research results at the end of training program. Lastly, the student will prepare and present a poster of their work at the Summer Research Fellow Poster Day.

This work will be conducted at NEOMED in F-205A.

Submit your application to Dr. Adam Goodwill – project 1 of 2

PROJECT DESCRIPTION

Project Title: Identifying the Coronary Metabolic Dilator
Principal Investigator: Adam G. Goodwill, PhD, FCVS; Assistant Professor Integrative Medical Sciences
Research Location: Northeast Ohio Medical University College of Medicine
RGE-300 RGE-308 Comparative Medicine Unit

Abstract
Owing to the need of the heart to constantly oscillate between contraction and relaxation, the metabolic demands of the heart are amongst the highest of any tissue in mammalian physiology. To meet these demands, cardiac tissues rely almost exclusively on aerobic metabolism. Accordingly, the heart must have mechanisms in place to rapidly increase coronary blood flow (oxygen delivery) in response to increases in myocardial demand. While this need is known, the specific mechanism linking these processes remains undefined. In collaboration with other NEOMED faculty including Dr. William Chilian and Dr. Xinwen Wang, we believe that we have developed an experimental approach to allow for novel insights into metabolically driven coronary dilator responses. Our hypothesis is that any metabolic dilator must be initially secreted by cardiac tissue and that increases in myocardial demand will result in proportional increases in this secreted compound. Using a large animal model, we intend to place custom biocompatible catheters directly into the left ventricular free wall of an anesthetized swine model and vary myocardial demand through alterations in cardiac electrical activity and/or chemical stimulation of contractility. We will collect samples of extracellular fluid at specific and carefully controlled levels of myocardial oxygen consumption. This extracellular fluid will then be submitted for metabolomic analyses (Dr. Wang's group). It is our belief that these analyses will allow for initial identification of plausible, physiologically relevant metabolic dilators.

Significance
The treatment of all diseases is predicated on the notion that we understand how a tissue/organ works under normal conditions. Identifying what mechanism tie cardiac metabolism to coronary blood flow is central to identifying pathways for pharmacologic interventions in cardiac ischemic disease.

Goals and Objectives for the Research Project
The goal of this research is to create a dataset that will allow for identification of extracellular compounds that increase/decrease in proportion to myocardial demand.

Research Methods
All sample collections will be performed in a large animal model. Studies will be performed in the comparative medicine unit. All animal procedures will be performed under the direct supervision of Adam Goodwill PhD. In brief, animals will be anesthetized using an induction cocktail prior to intubation. Once intubated, general anesthesia will be maintained using a combination of □-chloralose and an approved schedule II analgesic. While under general anesthesia, arterial and venous access will be obtained and the animal rotated into lateral recumbency and a left lateral thoracotomy performed. After breaching the body wall, the pericardium will be opened and perivascular flow probes placed around the left anterior descending and left circumflex coronary arteries. An intraventricular venous catheter will be placed, a left ventricular pressure-volume catheter may be placed and a custom micro-renethane catheter will be passed through the epicardium. This combination of instrumentation will allow for real-time measurement of all left ventricular coronary flow, myocardial oxygen consumption and myocardial mechanics. Systemic parameters (ECG, blood pressure, respiration) will also be monitored through the procedure. Finally, an epicardial cardiac pacing lead may be placed to allow for external pacing of the heart.

Upon completion of instrumentation, baseline measurements will be taken and extracellular fluid collected. Myocardial demand will then be increased in a step wise manner using the external pacing device, and/or pharmacologic intervention (commonly dobutamine). Myocardial demand may also be

stepwise decreased via direct electrical stimulation of the Vagus nerve. Functional parameters, blood gasses and extracellular fluid will be collected at each level of demand. All samples will be snap frozen for later molecular analyses.

At the conclusion of the protocol, one final metabolic challenge may be administered and cardiac biopsies obtained at each level of demand. All above procedures are performed under general (surgical grade) anesthesia. Once all studies are completed, each animal will be humanely euthanized by direct application of a 9v battery to the heart.
Samples will be provided to our collaborator at the conclusion of each individual experiment. Data from *in vivo* studies will be integrated with molecular data for analyses of relevant molecular pathways.

Methods of Data Analysis
Data analysis will be performed using commercially available software (SigmaPlot & GraphPad). At this time no specific analysis has been designated as singularly appropriate. Based on the study design, the vast majority of data will be analyzed using repeated measures ANOVA and multivariate regression analyses.

Anticipated Findings
We anticipate that we will be able to simulate the intact condition using our experimental approach. Accordingly, we anticipate increases in coronary flow to occur in proportion to myocardial demand. In systems that match well, coronary venous oxygen content remains relatively constant across a range of demand (the same amount remains after what is needed is extracted). Since we use a large animal model, we are uniquely able to monitor this parameter to assure that there is not an experimental perturbation that results in demand:perfusion mismatching. We assert that the metabolomics will likely reveal one or more compounds that increase in proportion to myocardial demand, each of which can/will serve as avenues of future investigation.

STUDENT FELLOW TRAINING/MENTORING PLAN

Training Plan
All learners will participate in necessary CITI training and CMU training in accordance with IACUC protocols. Additionally, all animal studies will be performed under the instruction and supervision of Adam G. Goodwill Ph.D. Every member of the laboratory will cross-train in every function of the laboratory from data acquisition to analysis to dissemination. Dr. Goodwill intends to work with every member of the laboratory on a daily basis. Learners will participate in daily informal meetings with Dr. Goodwill and in weekly joint lab meetings with Dr. William Chilian's research group. Dependent on the number of summer laboratory members, we tentatively plan to host a student led cardiovascular journal club for the duration of the summer training experience. These journal clubs will be open to all other NEOMED faculty, staff and students. Trainee's will be encouraged to assist our collaborators in any studies, at the discretion of those laboratories, and will be encouraged to explore the techniques and approaches employed by other faculty in the cardiovascular center. Trainee's will be encouraged to actively participate in any available seminars.

Data analysis software will be made available to all trainees. This software will be used in a see one, do one, teach one approach wherein students will be guided through the software and then encouraged to explore data analyses and visualizations. Dr. Goodwill will be available for questions and guidance through this process and will verify all analyses before any presentation.

All data presentations will be developed in coordination with Dr. Goodwill and rehearsed first with the laboratory group, then our cardiovascular center before any public presentation.

Finally, trainees will be encouraged to disengage from research at the end of the day. Our laboratory believes that mental health is health and it should be safeguarded accordingly. While we assert that our work is important, we recognize that it will be there in the morning.

Available Resources

- Assorted Surgical Equipment
- ADInstruments Powerlab C with 16 inputs
- Dell XPS Computer for data acquisition
- Grass Amplifier (Multiple)
- Harvard Apparatus Perfusion Servo Controller (2)
- Harvard Apparatus Syringe Pumps (Multiple)
- Harvard Apparatus Transducer Amplifier (8)
- Haake K20 Heated/Cooled Circulating Water Bath
- iWorx Biopotential Amplifier for ECG
- Lifepak 20 Defibrillator with Internal/External Paddles
- Masterflex L/S Peristaltic Pumps (2)
- Stryker 810 Autopsy Saw
- Transonic ADV-550 Admittance Pressure Volume System
- Transonic TS410 Tubing Flow Module (2)
- Transonic TS420 Transit Time Perivascular Flow Module (3)
- Werfen Gem Premier 5000 Blood Gas Analyzer

Research Location:
Northeast Ohio Medical University College of Medicine
RGE-300 RGE-308 Comparative Medicine Unit

Submit your application to Dr. Adam Goodwill – project 2 of 2

PROJECT DESCRIPTION

Project Title: Coronary Hypoxemia is a Sufficient Stimulus for Cardiac Effects of Sodium Glucose Cotransporter Type 2 Inhibitors
Principal Investigator: Adam G. Goodwill, PhD, FCVS; Assistant Professor Integrative Medical Sciences
Research Location: Northeast Ohio Medical University College of Medicine
RGE-300 RGE-308 Comparative Medicine Unit

Abstract
Since their initial FDA approval in 2013, sodium-glucose cotransporter type 2 inhibitors (SGLT2i) have become an increasingly prescribed drug category for glycemic control in patients with type 2 diabetes mellitus. Through therapeutically induced glycosuria, SGLT2i have been demonstrated to be well tolerated and efficacious in lowering a long-term measure of glucose regulation. Interestingly, numerous outcome studies have demonstrated unexpected and potent decreases in major adverse cardiac effects (MACE) with SGLT2i therapy independent of effects on glucose levels. Understanding of SGLT2i mediated cardioprotection is confounded by the consistent observation that neither SGLT2 mRNA nor protein are measurable in cardiac tissue. Although myriad molecular mechanisms of cardioprotection have been proposed, no mechanism has received general support. Work from our research group has provided compelling data that SGLT2i can act directly on the heart and these actions specifically only occur when the heart is in a condition of disease/damage. It is our assertion that SGLT2i act through a mechanism that is dependent on factors released during myocardial ischemia. We propose a study design that will allow us to better understand the differential role of ischemia vs hypoxemia and stands to provide a dataset for identification of the molecular mechanisms at play in SGLT2i mediated cardioprotection.

Significance
The cardioprotective effects of SGT2 inhibitors are well documented while the mechanisms remain fully undefined. Insight into mechanism will provide insight into more targeted therapies specific to enhancing cardioprotection.

Goals and Objectives for the Research Project
The goal of this research is to identify the relative contributions of coronary oxygen delivery vs delivery of other humoral factors in mediating SGLT2 inhibitor associated cardioprotection.

Research Methods
All studies will be performed in a large animal model. Studies will be performed in the comparative medicine unit. Animals will be divided into two groups: Control & Treatment. All animal procedures will be performed under the direct supervision of Adam Goodwill PhD. In brief, animals will be anesthetized using an induction cocktail prior to intubation. Once intubated, general anesthesia will be maintained using a combination of □-chloralose and an approved schedule II analgesic. While under general anesthesia, arterial and venous access will be obtained. Large bore arterial catheters will be advanced through the arterial access points into the aorta to provide an aortic blood supply. The animal will then be rotated into lateral recumbency and a left lateral thoracotomy performed. After breaching the body wall, the pericardium will be opened. The left anterior descending and left circumflex coronary arteries will be carefully isolated from the surrounding tissue. Each of the left ventricular epicardial coronary arteries will be cannulated and blood will be supplied from the arterial via a custom servo-controlled peristaltic pump drive extracorporeal perfusion system (similar to bypass equipment). A custom extracorporeal oxygenation/deoxygenation device will be connected in series to the perfusion circuit. An intraventricular venous catheter will be placed, and a left ventricular pressure-volume catheter may be placed. This combination of instrumentation will allow for real-time pressure-clamp control of all left ventricular coronary flow, measurement of myocardial oxygen consumption and myocardial mechanics. Systemic parameters (ECG, blood pressure, respiration) will also be monitored through the procedure.

Upon completion of instrumentation, baseline measurements (coronary perfusion pressures clamped at 100mmHg) will be taken. Following collection of baseline data, coronary autoregulatory responses will be assessed by adjusting servo-control settings step wise, assessing flow at coronary perfusion pressures from 50-150 mmHg. Samples will be collected at each stage.

Using the extracorporeal membrane oxygenator/deoxygenator, plasma oxygen content will be modulated to create hypoxemic and hyperoxemic conditions. Autoregulatory responses will again be assessed with the heart in hypoxemic conditions and hyperoxemic conditions. Functional parameters and blood gasses will be collected at each step.

