NEOMED

Office of Research and Sponsored Programs' Summer Research Fellowship Program

2018 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 POLICY

Phase 1, M1, M2, P1 and P2 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 summer 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 summer research fellowship will be $3,000. Summer research fellows are considered to be NEOMED employees and will be paid on a monthly basis through NEOMED's payroll system and will be subject to withholding tax and OPERS withholdings. A W-2 will be issued to the summer research fellow the following January for use in filing their tax return.

(**A special note** – a student can claim exempt on their tax withholdings if they believe they are eligible for a tax refund on their tax return. A student can also apply for a refund for the withholding paid to OPERS after they leave NEOMED's employment. However, the 1.45% withholding for Medicare is not exempt and cannot be refunded after end of employment.)

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.

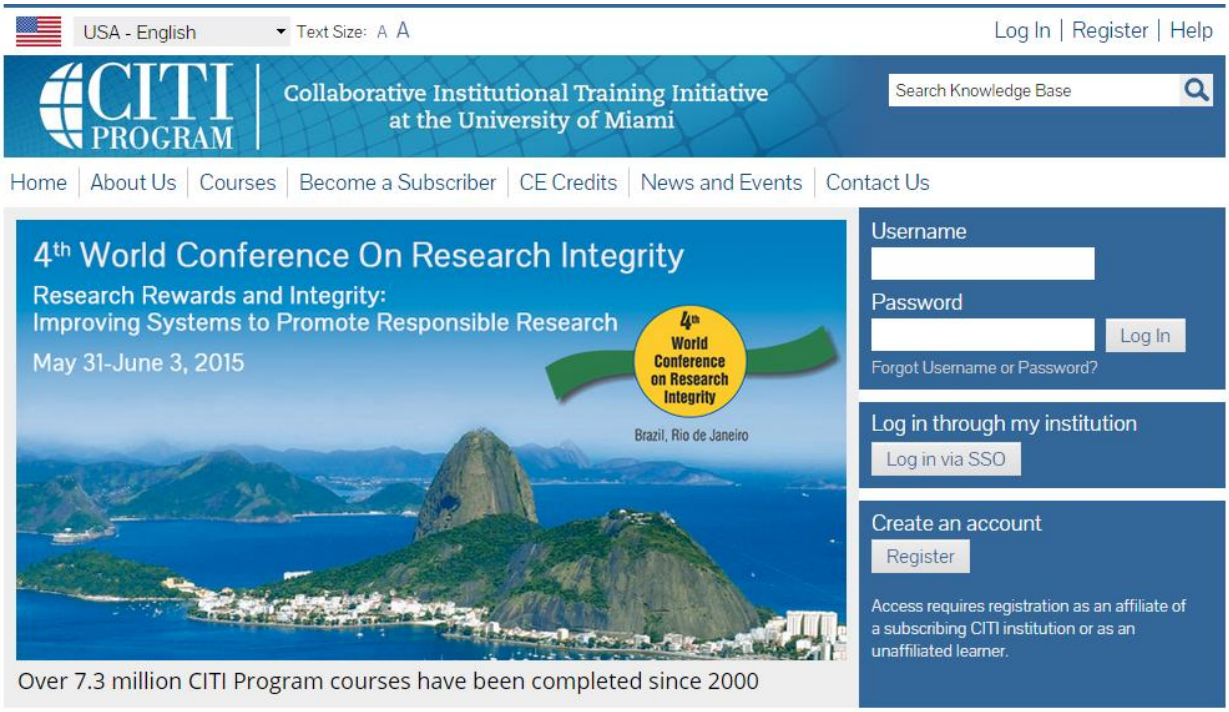
2. Description of course from the CITI Program:

   "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 permitted to participate in any research without this certification.


USA - English
Text Size: A A
Log In | Register | Help
CITI PROGRAM
Collaborative Institutional Training Initiative at the University of Miami
Search Knowledge Base
Home | About Us | Courses | Become a Subscriber | CE Credits | News and Events | Contact Us
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.

Summer Research Fellowship Project Poster Day

Friday, August 24, 2018

Northeast Ohio Medical University Campus
NEW Center Ballroom

All ORSP sponsored fellows are **required** to participate in Poster Day.

All other students are invited and encouraged to participate but need to contact Nona Hose in the ORSP before August 1, 2018 to reserve a spot at Poster Day as space is limited.

Easels, poster boards and push pins will be provided by the ORSP for each poster. Students are responsible to set up and take down their own posters.

Details as to preparation, deadline, etc., for posters will be provided to the students who are chosen to participate in the program. The ORSP pays for poster printing for those students whose projects are being funded through the ORSP.

NEOMED
Office of Research & Sponsored Programs Summer Research Fellowship

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 project investigators have been asked to conduct interviews, select the student(s) who will be working on their project(s) by **Monday, April 30, 2018.**

1. Students who are required to complete a summer course remediation are strongly discouraged from participating in any summer 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 (page 8).
   b. Prepare a *curriculum vitae* (see page 9 for hints on CV preparation).
   c. Send the application form and *curriculum vitae* to all investigators from whom you are requesting an interview.

   - Follow the instructions on the contact list (pages 11-14).
   - You may put the in-house envelopes in the outgoing mailbox located in the Office of Research and Sponsored Programs (G-235) for pick up.

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.

   Nona will provide Human Resources (HR) the names of the students who have been selected. HR will contact each student to provide the necessary paperwork and to set up an appointment for onboarding if necessary. 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 Officer, Mechelle Gehle-Wann.

   All 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; Fax: 330-325-5926
   E-Mail: nhose@neomed.edu

2018 SUMMER FELLOWSHIP PROGRAM

APPLICATION FORM

Submit application form directly to investigator and set up interview.

NAME: _____

ADDRESS: _____

_____

PHONE: _____

	Phase I				Phase II			
YEAR IN BS/MD PROGRAM:	C1	C2	C3	C4	M1	M2	M3	M4

	Phase II			
	P1	P2	P3	P4

PROJECT INVESTIGATOR: _____

Briefly describe all previous research and laboratory experiences. Use additional pages if necessary.

_____

_____

_____

_____

_____

_____

List chronologically all employers during the past three years and briefly describe your job responsibilities.

	Employer	Job Responsibilities
1.	_____	_____
2.	_____	_____
3.	_____	_____
4.	_____	_____
5.	_____	_____

THE CURRICULUM VITAE

What does that mean?

The *Curriculum Vitae*, Latin for "course of (one's) life," is more commonly referred to as a "C.V." In your C.V. you will give a brief account of your education, experiences, qualifications, and career course.

C.V. or Resume?

A *Curriculum Vitae* appears similar to a resume. The phrase C.V. is used primarily for professionals – doctors, lawyers, Ph.D.'s – and contains more detailed information than a standard resume.

How do I begin?

If you have never pulled together a resume or C.V., use the following checklist as a starting point, and write down the information for each area. Remember to keep your content concise and accurate. This will be brainstorming. You will be striving for eventually editing your document to one page.

- Name, address where you can be contacted quickly, phone number, e-mail address. This usually goes on top, with the name in a slightly larger font than your address (you want your name to stand out).
- Education: graduate, and undergraduate, years attended, degrees earned.
- Research experience (fellowships and/or volunteer, list principle investigators' name)
- Publications (full citation of publication, so the reader could look it up)
- Honors/awards/scholarships
- Society memberships/leadership experience
- Employment experience (even if not medically related, as this conveys your work ethic and willingness to do non-glamorous jobs)
- Interests outside of medicine (list no more than 3-5 items)

What format should I use?

In the Microsoft Word computer program, there is a resume template, which can help you format your C.V. Usually, information is listed in reverse chronological order, with the most recent experiences listed first under headings. If the fellowship is asking for specific skills, you can use those skills as headings listing your experiences under that heading. On the next page is an example C.V. for a first year medical student. The library also has reference books with example C.V.s.

1313 Mockingbird Lane
Kent, Ohio
330.555.1313
max@neomed.edu

Max Benson

Education

Northeastern Ohio Medical University
Rootstown, OH 2014-present
- M.D., anticipated graduation May 2020

Kent State University
Kent, OH 2010-2014
- B.S. in Integrated Life Sciences
- Dean's list and President's Scholarship
- Combined B.S./M.D. program with NEOUCOM

Experience

Summer Practicum Research, NEOMED, 2017
- Researched causes of youth drug abuse
- Developed program with group to reduce incidence of drug abuse for grades eight through twelve.
- Presented research to Mock State Assembly.

Activities

- Student National Medical Association
- Ohio State Medical Association – Medical Student Section

Community

Habitat for Humanity
Akron, Ohio 2016-present
- Volunteered doing various house construction tasks

Childhood Cancer Program Buddy
NEOUCOM and Akron Children's Hospital, 2017-present
- Paired with child with cancer, and meet regularly with her and her family.

Interests

Guitar, Fishing, and Cleveland Indians baseball.

CONTACT LIST FOR PROJECT APPLICATIONS

Applications may be submitted electronically or by mail. If you are mailing hard copies of applications to NEOMED from **off campus** use the following mailing address:

PI Name
Department/Room Number
NEOMED
PO Box 95
4209 State Route 44
Rootstown OH 44272

If you are submitting an application by hard copy and are on the NEOMED campus, it is recommended that you deliver your applications to the project investigator's office. You can also drop off your addressed envelopes to Nona Hose in the Office of Research and Sponsored Programs in Room G-235. The mail is picked up at 1 p.m. by the NEOMED mailroom personnel for in-house delivery.

Project Investigator contact information for those on NEOMED campus:

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

Lisa Cooper, Ph.D.
Anatomy and Neurobiology, RGE-242
Email: lcooper@neomed.edu

Rebecca German, Ph.D.
Anatomy and Neurobiology, D-106
Email: rgerman@neomed.edu

Catherine Mattinson, Ph.D.
Anatomy and Neurobiology, E-132
Email: cmattinson@neomed.edu

Jeffrey Mellott, Ph.D.
Anatomy and Neurobiology, E-110
Email: jmellott@neomed.edu

Dana Peterson, Ph.D.
Anatomy and Neurobiology, E-138
Email: dpeterson@neomed.edu

Merri Rosen, Ph.D.
Anatomy and Neurobiology, D-114
Email: mrosen@neomed.edu

Sharad Shanbhag, Ph.D.
Anatomy and Neurobiology, E-106
Email: sshanbhag@neomed.edu

Hans Thewissen, Ph.D.
Anatomy and Neurobiology, E-142
Email: thewisse@neomed.edu

Chris Vinyard, Ph.D.
Anatomy and Neurobiology, D-104
Email: cvinyard@neomed.edu

Jeff Wenstrup, PhD
Anatomy and Neurobiology, E-116
Email: jjw@neomed.edu

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

Michael Appleman, M.A. Ed.
Family and Community Medicine, G-149
Email: mappleman@neomed.edu

Rebecca Fischbein, Ph.D.
Family and Community Medicine, G-112
Email: rfischbein@neomed.edu

Stacey Gardner-Buckshaw, Ph.D.
Family and Community Medicine, G-141
Email: sgardnerbuckshaw@neomed.edu

Amy Lee, M.D., M.P.H., M.B.A.
Family and Community Medicine, G-118
Email: afl@neomed.edu

Janice Spalding, M.D.
Family and Community Medicine, G-128
Email: jmspaldi@neomed.edu

Integrative Medical Sciences department's main office is located in RGE-333

Yeong-Renn Chen, Ph.D.
Integrative Medical Sciences, RGE-338
Email: ychen1@neomed.edu

William Chilian, Ph.D.
Integrative Medical Sciences, RGE-335
Email: wchilian@neomed.edu

Vahagn Ohanyan, M.D., Ph.D.
Integrative Medical Sciences, RGE-331
Email: vohanyan@neomed.edu

Charles Thodeti, Ph.D.
Integrative Medical Sciences, RGE-343
Email: cthodeti@neomed.edu

Liya Yin, Ph.D.
Integrative Medical Sciences, RGE-336
Email: lyin@neomed.edu

Pharmaceutical Sciences department's main office is located in RGE-116

Christine Crish, PhD
Pharmaceutical Sciences, RGE-132
Email: ccrish@neomed.edu

Sheila Fleming, PhD
Pharmaceutical Sciences, RGE-140
Email: sfleming1@neomed.edu

Muhammad Hossain, D.V.M., PhD
Pharmaceutical Sciences, RGE-139
Email: mhossain@neomed.edu

Takhar Kasumov, PhD
Pharmaceutical Sciences, RGE-100
Email: tkasumov@neomed.edu

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

Natalie Bonfine, PhD
Psychiatry, A-144A
Email: nbonfine@neomed.edu

Project Investigator contact information outside of NEOMED:

Julia Jones Huyck, Ph.D.
Assistant Professor, Speech pathology and Audiology
Kent State University
Email: jhuyck@kent.edu

NEOMED

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

Project Descriptions

Submit your application to Dr. Lisa Cooper

Project Description

1) **Title**: Age-Related Changes to the Joints, with an Emphasis on Articular Cartilage

PI: Lisa Cooper, Assistant Professor, Department of Anatomy and Neurobiology

LOCATION: NEOMED, RGE-200

2) **Abstract:** For most mammals, joint deterioration is a naturally-occurring consequence of aging. In humans, these age-related shifts include modification to the cartilage including cell death, fibrillation, matrix loss, fissures, and/or osteophytes. These morphological changes leave many elderly people vulnerable to painful joint immobility, and in need of expensive and painful joint replacements. No research to date has shown how to reliably prevent the process from beginning. This study validates the concept that bats have the potential to be a model for the prevention of age-related cartilage deterioration. Unlike other mammals, our studies of comparative gene expression showed that elderly bats compared to young bats, increased expression of genes that are critical for joint lubrication and articular cartilage survival and health. However, little is known of how the morphology and cellularity of joint phenotypes change with age in bats and mice. This study utilizes standard bench techniques to evaluate shifts in morphology and gene expression throughout age in bats and mice. The Cooper Lab has ontogenetic samples of bats and mice that will form the basis for this study. These specimens will lay the foundation for a summer fellowship in which a qualified applicant will quantify and compare cartilage-specific histomorphometry and transcript levels. Results will be presented at the ORSP Summer Research Fellowship Program, and potentially result in a co-authorship in a resultant publication.