At the conclusion of the protocol, one final metabolic challenge may be administered and cardiac biopsies obtained at each level of demand. All above procedures are performed under general (surgical grade) anesthesia. Once all studies are completed, each animal will be humanely euthanized by direct application of a 9v battery to the heart.

Methods of Data Analysis

Data analysis will be performed using commercially available software (SigmaPlot & GraphPad). At this time no specific analysis has been designated as singularly appropriate. Based on the study design, the vast majority of data will be analyzed using repeated measures ANOVA and multivariate regression analyses.

Anticipated Findings

Historic data has demonstrated that the onset of acute coronary ischemia is associated with a near instantaneous decrease in cardiac efficiency in control animals whereas efficiency is maintained in treated animals. While interesting, this response only allows for binary analyses: total regional myocardial ischemia vs control conditions. We believe that the response to therapy will be proportional to the magnitude of supply:demand imbalance and that this will specifically be an oxygen sensitive response. As we are one of the few research groups in the world trained and equipped to perform these studies, we are in the unique position to be able to create coronary specific hypoxemia at normal perfusion pressures. By comparing the effects of ischemia (inadequate perfusion) vs non-ischemic hypoxemia, we will be able to establish whether effects are mediated by plasma oxygen concentrations or some other humoral factor. Moreover, while our hypothesis is centered on the notion that effects are oxygen specific, the study design should allow us to identify the relative contribution of oxygen delivery. Even if oxygen does not play a role, that data is informative and could guide us to future studies, aided by analyses of samples collected at each level of oxygen tension and coronary perfusion pressure.

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- Werfen Gem Premier 5000 Blood Gas Analyzer

Research Location:
Northeast Ohio Medical University College of Medicine
RGE-300 RGE-308 Comparative Medicine Unit

Submit your application to Dr. James Hardwick – project 1 of 2

Title: Cytochrome P450 production of dicarboxylic acid leads to synthetic lethality of hepatocellular carcinoma.

Dr. James P. Hardwick
Professor of Biochemistry and Molecular Pathology
Department of Integrative Medical Sciences
NEOMED, F141
jph@neomed.edu

Abstract of Project

Cancer is the second leading cause of mortality worldwide and is suspected to be the foremost killer in the coming decades by the World Health Organization. Cancer treatments, including surgery, chemotherapy, and radiotherapy, have achieved considerable therapeutic efficacy, but damage to the normal tissue and the subsequent side effects are inevitable. Accordingly, besides the conventional therapy modalities, it is crucial to identify other assistant treatment methods to enhance the therapeutic efficacy further, reduce side effects, and improve prognosis. To improve chemotherapeutic effectiveness, multiple tumor pathways are targeted by drugs that show synthetic lethality (SL). Synthetic lethality is a novel strategy for anticancer therapies, whereby mutations of two genes will kill a cell, but mutation of a single gene will not. A growing number of recent studies in cancer treatments have suggested that factors in the categories of naturopathic medicine profoundly affect the initiation and treatment outcomes of cancer. Fasting therapy is a naturopathic treatment method used as a valid therapeutic modality for acute and chronic diseases in medicine worldwide. In cancer-bearing models, fasting therapy was reported to be a reproducible and efficient intervention in protecting mammals against tumors and prolonged overall survival. The chemotherapy-protection effects of fasting therapy in reducing chemotherapy side effects and related death were also shown in human clinical trials. There is little knowledge of how synthetic lethal chemotherapeutic drugs and fasting interplay improve drug efficacy and reduce systemic toxicity. We hypothesize that induction of omega fatty oxidation cytochrome P450 gene by fasting and inhibition of peroxisomal acyl-CoA oxidase (ACOX) will increase tumor dicarboxylic acids, causing synthetic lethality.

Significance of overall research

Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer. Hepatocellular carcinoma occurs most often in people with chronic liver diseases, such as cirrhosis caused by hepatitis B, hepatitis C infection, or non-alcoholic fatty liver disease. Systemic chemotherapy for HCC is usually not well tolerated by patients with significant underlying hepatic dysfunction, and chemotherapy may also be less effective in patients with substantial cirrhosis. Although combination therapies such as the Tyrosine kinase inhibitor Sorafenib and the anti-vascular endothelial growth factor inhibitor bevacizumab prolonged survival, they failed to increase the remission rate. Therefore, it is imperative to design new therapies to treat advanced liver cirrhosis and HCC. We have shown that the fatty acid omega hydroxylase P4504A11 is increased in patients with liver cirrhosis and HCC, producing vasoconstrictive 20-arachidonic acid and 12-hydroxylic acid. These fatty acids are converted to dicarboxylic acids, metabolized by peroxisome β-oxidation to acetate that feeds lipogenesis. Therefore, inhibition of peroxisome acyl-CoA oxidase will decrease acetate production for lipogenesis and increase dicarboxylic acid that can un-couple mitochondria respiration leading to cell death. These studies will provide insight into the beneficial effects of fasting in increasing the efficacy of chemotherapeutic drugs and reducing their subsequent side effects.

Goals and Objectives of the summer research project:

This research aims to determine the absolute expression levels of human ***CYP4*** genes in hepatocellular cell lines. This proposal seeks to determine if induction of CYP4 P450 and inhibition of ACOX1 can lead to synthetic lethality (SL) of hepatocellular cancer cell (HCC) lines through increased production of dicarboxylic acids.

Investigative Research methods:
Seven different HCC cell lines with different levels of cell proliferation and metastatic potential will be used throughout this study. These cell lines will be maintained in cell culture and treated with other chain-length dicarboxylic acids, and the rate of cell death and proliferation will be measured. The cell lines will be treated with ACOX1 inhibitors under fasting conditions, and the rate of cell and death and cell proliferation will be determined again. The absolute expression of CYP4 genes will be measured by real-time PCR and western immunoblot analysis.

The proposed method of data analysis:

- The student will be responsible for the following:
- Maintaining seven hepatocellular cell lines in culture
- Isolate RNA from cell lines and produce cDNAs for determining expression levels of CYP4 genes and protein.
- Incubate HCC cell lines with different chain-length saturated dicarboxylic acids
- Determine the rate of cell proliferation in response to dicarboxylic acids
- Determine the rate of cell death in response to dicarboxylic acids
- Determine the effect of ACOX1 inhibitor on cell proliferation and death. Isolate total protein and RNA from cell cultures
- Synthesis of cDNA
- Determine absolute mRNA content by real-time PCR
- Determine the level of CYP450 protein by Western immunoblot
- Determine the rate of cell proliferation in cell culture
- Determine the rate of apoptosis cell death

The techniques and procedures the student will learn
The student will meet daily with the PI to discuss the objective of the day's experiment and will be taught by the PI and laboratory technician how to perform each experiment. The student will be taught how to record and interpret experimental results using prism statistical programs. Finally, the students will attend weekly meetings of the liver focus group and present results at least once to the group over the eight weeks.

Significance of anticipated findings:
These results may lead to novel, innovative treatments for hepatocellular carcinoma by identification of synthetic lethality pathways of induction of fatty acid omega hydroxylase genes with the inhibition of peroxisomal acyl-CoA oxidase. The results may explain why intermittent fasting improves chemotherapeutic efficacy in patients and reduces the toxic side effects of chemotherapy. These studies may also provide evidence of a new synthetic lethality pathway in treating liver cirrhosis and HCC.

Submit your application to Dr. James Hardwick – project 2 of 2

Title: Cytochrome P450 production of 20-HETE leads to liver cirrhosis and hepatocellular carcinoma.

Dr. James P. Hardwick
Professor of Biochemistry and Molecular Pathology
Department of Integrative Medical Sciences
NEOMED, F141
jph@neomed.edu

Abstract of Project

Cirrhosis is the outcome of chronic liver disease due to progressive liver injury and fibrosis. Cirrhosis leads to portal hypertension and liver dysfunction, progressing to complications such as ascites, variceal bleeding, hepatic encephalopathy, hepatorenal syndrome, hepatopulmonary syndrome, cirrhotic cardiomyopathy, sarcopenia, hepatocellular carcinoma, and coagulation disorders. The cause of increased portal hypertension and ascites is believed to be vasoconstriction of the portal vein and vasodilation of the splanchnic arterial system. We have recently found the increased cytochrome P450 4a11 mediated increase in the vasoconstrictive 20-HETE eicosanoid in the progression of non-alcoholic fatty liver disease (NAFLD) in human patients. It is believed that 20-HETE mediates its vasoconstrictive effect by activating the GPR75 receptor in endothelial cells and hepatocytes, leading to vasoconstriction and hepatocyte proliferation respectively. This study aims to determine levels of 20-HETE and 12-HETE in human livers from patients with cirrhosis and hepatocellular cell lines, and whether blocking CYP4A11 production of 20-HETE or 20-HETE activation of the GPR75 receptor inhibits hepatocyte cell proliferation.

Significance of overall research

Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer. Hepatocellular carcinoma occurs most often in people with chronic liver diseases, such as cirrhosis caused by hepatitis B, hepatitis C infection, or non-alcoholic fatty liver disease. Systemic chemotherapy for HCC is usually not well tolerated by patients with significant underlying hepatic dysfunction, and chemotherapy may also be less effective in patients with substantial cirrhosis. Although combination therapies such as the Tyrosine kinase inhibitor Sorafenib and the anti-vascular endothelial growth factor inhibitor bevacizumab prolonged survival, they failed to increase the remission rate. Therefore, it is imperative to design new therapies to treat advanced liver cirrhosis and HCC. We have shown that the fatty acid omega hydroxylase P4504A11 is increased in patients with liver cirrhosis and HCC, producing vasoconstrictive 20-arachidonic acid and 12-hydroxylic acid. These fatty acids are converted to dicarboxylic acids, metabolized by peroxisome β-oxidation to acetate that feeds lipogenesis. Therefore, inhibition of peroxisome acyl-CoA oxidase will decrease acetate production for lipogenesis and increase dicarboxylic acid that can un-couple mitochondria respiration leading to cell death. These studies will provide insight into the beneficial effects of fasting in increasing the efficacy of chemotherapeutic drugs and reducing their subsequent side effects.

Goals and Objectives of the summer research project

This research aims to determine the level of 20-HETE and 12-HETE in human samples of patients with Non-alcoholic fatty liver disease (NAFLD) and hepatocellular cell lines by high-pressure liquid chromatography and GC-MS/MS.

Investigative Research Methods

Seven different HCC cell lines with different cell proliferation and metastatic rates will be used throughout this study. These cell lines will be maintained in cell culture and treated with 20-HETE, and the cell death and proliferation rate will be measured. 2

The proposed method of data analysis

The student will be responsible for the following:

- Maintaining seven hepatocellular cell lines in culture
- Determine eicosanoid levels in NAFLD patient liver samples by HPLC/mass spectrometry. Isolate total protein and RNA from cell cultures
- Separation and of eicosanoid levels in human tissues.
- Determine absolute mRNA content by real-time PCR
- Determine the level of CYP450 protein by Western immunoblot
- Determine the rate of cell proliferation in cell culture
- Determine the rate of apoptosis cell death

The techniques and procedures the student will learn

The student will meet daily with the PI to discuss the objective of the day's experiment and will be taught by the PI and laboratory technician how to perform each experiment. The student will be taught how to record and interpret experimental results using prism statistical programs. Finally, the students will attend weekly meetings of the liver focus group and present results at least once to the group over the eight weeks.

Significance of anticipated findings

These results may lead to novel, innovative treatments for liver cirrhosis by targeted inhibition of vascular vasoconstriction and hepatocyte proliferation through CYP4A11-mediated production of 20-HETE.

Submit your application to Dr. Heather O'Leary

OJECT DESCRIPTION

Project Title: Preservation of endogenous low oxygen signaling facilitates hematopoietic cell phenotype, function, and clinical utility

Principal Investigator: Heather A. O'Leary, PhD; Assistant Professor Integrative Medical Sciences
Research Location: Northeast Ohio Medical University College of Medicine
RGE-244 RGE-100
RGE-110 Comparative Medicine Unit

Abstract:
Hematopoietic stem cells (HSC) are derived in the bone marrow (BM), give rise to all lineages of cells of the immune system throughout life, and can be transplanted to reconstitute the immune system. The bone marrow niche provides critical signals for hematopoietic stem and progenitor cell (HSC/HSPC) maintenance, self-renewal, and differentiation. Previous studies identified retaining HSC/HSPC in endogenous low Oxygen (low O_2, ~1-4% O_2) retains stem cell number, phenotype, and function, which is blunted when HSC/HSPC are exposed to ambient air (20% O_2). Despite this knowledge, most studies are performed in air, leaving endogenous signaling mechanisms unidentified, HSC/HSPC phenotype/function diminished, and transplant efficiency sub-optimal leading to high costs and patient complications. To address these knowledge gaps, we have generated and patented novel equipment (US-11633730-B2 Issued 04/2023 (O'Leary)) to isolate/sort/analyze and transplant HSC/HSPC under endogenous, continuous low O_2 conditions. Using this novel technology, we generated the first reference landscape of endogenous low O_2 HSC/HSPC phenotype/signaling/function. These studies identified important roles for multiple mechanistic pathways including but not limited to; serine peptidase (Dipeptidylpeptidase-4, DPP4) and cytosolic/mitochondrial Calcium (Ca^{2+}) in the optimal regulation of HSC/HSPC phenotype/function in low O_2, during health and disease states, and set the foundation for discovery of additional novel regulatory pathways. Retaining HSC/HSPC in low O_2 for optimal clinical utilization is technically challenging and brief exposure to ambient air ablates the low O_2 enhancement in numbers, phenotype, and function. Therefore, our goals for this study are to identify, and analyze, the differential regulation of endogenous low O_2 pathways to facilitate the pharmacologically mimicking of the low O_2 HSC/HSPC signaling, phenotype, and function, in air, enhancing transplant efficiency/utility.

Significance:
Hematopoietic cells are the only organ tissue that can be consistently transplanted with minimal or no risk to donor, autologously (self) or allogeneically (to another person), and result in reconstitution of the entire immune system, and cure of some diseases (bone marrow failure, sickle cell anemia, HIV with CCR5 mutated cells). The full potential of these cells has not been achieved due to a lack of understanding of signaling and function under endogenous low O_2 conditions. Manipulation of cells in air facilitates diminished numbers, and quality, of HSCs resulting in inefficient engraftment/complications, enhanced numbers of cells needed for transplant, requiring multiple donors for utilization of cord blood, and increased financial burden with most allogeneic transplants costing between $400,000-$800,000. Identification, and replication, of critical endogenous HSC signaling will facilitate enhanced phenotype, function, and interaction with the BM microenvironment resulting in enhanced cell utility/transplant efficiency, decreased cost, and diminished complications.

Goals and Objectives for the Research Project
Our goals for this project are to identify, and analyze, the differential regulation of endogenous low O_2 pathways to facilitate the pharmacologically mimicking of the low O_2 HSC/HSPC signaling, phenotype, and function, in air, enhancing transplant efficiency/utility.

Research Methods and Resources
Samples utilized will be isolated from animal models (C57 BLK/6 background mice that are control or have mutations to mimic human disease states, blood and bone marrow) as well as from primary patient samples (umbilical cord blood, peripheral blood of patients with and without hematologic disease).

Phenotypic and functional studies will consist of flow cytometric analysis and sorting of cell populations (BD Melody, FACS Chorus, patented equipment), pharmacological and molecular analysis, generation of additional omics data (single cell RNA sequencing, proteomics etc.) replating studies to *in vitro* determine stem cell capabilities, and *in vivo* engrafting studies. High level statistical and bioinformatic analysis is completed in collaboration with additional expertise. Please see publications for in-depth overview and example details (PMID: 37051890, PMID: 28344320, PMID: 26073944).

Methods of Data Analysis

Flow cytometry and statistical data analysis will be performed using commercially available software (FLOWJO, SigmaPlot, & GraphPad). Based on the study design, many data will be analyzed using paired t-tests and repeated measures ANOVA.

Anticipated Findings

Our published and preliminary data suggest that combined pharmacological inhibition will enhance phenotype and signaling in HSC/HSPC populations, we anticipate these further studies will facilitate the identification of additional pathway interactions to modify and lead to determination of functional, clinically relevant, alterations.

STUDENT FELLOW TRAINING/MENTORING PLAN

Training Plan

All learners will participate in background literature review as well as required CITI training and CMU training in accordance with IACUC or IRB protocols. Resources in the form of webinars, lectures, and access to American Society of Hematology (ASH) education materials are made available for those that want additional insight. As Dr. O'Leary has a long-standing history with ASH and their programs/awards, those interested in hematology will have support to help increase their interaction with ASH and their programs, if desired. Training is structured on an individual basis to ensure support of the trainee for their long-term overall goals as well as their lab goals. All trainees are taught through a "see one, do one, teach one" model and every member of the laboratory will cross-train in every function of the laboratory from sample collection to data acquisition and analysis to dissemination/write up of information under the instruction and supervision of Heather A. O'Leary Ph.D. and additional lab staff. Each member of the laboratory will have bi-weekly individual meetings in addition to group/lab meetings to discuss short/long term goals, current experimental data, intellectual scientific development, individual strengths and where the trainee needs additional support etc. Trainees will also participate in departmental and other research group meetings, seminar speakers etc. Trainees will be encouraged to assist our collaborators in any studies, at the discretion of those laboratories, and will be encouraged to explore the techniques and approaches employed by other faculty. Dr. O'Leary makes herself readily available for questions and consistent guidance as well as assists in learning data analysis, weekly data analysis, generation of data figures, presentations, and practice for presentations. The lab goal is to have a fun, inclusive, safe, and productive environment for learning where people feel comfortable asking questions and are willing to work as a team to support each other. If desired, Dr. O'Leary is happy to connect you with former lab members to discuss the mentoring and laboratory environment.

Submit your application to Dr. Priya Raman

PROJECT TITLE: Effect of cytokine storm on vascular smooth muscle cell phenotype under healthy vs. diseased conditions
PRINCIPAL INVESTIGATOR: Priya Raman, Ph.D., Associate Professor, Integrative Medical Sciences
LOCATION: NEOMED-Rootstown

ABSTRACT OF PROJECT

Recent Covid-19 pandemic has provided evidence for a potential link between Covid-19 infection and future risks of cardiovascular complications. Atherosclerosis is a major player in the development of several cardiovascular complications including myocardial infarction, heart failure and stroke. Elevated serum cytokine levels or systemic 'cytokine storm' (CS) is one of the most common clinical manifestations of severely ill hospitalized Covid-19 patients. Clinical studies have suggested that pre-existing risk factors for vascular disease, such as diabetes, obesity, and dyslipidemia, can exacerbate the inflammatory responses of the vasculature to CS prompting atherosclerotic complications. However, the molecular mechanism(s) by which CS may trigger atherosclerosis are unknown. De-differentiation of vascular smooth muscle cells (VSMC) from 'quiescent' contractile to 'synthetic' proliferative phenotype is a key event for development of atherosclerosis. This project aims to study whether CS conditions may induce VSMC phenotypic transition to a diseased proatherogenic phenotype, and further interrogate whether this effect is more pronounced under diabetic conditions. For these studies, we will utilize murine aortic SMC (MASMC) primary cultures isolated from healthy and diabetic mice. MASMCs will be exposed to different concentrations of CS cocktail in vitro for varying periods of time; VSMC signaling, and differentiation marker expression as a readout of VSMC phenotypic transition will be assessed using immunoblotting and immunocytochemistry.

BACKGROUND AND RATIONALE

Cumulative evidence from basic sciences and clinical observations highlights a strong correlation between Covid-19 exposure and risks of accelerated vascular complications. 'Cytokine storm', a hallmark of host-immune responses and immune dysregulation induced by the deadly and pathogenic coronavirus, SARS-CoV-2, is a typical manifestation of critically ill Covid-19 patients. Systemic cytokine storm relevant to Covid-19 infection is clinically marked by elevated levels of serum inflammatory cytokines (e.g., TNF-□, IFN-γ, CXCL9 and CXCL10, chemokines induced by IFN-γ), interleukins (IL)-1□, 6, 10, and soluble IL-2 receptor alpha. Clinical studies suggest that individuals with pre-existing risk factors for vascular disease, such as diabetes, obesity, and dyslipidemia, are more vulnerable to the onslaught of 'cytokine storm', characteristic of hospitalized Covid-19 patients, regardless of race, socioeconomic disparities, and ethnicity. However, the molecular mechanisms by which 'cytokine storm' exacerbates the inflammatory responses of the diseased vasculature, triggering accelerated vasculopathy in individuals with pre-existing cardiovascular risk factors remain poorly understood.

Atherosclerosis is a major player in the development of numerous cardiovascular complications and accounts for increased morbidity and mortality in individuals with pre-existing risk factors for vascular disease. Recent studies provide strong evidence for the direct involvement of vascular smooth muscle cells (VSMC), a major cell type in the blood vessel, in atherosclerotic lesion formation. Growing literature indicates that VSMC de-differentiation from 'quiescent' contractile to 'synthetic' proliferative phenotype is a critical mediator of augmented atherosclerotic lesion progression.

The proposed summer project is part of a larger research program that aims to interrogate whether pre-existing metabolic or vascular anomalies can stimulate VSMC proatherogenic phenotype prompting atherosclerotic complications following exposure to elevated concentrations of a cytokine cocktail, mimicking the systemic 'cytokine storm' manifested in hospitalized Covid-19 patients requiring intensive care. In the proposed project, we will determine the impact of 'cytokine storm' conditions *in vitro* on the cellular and molecular phenotypic properties of VSMCs isolated from healthy and diseased mice.

GOALS AND OBJECTIVES

Goal: We will investigate the effect of 'cytokine storm' conditions *in vitro* on the smooth muscle phenotypic characteristics of murine aortic smooth muscle cell (MASMC) primary cultures isolated from healthy and diabetic mice.

Objectives:

1. To compare expression of VSMC contractile and synthetic markers in healthy vs. diabetic MASMC primary cultures exposed to 'cytokine storm' cocktail *in vitro*.

2. To compare expression of signaling mediators of VSMC growth and migration in healthy vs. diabetic MASMC primary cultures exposed to 'cytokine storm' cocktail *in vitro*.

3. To compare expression of key transcriptional regulators of VSMC differentiation in healthy vs. diabetic MASMC primary cultures exposed to 'cytokine storm' cocktail *in vitro*.