3) **Significance of Anticipated Findings:** Insectivorous bats will die if they are unable to capture prey on the wing. As such, maintenance of joint health is critical for the survival of these bats. Curiously, insectivorous bats life 3 times longer than expected, suggesting their joint health is preserved throughout their exceptional lifespans. It could be that by delaying senescence of their joints, bats are able to at least partially achieve exceptional longevity. This study is the first to characterize *in vivo* gene expression of cartilage cells throughout the lifespan of long-lived bats and standard laboratory mice. By characterizing patterns of gene expression, this study identifies the molecular events critical for establishing and maintaining the cartilaginous matrix of bat wings.

Our contribution is expected to further our goal of validating the concept that bats have the potential to be a prevention model for osteoarthritis. If the concept proves out, the contribution will be significant because data will assist in validating a new model that would inform research that would likely transform how the problem of age-related joint deterioration is approached: prevent the process from even starting. It is highly likely that such a model would help to vertically advance clinical thinking about how to overcome the problem of age-related joint deterioration.

4) Background and Rationale: Modifications to cartilage cell activity partially result in age-related joint deterioration. Phenotypic changes with age are well characterized in humans and laboratory mice. However, nothing is known of the age-related changes in the genetic expression and morphology of the joints of bats. For instance, cartilage cells of fetal bats are exceptionally active compared to terrestrial mammals, but nothing is known of the activity of these cells in middle-aged, or elderly bats. This study departs from these previous efforts as we quantify age-related changes in cartilage cell activity *in vivo*. By quantifying age-related changes

in gene expression, our study will identify shifts in the molecular regulation of cartilage phenotype in bats.

5) **Goals and Objectives:** The overall objective of this study is to characterize the *in vivo* differences in expression patterns for genes critical to cartilage and joint health, and their phenotypic consequences. Based on preliminary data, the working hypothesis for this study is that bats, compared to mice, display novel *in vivo* cartilage cell signaling and activity rates in those genes crucial for cartilage maintenance and health.

6) **Research Methods:** Based on results of our previous analyses and new RNASeq analyses, this study quantifies differences in mRNA levels of candidate genes in the cartilage and bones of bats and mice. Based on our published methods for RT-qPCR analysis, a summer fellow will characterize differences in the spatiotemporal distribution of mRNA expression as it allows for direct inter- and intra-specific comparisons. Products will be amplified from bat and mouse cDNA using mouse-specific primers only if there is at least 95% sequence homology between published genes of bats and mice. If homology is lower, species-specific primers will be used.

The fellow will include three replicates for each species and age. Genes and species-specific standard curves will be constructed to detect transcript number variations between and among samples, as per our published methods. By characterizing the spatiotemporal expression of genes, we expect results to establish a critical understanding of novel targets that driving chondrogenesis and joint maintenance in bats.

The fellow will also be required to generate and analyze data from paraffin-based sections of articular cartilage. The student will receive fresh tissues that will then undergo a series of dehydration and clearing washes before being embedded in paraffin wax and sectioned between 6 and 10 µm and mounted on glass slides. Sectioned tissues will then be stained via standard dyes (e.g., toluidine blue, hematoxylin) and cover slipped. After slides are scanned using NEOMED's high-resolution slide scanner, students will then be expected to count cells, and identify other degenerative-related morphologies (e.g., fissures, etc.). Finally, students will be required to take publication-quality photos.

7) **Proposed Method of Data Analysis**: Significance of transcript number differences will be based on 95% confidence interval analyses and a significance of cycle threshold values analyzed with ANOVA.

8) **Student Fellow Contribution:** The student fellow will provide critical evidence as to the age-related changes in gene expression and their association with changes in joint morphology. By linking gene activity levels and joint morphology, the student will be able to test if bats undergo age-related joint deterioration as in other mammals. We expect the student will show that cartilage gene expression and cartilage morphologies are maintained throughout the lifespan in adult bats, regardless of age. These data will then further the field of skeletal biology upon age-related joint deterioration.

Student Fellow Training/Mentoring Plan (1/2 page):

Funding is requested to support one summer research fellow. PI Cooper and Postdoctoral Fellow Dr. Hope Ball, are committed to fostering the researcher's development for the summer. This goal will be achieved through a structured mentoring program, as described below.

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. Besides benefiting from working alongside the PI and Postdoctoral Fellow, the student will be required to attend and present at bi-monthly 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 regular brown bag seminar and journal club on the general topic of "Evolutionary Morphology", 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. Rebecca German

1. The Effect of Preterm Birth and RLN Damage on Airway Protection and Maturation

2. Abstract: The overarching objective of my research group is to understand the biomechanics and neural control in normal and pathophysiologic swallowing (dysphagia) and to develop rehabilitation strategies for infants with this condition. Coordination among the functional components of the aerodigestive system, particularly between swallowing and respiration, is critical for successful airway protection in infants. Disruption of this coordination can produce failure of airway protection, manifest as pulmonary aspiration. These problems may be compounded with damage to the recurrent laryngeal nerve (RLN) resulting from cardiovascular repairs necessitated by prematurity. Because the current understanding of the pathologies in these fragile patients is based largely on non-invasive technologies, the causal relationship between disordered coordination and airway protection, including how development impacts this system, is unknown. This project will determine the kinematic and biomechanical deficits in an animal model of such infants, and how those deficits change longitudinally over the course of development. Such data will change our understanding of the potential for recovery and be the basis for designing intervention strategies for preterm infants.

3. Significance: Preterm infants constitute 11% of all infants born (Martin et al. 2015). In those infants, pulmonary aspiration occurs frequently, and is correlated with pneumonia and associated lung injuries (Hegde and Greenberg 2015). Given the fragile nature of preterm infants, existing data on airway protection is frequently qualitative (Lau and Smith 2012). Even less is known about airway protection in preterm infants with recurrent laryngeal nerve (RLN) damage due to patent ductus arteriosus (PDA) ligation or other cardiovascular surgery (Tashiro et al. 2014), including any interaction of gestational age (GA) and nerve damage.

4. Goals and objectives: We have created an animal model to measure the coordination between respiration and swallowing in infants, and test the impact (1) of prematurity, (2) RLN sensory damage, and (3) the recovery and maturation over time of that coordination. We will be collecting several modalities of data (outlined below), each of which needs to be independently analyzed, and ultimately integrated into a larger picture. A trainee will participate in all aspects of data collection over the summer, but will design and carryout a project that will include hypothesis formulation and testing for a portion of these data. This strategy has been successful with numerous trainees.

5. Research Methods used/learned by students: The methods in this project include electrophysiology and highspeed fluorographic imaging. We will measure (1) performance or the degree of aspiration; (2) the kinematics, movements of oropharyngeal and laryngeal structures, using high-speed bi-planar videofluroscopy; (3) the kinematics of respiration and airflow. Because we can measure these factors simultaneously in an animal model, we will determine the causal links amongst these l evels, and thus be able to translate our results to patients with dysphagia. *Data Collection Methodology:* We will film at 100 fps movement of the bolus, as well as surgically implanted markers in the tongue, hyoid bone and epiglottis. We will

simultaneously measure respiration at both the thorax and the nares, which will permit us to infer laryngeal function as a valve controlling respiration.

6. Proposed method of data analysis This project will use the data analyses used in previously published work (Thexton 1996; German et al. 1997; Thexton et al. 2007; Thexton et al. 2009; Gould et al. 2015a; Gould et al. 2015b). We will compare the timing of the swallow to the respiratory traces, and determine the precise (msec) coordination for each swallow. Groups of swallows will be compared over time (longitudinal design), as well as to control infant data collected last year. We will also analyze bolus movement, as well as movements of the hyoid, thyroid, epiglottis and tongue. Relative timing of the movement among structures is possible with cross-correlation analyses. For each feeding session, we will calculate the average frequency of sucking, swallowing and the volume per swallow.

7. How the anticipated findings from the summer research fellow will contribute to the success of the overall research being investigated:
The results from these experiments will determine how the developmental course of airway protection differs in preterm infants from term controls. The students involved in this research will be the primary data to address the aims outlined above. The students will be involved in all phases of this work, from pre-experiment planning, through surgery, animal care, data collection on these infants, data reduction, and data analysis. Given that RLN injury often occurs in preterm infants, this model and design will permit us to understand the interaction between gestational age and iatrogenic insults. The data collected and analyzed by the trainees will provide a physiologic basis for decisions about care and intervention in these delicate patients that are informed by developmental changes.

Summer Research Fellow Training/Mentoring Plan

The student research fellow will be involved in all phases of the project. Initially, the fellow will be exposed to the larger research program through individual meetings with the PI, weekly lab meetings, and bi-weekly Biomechanics Laboratory Group Meetings (three PI's plus labs, discussion of ongoing projects). The fellow will be given several papers prior to the start of the project. There are several parts of this project that are appropriate for a summer project. The trainee and PI will discuss these sub-projects, and select one that is appropriate. The trainee will be responsible, working with the PI, for developing a hypothesis, alternatives, and outlining the necessary data to test the hypotheses. Because significant amounts of data for multiple projects are collected within a single "experiment", the trainee will work with other lab members in each stage of data collection. The project selected by the trainee will be sufficient to result in a publication at the end of the summer.

Complete resources for this project are available in the PI's lab which is part of the Anatomy & Neurobiology Biomechanics Laboratory at NEOMED (PI's: German, Vinyard, Young). The PI's lab includes 2 other trainees and 2 technicians. This project is a sub-project of a currently funded NIH R01 grant, with sufficient funds to cover the experimental costs.

Literature Cited

German, R. Z., A. W. Crompton, D. W. Hertweck and A. J. Thexton (1997). "Determinants of rhythm and rate in suckling." Journal of Experimental Zoology **278**: 1-8.

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Submit your application to Dr. Catherine Mattinson

1. PROJECT TITLE: Gross Anatomy Electronic Educational Resource Development

2. ABSTRACT OF PROJECT

This fellowship will assist with the development and implementation of electronic educational teaching resources for the NEOMED Yassine Gross Anatomy Laboratory. Specifically, the fellow will have the opportunity to assist in creating a virtual dissector, producing interactive review quizzes, developing images and videos for use in the laboratory, and learning the pedagogical basis for multimedia content delivery.

Over the past decade, the implementation of computer and video technology in gross anatomy labs has become the norm in medical education throughout the United States. This past fall, multimedia technology was installed in the gross anatomy lab. To enhance our traditional cadaver dissection experience, we are working to integrate computer and video resources into our course dissector. The primary goal of the electronic educational resource implementation is to integrate dissection with other learning modalities. By enhancing the student learning experience in Human Development and Structure (HDS), we hope to further NEOMED's educational mission and promote student success in their health care careers.

The multimedia resources developed here will be integrated into the HDS course for future medical students. The resources developed in the fellowship will enhance dissections by tailoring the dissection instructions to student needs, illustrating anatomical relationships through a variety of images, illustrations, and photographs, offering students the chance to practice the identification of structures through interactive quizzes, providing students with video-based dissection examples, fostering self-teaching through multimedia resource use and providing access to other teaching modalities used in the HDS course.

Fellow responsibilities may include: virtual dissector development, image and quiz editing and enhancement, cadaver dissection, video creation and editing, and teaching resource implementation. Previous completion of the NEOMED HDS course is required.

3. BACKGROUND AND RATIONALE

The development of computer and video educational technology has changed small group teaching in medical education. Integrated multimedia technology is now commonplace in gross anatomy labs throughout U.S. medical schools. The Yassine Gross Anatomy Laboratory at NEOMED has recently been upgraded to include computers, wide-screen monitors and video cameras at each cadaver station. With the availability of these new technological advancements, we are now in the process of integrating computer and video resources into our traditional laboratory experience for medical students in the HDS course. By incorporating electronic educational resources with our traditional cadaver dissection, we will foster integrative learning in the lab linking the unique dissection experience with lecture content, clinical correlates and other educational resources.

4. GOALS AND OBJECTIVES

Our primary goal is to develop and implement electronic educational resources in the gross anatomy lab experience for first year medical students. As part of this goal, we have the following specific aims: 1) to create a virtual dissector for cadaver dissection, 2) to identify and edit appropriate images, illustrations and photographs for specific dissection experiences, 3) enhance current resources, such as image quizzes, through the addition of interactive elements, 4) begin to develop videos for use in the laboratory and 5) establish the pedagogy for how to best deliver multimedia content during laboratory sessions. The fellowship student will have the opportunity to participate in each of these goals.

5. INVESTIGATIVE METHODS TO BE USED

This project primarily involves the development of electronic educational resources for use in gross anatomy laboratories. Specific summer opportunities will include the following educational tasks. *1) Virtual dissector*. The HDS course is migrating to a virtual (computerized) dissector that incorporates current dissection activities with computerized versions of a traditional (e.g., Grant's Atlas) and photographic atlas, dissection videos as well as potential new dissection activities (as appropriate). This customizable atlas will allow focused explanations of dissections as well as specific development of terms students are responsible for identifying during labs. The summer fellow will have the opportunity to contribute to specific dissection modules for labs. *2) Image identification and editing.* A significant component of the virtual dissector is linked images that students will use to better their understanding of three-dimensional relationships among structures. The fellow will have the opportunity to help identify appropriate images, illustrations, and photographs to supplement dissection labs, and then edit these images to emphasize important course terms and concepts. *3) Interactive image quizzes.* The summer fellow will have the opportunity to build upon existing cadaveric image quizzes by adding interactive elements to help further engage students as they study and review material from each lab. *4) Dissection videos*. The summer fellow would have the chance to assist in dissection and creating short dissection videos targeted at difficult-to-dissect regions throughout the dissection experience (e.g., ear, pterygopalatine fossa). These instructional/review videos would be available to assist students during laboratories and in studying for exams. *5) Establish pedagogy for laboratory multimedia.* In working on these specific tasks, the summer fellow will have a direct role in helping to establish the best practices for electronic educational resource delivery during HDS anatomy laboratory experiences. The summer fellow will develop an understanding of educational pedagogy as well as how content development and delivery is accomplished.

6. PROPOSED METHODS OF DATA ANALYSIS

Tasks will not be analyzed as typically occurs in basic science research. Alternatively, results will be iteratively evaluated by the fellow and anatomy faculty to optimize content before implementation in the lab.

7. SIGNIFICANCE OF ANTICIPATED FINDINGS

Addition of electronic educational resources to the gross anatomy labs will provide students with the opportunity to consult multiple multimedia resources concurrently with dissection, creating the first tangible link between lab learning and the eventual clinical experience; the ability to self-teach through dissection videos, and to assess their learning through interactive electronic quizzes; and access to a customized dissector with integrated virtual atlases, salient content from course lectures, and clinical vignettes relating dissections immediately to related health care outcomes. Integrated multimedia is now common in gross anatomy labs throughout medical schools. Students realize and understand the opportunities, as this initiative is designed around their feedback for improving their medical education in the anatomy lab. Development of these electronic educational resources will promote NEOMED's educational mission by furthering our students' ability to succeed in their health care careers.

STUDENT TRAINING/MENTORING PLAN

The fellow will work with the faculty sponsor to become familiar with and proficient in educational development tasks. They may also work with IT staff to develop video resources and implement educational modules. The fellow will build skills related to educational resource development, image identification and editing, the addition of interactive elements to existing resources, dissection, videography, and educational planning. As part of this training, the fellow will be exposed to the fundamentals of pedagogy in medical education. The fellow will have the opportunity to leverage specific tasks to complete during the experience based on their perceived benefits to the student's career goals. It is anticipated that the fellow will participate in the weekly Summer Student Fellow Training/Mentoring opportunity sponsored by Dr. Aultman and the Department of Family Medicine, as has been offered in previous years. Finally, the fellow will present their work at the NEOMED Research Symposium at the start of the 2018-19 academic year.

All required materials and equipment will be made available through the College of Medicine and the Department of Anatomy & Neurobiology, NEOMED or are freely available online.

The work will be primarily conducted in the Department of Anatomy & Neurobiology – NEOMED.

Submit your application to Dr. Jeffrey Mellott

1. **Project Title:** Neurotransmitter Changes in the Auditory System during Age-Related Hearing Loss.

2. **Abstract**: Age-related hearing loss (ARHL) is one of the most common maladies of industrialized populations. Essentially, as the cochlea (periphery nervous system) ages, excitatory input is lost to the central nervous system. To compensate for the lost excitation, the central nervous system will down-regulate inhibition. Eventually this inhibitory downregulation becomes problematic as the lost inhibition leads to a variety of hearing deficits. These deficits include difficultly interpreting speech and detecting salient signals from noisy environments. We investigate age-related inhibitory changes in the inferior colliculus (IC) during ARHL. The IC is 1) pivotal for the processing of complex acoustic stimuli, 2) receives inhibitory input from a diverse set of nuclei, 3) contains a vast population of inhibitory cells and 3) downregulates inhibition during aging.

3. **Significance:** The project will identify the circuits in the auditory midbrain that undergo age-related changes and if those changes are correlated to increases in hearing thresholds.

4. **Goals and objectives:** The student will determine IC circuits that lose inhibitory input during aging.

5. **Methods used:** We will use immunohistochemistry, fluorescent microscopy, viral and traditional tract-tracing, neuron reconstruction.

6. **Proposed methods of data analysis:** Most analysis will be conducted with Neurolucida Explorer (MicroBrightField) and chi-squared tests.

7. **Impact of findings:** Our long-term goal is to understand how aging affects auditory midbrain circuits. Findings from this project will help determine the auditory circuitry that attempts to compensate for age-related inhibitory changes. Furthermore, findings will help determine when these changes begin to occur and if these central changes can be correlated to the changes seen in the periphery.

Student Mentoring Plan

1. The student will be expected to attend weekly lab meetings. As a part of the Hearing Research Group, they will be expected to attend Friday morning Journal Clubs. The student will likely be asked to present their findings during one of the Journal Clubs.

2. Most of the tissue necessary to conduct the data collection and analysis is already “on slide”. Generating the cases needed will be minimal or absent from the 8-week term, which will help maximize the student’s time. All needed elements to complete the study are fully function and routinely used in the lab. Microscopy training will come from the PI. Training on the needed software will be a combination of the PI and Research Assistant.

3. All experiments will be conducted and analyzed in E-147/E153.

Submit your application to Dr. Dana Peterson

Project Title: "*Histology Across the Human Lifespan: A Photographic Atlas Project*"
Number of 2018 Summer Research Fellows that will be supported: Two
Number of hours per week/#weeks: 40hrs/week for 8 weeks.

1. Abstract:

This project will continue the development of an educational resource for students of higher education pursuing histology coursework in basic medical science programs. The electronic photographic atlas will include an innovative, learner-centered curriculum designed to guide students in the identification of predictable microscopic changes that occur in human tissues across the lifespan of an individual. The images and curricular activities will prepare students to recognize disruptions in the sequence and/or timing of these age-normal histological changes as pathological indices for medical intervention.

2. Background and Rationale:

In 2013 less than 25% of all graduating medical students in the U.S. reported that they had received excellent preparation in microanatomy (histology) for their clinical clerkships or senior electives. At this institution, less than 17% of the 2013 graduating medical students reported that they received excellent preparation in histology during their basic sciences curriculum. A frequent complaint of these students, is the (perceived) lack of medical relevance for histology in the education and training of physicians.

The pivotal design component for "*Histology - Across the Human Lifespan,"* is the presentation of multiple samples of a single tissue type to novice learners. Students examine microscopic samples of the same tissue type from individuals of different ages that include a pediatric exemplar, an adolescent exemplar, plus adult and geriatric samples. Medical science programs typically do not include age progression observations for students in traditional histology courses. However, comparisons made between tissue samples of individuals of different ages can reinforce similarities, and highlight differences, between tissues/organs. In addition, this atlas project will provide comparisons of adult tissue exemplars from all major organs between individuals with a normal body mass index (BMI) and those with an abnormally high BMI. Individuals with a BMI >30 are considered obese, and are at-risk for serious health complications. Laboratory exercises embedded in the electronic atlas will be designed to actively engage students in the analysis and interpretation of profound tissue changes that can occur in obese individuals. These simple comparisons are not possible when students examine only a single tissue sample/photomicrograph. Their cognitive skills are restricted to recognition/recall of the single sample that they have observed. Students examining a single tissue exemplar are deprived of opportunities to practice more advanced cognitive operations like analysis, synthesis and evaluation, described by Benjamin Bloom (1956) in his taxonomy of cognitive domains.

Scope of Project: Students will be trained in all phases of tissue processing, paraffin-embedding, sectioning and tissue staining. Students will also be trained to operate the automated slide-scanning system that creates digital images from the prepared tissue slides. Students will learn to utilize the proprietary cell quantification software modules

to determine changes in cell numbers and cell types in the analyzed tissue samples across all age and weight categories for organs of the endocrine system, reproductive systems and integumentary systems. The primary goal for the summer of 2018 is to process approx. 1,000 autopsied tissue samples.

Investigative Methods

Previously, autopsy records from 2000 to 2009 were collected from Akron Children's Hospital and St. Elizabeth Youngstown Hospital. These records were screened for tissue exemplars of normal, non-pathologic samples from males and females for all organ systems, in all age categories. The tissue sample inventory to date has catalogued over 200 individual samples that could be utilized for the atlas project. This summer, autopsy samples of interest will be procured from both hospitals. New 5 µm sections will be cut from the existing paraffin blocks using a microtome, mounted, and stained using a modified hematoxylin and eosin staining protocol. Finished slides will be batch imaged with an Olympus VS120 Slide Scanner Microscope at 40x magnification using a high density focal map.

Proposed Method of Data Analysis

Automated multiple cell type quantification and analysis will be conducted on standardized regions of interest for each digital slide image using the Olympus VS-Desktop quantification software.

Significance of Anticipated Findings

Based on results documented last summer by NEOMED fellows working on this atlas project, there are **recognizable age-related** changes that occur in cell size, cell number and cell types in a single organ – the thyroid gland. These differences appear to be normal and predictive along an age progression continuum. However, there were observable changes in cell types and cell numbers when thyroid gland samples from obese and non-obese individuals (same gender/same age category) were compared that may be indicative of subtle pathological changes. In expanding the scope of the project for this summer to include multiple organs of the cardiovascular, pulmonary and digestive, it is anticipated that similar histological age-related patterns will be identified. A recent review of the literature and search for available curricular materials for students in the medical sciences that quantitatively and qualitatively documents age-related histological changes for multiple body systems across the human lifespan did not reveal any groups pursuing similar lines of investigation. The continuation of the atlas project this summer will allow student researchers to make substantial contributions to a growing body of evidence linking important health-related changes to aging and obesity. 2017 summer fellows attended the national pathology conference in January and were awarded the *Distinguished Poster Presentation* award based on their contributions to this project.

Student Fellow Training/Mentoring Plan

Individual Training

All of these student researchers will be introduced to the fundamental concepts of scientific recordkeeping, appropriate histological processing and staining techniques, photomicrograph imaging and image analysis using the new Department of Anatomy and Neurobiology slide scanning system.

Submit your application to Dr. Merri Rosen

1. Project Title

The interactive effects of developmental stress and hearing loss on auditory perceptual abilities

2. Abstract of Project

Conductive hearing loss (CHL) during childhood, such as that accompanying otitis media with effusion, produces deficits in performance that can endure for years after hearing thresholds have returned to normal. Speech processing skills are directly at risk, as language deficits correlate with the severity of hearing loss in children. An additional risk factor that leads to poor language skills are stress-inducing environmental factors in childhood. The ability to process sounds that change rapidly over time is a useful metric of these temporal processing abilities, and in pre-lingual babies is highly predictive of future language abilities.

This project will use earplugs to mimic CHL during development in gerbils, and will induce stress via intermittent maternal separation. Current work in our lab has revealed behavioral and neural sound processing deficits in early CHL animals, and behavioral deficits as a result of early stress. In this project, we will measure thresholds to detect and discriminate short amplitude-modulated sweeps, because such elements make up human speech, and deficits in discrimination of rapidly changing amplitudes correlates with problems with speech perception. Our lab has shown that earplugging causes behavioral deficits in perceiving rapidly changing sounds, but the interaction of early stress with hearing loss in regards to auditory processing is yet to be determined. Here, sound processing abilities will be assessed behaviorally and neurophysiologically, to determine the independent and interactive effects of hearing loss and stress.

3. Significance of anticipated findings

Early hearing deprivation, such as recurrent conductive hearing loss (CHL) during childhood, produces deficits in performance that can endure for years after hearing thresholds have returned to normal. Speech processing skills are directly at risk, as both perceptual and productive language deficits correlate with the severity of hearing loss in children. Early life stress is also a risk factor for children's cognitive and emotional development. In children with early hearing loss, early stress increases the risk of sustaining long-term speech and language deficits. Furthermore, the prevalence of recurrent otitis media is significantly higher in low socio-economic populations in which children are likely to suffer from social isolation and stressful events.

Ongoing work in our lab has documented several perceptual changes induced by early hearing loss, and has linked them to changes in auditory cortex. A recent paper we published shows similar perceptual changes induced by brief maternal separation, which directly suggests effects on the same auditory Cortical regions affected by early hearing loss. These findings prompted the hypothesis that the present study will address: Does early stress worsen perceptual deficits induced by early hearing loss, and is this effect due to synergistic changes at the level of auditory cortex? To this end, we will measure perceptual abilities for rapidly changing sounds in 4 groups of Mongolian gerbils: Controls, Early Stress, Early Earplugs, and Earplugs+Stress. We will also measure

the ability of auditory cortical neurons to respond to these sounds. This will allow us to determine the correlations between an animal's behavioral performance and its neural representation of the same auditory stimuli.

To date, no papers aside from ours have reported on the effect of early life stress on development of basic auditory temporal processing abilities, and none have examined the mechanisms of interactions between hearing loss and stress that are strongly suggested by the clinical literature. As both stress and hearing loss affect ascending regions that encode sound, it is important to understand the interactions between these effects that may underlie long-term perceptual difficulties. If we are able to clarify how early life stress could exacerbate auditory deficits in children with recurrent otitis media, we can draw attention to the importance of aural rehabilitation, particularly in low socio-economic populations.

4. Goals and objectives

The overall research question that the student's work will address is whether early life stress induces perceptual deficits for basic temporally-varying auditory stimuli.

The goals of this summer project are as follows:

The student will become familiar with the background related to this project. This includes literature regarding 1) the development of auditory temporal processing in humans and animals, as assessed behaviorally, and 2) the cortical neurobiology and physiology of auditory temporal processing.

The student will learn animal handling techniques so as to perform behavioral testing with minimal distress to the animals. S/he will learn to set up and run the pre-pulse inhibition (PPI) tests and/or to train and test animals using behavioral operant conditioning approaches. Depending on time available, s/he may also learn to operate the neurophysiology rig, including electrode placement, searching for and isolating individual auditory neurons, and recording those neurons' responses to complex sound stimuli. These tasks require dexterity and a learned intuition to recognize subtle auditory and visual patterns.

The student will acquire a basic understanding of how sound is processed by the auditory system, both peripherally and more centrally in the brain.
The student will learn methods for analyzing behavioral data, and how to compare results from within individual animals, across individuals, and across groups.

5. Investigative methods to be used

Behavioral Experiment 1 – AM detection: Animals will be trained to detect an amplitude modulation (AM) emerging from an unmodulated, constant noise background. The depth of the AM signal will vary, allowing us to determine an animal's detection threshold. This requires working with individual animals to train and test them on the task. The animals will be water-deprived and receive a combination of water reward and aversive feedback during training and testing.

Behavioral Experiment 2 – gap detection: Pre-pulse inhibition (PPI) of the acoustic startle response (ASR) can be used to measure auditory processing skills, such as

recognizing frequency modulated sweeps. It assesses the unconscious, reflexive response to a loud, startling stimulus and how much that response is reduced when the sound is preceded by a specific sound. A reduction in the response indicates detection of the sound. Since it does not require task-specific training, it allows us to test animals of any age and gain useful data immediately. Here, the pre-pulse stimuli will be short gaps of varying durations in an ongoing noise background. The testing is fully automated and the startle amplitude, as assessed using a force-sensitive plate, is recorded for further analysis.