Experimental Design. We will use MASMC primary cultures, isolated from healthy wild-type and diabetic agouti KKAy+/- mice, from our in-house cell culture banks for these studies. A subset of the wild-type MASMC cultures will be incubated with high glucose (30mM) to mimic the diabetic milieu *in vitro*. To induce systemic inflammation *in vitro* consistent with Covid-19-induced cytokine storm (CS), wild-type and diabetic MASMC cultures will be incubated with a cytokine cocktail mix containing the following components: IL2 (2ug), IL6 (1ug), IL10 (0.5ug), TNF-alpha (1ug), IFN-gamma (2ug), IL-4Ralpha (2ug), ACE2 (1ug), IL4 (1ug), IL13 (2ug). Our recipe for the CS cocktail is based on a recent publication where different CS concentrations were injected in murine models to study the cytokine storm-based mechanisms for extrapulmonary manifestations of SARS-CoV-2 infection (JCI Insight. 2023;8(10):e166012. https://doi.org/10.1172/jci.insight.166012). Cells will be exposed to different concentrations of CS cocktail (low, medium, high) for varying periods of time (short-term: 1d, 3d; long-term: 7d, 10d). This will be followed by cell harvests for the following in vitro assays.

INVESTIGATIVE METHODS TO BE USED

Cell Culture: We have already isolated MASMC primary cultures from wild-type and diabetic KKAy+/- mice in our lab and have multiple frozen vials stored at different passages. MASMC primary cultures will be grown in DMEM/F12 media supplemented with 10% FBS and appropriate antibiotic and antimycotic solutions. About 80% confluent cells will be allowed to grow overnight in serum-starved media. This will be followed by incubation of cells with different concentrations of CS cocktail for short-term (1-3 days) and long-term (7-10 days) duration. In a parallel study, following overnight serum-starvation, 80% confluent wild-type cells will be incubated with 30 mM glucose to mimic the diabetic milieu *in vitro* ± CS cocktail to induce systemic inflammation *in vitro*. Following these treatments, cells will be harvested and utilized for immunoblotting and immunocytochemistry experiments as outlined below.

Immunoblotting: Whole cell lysates will be prepared in 1X RIPA lysis buffer containing protease and phosphatase inhibitors. Protein content will be measured using the BCA protein assay. Equal concentrations of protein lysates (10-20 µg) will be resolved on 8-12% SDS-PAGE followed by wet transfer to PVDF membranes. Immunoblotting will be performed using the following antibodies: ACTA2, CNN1, LMOD1, SM22 (SM contractile markers); VIM, OPN (SM synthetic markers); p-ERK1/2, t-ERK1/2, p-p38, t-p38, p-AKT, t-AKT (VSMC signaling mediators); SRF, YY1, ELK1, KLF4 (transcriptional regulators of SM differentiation). Equal protein loading of samples will be confirmed by staining the membranes with Ponceau S and probing with tubulin or β-actin antibodies (loading controls).

Immunocytochemistry: Cells will be grown on coverslips in 6-well cell culture clusters and treated as described above under "Cell Culture". At endpoint, cells will be fixed and permeabilized in a solution containing 4% PFA and 0.2% TTX. Following this, cells will be blocked in 5% donkey serum followed by incubation with primary antibodies against SM contractile markers (ACTA2, CNN1, SM22). After a brief PBS wash, cells will be incubated with appropriate Alexa Fluor 594 or 647 secondary antibodies.

Coverslips will then be mounted on DAPI-containing mounting media for cell nucleus visualization. To control for non-specific staining, identical set of cells will be incubated with species-specific IgG control antibody in the absence of the corresponding primary antibodies or no primary antibody. Coverslips will be observed using the Olympus fluorescence IX71 microscope (10× magnification) and images will be digitally captured using an identical set of parameters across all samples, specific for each antibody.

PROPOSED METHOD OF DATA ANALYSIS

Each experiment will be repeated at least three times with two to three replicates for each treatment within an independent experiment. For immunofluorescence experiments, six to eight images will be collected for each individual treatment within an independent experiment. Densitometric quantification of immunoblots and immunofluorescence quantification will be performed using ImageJ software. All data will be presented as Mean ± SEM; statistical significance will be analyzed by one-way ANOVA (using GraphPad Prism Software) followed by post-hoc Tukey HSD test or unpaired Student's t-test (two-tailed), as appropriate. Any data that does not meet the assumptions of ANOVA will be analyzed using non-parametric statistics; $p \leq 0.05$ considered statistically significant.

SIGNIFICANCE OF ANTICIPATED FINDINGS

Expected Outcome. We predict that exposure to CS cocktail will induce VSMC phenotypic transition to a disease proatherogenic phenotype and this effect will be more pronounced in diabetic MASMC cultures compared to healthy MASMC.

Impact. The proposed studies will provide key pilot data that will lead to future studies aimed to interrogate the role of Covid-related cytokine storm in VSMC phenotypic transformation and development and progression of atherosclerotic complications under conditions of diabetes, obesity and hyperlipidemia, and further elucidate the underlying molecular mechanisms.

SUMMER RESEARCH FELLOW TRAINING/MENTORING PLAN

Plan for Training/Mentoring: The summer research fellow will be supervised and mentored by Dr. Priya Raman. The student will receive hands-on training from Dr. Raman and her laboratory personnel during the first 2-3 weeks of the program. Upon demonstration of adequate independence, the summer fellow will be expected to run independent experiments under close supervision by Dr. Raman and her team. Dr. Raman will be responsible for student training on all aspects of this project, including data analysis and graphical presentations, interpretation of data and poster preparation and presentation. After the initial period involving casual interactions, Dr. Raman will meet with the student one-on-one at least once per week to discuss progress/data and will be trained in scientific reading relevant to the field of study. The student is also expected to participate in departmental seminars and weekly group meetings. These meetings will develop the student's research horizons and enhance his/her scientific presentation and perception skills. At the end of the training period, the student will be expected to submit a brief report summarizing the project and results as well as present work during NEOMED's Annual Poster Day. Description of Resources available: The student will have access to Dr. Raman's laboratory, including all necessary supplies and reagents in her lab as well as other departmental core facilities, as needed for completion of the proposed studies. The summer fellow will also have access to graphing and imaging software, as needed.

Site where the research will be conducted: This project will be conducted in Dr. Raman's laboratory within the department of Integrative Medical Sciences at NEOMED.

Submit your application to Dr. Liya Yin

Title: The regulation of mouse coronary collateral growth
Principal Investigator: Liya Yin, Associate Professor, Integrative Medical Sciences
Location: NEOMED

Abstract. Ischemic heart disease continues to be a leading cause of death, and ill-health in the United States. The presence of coronary collateral vessels—the naturally occurring vessels that supply flow to an area of the heart to bypass a blocked vessel—confers a significant benefit to patients. The incidence of death decreases. The ability to survive a heart attack is better. And the amount of tissue that dies following a heart attack is less. However, the presence of such collateral vessels occurs in only 10-15% of all patients, so that the vast majority suffer the full consequences of death and ill-health in the event of a blockage in a vessel supplying the heart muscle. Currently, our understanding of coronary collateral growth (also termed coronary arteriogenesis) is based on studies in live animals, in which certain inhibitors are administered to reduce the vascular growth. A limitation of such "loss of function" studies is the cellular "target" of the inhibitor is unknown. The inhibitor could be acting on endothelial cells, smooth muscle cells, cardiac myocytes, inflammatory cells, and/or fibroblasts. Currently there is no way to decipher the cell-based mechanisms of coronary blood vessel growth. Moreover pharmacological inhibitors suffer from the problem on non-specificity. To overcome these deficiencies, we use the transgenic mouse model to interrogate many questions regarding regulation of process of coronary arteriogenesis in normal or diseased model (obesity and diabetes) and which cell types may be involved in this adaptive vascular growth. We hope that these studies will eventually lead to new therapies designed to help patients with ischemic heart disease grow new blood vessels in their hearts.

The significance of the overall research
The patients with coronary collaterals have nature bypass during ischemia and have better prognosis after heart attack. If we understand the mechanism of regulation of coronary collateral growth, we can stimulate coronary collateral growth and amplify the effect of coronary collateral growth, especially for the patients who have impaired coronary collateral growth such as patients with metabolic syndrome.

Goals and objectives. The goal of this summer research is to study the mechanism of coronary collateral growth and how to stimulate and amplify the effect of CCG.

Research Methods. Mice will be anesthetized, intubated, and prepared for sterile surgery (involved areas will be shaved and scrubbed with betadine). In all animals an incision will be made through the sternum, and a special occluder will be situated on the surface of the heart around the left anterior descending artery. The wounds will be repaired and the chest evacuated and closed. Post-operative pain will be treated by injection of an analgesic for the first day post-op, and then as needed (we will defer to the attending veterinarian's advice). At several points after implantation of occluder, we will perform non-invasive echocardiography to evaluate cardiac function. In some animals, terminal experiments will be made at intervals (days 3, 7, and 14) up to 21 days after implantation of the occluder using contrast echocardiography to measure flow. These measurements will be made while the animals are anesthetized using gas anesthesia. Final measurements blood flow to the heart and blood pressure will be made in anesthetized mice 21 days following implantation of the occluder using contrast. This is done in anesthetized mice (isoflurane) in which arterial pressure is measured from a femoral catheter, and contrast microbubbles and drug infusions are done via a tail vein catheter or a catheter inserted in a jugular vein. After completion of the measurements, the mouse will be euthanized and the hearts will be removed for various in vitro and imaging studies. We will digest the heart and isolate cells

for single cell RNA-sequecing. We also use the microfil to perfuse the heart for micro-CT to analyze the coronary collateral growth. We also use immunohistology and molecular biology techniques to study the genes/proteins involved in CCG.

Proposed method of data analysis. The analysis will involve only unpaired t-tests as we will compare shams to animals instrumented with the occluder. $P<0.05$ will be accepted for statistical significance.The bioinformatic data analysis will be used for single cell -RNA -sequecing for temporal difference at different stage of CCG.

Significance of anticipated findings. If the study is successful, it will accelerate our understanding of mechanisms underlying coronary collateral growth as we will be able to use better link particular genes with this adaptive process. This will lead to the novel therapeutic approach to patients with ischemic heart diseases.

Summer Research Fellow Training/Mentoring Plan. The plan we have devised is arranged in a hierarchical manner.

- First, the student will interact in a 1:1 manner with Dr. Liya Yin for experimental design, the protocols, data collection and reviewing and the interpretations.
- Second, the student will interact with other lab personnel including graduate students, postdoc, research assistant for surgery, transgenic mouse breeding, phenotyping, contrast echo calculation and image analysis of micro-CT.
- Third, the student will present in our lab meetings and the Cardiovascular Interest group (a combined lab meeting of the faculty with interest in cardiovascular research (Drs. Chilian, Penn, Chen, Bratz, Raman, Thodeti, Meszaros, Yin, Ohanyan, Dong, Mayorga, and Yun) and will present her results in this weekly meeting.
- Fourth, the student will participate in a summer journal club that will involve all the summer research students and faculty. Each summer student will be expected to participate.
- Fifth, the student will be expected to present a poster at the research day when all summer fellows present a synopsis of their work.

All the necessary resources (echocardiographs, anesthesia machines, computer for measuring valuating echo images, mice, surgical instruments, surgical supplies, ultrasonic contrast, micro-CT, Fluorescent imaging system including multiphoton and confocal scope) and financial resources for completing the research are available.

The research will be completed at NEOMED.