Electrophysiology: The electrical responses of individual neurons within the auditory cortex of awake or anesthetized gerbils will be recorded using tungsten microelectrodes. Auditory stimuli similar to those used in the behavioral testing will be presented to animals during the recording procedure.

6. Proposed method of data analysis

For behavioral performance, the shortest duration of a detectable gap in a background noise (gap detection thresholds, GDTs) or the smallest AM depth will be calculated per animal per session. These analyses will be performed using pre-prepared scripts in the Matlab programming language.

Neural thresholds for gaps and AM depth will also be calculated for each individual neuron by calculating changes in action potential firing in response to these stimuli. Again, existing Matlab code will be used.

Correlations between neural and behavioral data within individual animals, across individuals and across groups will be determined.

7. Relevance of summer project to lab goals

The anticipated findings from the summer fellow will be key to providing baseline data for the effects of early life stress on perception. This will let us move on to experiments that will determine the mechanisms underlying these effects.

8. Training / Mentoring Plan

Early on, I will spend several hours weekly with the student, explaining the details of the background and project. My postdoctoral and graduate students will be primarily working with the research fellow on the details of conducting the experiments. I will conduct individual research meetings with the research fellow weekly. We also have regular lab meetings which the student will be a part of, and will present her/his results as they are acquired and analyzed.

The Auditory Focus Group at NEOMED is a robust active group of researchers, and we have weekly Group meetings. The fellow will attend these meetings and will be encouraged to engage in our discussions.

Resources: I have an R01 research grant to fund my lab, a technician, a graduate student, and a postdoc who are available to help in training the student. The experimental protocol is worked out and the student can begin collecting data as soon as s/he is comfortable. The research will be conducted in my laboratory, D113-115 on the NEOMED campus.

Submit your application to Dr. Sharad Shanbhag

1. Project information:

Title: Brain Circuitry and Neurochemistry Underlying Hearing and Emotions
Principal Investigator: Sharad Shanbhag, Ph.D., Research Assistant Professor
Co-Investigator: Jeffrey Wenstrup, Ph.D., Professor and Chair
Location: Department of Anatomy and Neurobiology, NEOMED

2. Abstract

Our work investigates neural mechanisms underlying the process by which emotional centers in the brain assign meaning to social vocalizations. Past experiments in our lab have found neurons in the amygdala that respond selectively to social vocalizations. We have examined how contextual cues associated with a social vocalization alter the interpretation of that vocalization by the individual and by neurons in the amygdala. Recently we have shown that the behavioral and amygdalar response to a vocalization is differentially altered by exposure to olfactory cues associated with either mating or predators. We now propose to examine the neural connections and neurochemicals that contribute to selective auditory responses and that provide this type of contextual information to amygdalar neurons. For tracing connections, we will place deposits of retrograde tracers in the basolateral amygdala of mice and then examine the labeling in other parts of the brain. Of particular interest will be non-primary auditory cortical areas, frontal cortical areas and olfactory centers that might provide input to the basolateral amygdala. To study neurochemistry, microdialysis and liquid chromatography-Mass Spectrometry (HPLC) will be used to collect, identify and quantify neuromodulators (e.g., serotonin, dopamine, acetylcholine) released under different behavioral and acoustic contexts. We will also analyze vocalizations and the behaviors in response to playback of social vocalizations.

3. Background and rationale

Our long-term goal is to improve the understanding of neural mechanisms that underlie acoustic communication. This project focuses on the amygdala, a structure known for its role in auditory fear conditioning. For this role, it receives auditory input from the thalamus and cortex, contributes to identifying a stimulus as aversive, and provides for appropriate emotional responses (e.g., autonomic responses, freezing). Recent work demonstrates a broader role for the amygdala in auditory behavior, including acoustic communication. Our view is that the amygdala plays a critical role in acoustic communication through participation in several processes. 1) The amygdala “decides” whether a vocal signal is salient and whether that salience is positive or negative, based on contextual information from the vocal sequence, other sensory information, and the animal’s internal state, 2) It orchestrates emotional responses that are appropriate for the received vocal communication signals and their context, 3) It modulates responsiveness to subsequent vocal signals through its direct and indirect projections to cortical and other auditory structures. In other words, the amygdala is likely to influence how we hear and respond to vocal communication signals. Dysfunction in the amygdala may play a critical role in abnormal relationships between acoustic inputs and emotional responses in disorders such as autism, schizophrenia, post-traumatic stress, and tinnitus.

In our current work, we examine how contextual cues associated with a social vocalization alter the interpretation of that vocalization. We have shown that contextual cues modify acoustically guided behavioral responses to social vocalizations, and that physiological measures (heart rate) in response to sound are modified by the meaning of a sound. We have further shown that contextual cues modify the response of amygdalar neurons to social vocalizations. Two fundamental questions are of interest. First is the question concerns of which neural inputs provide contextual information necessary for interpretation of acoustic stimuli. The second question concerns the particular neurochemicals that facilitate this communication. This is the next step in our overall goal of understanding how these neural inputs act on amygdalar neurons to influence behavior.

4. Goals and objectives

Our long-term goal is to improve the understanding of neural mechanisms that underlie acoustic communication. The ***specific aims*** of the project are: (1) to identify the cortical and subcortical structures that provide information to the basolateral amygdala for contextual processing of social vocalizations, and (2) to identify and quantify the neurochemicals responsible for contextual modulation. We hypothesize that discrimination and selectivity in response to social vocalizations arises from projections of secondary auditory cortical areas. We further hypothesize that inputs from the prefrontal cortex, ventral tegmental area and hippocampus underlie contextual modulation of auditory responses. This work will provide guidance for future studies that investigate the origin of social vocalization selectivity in the basolateral amygdala. Of further interest is whether the stimulus selectivity found in BLA neurons is inherited from inputs or arises from local circuit interactions with inputs.

5. Investigative methods to be used

To identify inputs to the basolateral amygdala, we combine neurophysiological recording with retrograde tract-tracing. After surgical preparation, animals are placed in a stereotaxic restraint to record neurophysiological responses to sound. Once responses match those expected from the amygdala, a deposit of neural tracer is made. The electrode is then removed from the animal and the animal is allowed to recover. A five-day post-deposit time will be used to allow for successful transport of tracer along neuron fibers. After this period, animals will be deeply anesthetized and transcardially perfused with saline followed by a paraformaldehyde solution. Brains will then be blocked, sectioned and mounted on slides for neuronal tracing.

The neural tracers deposited into the basolateral amygdala are taken up by synaptic terminals of neurons that project to the amygdala from other brain regions, then transported back to their cell bodies. Inspection of brain sections will reveal labeled neurons that project to the amygdala. We will evaluate the numbers, types, and distribution of labeled cells in all locations. However, we will concentrate our study to labeling in areas that project specific auditory or contextual information to the basolateral amygdala.

To identify and quantify neuromodulators, we will surgically implant a guide cannula in the BLA. After recovery, the animal will be habituated to the test chamber for two days. The mouse will then be to one of the two social contexts (mating or stress context) for 1 hour. On the day of the experiment to measure neurotransmitters, we will insert a

microdialysis probe in the guide cannula and a heart rate lead will be connected to the animal. Samples of microdialysis fluid will be collected every 15 minutes for 3 hours. Samples from the first 90 minutes will be used to assess baseline neurotransmitter levels. Then, vocalizations related to the previously experienced context will be presented for 30 minutes and two samples will be collected. After that, 3-4 more samples will be collected to assess the post-exposure return to baseline. Microdialysis samples will be analyzed using HPLC in order to measure the concentration of neuromodulators (acetylcholine, dopamine, norepinephrine, serotonin and corticotropin-releasing factor) in each sample. These results will be compared to the animal's behavior and heart rate prior to, during and after the vocalization presentation. This experiment will allow us to assess how neuromodulator release in the amygdala is related to the behavioral response to contextual vocalization. In addition, it will provide insight into the internal state of the receiver of auditory information.

6. Proposed method of data analysis

Slide-mounted and cleared tissue sections will be examined using fluorescence microscopy. Sections containing fluorescently-labeled neurons or fiber tracts will be digitally photographed and the images stored for further off-line analysis.
Analysis will include, but not be limited to, counts of retrogradely-labeled cell bodies in areas of interest (association and auditory cortices, auditory thalamus, olfactory cortices, substantia innominata; parietal cortex) and reconstruction of projection pathways to the BLA. The tracer deposit site will be analyzed to ensure that fiber tracts bordering the BLA are not directly labeled.

For several regions of interest, we will reconstruct the distribution of labeled cells in three dimensions, a feature provided for by NeuroLucida.
Microdialysis samples will be analyzed using HPLC to both identify and quantify specific neuromodulators (acetylcholine, dopamine, norepinephrine, serotonin and corticotropin-releasing factor). The concentration of neuromodulators in each sample will be related to the animal's behavior and heart rate.

7. Significance of anticipated findings

These findings will be significant in several ways:

• The results will provide the first description of the convergence of auditory, olfactory, and other sensory information in amygdalar sub-regions responsive to social vocalizations. This helps to establish the neural circuitry underlying contextual modulation of auditory responses.
• The results will identify the regions of auditory cortex that may endow amygdalar neurons with responsiveness to social vocalizations. The results will guide subsequent neurophysiological studies of these auditory cortical regions, and seek to determine how auditory responses in amygdalar neurons arise.

• The results will identify brain regions that project to the amygdala to modulate auditory responses. By doing so, these results will guide subsequent deposits of viral tracers that allow selective stimulation of these input neurons by optical methods. This work will explain how specific inputs to the amygdala contribute to behaviors associated with social communication by sound.

• The results will identify the neuromodulators in amygdala that modulate an animal's behavior in response to vocalizations emitted in a particular social context. This will provide an important measure of the internal state of the animal. It will provide crucial insight into the contextual modulation of activity in the amygdala and can inform future experiments using voltammetry to measure finer time scales of neuromodulator release. In addition, these experiments will guide future research into neuron subtypes that are involved in modulating social.

B. Summer Research Fellow Training/Mentoring

All research will be conducted in the Acoustic Communication and Emotions Laboratory, which is part of the Department of Anatomy and Neurobiology at NEOMED. The laboratory includes three faculty, one research associate, and three graduate students. The student will work closely with Dr. Shanbhag, but interact extensively with other laboratory members. In particular, Zahra Ghasemahmad, a fifth year Biomedical Sciences graduate student, will supervise the microdialysis experiments as well as behavioral and vocalization analysis.

The laboratory emphasizes collaborative interactions, high expectations and enthusiasm. The group meets in weekly laboratory meetings where ideas are developed and technical issues and results discussed. Our laboratory has an extensive record of mentoring undergraduate and professional student trainees since 2009.

The fellow will be trained in many of the procedures associated with this project, commensurate with their skill and ability. The student will participate in neural recording experiments, in which small microelectrodes are placed into the amygdala of mice and tracers injected. The student will participate in histological processing to prepare brain sections for subsequent neural imaging and tracing. The student will also participate in analyzing the results of neural tracing studies. From these methods, the student will gain experience in identifying brain regions, histological and electrophysiological techniques, microdialysis and data analysis. The laboratory is well-equipped for neurophysiological studies of brain centers, behavioral testing, and neuroanatomical research. It is equipped with four experimental sound booths, a wet laboratory for histological processing, small animal surgery room, electrode fabrication room, and high end microscopy.

The fellow will also attend the weekly journal club of the Auditory Neuroscience Group (ANG). The highly interactive ANG is composed of members of nine hearing neuroscience laboratories with a wide range of experimental approaches. The fellow would be expected to present a summary of their summer project to this group.

Submit your application to Dr. Hans Thewissen

Title: CT investigation of cetacean fetuses

Abstract: This project aims to document the radiological morphology of fetuses of cetaceans (whales, dolphins, and porpoises). The fetuses that will be used have already been CT-scanned. Individual CT slices will be studied, and organs of interest identified and marked. The computer programs AMIRA and AVIZO will be used to make three-dimensional reconstructions of specific organs of these fetuses. The organs of interest are the sense organs: olfaction, vision, hearing, and balance.

Most postnatal cetaceans do not have olfactory organs, rudimentary olfactory organs, or small but functional ones. This project will help us determine whether some of small olfactory organs of cetaceans could still be functional. Study of the anatomy of the fetuses will help us understand the adult anatomy and guide dissections of cetaceans (which are not part of the project).

The position of the eyes is very different in cetaceans versus other mammals. An anatomical study of the eye of cetaceans (not part of this project) is investigating the topography of the retina: its shape, the location of the fovea, location of the optic papilla, shape of the globe and the distribution of ganglion cells. CT-scans of the eyes under the proposed project will help to place the retina in its anatomical context in the head.

The cochlea of the ear is oriented differently in cetaceans and this position may match the unusual orientation of the ear ossicles. In fact, it has been shown that cetacean ear ossicles rotate in ontogenetic development. However, the orientation of the cochlea has not been studied in this context. This study will document the position of the cochlea and its relation to the middle ear ossicles, and determine whether that position differs from that in postnatal individuals.

The organ of balance is located in the inner ear, and part of this organ is easily imaged. The semicircular canals are much smaller in cetaceans than in other mammals. These canals are oriented in very consistent planes in mammals, but their orientation and relative size in cetaceans is different. This part of the project will document whether the semicircular canals of the fetus are already similar to the postnatal individual or whether they undergo a change in morphology and orientation.

Significance: The sense organs of cetaceans are very different from those of other mammals. The ontogenetic development of these organs is poorly known. This project contributes to more detailed understanding thereof.
Goals: To produce three dimensional reconstructions based on CT data of organ systems studied in the lab, especially the sense organs (olfaction, vision, hearing, balance). The specific research question is to determine the shape differences in the sense organs between the fetuses studied and younger fetuses, as well as postnatal individuals.

Research Methods: Computer-based three-dimensional reconstruction of CT data using the programs AMIRA and AVIZO. Student will learn to use these programs and produce anatomical reconstructions based on them.

Relation of Other Research in the lab: The Thewissen lab studies the embryology of cetaceans using histological, anatomical, and developmental biological means. This project will focus on some of the larger fetuses and anatomically reconstruct organ systems that are also studied in smaller embryos. All sense organs are studied in the lab using histological, anatomical, and molecular techniques, the current project, using radiological data, will provide the context for all of these.

Student involvement: training on use of computer programs; attending all lab meetings with entire lab group (every other week). At least twice weekly meetings with PI, daily meetings with CT technicians. Research will take place in imaging lab of the Anatomy and Neurobiology Department.

Submit your application to Dr. Chris Vinyard

1. PROJECT TITLE: Oral processing and performance of mouth behavior groups during feeding.

2. ABSTRACT OF PROJECT:

This fellowship will be in support of an ongoing project aimed at evaluating the relationship between food preference and chewing physiology in humans. Recently, four major types of chewers have been identified based on their choices of foods to eat. It is likely that members of these groups differ in the way they chew their foods too, yet this assumption is untested.

This summer we will collect and analyze physiological data that will assess how different types of chewers process foods of varying textures. We will utilize surface electromyography (EMG) to record jaw-muscle activity patterns and a jaw-kinematic device to document the 3D movements of the jaw in up to 40 subjects. Together, these techniques will provide a thorough analysis of the masticatory process during consumption of different food types.

The data collected in this project will facilitate a more complete understanding of 1) variation in oral physiology across humans, 2) how this variation may relate to food preference and 3) how chewing behavior grouping may impact oral physiology.

3. BACKGROUND AND RATIONALE:

Recent work has tied food texture preference to food choice, described as mouth behavior (MB) groups, in a broad sample of humans. In these studies, consumers were asked to indicate preferences for various types of foods. Four mouth behavior groups were identified that describe most consumers: chewers, crunchers, smooshers, and suckers. These behavioral preferences for chewing styles are thought to play a significant role in consumer food choices and decisions in purchasing. While these behavioral groups play a role in food selection, it is unknown how closely they relate to oral processing during chewing.

To date, no one has systematically assessed how behavioral preference in food choice (e.g., MB groups) affects oral processing in humans. This absence exists despite evidence from non-human primates that preference can impact oral processing suggesting an evolutionary predisposition of preference impacting oral processing. The significance of this absence is further highlighted by our ability to accurately assess preference in humans (compared to other animals) and knowledge that oral processing is impacted by food material properties, satiety and satiation.

4. GOALS AND OBJECTIVES:

The primary goal of this project is to conduct a series of assessments to determine how preferred mouth behavior relates to oral processing during mastication of various foods. Oral processing will be evaluated using EMG and a jaw-tracking device, which will measure jaw-muscle activity and jaw movement patterns, respectively. These techniques will be employed in three different scenarios. In the first scenario, we will assess how members of the four mouth behavior groups chew a series of different foods

that are behaviorally linked to each of the groups (e.g., chocolates including nuts [crunchers/chewers] vs softer, melt-in-your-mouth chocolates [smooshers/suckers]). Second, we will assess how members of the four groups chew different food-grade gels of known material properties to evaluate physiological variation while holding food material properties constant. Third, we will assess any behavior by oral processing interaction during feeding by allowing participants to choose various foods that are behaviorally linked to the mouth behavior groups.

5. INVESTIGATIVE METHODS TO BE USED:

This project will involve collecting and analyzing data on jaw-muscle activity and jaw movements during oral processing of food items in up to 40 participants. Surface EMG will record the electrical activity of six muscles (left and right anterior temporalis, masseter, anterior belly of digastric) as they contract during chewing. Specifically, EMG will indicate when and how long a muscle is active during a chewing cycle as well as provide a measure of relative recruitment level (a correlate of muscle force production). Mandibular movements will be collected using a Jaw Tracker device that records 3D movements during chewing. This will provide information regarding the complex kinematics of the jaw during mastication and such data can be related to muscle activity patterns. The fellow will have the opportunity to participate in all phases of data collection.

6. PROPOSED METHODS OF DATA ANALYSIS:

We will rectify and integrate the recorded EMG signal to produce a single waveform estimate of muscle activity. We will then scale these waveforms to provide relative estimates of muscle activity. These relative estimates can be compared across jaw muscles and experiments. Similarly, we will measure the time of peak activity of the different jaw muscles during a single chew relative to the peak activity of the chewing-side superficial masseter. We will quantify jaw movements in three dimensions as well as estimate the length of the chewing cycle (e.g., chewing speed), the length of occlusion as well as the total distance the jaw travels during a single chewing cycle. We will also calculate the velocity of the jaw in various directions during chewing. Finally we will calculate the number of chews per chewing sequence (i.e., chews per bite of food) and the length of the sequence. These data will be compared via parametric statistics (e.g., ANOVA) across mouth behavior groups and foods.

7. SIGNIFICANCE OF ANTICIPATED FINDINGS:

We are not addressing a particular clinical pathology but rather asking a basic science question about the relationship between chewing behavior and oral physiology in humans. We anticipate that the basic science information gathered here will further inform our understanding of the physiological variation that occurs during mastication foods of different textures, which may be relevant to product testing and consumer preference.

8. APPENDIX:

We have already obtained IRB approval for this work and the fellow will complete the mandatory IRB training prior to any involvement in the study. The PI will also fill out the necessary amendment form to add the fellow to the protocol.

STUDENT TRAINING/MENTORING PLAN

The fellow will work closely with the faculty sponsor and Dr. Erin Franks, a postdoctoral researcher playing a lead role in the project, to become familiar with and proficient at all relevant techniques. Initially the student will be given relevant background readings that will then be discussed with the PI and postdoctoral researcher. The fellow will then be taught the appropriate data collection and analytical techniques. The student will have opportunities to learn about theoretical and technical aspects of electromyography and kinematics, in vivo data recording, statistical analysis of waveform and kinematic data, and the functional morphology of the human feeding apparatus. The importance of statistical hypothesis testing will be emphasized in this fellowship. Fellows will participate in the weekly biomechanics journal club and the end of summer research poster presentation at NEOMED. Fellows will have the opportunity to participate in the summer research series presented by Dr. Aultman. Furthermore, they can continue in this research after the 8 week term of the fellowship and be part of publications in peer-reviewed journals if so motivated.

All required materials and equipment for data collection and analysis are available in the faculty sponsor's lab facility.

The study will be conducted in the Department of Anatomy & Neurobiology – NEOMED.

Submit your application to Dr. Jeff Wenstrup

1. Project title, Principal Investigator name, title and location

Project: Determining the Meaning of Mouse Vocalizations.
Supervisor: Jeffrey Wenstrup, Ph.D., Professor and Chair of Anatomy and Neurobiology, NEOMED.

2. Abstract of project

Both human and animal vocal communication require the recognition and interpretation of sounds. The long-term goal of this research is to elucidate the mechanisms that underlie acoustic communication and develop strategies for treatments of communication disorders. Amygdalar dysfunction, and abnormal inhibition, has been linked to a range of disorders where the coding of a sound's context is abnormal. These disorders include; schizophrenia, autism, and posttraumatic stress disorder. Previous summer students in the lab have collected and analyzed data determining how context is encoded in mouse vocalizations. Their level of involvement gained them co-authorship on a peer-reviewed research article (Grimsley JMS, Sheth S, Vallabh N, et al. Contextual Modulation of Vocal Behavior in Mouse: Newly Identified 12 kHz "Mid-Frequency" Vocalization Emitted during Restraint. *Frontiers in Behavioral Neuroscience*. 2016;10:38. doi:10.3389/fnbeh.2016.00038). Their study showed that mice use different vocalizations and adapt the features of sounds depending on the context they are in. This included the discovery of a new mouse vocalization category emitted by mice in stressful situations. This project will go on to determine the meaning of these calls to the listener using behavioral and hormonal methods.

3. Background and rationale

Understanding how mice interpret this contextual information is critical for studies investigating the neural mechanisms of communication disorders. This project used behavioral and physiological methods in mice to determine the meaning of vocalizations in the listener. **The aim is to determine the contextual meaning of mouse vocalizations.** We have shown that mice emit four major categories of vocalizations in different situations. A mid frequency vocalization is emitted by mice in certain stressful situations. A broadband vocalization is emitted by animals in isolation. Ultrasonic song and an audible squeak are emitted by mice during mating. The selected student will investigate if these vocalization types have different effects on the behavior and physiology of the listener. The types of data collected include behavioral and hormonal assessments of stress. These results will be used to interpret the meaning of the vocalization types to the listener.

4. Goals and objectives

The goal is to determine the emotional meaning of the four major classes of mouse vocalizations.

Learning objectives:
The student will learn to assess stress levels in mice behaviorally.
The student will learn to analyze mouse vocalizations emitted in response to playback of conspecific vocalizations

The student will become familiar with the measurement of hormones in blood.
The student will become familiar with the medical research environment by actively participating in lab meetings and departmental journal clubs

5. Investigative methods to be used

Animals: All procedures are approved by the Institutional Animal Care and Use Committee at the Northeast Ohio Medical University (Approval ID number 15-034). Adult CBA/CaJ mice ranging between ages p90 to p150 will be used for this study (12 males, 12 females).

Behavior: Animals will be presented with one of four vocalization types in a counterbalanced order. Animals will hear only one vocalization type per day. Mice will be situated in an open field arena and their locomotive behavior will be recorded using DataWave Videobench.

Vocal behavior: The vocal behavior of the mice in response to the four different vocalization types will be recorded using an ultrasound microphone (Avisoft technologies).

Hormonal measurements of stress: Corticosterone levels will be used to compare the levels of stress in response to the different vocalization categories. Within 10 minutes of completion of exposure testing, animals will be anesthetized with Isoflurane (4%, Abbott Laboratories, Abbott Park, IL) and blood will be collected from the submandibular vein.

6. Proposed method of data analysis

Behavior: Video tracking will be used to monitor the locomotor activity of the animals. Stress levels will be monitored by assessing the animals' position within the arena and by scoring stress-related behaviors (rearing, grooming, tail rattling).

Vocal behavior: Individual vocalizations will be identified via analysis of the sound spectrogram using Avisoft Bioacoustics SAS lab. This will identify whether mice emit different vocalization types in response to vocalizations from their conspecifics.

Hormonal measurements of stress: Plasma corticosterone will be measured using a corticosterone enzyme-linked immunosorbant assay (EIA) kit. Multifactorial ANOVAs will be used to determine if hormone levels are affected by the sound type.

7. Significance of anticipated findings

The outcome of this study is of broad interest to scientists using mouse vocalizations as biomarkers for disease and those investigating communication disorders. A full understanding of acoustic communication systems requires an in-depth assessment relating vocalizations to caller and listener states. This study relates vocalizations to the state of calling mice and measures the effect they have on the listener. Mice have become an important model of vocal behavior and social communication, with research focusing both on the communication system within the brain and on the use of vocalizations as biomarkers of animal state in health and disease.

Summer Research Fellow Training/Mentoring Plan:
The lab is situated in the department of Anatomy and Neurobiology at NEOMED. It is well equipped for the undertaking of behavioral research in mice. Experiments will be performed within large sound attenuating chambers lined with anechoic foam. Mice communicate in the ultrasonic range thus acoustic stimuli will be presented with a speaker capable of accurately presenting signals at up to 100 kHz. The student will be trained to analyze mouse behavioral data by the skilled investigators within the laboratory; we are at the cutting edge of these methods and have recently published techniques for this analysis. The student may have the opportunity to observe corticosterone enzyme-linked immunosorbent assays as well.

The student will work closely with other members of the laboratory and will be trained on techniques one-to-one. We are a large lab (6 members) with a great team atmosphere that provides an exciting research environment. The selected student will partake in weekly lab meetings where ideas are developed, and results are discussed. The student will also attend a weekly journal club run by the Hearing Research Group and will present a summary of their summer project at this meeting.

Submit your application to Mr. Michael Appleman

1. Project Title:

Enhancing Students' Perceptions of the Learning Environment: Peer Education and Learning Community Program Models

2. Abstract:

Medical student success and well-being are of utmost importance to medical school faculty and staff, yet medical students' mental health tends to decrease as they progress through their education. One potential solution to addressing medical students' mental health is increased understanding of how they perceive, define and make meaning of their learning environments. Much research has been done on the subject in undergraduate education, although generalizations are narrow given the unique contexts of higher education environments. This study will investigate how students' perceptions of their learning environments influences their satisfaction during medical school. Further, this study will investigate programmatic methods to address low satisfaction and increase medical student well-being overall.

3. Significance of the Overall Research:

The categorization of learning environments is an important step toward understanding strengths and weaknesses of educational contexts. Much research is available in the general higher education literature, yet little has been transferred to medical education contexts. This is a gap in the literature ripe for attention. Further, methods for enhancing or improving students' perception of the learning environment are important for increasing congruence between perception and reality and increasing medical student well-being.

4. Goals and Objectives:

1. Determine whether peer education models are effective for improving students' perception of the learning environment
2. Determine whether learning communities are effective for improving students' perception of the learning environment.
3. Investigate medical school curricula and educational methods to increase medical student satisfaction and well-being overall.
4. Research question: What methods improve students' perceptions of medical education learning environments?

5. Research Methods:

1. Conduct a comprehensive literature review on the topic of students' perception of the medical education learning environment.
2. Conduct a comprehensive literature review to determine the characteristics of peer education programs and learning community models in US medical schools.
3. Working with the research team create a final report with recommendations to include the following:
   a. Internal and external resources for developing peer education programs at NEOMED.
   b. Internal and external resources for developing learning communities at NEOMED.

c. Challenges and barriers to offering medical students with peer education programs and learning communities.
d. Recommendations for the NEOMED medical education context.