Submit your application to Dr. Yanqiao Zhang

The Role of Intestinal Hepatocyte Nuclear Factor 4α in Alcoholic Liver Disease

PI: Dr. Yanqiao Zhang
Professor of Integrative Medical Sciences, NEOMED

Abstract: Hepatocyte nuclear factor 4α (HNF4α) plays an important role in glucose and lipid metabolism. So far, the role of intestinal HNF4α in the pathogenesis of alcoholic liver disease (ALD) is unknown. In this project, we plan to use mice lacking intestinal HNF4α to investigate the role of HNF4α in ALD. The mice will be subjected to an NIAAA alcohol diet for 2 weeks. We will investigate whether loss of intestinal HNF4α protects against or aggravates ALD as well as the underlying mechanisms.

Background and rationale: ALD is one of the most common liver diseases worldwide. So far, the underlying mechanism is not well elucidated. HNF4α is expressed in the liver, pancreas, and intestine. Given that intestinal HNF4α regulates chylomicron secretion, intestinal HNF4α may also regulate alcohol absorption or metabolism.

Goals and objectives: We will investigate whether intestinal HNF4α plays a role in the pathogenesis of ALD and will also determine the underlying mechanism.

Investigative methods to be used: We will use the following mice: *Hnf4α*$^{fl/fl}$ mice and *Hnf4α*$^{fl/fl}$ X vilin-Cre (*Hnf4α*$^{INT-/-}$) mice. The mice will undergo the following protocol: mice will be fed a liquid control diet for 5 days, and then fed either a liquid control diet or a diet containing 5% alcohol for 10-15 days. On the last day of the study, the mice will be gavaged with alcohol and then euthanized after 9 h. Plasma and liver will be collected. Plasma levels of triglycerides (TG), cholesterol, AST, and ALT will be determined. Hepatic TG and cholesterol levels will also be determined. In addition, liver sections will be used for oil red O staining, H & E staining, and Sirius red staining (for staining of fibrosis). Hepatic mRNA levels of genes involved in lipid metabolism, inflammation, and fibrosis will be determined by qRT-PCR, and hepatic protein levels will be determined by Western blots. We predict that hepatic FOXA3 will protect against ALD.

Proposed method of data analysis: Student two-tailed t-test and one-way or two-way ANOVA (GraphPad Prism 10 software).

Significance of anticipated findings: The proposed studies will provide evidence regarding whether intestinal HNF4α plays a role in the development of ALD, and may lead to the identification of intestinal HNF4α as a novel target for treatment of ALD.

Mentoring plan

The summer research student will be mentored by the lab's PI or a research scientist in the lab. The postdoctoral fellows and graduate students in the PI's lab will provide technical support for the student.

The PI will provide the summer research student with the background information and will work with the summer research student to develop the rationale, hypothesis, and approaches to test the hypothesis. The PI and his lab staff will work closely with the summer research student to make sure the project will move forward as expected.

The student will also attend the PI's weekly lab meeting and report the progress of the project in the lab meeting. The student will also present his/her data on the NEOMED's research/poster day.

Through this training, the student is expected to learn how to design studies to test a hypothesis. He/she will also learn lots of experimental techniques and how to work with animals, including animal gavage, RNA extraction, Western blots, Oil Red O staining, H & E staining, Sirius red staining, lipid extraction, measurements of AST, ALT, and lipids, etc.

Resources All the mice and reagents are available for this study.

Performance site NEOMED (PI's lab – F-205 and CMU for animal study).

Submit your application to Dr. Christine Crish

Project Title: Colocalization of αVβ integrins with neurons and glia in hippocampus of Alzheimer's model mice
PI: Christine M. Crish, Assistant Professor
Location: Dept. of Pharmaceutical Sciences, NEOMED

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, tau pathology begins to accumulate in the brain, yet in fully symptomatic disease stages, spread of brain tauopathy closely correlates with progression of cognitive decline. There is a critical need to understand the factors fueling progression of tauopathy across these disease stages, and this defines the overall purpose of our research to identify early disease mechanisms that can be targeted to prevent onset of dementia.

A potential contributor to tauopathy progression involves the dysfunctional signaling of cell surface integrins. In the healthy adult brain, integrins are expressed ubiquitously and play important roles in neural plasticity. Recent studies have suggested that changes in expression levels and function of specific integrin subtypes may be associated with onset and spread of Alzheimer's brain pathology. Our lab has preliminary data showing that αVβ1 and αVβ5 integrins are dramatically upregulated in hippocampus of transgenic tauopathy model (htau) mice, and this increase occurred over a 5-month period as these animals aged from presymptomatic (5mo.) to symptomatic ages (10 mo.). The proposed project will work to determine whether αVβ1 and αVβ5 integrins in the hippocampus colocalize with neurons, astrocytes, or microglia in presymptomatic and symptomatic aged htau Alzheimer's disease model mice. Student fellows will be expected to work with brain tissue obtained from mice to conduct immunofluorescence assays to label αVβ integrins in target brain regions and use microscopy imaging techniques to identify cell types and quantify integrin distributions.

Significance:
Dysfunctional integrin signaling has been widely studied as a catalyst for various cardiovascular diseases and cancers, but its relevance to neurodegenerative disease is only just emerging. Integrins are highly accessible, druggable targets for which many modulating agents already exist and are even FDA-approved. Thus, our research proposes to grow the overall understanding of how aberrant integrin signaling contributes to tauopathy.

Goals & objectives:
Goal A: Prepare tissue for immunofluorescent assays.
Students will learn how to section fixed mouse brain sections on a microtome, store sections properly, and use knowledge of neuroanatomy to identify hippocampal brain sections for assays.

Goal B. Perform histological immunofluorescent labeling assays on brain tissue to identify integrins, neurons, and glia. 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 C. **Perform microscopy and analyze images to determine integrin expression patterns**
Students will use microscopy imaging to identify the extent to which αVβ1 and αVβ5 integrins colocalize with neurons, astrocytes or microglia in sections of hippocampus, and provide both qualitative and quantitative reports describing these findings.

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 colabel expression of αVβ1 and αVβ5 integrins with specific cell types in hippocampal sections of mice. We will compare integrin distribution between groups of age- and sex-matched Alzheimer's model htau 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.

Proposed method of data analysis

Our goal is to quantify the number of integrin co-localized neurons, astrocytes and microglia. These data will be analyzed for significant differences using SPSS for IBM Statistical Software. The PI will directly guide the student fellow in the use of this program to perform simple analyses describing mean differences from groups. The student will also be required to generate figures and illustrations depicting important findings using Prism and Adobe Illustrator.

Outcomes of research findings

This project will generate fundamental data on integrin expression patterns in tauopathy, which is only just beginning to be understood, and holds promise as an important etiological mechanism in early stage dementia. 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.

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 not work directly with animals, only brain tissue obtained from animals, and therefore, will not be required to complete any CITI-training for working with live animals. 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 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.

Submit your application to Dr. Sheila Fleming – project 1 of 2

Title: Gene-Environment Interactions in Parkinson's Disease
PI: Sheila Fleming, Ph.D. Assistant Professor in the Department of Pharmaceutical Sciences
Location: Research and Graduate Education Building, RGE-400

Abstract: Parkinson's disease (PD) is the most common neurodegenerative movement disorder and is characterized by the loss of dopaminergic neurons in the substantia nigra and the development of lewy bodies and lewy neurites in the brain and periphery. While the cause of the majority of cases is unknown, it is generally considered that gene-environment interactions underlie most cases of PD. Therefore, the identification of gene-environment interactions associated with PD-like pathology and neurodegeneration is an important goal in the field. ATP13A2 is a P5-ATPase of the P-type ion transport ATPase superfamily and loss of function mutations cause the neurodegenerative condition Kufor-Rakeb Syndrome, an autosomal recessive form of PD. The function of ATP12A2 is unclear but *in vitro* studies suggest it may be involved in the lysosomal degradation of proteins, polyamine and heavy metal transport (manganese and/or zinc), and mitochondrial function, all mechanisms that can overlap with PD. An important next step is to determine how loss of function of ATP13A2 *in vivo* interacts with environmental factors such as heavy metals and toxicants that interfere with cellular transport, protein degradation, and mitochondrial function. It is hypothesized the loss of ATP13A2 function causes an increased vulnerability to the toxic effects of certain heavy metals and pesticides associated with PD. This hypothesis will be tested using Atp13a2-deficient mice that have been shown to develop age-dependent motor impairments, enhanced accumulation of lysosomal storage material, and increased accumulation of the PD protein alpha-synuclein. Wildtype and Atp13a2-deficient mice will be exposed to different metals and toxicants associated with PD (ex. manganese). Sensorimotor function will be measured and in the brain accumulation of the PD protein alpha-synuclein and neurodegeneration will be determined. A combination of behavioral, cellular, and molecular techniques will be employed.

Background and Rationale: PD belongs to a group of diseases known as synucleinopathies, where the presynaptic protein alpha-synuclein abnormally accumulates in the brain and periphery. Alpha-synuclein is a major component of lewy bodies, the pathological hallmark of synucleinopathies and a key protein in the study of PD. Inherited forms of PD show that mutated or increased alpha-synuclein can lead to the development of PD. Thus, the identification of genetic and environmental factors that can increase alpha-synuclein accumulation and toxicity could have a major impact on the development of therapeutics for the disease. P-type ATPases are a large family of proteins involved in the transport of cations and other substrates across cell membranes through the utilization of energy from ATP hydrolysis (Schultheis et al., 2004; van Veen et al., 2014). Functionally, they are involved in essential cellular processes including vesicular transport and excitability. ATP13A2 is most abundant in the brain and loss of function mutations in humans causes Kufor-Rakeb Syndrome, an autosomal recessive form of PD. More recently, ATP13A2 polymorphisms have been linked to an enhancement of the neurotoxic effects of manganese in an elderly population. Loss of ATP13A2 function in mice causes age-related sensorimotor impairments, gliosis, enhanced lysosomal storage material, and increased alpha-synuclein accumulation (Schultheis et al., 2013; Kett et al., 2015). This suggests ATP13A2 could be an important factor in gene-environment interactions associated with PD.

Goals and Objectives: The goal of these studies is to understand the role of ATP13A2 in cellular dysfunction and neurodegeneration. The objective is to characterize ATP13A2 x environmental exposure interactions and determine the mechanisms by which they contribute to behavioral dysfunction and neurodegeneration *in vivo*.

Investigative Methods: A combination of behavioral, cellular, molecular, and genetic methods will be employed to determine the effect of different environmental exposures in Atp13a2-deficient mice.

Environmental Exposures. Separate cohorts of wildtype and Atp13a2-deficient mice will be exposed to manganese. Mice will then be behaviorally tested to determine the effect of the exposures on sensorimotor function and cognition. In the brain alpha-synuclein accumulation, mitochondrial bioenergetics, and neurodegeneration of the nigrostriatal dopaminergic system will be determined.

Behavioral methods. Sensorimotor function will be assessed using a battery of tests shown to be sensitive in genetic mouse models of PD (Schallert et al., 1978; Fleming et al., 2004; Schultheis et al., 2013). Cognitive function will be determined using tests that measure aspects of attention, memory, and executive function.

Alpha-Synuclein Accumulation (brain). Soluble and insoluble alpha-synuclein protein will be measured using both immunoblot and immunohistochemistry techniques.

Mitochondrial Bioenergetics. Mitochondrial bioenergetics will be measured in multiple brain regions using Seahorse analysis.