6. Methods of Data Analysis:

- Literature review
- Review and analysis of educational policy

How anticipated findings will contribute to the success of the overall research:

1. A document that summarizes peer education and learning community opportunities at NEOMED
2. Recommendations for new educational opportunities
3. A Poster or presentation at a regional or national meeting
4. A brief report to be submitted to an educational journal

7. <u>Student Fellow Training/Mentoring Plan</u>

1. The student will meet with Dr. Janice Spalding, vice chair of the Department of Family and Community Medicine, and/or Mr. Appleman on a weekly basis.
2. All students working in the Department of Family and Community Medicine will participate in for discussion sessions including
    a. establishing a research question
    b. conducting a gap analysis/literature review
    c. protection of human subjects
    d. posters and presentations
3. Students will work with Dr. John Boltri, chair of Family and Community Medicine, and his team to learn how to submit a regional or national presentation of their work.

8. Resources Available: the student will be provided space in the Department of Family and Community Medicine. The student will also be provided research and statistical support as needed.

Site Where Research Will Be Conducted: the research will be conducted at the Department of Family and Community Medicine.

Submit your application to Dr. Stacey Gardner-Buckshaw

1. **Title:** Primary Care Implementation of MAT for Substance Use Disorder in Northeast Ohio

   Principal Investigator: Stacey Gardner-Buckshaw, Ph.D., MPA, Director of Community Engagement, Department of Family and Community Medicine, NEOMED

   (John Boltri, M.D., Chair, Department of Family and Community Medicine is the PI of the HRSA PCTE grant and will provide support for this project)

   Location: Department of Family and Community Medicine, G-141. Research will be conducted at NEOMED, with potential travel to Metro Health for meetings with collaborating faculty.

2. **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 are collaborating to offer Medication-Assisted Treatment (MAT) training designed specifically for PCPs. The recent opioid crisis has created an increased need for addiction treatment. Primary care residency programs offer limited exposure to patients suffering from addiction relative to the demand. Concern regarding opioid misuse remains perhaps the most significant barrier to the optimal use of opioids in patients with chronic, noncancer pain. This includes the risk of addiction in patients, drug diversion by patients (or members of their household) for nonpain purposes, the fear of attracting substance abusers to one's practice, and fear of legal/regulatory authorities.

   To assist PCPs in the successful incorporation of MAT into graduate medical education and primary care practice, NEOMED and Metro Health will host four training sessions from November 2017 – April 2018. 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 will teach 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.

   To attract PCPs, resources from HRSA enable us to reimburse providers for time in training. This program model gives access to critical MAT information, without pressure to apply for the X waiver. PCPs will have the qualifications to apply, but will not be required to do so to receive compensation. To sustain the activities after the funding period has ended, program participants will receive a list of resources to reference as they champion the effort at their sites and acclimate the residents, that compliments what they learned during training and certification. Dr. Christina Delos Reyes and Dr. Russell Spieth, will be available for ongoing program consultation, clinical consultation

and training to participants as needed to incorporate the content in their practices. Graduates of the MAT trainings are invited to participate in Project ECHO, in which NEOMED's Dept. of Psychiatry is a partner, which provides support to prescribers.

3. **Goals and Objectives:** The goal of this study is to assess how participation in the NEOMED/Metro Health MAT training for PCPs influenced implementation of MAT into graduate medical education and primary care practice 4 months after training. To accomplish this, the summer research fellow will develop and implement a 5-minute interview protocol aligned with the pre- and post-test evaluation tool administered at the MAT in-person training sessions. Results will be analyzed and disseminated in a report to participants, and in a poster presentation.

4. **Significance of Anticipated Findings:** Results of this study will inform best practices in MAT training for PCPs. Specifically, if remuneration for time in training is successful in attracting providers who are interested in the topic but unsure about committing to X waiver application, and if training ultimately resulted in MAT implementation in graduate medical education and primary care practice.

5. **Investigative Methods:** The student will develop and complete short survey interviews that require qualitative and quantitative methods.

6. **Proposed Method of Data Analysis:** This project requires simple descriptive statistics, frequencies, crosstabs and comparative means tests.

7. **Student Role:** Under direction of NEOMED and Metro Health faculty, 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 once weekly with collaborating NEOMED and Metro Health faculty as needed. With other student summer research fellows in the Department of Family and Community Medicine, the student will receive research training led by Dr. Julie Aultman.

   Mentoring Plan:
   1. The student will meet with Dr. Gardner-Buckshaw and/or Dr. Boltri weekly.
   2. All students working in the Department of Family and Community Medicine will participate in discussion sessions about:
      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. Students will work with Dr. Boltri's team and learn how to submit a regional or national presentation of their work.

8. **Resources Available:** the student will receive space in the DFCM, with access to computers and a telephone for survey interviews and data collection. The student will also receive research and statistical support as needed. Funds from the HRSA PCTE grant may be used for student travel to meetings/interviews/presentations, or for poster printing for dissemination.

Submit your application to Dr. Rebecca Fischbein

1. **Title:** Patient and Family Needs During a Medically High-Risk Pregnancy: A Qualitative Systematic Review

2. **Abstract:** The medically high-risk, or complicated, pregnancy can be a stressful experience, both physically and psychologically, for patients and families. The results of qualitative research afford researchers and practitioners the opportunity to better understand and address patient and family needs during these pregnancies. This research aims to find common disease experiences through a qualitative systematic review which will synthesize the results of studies that have examined different diseases, populations, and settings.

3. **Significance:** This research will explore the common, shared needs during a medically high-risk pregnancy. However, while uncovering shared experiences, this study will also provide the opportunity to explore differences across diseases, populations, and settings. This knowledge can be used to guide theory as well as provide practical recommendations to help address the needs of patients and families during a medically high-risk pregnancy.

4. **Goals and Objectives.** The objective of this study is to systematically review the needs of patients and families during a medically high-risk pregnancy. The summer research student will be involved in all aspects of the study. The iterative process of a qualitative systematic review will begin with refinement of the research question, identification of key search terms, review of the literature, grading of the literature, and synthesis of findings, and conclude with formulation of ratings of the relative confidence that researchers and practitioners can have in the developed findings.

5. **Research Methods:** The student will learn the process of conducting a rigorous qualitative systematic review, following the GRADE-CERQual process, a system designed with support from the Cochrane Methods group. GRADE-CERQual was developed to facilitate researchers' ability to identify, synthesize, and assign a confidence rating to findings based on four primary criteria: methodological limitations; coherence; relevance; and adequacy. The student will learn how to identify the key MeSH search terms, establish inclusion criteria, develop data extraction forms, conduct searches in multiple databases, identify articles for inclusion, extract key data, and develop confidence ratings for synthesized findings.

6. **Data Analysis**: Data analysis will include extraction, synthesis, and calculation of confidence ratings, based on the four GRADE-CERQual criteria shown above, relative to the qualitative research findings gathered from studies meeting inclusion criteria.

7. **Contribution to Overall Project Success**: This project is part of a larger set of research examining experiences related to medically high-risk pregnancies. This study will compliment a currently, in-progress quantitative systematic review.

8. **Student Fellow Training/Mentoring Plan**

Training for the summer research fellow will include one-on-one mentoring with Dr. Fischbein. Dr. Fischbein is collaborating with colleagues at other universities who have expertise in in qualitative systematic reviews and have received direct training and support from members of the international GRADE- CERQual group. The research fellow will also receive training and guidance from these individuals. The research fellow will also participate in the summer research series through the Department of Family and Community Medicine.

The student will conduct the research using Department of Family and Community resources, which includes access to desk space and computer and any necessary software.

Submit your application to Dr. Amy Lee

1. **Title:** Finding a Fetal Alcohol Spectrum Disorders (FASD) Screening Tool for Youths
Principal Investigators: Amy Lee, MD, MPH, Professor and Director, Consortium of Eastern Ohio Master of Public Health & Stacey Gardner-Buckshaw, PhD, MPA, Assistant Professor and Director of Community Engagement

Location: NEOMED Department of Family and Community Medicine. Meetings at Stark County organizations, possibly including Child and Adolescent Behavioral Health in Stark County, United Way of Greater Stark County, Stark County Board of Developmental Disabilities, and Stark County Educational Services Center.

2. **Abstract of Project**

Fetal Alcohol Spectrum Disorder affects up to 10-15 per 1,000 births in high risk populations. Research has shown that 60% of FASD youths become in trouble with the law, among other problems. Stark County is in the unique position, possessing one of three multi-disciplinary diagnostic clinics in Ohio, of being able to treat youths with FASD. However, the barrier is that the juvenile court is unaware of any rapid screening tool for FASD for youths. Existing tools screen parents, and many of the incarcerated youths have foster or adoptive parents who do not know the birth history. This project proposal is for a student to develop criteria for an FASD screening tool for youths based on input from Stark County stakeholders and to search and literature for appropriate tools. The student will interview key informants, based on the literature search, and categorize the screening tools according to the established criteria. Findings will be presented at an FASD committee meeting on August 10, 2018.

Results: The results will be shared with the Stark County FASD committee so that they can make a decision to implement any tools that are recommended based on pre-determined criteria.

3. **Background and Rationale**

Fetal Alcohol Spectrum Disorder (FASD) is estimated to affect 1-3 per 1,000 births. In high risk populations, such as foster care populations, the prevalence may be up to 10-15 per 1,000 births. Although most mothers may not consume alcohol in pregnancy, they may consume alcohol before they know they are pregnant in the first 12 weeks. Unfortunately, weeks 3-8 is a critical period of a pregnancy, in which the developing baby is sensitive to brain damage from FASD. Among children with FASD, research has indicated the 90% are also diagnosed with mental health problems, 60% have problems with school placement, 60% are in trouble with the law, 50% get confined in inpatient treatment or jail, 50% have inappropriate sexual behavior, 30% have drug and alcohol problems, and 80% have trouble with employment.

In Ohio, an estimated 114,000 Ohioans may have FASD, but only 300 have been clinically diagnosed. In Stark County, estimated prevalence of FASD based on 2017 birth rates is 2000 cases (for birth-50 years).

In Stark County, the Juvenile Court has a Behavioral Health unit that conducts an assessment within 24 hours of a youth being placed in the Attention Center. FASD is often considered in the incarcerated youth population. The director of the Juvenile Court Behavioral Health unit, Kimberly Genis, Med, LPCC, describes that current screening tools are designed for the parent; however, 80% of youths likely to live in a foster or adoptive home, which limits the ability to gather accurate historical information. The Ohio FASD State Steering Committee and other sources were unfamiliar with a tool to screen for FASD in youths. Based on this challenge, the Stark County FASD committee would like to find a screening tool for youths so that undiagnosed of misdiagnosed youths with FASD can access appropriate treatment. Stark County has a unique opportunity to treat these youths, since Child and Adolescent Behavioral Health in Stark County is one of only three multidisciplinary FASD diagnostic clinics in Ohio.

4. Goals and Objectives

The goal of the project is to identify existing screening tools for FASD that might be used or adapted for youths.

Objectives of the project are the following:

1. By July 31, 2018, list the evaluation criteria that Stark County stakeholders desire for an FASD screening tool for youths.
2. By August 31, 2018, identify potential screening tools to identify FASD in youths.
3. By August 31, 2018, provide recommendations, based on stakeholder criteria, for screening for FASD in youths both in presentation and paper format.

5. Investigative Methods to be Used

The student will conduct the following activities:

- Conduct a literature search on the FASD and FASD screening tools; review FASD problem in Stark County.
- Meet with pertinent stakeholders in Stark County to develop an evaluation template to identify a useful FASD screen tool criteria. Stakeholders will indicate criteria that will determine characteristics of the most effective tool.
- Conduct key informant interviews using the evaluation template to determine the usefulness of tools found in the literature. Example evaluation aspects that the student will develop as part of the interview components may include usefulness in the youth population, time needed to administer the tool, and ease of use.
- Compile a listing of possible FASD screening tools. Calculate positive/negative predictive values, sensitivity and specificity on each tool. Rate tools based on stakeholder criteria.
- Analyze the results.
- Write a research paper on background, methods and findings, providing recommendations.
- Present on findings.

Besides the research methods that the student will perform, the student will also be coached on project management, including creating a project charter, meeting agendas and summaries and following a project timeline, using a Gantt chart. The student will be interacting with professionals in the community knowledgeable about the FASD in the community, so will need to practice professional behavior.

6. Proposed Method of Data Analysis

The student will have both quantitative and qualitative results. The key informant interviews will be collected in a table format, using the evaluation template. For the evaluation template, the data analysis will involve frequency distributions. The qualitative data will be considered in the rankings for tool usefulness. The sample size will likely be too small to use statistical testing.

7. Significance of Anticipated Findings

If the student is able to identify a useful tool that can be used in Stark County that can quickly identify youths in juvenile court as having FASD, they may have access to the Child and Adolescent Behavioral Health clinic where they can be diagnosed and viewed through a neurodevelopmental lens, rather than as a juvenile with behavioral problems in need of punishment. Appropriate supports can be put in place, and they would have access to services through the Stark County Board of Developmental Disabilities.

8. Appendix

- *Plan for training/mentoring the summer research fellow—individual, group, lab meetings, journal clubs, seminars, etc.* The student will have regular meetings with the faculty advisors as part of the research "team." Part of the goal, besides learning research skills, is being able to demonstrate project management techniques, such as creating agendas and meeting summaries and adhering to a project timeline. The student will be invited to any FASD or relevant Stark County agency meetings occurring in the summer. In the past, the Department of Family and Community Medicine has had a research seminar series; if this series occurs this year, the student would be expected to attend.
- *Description of resources available.* The student will have a desk in the Department of Family and Community Medicine. In addition, one of the Stark County agencies in the FASD committee may be able to provide a stipend for student travel.
- *Site where the research will be conducted.* The research will partly be conducted at the NEOMED and some stakeholder interviews will occur in Stark County agencies. The final presentation will be at Child and Adolescent Behavioral Health on Fulton Dr. in Canton. The literature search, key informant interviews, and data analysis will occur at NEOMED.

References:

Adubato SA and Cohen DE. Prenatal alcohol use and fetal alcohol spectrum disorders: diagnosis, assessment and new directions in research and multimodal treatment. Bentham Books, 2011.

Baker T. Child psychologist, Child and Adolescent Behavioral Health, correspondence. February 16, 2018.

Blaisman L, Assistant CARE Team Director, correspondence, February 16, 2018.

Ohio Mental Health and Addiction Services. Not A Single Drop: Ohio's fetal alcohol spectrum disorders initiative. http://mha.ohio.gov/Portals/0/assets/Prevention/FASD/Ohio-FASD-Fact-Sheet.pdf Accessed February 16, 2018.

North Dakota Fetal Alcohol Syndrome Center. Prevalence and cost calculator. http://www.online-clinic.com/calcs/calc-prev-cost.aspx Accessed February 16, 2018.

Submit your application to Dr. Janice Spalding

1. **Project Title:** Primary Care Medical Education: An Investigation of Direct Primary Care Services and Educational Opportunities

2. **Abstract:**

According to Healthy People 2020, a seminal report published by the Department of Health and Human Services, access to health services is the most pervasive health care problem in the US. Barriers to health services include: the high cost of care, inadequate or no insurance coverage, lack of availability of services and culturally competent care. These barriers to accessing health services lead to: unmet health needs, delays in receiving appropriate care, inability to get preventive services, financial burdens, and preventable hospitalizations. In terms of access, primary care is a vital component of the US health care system. When access to primary care services is high, the health of populations improves. Unfortunately, there are numerous impediments to greater access to primary care in the US, and the US rates poorly on many health outcome measures. This creates a need for better care quality, efficiency, and decreased costs. Alternative care models such as direct primary care present possible solutions to the need for greater primary care access. This study will investigate the prevalence and efficacy of the direct primary care model in the US. Additionally, medical education opportunities will be investigated.

3. **Significance of the Overall Research:**

Access to health services is the most significant problem in the US health care system. Direct Primary Care is a growing care model which addresses barriers to health services. Increased knowledge of the Direct Primary Care model is the first step in understanding what educational opportunities will best prepare students to make career decisions as it relates to their primary care skills, business acumen, and advocacy for underserved populations.

4. **Goals and Objectives:**

1. Determine the prevalence and location of Direct Primary Care models in the US
2. Determine the efficacy of Direct Primary Care
3. Investigate medical school curricula and educational methods to expose medical students to Direct Primary Care.
4. Research question: What opportunities in Direct Primary Care will benefit aspiring primary care physicians?

5. **Research Methods:**

1. Conduct a comprehensive literature review to determine the prevalence of Direct Primary Care in the US.
2. In concert with the research team, conduct interviews and/or disseminate surveys to internal and external stakeholders regarding Direct Primary Care in northeast Ohio.
3. Working with the research team create a final report with recommendations to include the following:
   a. Internal and external resources for offering Direct Primary Care experiences
   b. challenges and barriers to offering Direct Primary Care experiences

c. recommendations for overcoming barriers to offering Direct Primary Care experiences

6. Methods of Data Analysis:

- Literature review
- Analysis of semi-structured interviews
- Textual analysis of survey data

7. How anticipated findings will contribute to the success of the overall research:

1. A document that summarizes Direct Primary Care in the US and northeast Ohio
2. Recommendations for educational opportunities
3. A Poster or presentation at a regional or national meeting
4. A brief report to be submitted to an educational journal

8. Student Fellow Training/Mentoring Plan

1. The student will meet with Dr. Spalding and/or her research team on a weekly basis.
2. All students working in the Department of Family and Community Medicine will participate in for discussion sessions including
   a. establishing a research question
   b. conducting a gap analysis/literature review
   c. protection of human subjects
   d. posters and presentations
3. Students will work with Dr. Spalding's team and learn how to submit a regional or national presentation of their work.

Resources Available: the student will be provided space in the Department of Family and Community Medicine. The student will also be provided research and statistical support as needed.

Site Where Research Will Be Conducted: the research will be conducted at the Department of Family and Community Medicine.

Submit your application to Dr. Yeong-Renn Chen

1. Project title: Cardiac metabolism in the disease of chronic myocardial infarction and heart failure
Principal Investigator Name: Yeong-Renn Chen, Ph.D.
Title and location: Professor, RGE338.

2. **Abstract of Project:**

The objective of this proposed research project is to study the disease biomarkers related to metabolic syndrome, associated mitochondrial dysfunction in the disease mechanism of chronic myocardial infarction. The project will be focused on one disease model of chronic myocardial infarction, one major metabolic pathway of fatty acid oxidation (FAO) in myocardium, and phenotypic switch of FAO pathway to glucose oxidation (GO) pathway in the disease progress of heart failure. Catabolic pathway of FAO as the major source of fuel oxidation in heart and a major target of oxidative damage occurred during chronic myocardial infarction and consequent heart failure. Impairment and down-regulation of cardiac FAO is closely related to mitochondrial dysfunction caused by chronic myocardial infarction. The disease model of chronic infarction associated heart failure will be created by the animal surgery of in vivo occlusion of rat heart and followed by in vivo reperfusion. Dimethyl stable isotope will be used to label key enzymes controlling FAO and GO in cardiac mitochondria, and gluconeogenesis in cytosol. Chronic post-ischemic effect on the major metabolic pathways of FAO and GO will be assessed by tandem mass spectrometry (LC-MS/MS). The progress of this project will advance our knowledge toward understanding disease pathogenesis of chronic reperfusion injury and promote diagnosis as well as developing therapeutic intervention of heart failure.

3. **Background and rationale:**

Myocardial infarction is known as heart attack. More than 0.7 million Americans suffer a heart attack every year with 50% mortality rate. Mitochondria as the major source of energy generation are essential for proper cellular function in heart. There is considerable evidence supporting the key role of mitochondrial dysfunction and metabolic syndrome in the disease pathogenesis of chronic myocardial infarction. At the myocardial level of the post-ischemic heart, a defect in energy metabolism associated with mitochondrial dysfunction was marked. Downregulation of FAO has been further detected in the mitochondria of the disease model of acute myocardial infarction. The above decreased FAO is closely associated to metabolic switch to GO and gluconeogenesis in disease development of heart failure. This project is proposed to test the hypothesis of that metabolic switch of the marked FAO downregulation to dominant glucose-based metabolism in the myocardium mediates the disease progress of heart failure resulted from chronic myocardial infarction and post-ischemic injury.

4. **Goals and objectives**

The objectives of this research are to assess the role of metabolic switch in the pathogenesis of chronic myocardial infarction and to explore new insights into the specific disease biomarker of mitochondria in order to more fully understand the mechanisms of cardiovascular disease. As metabolic syndrome associated FAO downregulation in the mitochondria is likely to have an impact on fuel homeostasis in

myocardium, it is desirable to obtain further information on how metabolic switch affects the function of mitochondria, overall cardiac function, and regulation of the heart remodeling and related pathogenesis of heart failure development. To partially address this issue in 8 weeks and optimize the efficacy of summer fellowship training in biomedical research, the proposed studies have been designed to narrow the scope of investigation focusing on the mapping the metabolic pathways of FAO and GO in the mitochondria from the disease model of chronic myocardial infarction using proteomic approach.

5. Investigative methods to be used

In vivo disease model of myocardial infarction using rat - The procedure for the in vivo myocardial ischemia and reperfusion to create the disease model of heart attack will be performed by the technique reported in our previous publications [Chen, Y-R (2007) J. Biol. Chem., 282, 32640-54, and Chen, C-L (2008) J. Biol. Chem. 283, 27991-28003]. Sprague-Dawley rats (~300-350 g and 10-12 weeks old) will be anesthetized, and subjected to 30-min of in vivo coronary artery ligation followed by 3 weeks reperfusion. At 3 weeks post-infarction the rat will be placed under deep anesthesia. Rat will then be sacrificed, and the rat heart will be excised. The infarct area of myocardium will be identified by 2,3,5-triphenyltetrazolium chloride (TTC) staining. The risk region of myocardium will be excised for biochemical analyses.

Mitochondria preparation and oxygen consumption measurements - Mitochondria will be prepared from the non-ischemic and infarct tissues by differential centrifugation described in the published method [Chen, Y-R (2007) J. Biol. Chem., 282, 32640-54; Chen, C-L (2008) J. Biol. Chem. 283, 27991-28003; Lee, H-L (2012) AJP-Heart and Circulatory Physiology 302, H1410-1422]. Mitochondrial respiration will be measured by the polarographic method using a Clark-type oxygen electrode (Oxytherm, Hansatech Instruments) at 30 °C. The NADH-linked respiration will be induced by malate/glutamate.

In-solution proteolytic digestion – The mitochondrial preparation will be subjected to in-solution proteolytic digestion with sequencing grade trypsin/LysC by following our recent published approach [Zhang L (2017) Free Radic. Biol. Med. 108, 595-609].

Dimethyl stable isotope labeling of the tryptic digests and LC-MS/MS analysis – Tryptic digests of mitochondrial preparation will be subjected to dimethyl stable isotope labeling according to our published method [Zhang L (2017) Free Radic. Biol. Med. 108, 595-609]. Digested samples will be analyzed with LC-MS/MS via capillary liquid chromatography coupled with a LTQ-Orbitrap mass spectrometer. These experiments will be conducted by following our publishing approach [Zhang L (2017) Free Radic. Biol. Med. 108, 595-609; Chen, Y-R (2007) J. Biol. Chem., 282, 32640-54; Chen, C-L (2008) J. Biol. Chem. 283, 27991-28003; Zhang L (2010) Biochemistry 49 2529-2539; Kang PT (2012) Free Radic. Biol. Med. 53, 962-73].

6. Proposed method of data analysis

(1) Data analysis of mitochondria-mediated oxygen consumption rate will be accessed by the first derivative of kinetic curve of oxygen consumption rate and NADH oxidation rate.

(2) Mitochondrial preparations (30 μg) from the healthy tissue and infarct tissue will be subjected to in-solution trypsin digestion at 37 °C for 12 h. Mixtures of tryptic peptides were then globally dimethyl labeled at the N-terminus and –amino group of lysine by formaldehyde (HCHO for healthy mitochondria and DCDO for infarct mitochondria) and subjected to reductive amination with sodium cyanoborohydride (NaBH3CN) prior to LC-MS/MS and MaxQuant software analysis. The labeling strategy will produce peaks differing by 28 mass units for each derivatized site relative to its non-derivatized counterpart and 4 mass units for each derivatized isotopic pair.

(3) Sequence information from MS/MS data will be processed by converting the raw data files (.raw) into a merged file (.mgf) using an in-house program, RAW2MZXML_n_MGF_batch (merge.pl, a Perl script). The resulting mgf files will be searched using the quantitative proteomics software package of Mascot Daemon by Matrix Science. The raw data of MS/MS analysis will be further searched using additional software package of MaxQuant to ensure reproducibility and accuracy. The database will be searched against the SwissProt rat database.

(4) All data will be reported as group averages ± SEM. Statistical analyses and comparison between two groups will be assessed by Student's t test and among three groups will be assessed by one-way analysis of variance followed by the Least Significant Difference, Tukey's HSD or Games-Howell post hoc tests.

7. Significance of anticipated findings

By combining the biochemical and proteomic approaches with unique in vivo disease model, new information will be elucidated toward understanding metabolic switch in regulating the mitochondrial dysfunction and disease process of heart failure. The studies will be potentially translated to establishing a useful disease biomarker and metabolic profiles available in the key pathways of energy homeostasis during disease progress of chronic myocardial infarction. Results from these studies will increase the depth of understanding of disease pathogenesis and could be potentially translated to clinical diagnosis and therapeutic intervention for ischemic disease and heart failure.

8. Summer Research Fellow Training/Monitoring Plan. The plan I have for the student fellow is arranged in a hierarchical manner.

a. First, the student will interact with Dr. Chen, and Dr. Chen's lab members, who will explain the rationale, then teach the techniques using in vivo myocardial ischemia and reperfusion system and mitochondrial preparation, the assay procedures, in-solution trypsin digestion, dimethyl stable isotope labeling, data analysis and interpretations. LC-MS/MS analysis will be conducted in the facility of mass spectrometer available in the Department of Pharmaceutical Sciences/NEOMED under the supervision of Dr. Kasumov. There will be 1:1 meetings between the student and the mentor as well as 2:1 meetings involved in Dr. Chen, Dr. Kang (a research scientist of Dr. Chen. Dr. Chen will serve as mentor and Dr. Kang will serve as technical mentor). Training of disease model, mitochondrial biology, in-solution trypsin digestion, stable isotope labeling and LC-MS/MS will be completed within first 6 weeks. Training in the final two weeks will be working on mapping metabolic profiles of FAO and GO using the software package of MaxQuant.

Second, the student trainee will attend the biweekly lab meeting of the Cardiovascular Interest group (a combined lab meeting of the faculty with interest in cardiovascular research including Drs. Chen, Chilian, Dong, Meszaros, Ohanyan, Raman, Thodeti, Yin, and Yun), and will present results in this meeting.

Third, the student will participate in a summer journal club that will involve in all the summer research students and faculty. Each summer student will be expected to participate in discussion.

Fourth, the student will be expected to present a poster at the research day when all summer fellows present a synopsis of their summer work.

b. I have an active RO1 research grant to fund my lab. All the necessary sources, equipment, and finances are available. Furthermore, a research scientist and a research technician are available to help in training student fellow.

Submit your application to Dr. William Chilian

1. **Title:** The Role of Bone Marrow Derived Stem Cells in Coronary Collateral Growth.
Co-Principal Investigator: Liya Yin, Associate Professor, Integrative Medical Sciences
Co-Principal Investigator: William M. Chilian, Professor and Chair, Integrative Medical Sciences
Location: NEOMED

2. **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, because 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. The purpose of this project is to define the role of endogenous stem cells in coronary collateral growth. Currently, there is no evidence (pro or con) that endogenous stem cells are even involved in coronary collateral growth. The goal of this project is to rectify this deficiency by establishing if stem cells participate in coronary collateral growth and then the mechanisms underlying this effect. Within this context, we propose to establish the sub-type(s) of stem cells that home to the heart and coronary vasculature. Then will we study if depletion or enrichment of these cells (during the creation of a chimeric model) will blunt or magnify, respectively, coronary collateral growth. We hope to establish the fate of the stem cells and determine if they engraft in collateral arteries and differentiate into smooth muscle or endothelium.