Neurodegeneration (brain). Neuron counts will be measured using immunohistochemistry in the substantia nigra. Dopamine neurons in substantia nigra pars compacta and dopamine terminals in the striatum will be identified utilizing tyrosine hydroxylase immunohistochemistry protocols routinely used in the lab.

Proposed Method of Data Analysis: A combination of parametric and non-parametric statistics will be used to analyze the behavior and tissue data. For parametric statistics, 2X2 randomized ANOVA will be used to analyze genotype (wildtype and Atp13a2-deficient) and treatment (vehicle and manganese). Post hoc comparisons will use the Bonferroni corrected factor when multiple comparisons are being made. For scores that do not meet the assumptions of ANOVA nonparametric statistics will be used to compare genotypes and treatment.

Significance of Anticipated Findings: It is anticipated that Atp13a2-deficient mice will be more sensitive to the toxic effects of environmental exposures compared to wildtype mice. It is anticipated that exposed Atp13a2-deficient mice will show more severe alterations in behavior than exposed wildtype mice and vehicle-treated Atp13a2-deficient mice. In the brain it is expected that exposed Atp13a2-deficient mice will have increased alpha-synuclein accumulation, impaired mitochondrial function, and nigrostriatal cell loss compared to exposed wildtype mice and vehicle-treated Atp13a2-deficient mice. These findings will be significant because they will reveal a novel gene-environment interaction that could lead to neurodegeneration in humans. This would also identify ATP13A2 as a potential target for neuroprotection or therapeutic intervention.

Appendix:

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Schallert T, Whishaw IQ, Ramirez VD, Teitelbaum P. 1978. 6-hydroxydopamine and anticholinergic drugs. Science. 1978; 202(4373): 1216-1217.

Schultheis PJ, Fleming SM, Clippinger AK, Lewis J, Tsunemi T, Giasson B, Dickson DW, Mazzulli JR, Bardgett ME, Haik KL, Ekhator O, Chava AK, Howard J, Gannon M, Hoffman E, Chen Y, Prasad V, Linn SC, Tamargo RJ, Westbroek W, Sidransky E, Krainc D, Shull GE. Atp13a2-deficient mice exhibit neuronal ceroid lipofuscinosis, limited α-synuclein accumulation and age-dependent sensorimotor deficits. Hum Mol Genet. 2013; 22(10): 2067-2082.

Schultheis PJ, Hagen TT, O'Toole KK, Tachibana A, Burke CR, McGill DL, Okunade GW, Shull GE. Characterization of the P5 subfamily of P-type transport ATPases in mice. Biochem Biophys Res Commun. 2004; 323(3): 731-8.

van Veen S, Sørensen DM, Holemans T, Holen HW, Palmgren MG, Vangheluwe P. Cellular function and pathological role of ATP13A2 and related P-type transport ATPases in Parkinson's disease and other neurological disorders. Front Mol Neurosci. 2014; 7:48.

Student Fellow Training/Mentoring Plan:

This is a large project that is ongoing in the lab. The PI will work with the student to determine what aspect of the project best suits his/her interests, abilities, and goals. The student would have the option to work mainly on one aspect of the project (such as behavioral testing and analysis or tissue processing and immunohistochemistry) or multiple aspects of the study. The student will meet with the PI on a weekly basis to discuss project progress and literature in the field. In addition to individual meetings the student will attend regular lab meetings where each person in the lab discusses the project they are working on and the progress or setbacks they have encountered. Short PowerPoint presentations are encouraged during these meetings as they will keep the student on track for the final poster session at the end of the summer.

*Resource*s. The lab has all resources necessary for the student to complete a summer project. Mutant mice are available and behavioral testing protocols are already established. Supplies and space for tissue processing are also available.

Location. The experiments will be conducted primarily in the laboratory area in RGE-200. There is behavioral testing space in C-129 where motor and cognitive testing will take place. The student will have a desk and access to a computer in the write-up area for data analysis and presentation.

Submit your application to Dr. Sheila Fleming – project 2 of 2

Title: The effect of exercise in Parkinson's Disease
PI: Sheila Fleming, Ph.D. Assistant Professor in the Department of Pharmaceutical Sciences
Location: Research and Graduate Education Building, RGE-100

Abstract: Parkinson's disease (PD) is the most common neurodegenerative movement disorder and is characterized by the loss of dopaminergic neurons in the substantia nigra and the development of alpha-synuclein positive lewy bodies and lewy neurites in the brain and periphery. The cardinal motor symptoms in PD (rigidity, resting tremor, bradykinesia, postural instability) are well studied and can be managed to a certain extent with dopamine replacing therapies. However, there are also a host of non-motor symptoms that can negatively impact the quality of life for people with PD. Cognitive dysfunction is one of those symptoms. It is common and can progress to dementia over time. Unfortunately, how dementia develops in PD is unclear. In this project we are working to develop a new model of PD that develops dementia. In addition, we will examine whether exercise therapy using treadmill running can protect against the development of cognitive dysfunction and dementia in our new rat model.

Background and Rationale: Cognitive dysfunction is a common and potentially debilitating non-motor symptom in Parkinson's disease (PD) that profoundly reduces quality of life for patients and caregivers. Although much is understood about the underlying pathology associated with motor symptoms in PD, less is known about the pathophysiology and mechanisms associated with cognitive impairment in PD. The estimated prevalence of cognitive impairment in PD ranges from ~20 to 40% in the early to mid-stages and is reported to dramatically increase in up to 80% of patients who have had PD for 20 or more years. PD patients typically develop executive dysfunction early in the disease that can affect attention, strategy switching, and cognitive flexibility and then dementia dominates in the later stages. Studies show midbrain dopaminergic (DA) dysfunction can contribute to executive function impairments in PD, however, dysfunction in other neurotransmitter systems and circuitry likely drive the progression to dementia. Indeed, cholinergic structures within the basal forebrain have been linked to the development of dementia in PD. In this project we will develop a new animal model of PD with dementia in order to identify novel mechanisms, pathways, and targets for therapy. In addition, we will test the effect of exercise on cognitive function in this model.

Goals and Objectives: The goal of these studies is to develop a novel rat model of PD that recapitulates the development of dementia seen in patients. In addition, we will then determine the therapeutic potential of exercise on cognitive function. The objective is to develop this novel model in order to better understand the pathology and circuits involved in dementia in PD so that we begin to identify novel targets for therapy.

Investigative Methods: A combination of behavioral, cellular, molecular, and genetic methods will be employed to determine the effect of exercise in the alpha-synuclein preformed fibril model of Parkinson's disease.

Development of a rat model of Parkinson's disease with dementia. Rats will be injected with alpha-synuclein preformed fibrils in the substantia nigra, striatum, or in the basal forebrain. At six or nine months post injection all rats will undergo a battery of cognitive testing to determine the impact of synuclein pathology on executive function, spatial memory, working memory, and habit learning.

Treadmill Training. Separate cohorts of alpha-synuclein monomer control or PFF-injected rats will be exposed to treadmill training. Rats will be tested during the dark cycle (rats are housed in a

reverse light/dark cycle) and will build up to a running rate of 10 meters/min for 20 minutes per day, 3x per week. Rats will then be behaviorally tested to determine the effect of the exercise on cognitive function. In the brain alpha-synuclein accumulation and neurodegeneration of the nigrostriatal dopaminergic system will be determined.

Alpha-Synuclein Accumulation (brain). Soluble and insoluble alpha-synuclein protein will be measured using both immunoblot and immunohistochemistry techniques. For immunoblot fresh frozen tissues will be homogenized and subjected to successive freeze-thaw cycles. Lysates will then be centrifuged and supernatants will be collected as the soluble fraction. The remaining pellet will be resuspended in a SDS-based lysis buffer, boiled and sonicated. Lysates will be centrifuged and the supernatants collected as the insoluble fraction. Protein from each sample is fractionated on gels and then transferred to membranes. The membranes are incubated with primary antibodies for alpha-synuclein. The membranes are developed using ECL Plus Western Blot Detection Kit. For immunohistochemistry, free-floating coronal sections will be collected for analysis. Sections will be processed with primary antibodies and for controls, sections will be incubated with the corresponding IgG at the same concentration as the primary antibody. The avidin-biotin complex method will be used to detect the secondary antibody and the reaction product visualized by DAB.

Proposed Method of Data Analysis: A combination of parametric and non-parametric statistics will be used to analyze the behavior and tissue data. For parametric statistics, ANOVA will be used to compare Monomer and alpha-synuclein preformed fibril injected groups on each of the cognitive tests. Similarly, for the exercise study rats receiving treadmill training or stationary on treadmill will be compared. Post hoc comparisons will use the Bonferroni corrected factor when multiple comparisons are being made. For scores that do not meet the assumptions of ANOVA nonparametric statistics will be used to compare genotypes and treatment.

Significance of Anticipated Findings: We will investigate the biological basis of cognitive dysfunction in PD combined with the impact of exercise on the same outcomes. Results from these experiments will yield essential data on the biological mechanisms contributing to cognitive decline in PD and the therapeutic potential of exercise as a disease-modifying intervention. In addition, this work has high translatability and will help to inform clinical trials and identify optimal intervention strategies for PD patients and at-risk populations.

Student Fellow Training/Mentoring Plan:

Plan. This is a large project that is ongoing in the lab. The PI will work with the student to determine what aspect of the project best suits his/her interests, abilities, and goals. The student would have the option to work mainly on one aspect of the project (such as behavioral testing and analysis or tissue processing and immunohistochemistry) or multiple aspects of the study. The student will meet with the PI on a weekly basis to discuss project progress and literature in the field. In addition to individual meetings the student will attend regular lab meetings where each person in the lab discusses the project they are working on and the progress or setbacks they have encountered. Short PowerPoint presentations are encouraged during these meetings as they will keep the student on track for the final poster session at the end of the summer.

*Resource*s. This project is funded by the Department of Defense and the lab has all resources necessary for the student to complete a summer project. The rats and treadmills are available and behavioral testing protocols are already established. Supplies and space for tissue processing are also available.

Location. The experiments will be conducted primarily in the laboratory area in RGE-400. There is behavioral testing space in C-133 where motor and cognitive testing will take place. The student will have a desk and access to a computer in the write-up area for data analysis and presentation.

Submit your application to Dr. Takhar Kasumov

Project title: Role of alcohol-induced acetylation in Alzheimer's disease (AD).
Principal Investigator: Takhar Kasumov, Ph.D.
Associate Professor, Department of Pharmaceutical Sciences
College of Pharmacy, NEOMED

Abstract: Alcohol (EtOH) consumption, prevalent in the United States, is strongly associated with an elevated risk of late-onset Alzheimer's disease (AD), a leading cause of dementia. Excessive alcohol intake increases the likelihood of AD by a staggering 300%, underscoring the urgent need to investigate the link between Alcohol Use Disorder (AUD) and increased AD risk.

A possible AUD-AD connection may stem from disrupted brain protein homeostasis due to EtOH metabolism. Post-translational acetylation at lysine side chain of proteins by acetyl-CoA (AcCoA) has emerged as an essential regulatory mechanism in protein stability, intermediary metabolism, and epigenetics. EtOH detoxification produces AcCoA and depletes NAD+, key factors involved in acetylation. Tau acetylation is implicated in tauopathy, accumulation of hyperphosphorylated microtubule-associated protein tau (p-tau), in AD. Yet, how alcohol metabolism is linked to altered acetylation of tau in AD remains unknown.

Site-specific tau acetylation dynamic in tauopathy is poorly understood, and the influence of alcohol on acetylation-dependent tauopathy remains entirely uncharted. EtOH metabolism-induced NAD+ deficiency may hinder brain deacetylation, potentially disrupting tau turnover and increasing p-tau accumulation. As EtOH-derived acetate contributes to mouse brain histone acetylation, it may also induce epigenetic alterations linked to tauopathy. Hence, the EtOH-induced shift in site-specific acetylation dynamics, rather than mere changes in acetylation levels, can influence brain function through epigenetic mechanisms and p-tau aggregation.

Our group developed a mass spectrometry (MS)-based method to examine acetylome dynamics in vivo. Here, we aim to employ this method to establish a connection between AUD and AD. *The central hypothesis is that alcohol-induced altered brain acetylation dynamics contribute to the accumulation of toxic acetylated tau*.

We will measure site-specific acetylation turnover of histones and tau in the hippocampus and cortex of alcoholic *htau* mouse model of tauopathy to determine whether the altered acetylation results from impaired acetylation or deacetylation. Utilizing ChiP-Seq, we will identify histone acetylation-regulated transcriptional changes to uncover modified signaling pathways.

The impact. This study will also establish the feasibility of the acetylome dynamics method, which also can be used to investigate the selectivity and specificity of deacetylase and acetyltransferase inhibitors or activators *in vivo* and motivate the development of new AD therapies.

Background and Significance: EtOH consumption in the United States is strongly linked to a higher risk of late-onset Alzheimer's disease (AD), a major cause of dementia. Among the 14 million Americans with AUD, 9% have concurrent brain disorders, including increased AD risk[1, 2]. A possible AUD-AD connection may stem from disrupted brain protein metabolism and EtOH-induced epigenetic changes. Post-translational acetylation at lysine side chain of proteins by AcCoA, a central metabolite, plays a pivotal role in protein stability and epigenetics. While the liver primarily metabolizes EtOH, acetate produced enters the brain, competing with glucose metabolism[3]. Acyl-CoA synthetase 2 (ACSS2) converts acetate to AcCoA, affecting histone acetylation in the brain, crucial for gene expression control in neural activity[4]. AD-related

tauopathy, characterized by tau protein accumulation, is associated with altered acetylation[5]. However, the link between EtOH metabolism and dysregulated acetylation in AD remains underexplored. Acetylation is a dynamic process impacting protein behavior through temporal changes in acetylation, deacetylation, and protein decay[6, 7]. Reversible acetyl transfer allows cells to adapt changes through transcriptional and enzymatic regulations. In general, histone acetylation activates transcription, while deacetylation silences it[8]. Importantly, an active acetylated histone marks have faster turnover than silent marks[9, 10]. Acetylation also regulates enzyme activity by influencing catalytic function and substrate accessibility[11]. Additionally, acetylation may enhance protein accumulation by influencing turnover. The dynamics of site-specific tau acetylation in tauopathy are poorly understood, and the impact of EtOH-dependent acetylation on tauopathy is entirely unexplored. EtOH-derived acetate, contributing to brain histone acetylation[4], may induce epigenetic alterations linked to tauopathy. Therefore, the EtOH-induced shift in site-specific acetylation dynamics, rather than mere changes in acetylation levels, can influence brain function through epigenetic mechanisms and tau aggregation. We have devised a mass spectrometry (MS)-based method to examine acetylome dynamics *in vivo*. Here, we will employ this method to establish a connection between AUD and AD.
Goals and Objectives: Our *objective* is to evaluate the link between EtOH-induced protein acetylation and AD in AUD. *Our central hypothesis posits that* EtOH *-induced changes in acetylation dynamics contribute to the accumulation of toxic acetylated tau.* We will this hypothesis through studies in the following Specific Aims:

Aim 1: Investigate the role of alcohol-regulated site-specific acetylation on brain protein stability *in vivo*. We will employ our in vivo $^{2}H2O$-labeling method to examine acetylome dynamics in the hippocampus and frontal cortex of normal and alcoholic htau mice. Additionally, we will evaluate the development of AD characteristics, such as p-tau accumulation and neurofibrillary tangle formation to understand the role of AUD in tauopathy.

Aim 2: Examine the effect of chronic alcohol consumption on brain protein acetylation turnover *in vivo*. We will measure site-specific acetylation turnover of histones and tau, to determine whether the altered acetylation in the mice from Aim 1 results from impaired acetylation or deacetylation. Through ChiP-Seq, we'll identify histone acetylation-regulated transcriptional changes to uncover modified signaling pathways in tauopathy.

A student will be involved in the analysis of collected brain samples. Specifically, a student will gain experience in proteomics sample preparation, MS acquisition, and data analysis using bioinformatic tools and statistical methods. In addition, a student will learn how to characterize tauopathy using Western blots.

Investigative Methods and Data Analysis: We'll conduct kinetic studies on the *htau* mice to investigate alcohol-dependent tauopathy using our lab's established EtOH-feeding paradigm. Age-, and weight-matched female *htau* mice will be randomized into control pair-fed (**PF**) and EtOH-fed (**EF**) groups and fed their respective diets for 25 days. To assess the impact of acetylation, mice will be treated with $^{2}H2O$ will start during the EtOH feeding experiment. To assess alcohol-related changes in acetylation stoichiometry, we will isolate nuclear cytosolic, and mitochondria fractions. Histones will be acid extracet from nuclear fraction. Cytosolic and mitochondrial acetylated peptides will be enriched using PTMScan kit for ^{Ac}K motif and analyzed by LC-MS/MS. We will use commercially available anti-tauK174ac and anti-p-tau (ser202 and ser416) to assess the role of site-specific tau acetylation in tau phosphorylation and tauopathy.

Raw MS data files including peptide ion masses and fragment spectra obtained from the Q-Exactive Plus mass spectrometry will be processed using MaxQuant against SwissProt mouse protein database. Trypsin-digested peptide sequences will be searched at up to a maximum of two missed cleavages. Database searching was performed with 6 ppm mass tolerance for

precursor ions and 20 ppm for fragment ions. The false discovery rate (FDR) will be set to a maximum of 1% false identifications from a reversed sequence database.

Significance of anticipated findings: We aim to uncover the influence of alcohol on brain proteome and acetylome dynamics in a mouse model of AD, offering essential insights into the mechanisms underpinning AUD-related AD. The acetylome dynamics method could be used to evaluate the efficacy of HDAC and KAT inhibitors and activators in vivo and catalyzing the development of innovative treatments for individuals with alcoholic AD.

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Student Fellow Training/Mentoring Plan:

The goal of the mentoring program is to provide skills, knowledge, and experience to prepare a student fellow to excel in mass spectrometry and bioinformatics technology. To accomplish this goal, the mentoring plan will follow the guidance of the National Academies of Science and Engineering on how to enhance the research experience, by providing a structured mentoring plan, career planning assistance, and opportunities to learn a number of career skills such as developing scientific presentation and writing skill.

Mutual expectations will be discussed and agreed upon in advance. The plan topics will include (a) interaction with coworkers, (b) work habits, and (c) documentation of research methodologies and experimental details so that work can be continued by other researchers and colleagues. Participation in the journal clubs in which graduate students and postdocs meet weekly, along with a faculty facilitator, to discuss and critique recent journal articles and to discuss how to write and submit papers.

Instruction in Professional Practices will be provided on a regular basis in the context of the research work and will include fundamentals of the scientific method in the design of research question, formulation of a hypothesis and description of defined approaches to test the hypothesis. She/he will learn to identify research questions, definition of objectives, description of approach and rationale and construction of a work plan and timeline. Success of this mentoring will be assessed by tracking the student fellow's progress through interviews to assess satisfaction with the mentoring program and tracking of progress through weekly discussions in group meeting. At the end of the internship, the results of the summer research will be presented in a poster session.

Submit your application to Dr. Xinwen Wang

Title: Remimazolam Pharmacogenetics
Principal Investigator: Xinwen Wang, Ph.D.
Assistant Professor, Department of Pharmaceutical Sciences
College of Pharmacy, NEOMED
Location: The study will be mainly conducted in Dr. Wang's lab on the RGE4th floor. The data analysis could be completed remotely.

Abstract:
Annually in the United States, over 100,000 deaths and 2 million hospitalizations occur due to drug adverse reactions. With nearly 40 million anesthetic and sedative administrations yearly, and over 2 million reported complications, optimizing these medications is crucial for patient safety, comfort, and procedural success. Remimazolam (Byfavo®), a recently approved ester-linked benzodiazepine for sedation by the US FDA, offers distinct advantages in sedation with its rapid action and predictable duration, highly suitable for widespread anesthesia use. However, its notable interindividual variability in patient responses, accompanied by an adverse effect rate surpassing 50%, poses a significant challenge, leading to patient distress and procedure failure. Consequently, there is an urgent clinical need for optimizing remimazolam use to improve the sedative/anesthetic outcomes.

The favorably short and predictable action of remimazolam is largely attributed to its fast metabolism, primarily involving the hydrolysis of its ester group. Thus, the hydrolysis of remimazolam plays a crucial role in its deactivation, which in turn significantly influences its action duration, overall efficacy, toxicity, and contributes to the variability in its response. Nevertheless, the enzyme(s) responsible for the hydrolysis of remimazolam remains controversial. Carboxylesterase1 (CES1) serves as the primary hepatic hydrolase in humans, participating in the metabolism of numerous therapeutic agents. Significant interindividual variability in expression and activity of CES1 has been consistently reported. Our previous publications have demonstrated that the *CES1* genetic polymorphisms and inhibitors are associated with significantly altered metabolism and/or efficacy of several selective CES1 substrate drugs. Given the ester structure of remimazolam, characterized by a small alcohol group and large acyl group, which aligns with the substrate preference of CES1, however, the experimental evidence identifying the enzymes responsible for remimazolam hydrolysis remains lacking.

The **overarching objective** of this proposal is to identify the enzyme responsible for remimazolam deactivation and to determine the impact of genetic polymorphisms on remimazolam deactivation. Our **central hypothesis** is that the remimazolam is deactivated by CES1 and functional *CES1* genetic polymorphisms can significantly affect the remimazolam deactivation in human liver. The central hypothesis will be examined in two specific aims:

Aim1: To identify the enzyme responsible for CES1 deactivation. Human liver and intestine s9 fractions, human plasma, CES1 and CES2 recombinant enzymes will be used to test the hypothesis that remimazolam is deactivated by hepatic CES1 specifically.

Aim2: To examine the impact of CES1 genetic polymorphism on the deactivation of remimazolam *in vitro*. We will assess the impact of CES1 G143E on remimazolam deactivation using wild type CES1, CES1 G143E, and vector transfected cell lines.

Significance:

There is an urgent need for researchers to probe the factors contributing to the interindividual variabilities in drug responses[1]. Every day, millions of individuals are prescribed medications that may not provide effective treatment. Shockingly, among the top ten highest-grossing drugs in the United States, their efficacy rate ranges from benefiting only 1 in 25 to 1 in 4 of those who take them. For some commonly used medications, such as statins, the benefit rate may decrease to as low as 1 in 50[2]. Undesired drug responses contribute significantly to the overall costs of health care, and the associated adverse drug reactions even lead to significant patient morbidity and mortality. Understanding the factors that shape a person's response to a particular treatment is crucial for developing more tailored and effective medical interventions[3].

Approximately 40 million short diagnostic and therapeutic procedures requiring sedation are performed in the US annually (FDA 2012)[4]. However, over 50% of the patients receiving anesthetics or sedatives experienced associated side effects or potential complications. These complications can lead to pain, discomfort, procedural failure, or even additional health issues.[5] Thus, the importance of precision anesthesia and sedation cannot be overstated, which ensures not only patient comfort and safety during interventions but also swift recovery, reducing monitoring needs and room occupancy time, consequently saving cost for healthcare system. Moreover, the ability to tailor anesthetic and sedative use significantly enhances the quality of health care during millions of procedures, ultimately contributing to increased procedural efficiency and optimal patient outcomes[6].

The development of remimazolam represents a remarkable advancement in sedatives and anesthetics, which is expected to be widely used in anesthesia and sedation procedures. In January 2020, remimazolam (Byfavo®), a novel benzodiazepine ester derivative, was approved as a general anesthetic in Japan firstly in the world. It received US FDA approval for induction and maintenance of procedural sedation in adults in July 2020. In comparison to current corner stone anesthetics and sedatives, like propofol and midazolam, remimazolam minimizes risk of respiratory depression and offers a smoother patient recovery. Due to its promising profile, remimazolam is increasingly being considered for a wide range of surgical and medical procedures. The extensive range of potential applications underscores the critical need for the precise use of remimazolam, given its capacity to significantly enhance patient care across various medical settings[7-9].

Significant interindividual variability in responses to remimazolam has been observed, which can lead to severe and potentially life-threatening adverse effect10. According to an analysis of 36 case reports and 73 trials involving 6740 patients who received remimazolam, common adverse reactions following remimazolam administration include hypotension (33-58%), hypertension (20-42%), respiratory acidosis (19%), bradycardia (4-11%), hypoxia (22%), respiratory rate increased (14%), tachycardia (8%), pyrexia (4%), nausea (4%), and headache (3%). Notably, 68 cases reported delayed emergence, ten cases reported anaphylaxis, and eight cases reported re-sedation[10]. However, the factors contributing to the interindividual variability in remimazolam response remain unclear. Therefore, there is a **critical clinical need** to identify the factors contributing to the interindividual variability in remimazolam response, so that anesthesiologists and medical professionals can tailor anesthetic use to minimize these unwanted consequences for patients.

Research methods:

Aim1: To identify the enzyme responsible for CES1 deactivation.

We will conduct *in vitro* incubation study to determine the enzyme and tissue responsible for the deactivation of remimazolam using human liver and intestine S9 fractions and human serum. Both CES1 and CES2 are expressed in human liver, while CES1 greatly exceeds CES2. In the human small intestine, only CES2 is present. Hydrolytic activity in human serum is attributed to several esterases, including butyrylcholinesterase (BChE), Paraoxonases (PONs), acetylcholinesterase

(AChE) and albumin, but neither CES1 nor CES2 is expressed in human serum. We will then validate the results using recombinant CES1 and CES2 enzymes.

Aim2: To examine the impact of CES1 genetic polymorphism on the deactivation of remimazolam in vitro. We will assess prepared s9 fractions from WT CES, CES1 G143E and vector transfected cell lines. Then we will compare the remimazolam deactivation rates among these three groups. The remimazolam inactive metabolite formation will be measured by LC-MS/MS after incubation using the method established in our lab.

Expected Results

We expected 1) remimazolam is only deactivated in the s9 fractions from human liver, but no appreciable hydrolysis in human intestine or serum. Remimazolam is only deactivated in CES1 but not CES2 recombinant enzyme. 2) Remimazolam deactivation rates are significantly lower in the G143E transfected cells compared to the WT CES1.

Contribution to the success of the overall research

At the completion of this study, it is our expectation that we will have established the critical role of CES1 genetic variants on remimazolam metabolism. The findings from this translational project will form a solid foundation for future clinical studies that will validate the impact of CES1 genetic polymorphisms and the drug-drug interactions on the outcomes of remimazolam use in humans. This line of research holds promise for improving the effectiveness of anesthesia by revealing an important gap in our understanding of interindividual variability in remimazolam therapy, which will ultimately optimize anesthetic options and dosing precision. Therefore, the findings will be invaluable for advancing tailored treatment strategies for patients receiving anesthetics, promoting more effective and individualized care.

Student Fellow Training/Mentoring Plan

Training Goal

The ORSP summer research fellowship program will provide the student fellow a great opportunity to broaden research horizons, gain deep knowledge of translational research, and develop skills in 1) CES1 pharmacogenetics, 2) *in vitro* drug metabolism assay 3) LC-MS/MS, 4) Scientific writing, 5) Scientific communication and oral presentation.

Mentoring Plan

With regarding to the science training for the proposed project, the key areas I would like to help the student improve are listed as below:

a) Pharmacogenomics

• We have Journal Club to share research trend/development and critically evaluate published articles in the field. In the Journal Club, the student will be participating in the discussion of current development in our fields. We will critically discuss the cons & pros of the research, and how the study may be designed differently to better answer the research questions.

• We have weekly lab meeting to discuss the research progress of the summer project and guide the students for trouble shooting.

b) *In vitro* drug metabolism assay

• One-on-one mentoring will be offered to help student learn how to conduct *in vitro* drug metabolism assay.

Presentation skills development

• The student will present the research updates weekly in our lab meeting and present articles

in our Journal Club. He will also have opportunity to present the whole project in our department seminar after summer.

Scientific Writing

a) Read publications on scientific Journal

• Reading well-written scientific articles is the key to learning and improving scientific writing. At the beginning, I will select some publications relevant to the summer project for the student to read. In the meantime, the student will also learn to search for references of interest using Google Scholar and PubMed. We will update the reading progress in Journal Club.

b) Practice and Revise

• Practice is necessary to improve writing skills. I find the writing-revision cycles between trainee and faculty is a very efficient and individualized way to polish writing skills. I will find opportunities for the student to improve writing skill through the personalized writing-revision cycles.

Submit your application to Drs. Natalie Bonfine and Stacey Barrenger

Project title: *"*Social Factors Associated with Health Service Utilization, Health, and Well-being among People with Serious Mental Illness*"*
Co-Principal Investigators: Natalie Bonfine, Ph.D., Associate Professor, Department of Psychiatry
Stacey Barrenger, Ph.D., Assistant Professor, Department of Psychiatry
Location of research: NEOMED campus, Department of Psychiatry

Abstract of project

Social status characteristics, including age, gender, race, and socioeconomic status, have been shown to affect health outcomes. In addition to examining the direct connections between social status and health outcomes, it is also important to assess intervening effects of stress, social support, and psychosocial coping resources on the association between social status characteristics and health outcomes. Much of the research done in these areas has focused on community samples of adults representing the general population and are not specific to people living with severe and persistent mental illnesses, such as schizophrenia, bipolar disorder, and major depressive disorder. This study will examine survey data collected from a community-based sample of people with serious and persistent mental illness to better understand how social factors are associated with mental health service utilization and health outcomes. Specifically, this project will examine the associations among social characteristics and social factors, such as exposure to stress, mental health service utilization, overall health status, and psychological well-being.

Significance of the overall research

It is well known that social status impacts health. Social status characteristics most often studied are age, gender, race and ethnicity, and socioeconomic status, including education, income, and occupation, or may include role-based social statuses (e.g., parent, worker, partner, student). The historic Whitehall II studies documented a strong, direct link between social status and health, noting patterns of social inequality and health such that changes in social standing were associated with direct changes in a variety of health outcomes and mortality (Adler et al. 1994; Link and Phelan 1995; Marmot 2005; Marmot et al. 1991; Pearlin et al. 2005). Other research has examined how social status and social conditions impact access to and utilization of health care services, as well as the experience of patients interacting with the health care system (Hall and Dornan 1990; Hendryx, Ahern, Lovrich and McCurdy 2002; Marmot, Friel, Houweling and Taylor 2008).

Social conditions, including social status and other factors, such as social support, psychosocial coping resources, and exposure to stressful life events, have been described as social determinants or fundamental causes of health because they put people "at risk of risks" (Link & Phelan, 1995). Some social conditions may be protective factors that prevent illness or reduce disease burden, e.g., by increasing access to health-promoting resources or health services, while other social conditions may increase exposure to risk factors that can exacerbate poor health outcomes (Link & Phelan, 1995). As such, existing research has examined not only the direct connections between social factors and health outcomes but has also examined how other elements of the social environment may exacerbate or mitigate the connection between social conditions and health outcomes.

It is important to assess patterns of association between social conditions and health outcomes. However, much of this research in this area has focused on community samples of adults representing the general population and are not specific to people living with severe and persistent mental illness. This project will explore the association of social factors, social status,

well-being, health services utilization, and physical and mental health outcomes among a sample of people with serious mental illness living in a community setting.

Goals and objectives
This research Fellowship is a student-directed exploration of social factors that relate to health and health services utilization for people with serious mental illness. The aim of this study is to complete secondary data analysis of existing survey data collected from a sample of people with serious and persistent mental disorders. The co-Principal Investigators will provide the Fellow access to the dataset and the student will develop and define specific, conceptually driven research questions. The general, guiding research objectives for this study are to:

1) describe the current literature on social factors that influence mental health service utilization, general physical health, and well-being for people with serious mental illness, and

2) analyze quantitative data to assess the association of key measures, including social status, health services, stress and psychosocial coping resources and physical and mental health among a sample of adults with serious mental illness.

Research methods to be used/learned
This Fellowship is a social epidemiological research project. The student Fellow will engage in multiple aspects of the research process and will complete a review of the literature, participate in the development of research questions, and will analyze existing data and summarize findings to an academic and general audience.

Proposed method of data analysis
This project involves quantitative data analyses, where the student Fellow will conduct descriptives statistical analyses, bivariate correlations, means comparisons and/or multivariate analyses (e.g., ordinary least squares and logistic regression). Data analyses will be completed using SPSS. The student Fellow will also participate in other aspects of the research process, including literature review and summary, interpreting results, and developing tables and figures to summarize findings. The Fellow will also gain experience preparing research results for public dissemination.

While it is possible that applicants for the student fellowship may have some experience engaging in quantitative data analysis, prior knowledge or experience is not required. The Faculty PI will provide hands-on training and instruction on how to engage in such research in a scientifically rigorous manner.

Significance of anticipated findings
This study will contribute to an improved understand of how social status characteristics (e.g., gender, age, race, education, employment, parental status) and other social conditions influence health services utilization and health outcomes for people with serious and persistent mental illness. By doing this research in connection with the Health Services Research Focus Area, students will meet with other collaborators and social science researchers who are examining the role that social factors have in influencing health, quality of life and other outcomes.

Student Fellow Training/Mentoring Plan
This Summer Fellowship experience will involve collaborative, team-based research. The student will be supervised by Drs. Barrenger and Bonfine and will also meet with other faculty in the Department of Psychiatry and the Health Services Research Focus Area. Weekly research team meetings and/or lab sessions will be scheduled between the faculty and summer research fellow. During these guided, mentored meetings or lab sessions, the student Fellow will receive experiential training on the following research activities: developing research questions informed

by the literature, conducting quantitative analyses using statistical analysis software, interpreting results, and disseminating findings. The student Fellow will have access to workspace within the Department of Psychiatry Research Lab, including access to workstations with computers and appropriate data analysis software (e.g., SPSS). Students will receive training on the ethical conduct of human subjects research and will be guided to ensure that all aspects of the project adhere to IRB protocols.