3. **Background and Rationale.** In this summer project, we will focus on defining the role that endogenous bone marrow-derived stem cells (BMCs) have in coronary collateral growth, with the ultimate goal of using such information to amplify this natural mechanism in patients. One enigmatic observation is that collateral growth, i.e., the expansion of pre-existing arterial-arterial anastomoses, requires additional vascular cells when a vessel expands from a 20-40 µm microvessel to a 100-200 µm small artery, but despite this, analyses of cell proliferation show a muted effect, i.e., examinations of cell proliferation shows a scant number of proliferating cells in the vascular wall. It is this enigma that has fueled our interest in the recruitment of BMCs in this adaptive process—perhaps collateral growth depends on recruitment of stem cells from the bone marrow for growth instead of proliferation of cells in the vascular wall. What also gives pause in this line of thinking is the consideration that if cells in the vascular wall proliferate, there would be a phenotypic switch (considering smooth muscle) from a contractile to a synthetic phenotype, which is thought to be one of the processes of neointimal proliferation in coronary disease—not an adaptive response of abluminal remodeling. Perhaps, to minimize risks of neointimal proliferation, our system has adapted a strategy for collateral growth, using the recruitment of BMCs (which may proliferate once in the heart), which is what we will interrogate this summer.

4. **Goals and objectives**. The goal of this summer research is to determine if recruitment of bone marrow-derived stem cells is critical for coronary collateral growth. For this area to advance it is important to decipher the role of BMCs in this adaptive process.

5. Investigative Methods. First, chimeric rats will be created using bone marrow from a donor rat (bone marrow is isolated from a transgenic rat in which GFP is expressed by the ubiquitin promoter so all cells are fluorescent [GFP^+]). The recipient rat is given a dose of radiation that would be fatal if not for the reimplantation of the GFP^+ bone marrow cells. Rats will have 4-6 weeks for engraftment and proliferation of bone marrow, then will be subjected to a protocol involving implantation of a pneumatic snare around the left anterior descending artery that can be used to produce episodic myocardial ischemia to stimulate coronary collateral growth. After recovery from the instrumentation surgery, measurements of collateral flow and heart function will be made prior to the onset of the episodes of ischemia, and then 10-12 days after the protocol of episodic ischemia. Measurements are completed in anesthetized rats (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 rat will be euthanized and the hearts will be removed for imaging analyses. In particular, we will analyze the heart for GFP fluorescence as these signals should be derived from BMCs.

6. Proposed method of data analysis. The analysis will involve only unpaired t-tests and ANOVA followed by a multiple comparison test. $P<0.05$ will be accepted for statistical significance.

7. Significance of anticipated findings. If our model is successful and reproducible it will accelerate our understanding of mechanisms underlying coronary collateral growth and facilitate the implementation of therapies using bone marrow-derived stem cells.

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

a. First, the student will interact in a 1:1 manner with Dr. Yin and with Dr. Chilian. Dr. Chilian's laboratory will teach the surgical and experimental procedures, including echocardiographic measurements and data analysis.

Second, the student will interact with Drs. Yin and Chilian in a 1:2 manner reviewing the data, the protocols, the rationale and the interpretations. Some of these meetings will include both Dr. Chilian and Dr. Yin, who will serve as co-mentors.

Third, the student will work with Dr. Yin to perform imaging analysis of the GFP^+ cells in the heart. The student will meet with Drs. Yin and Chilian to discuss the results.

Fourth, the student will attend the lab meetings of the Heart and Blood Vessel Disease Focus group (a combined lab meeting of the faculty with interest in cardiovascular research (Drs. Chilian, Penn, Chen, Raman, Thodeti, Meszaros, Yin, Ohanyan, Dong, Mayorga, and Yun) and will present the results in this weekly meeting.

Fifth, 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.

Sixth, the student will be expected to present a poster at the research day when all

summer fellows present a synopsis of their work.

b. All the necessary resources (echocardiographs, anesthesia machines, computer for measuring evaluating echo images, mice, surgical instruments, surgical supplies, ultrasonic contrast, irradiator, confocal and fluorescence microscopy) and financial resources for completing the research are available.

c. The research will be completed at NEOMED.

Submit your application to Dr. William Chilian

1. **Title:** The Mechanism of Coronary Microvascular Insufficiency in Takotsubo Syndrome
Co-Principal Investigator: William M. Chilian, Professor and Chair, Integrative Medical Sciences.
Co-Principal Investigator: Vahagn Ohanyan, Assistant Professor, Integrative Medical Sciences
Location: NEOMED

2. **Abstract.** The goal of this proposal is to test the hypothesis that Takotsubo Syndrome results from an impairment in the connection between myocardial blood flow and cardiac metabolism, because of inadequate production of certain metabolic dilators. In the heart, the connection of flow to aerobic metabolism is critical to maintain cardiac pump function, and we have found a certain redox-sensitive ion channel, Kv1.5, plays a key role in this link. We have also observed that when subjected to hypertension or catecholaminergic stress mice null for Kv1.5 channels rapidly develop a phenotype similar to the human condition known as Takotsubo Syndrome. This syndrome is also known as the "Broken Heart Syndrome" or "Apical Ballooning Syndrome and has a characteristic anomaly—when the heart contracts during systole, the apex of the heart dilates (it should contract) as the base of the heart contracts. Although infrequently fatal, this condition is debilitating to a patient and recovery can take up to 6 months. Interestingly, the prevalence of this condition is increasing (thought to be related to better diagnosis) and up to 8% of patients admitted for signs of an acute myocardial infarction, instead have Takotsubo Syndrome. Our goal is to determine if Takotsubo Syndrome is caused by differences in the production of vasoactive metabolites from the base and the apex of the heart.

3. **Background and Rationale.** We will interrogate the connection between coronary microvascular dysfunction in Takotsubo Syndrome by analyzing the production of vasoactive metabolites from the base and apex of the heart. In patients, Takotsubo Syndrome is a debilitating condition with no accepted standard of care. In order to develop a treatment, it is critical that the cause of the disorder is identified—only then could a treatment designed to correct the deficiency and produce a salubrious effect. Although our results show that Takotsubo Syndrome occurs in mice null for Kv1.5 channels, we emphasize that the loss of the Kv channels is complete in all cells and tissues. Accordingly, this loss cannot explain why the microcirculations in the apex and the base behave differently, i.e., why microvascular control mechanisms are incapable of supplying flow to the apical regions of the heart, while those mechanisms can provide the basilar regions of the heart with adequate perfusion. We hypothesize that the apical myocytes produce a different compliment of vasodilator substances than those in the base. The basis for this speculation is that the apex of the heart has some different developmental aspects than the base. Specifically, during cardiac development, the heart starts as a single tube, but then folds. At the point of the fold, the apex forms, and the ends of the tube form the base. There must be some specialization to have the folding at a specific point, which is why we hypothesize myocytes in the apex will produce a different compliment of vasodilators than the base. If our hypothesis is correct, it will represent an explanation for this disorder that is currently deemed a condition of unknown etiology.

4. **Goals and objectives**. The goal of this summer research is to test the hypotheses that cardiac myocytes in the apex and in the base of the left ventricle produce a different compliment (amount and type) of vasodilators. We believe this will explain the regional differences in microvascular function that occur in Takotsubo Syndrome in Kv1.5 null mice. We currently have all the resources necessary for this project, and believe the goal is attainable over the course of the summer.

5. **Investigative Methods.**

<table>
<tr><th colspan="4">METHODS (in vivo studies will be performed in anesthetized [isoflurane] mice)</th></tr>
<tr><th>Model</th><th>Measurement</th><th>Method</th><th>Groups</th></tr>
<tr><td rowspan="3">Wild type, Kv1.5$^{-/-}$ mice subjected to i.p. isoproterenol to induce Takotsubo Syndrome</td><td>Hemodynamics: Arterial Pressure, Heart Rate, Cardiac function</td><td>Solid state catheter in aorta via femoral M-mode Echocardiography</td><td rowspan="3">Groups 1 and 2: Wild type control ± isoproterenol or vehicle.
Groups 3 and 4: Kv1.5$^{-/-}$ mice ± isoproterenol.</td></tr>
<tr><td>Metabolite bioassay</td><td>Isolated coronary arterioles</td></tr>
<tr><td>Analysis of metabolites</td><td>Mass Spectrometry</td></tr>
<tr><th colspan="4">Protocol</th></tr>
<tr><td colspan="4">The Takotsubo phenotype will be induced by injection of isoproterenol or vehicle for a control (Intraperitoneal bolus, 50-400 mg/kg per day or equal volume of sterile saline) for 3 days. After the development of apical ballooning (Takotsubo phenotype), we will sacrifice the mouse (4% isoflurane to produce deep anesthesia) and excise the heart. The aorta will be cannulated and a solution of collagenase will be perfused for 5-10 minutes. After this time, the heart will be sectioned into thirds (apex, mid-wall, and base) and the apical and basilar regions will be subjected to further enzymatic isolation and mechanical agitation resulting in an enriched fraction of cardiac myocytes. After separation of the myocytes from other cell types, the myocyte fraction will be transferred to a stimulation chamber containing a physiological salt solution. Myocytes will be stimulated (electrical stimulation of 500 bpm) for 20 minutes and the buffer (termed conditioned buffer) will be collected. This conditioned buffer will then be applied to isolated coronary arterioles to determine vasoactivity (this is the bioassay procedure). The buffer will also be subjected to mass spectrometric analyses to determine the nature of the metabolites produced by the stimulated myocytes. We will determine if microvascular dysfunction in Takotsubo Syndrome is the result of inadequate production of metabolic dilators.</td></tr>
</table>

6. **Proposed method of data analysis.** One-way ANOVA and t-tests with the Bonferroni Inequality to determine intergroup and treatment differences. $P<0.05$ will be accepted for statistical significance.

7. **Significance of anticipated findings.** If our hypothesis is correct (we reject the null hypothesis), we will show that the impairment in myocardial perfusion in Takotsubo Syndrome is caused by inadequate production of metabolic vasodilators. Our results may lead to a new paradigm for the treatment of this disorder.

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

a. First, the student will directly interact with Drs. Ohanyan and Chilian (1:1 or 1:2). Dr. Ohanyan will teach the in vivo experimental procedures, including echocardiographic measurements; whereas, Dr. Chilian will provide instruction for the isolated vessel experiments.

Second, the student will interact with Drs. Chilian and Ohanyan in a 1:2 manner reviewing the data, the protocols, the rationale and the interpretations.

Third, the student will attend the lab meetings of the Heart and Blood Vessel Disease Group (a combined lab meeting of the faculty with interest in cardiovascular research (Drs. Chilian, Penn, Chen, Raman, Thodeti, Meszaros, Yin, Ohanyan, Dong, Mayorga, and Yun) and will present the 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.

b. All the necessary resources (echocardiographs, Millar system to measure arterial pressure, anesthesia machines, computer for measuring evaluating echo images, transgenic mice, stimulation chambers, isolated vessel systems, surgical instruments, surgical supplies, ultrasonic contrast) and financial resources for completing the research are available.

c. The research will be completed at NEOMED with the exception of the myocyte isolation and stimulation that will be completed at Kent State University in the laboratory of Dr. Derek Damron.

Submit your application to Dr. Vahagn Ohanyan

1. **Title:** Doxorubicin-induced cardiomyopathy: Prevention and treatment by a coronary specific vasodilator.
Co-Principal Investigator: Vahagn Ohanyan, Assistant Professor, Integrative Medical Sciences
Location: NEOMED

2. **Abstract.** Doxorubicin is an anthracycline class chemotherapeutic agent that is used alone or in conjunction with other medications to treat different types of cancer. Doxorubicin works by slowing or stopping the growth of cancer cells due to its toxic effects mediated through redox cycling that produces oxidative stress. One of the side effects of doxorubicin treatment, that restricts its use and efficacy, is a form of cardiomyopathy, termed doxorubicin-induced cardiomyopathy (DiC). DiC typically has the morphological and functional abnormalities of dilated cardiomyopathy, with all four cardiac chambers being dilated. This dilation occurs as a result of reductions in ventricular ejection fraction and contractile function, resulting in diastolic and systolic dysfunction. Eventually, congestive heart failure can result, which carries a 50% mortality rate. Currently there is no treatment or prevention for DiC. The goal of this proposal is to test the hypothesis that Chromonar, which is coronary specific vasodilator, will prevent and treat DiC. Previously we have shown, that Chromonar has beneficial effect for treatment of dilated cardiomyopathy caused by coronary microvascular insufficiency, i.e., inadequate blood flow to the heart. We also observed that Chromonar has a beneficial effect for treatment heart failure with non-obstructive coronary artery disease. We speculate that a cause of DiC is insufficient blood flow to the cardiac myocytes, which causes minute areas of damage that accumulate over time, eventually leading to the development of heart failure. Our goal is to determine, if improvement of myocardial blood flow will prevent and treat Doxorubicin- induced cardiomyopathy.

3. **Background and Rationale.** There is consensus mechanism explaining the pathophysiology of the doxorubicin-induced cardiomyopathy. Based on our interrogation of other models of heart failure, in which insufficient myocardial perfusion seems to be a causal mechanism, we propose a similar cause for development of DiC. We postulate that Doxorubicin treatment produces coronary microvascular dysfunction and decreased myocardial blood flow. Similar to other types of heart failure, if myocardial blood flow is insufficient, even only a slight degree of insufficiency, that over the time dilated cardiomyopathy will develop. We hypothesize, that improvement of myocardial blood flow by the coronary-specific dilator, Chromonar, prevents and reverses (depending on the time of treatment) doxorubicin-induced cardiomyopathy. The bases of this this hypothesis is that doxorubicin treatment leads to coronary microvascular dysfunction, micro-areas of ischemia, cardiac dysfunction and heart failure. If our hypothesis is correct, it will represent an explanation, and treatment, for DiC that is currently has no treatment or prevention method.

4. **Goals and objectives**. The goal of this summer research is to test the hypothesis that heart failure occurring after Doxorubicin treatment is caused by microvascular dysfunction. We will test whether Chromonar treatment will prevent and reverse the

consequences of doxorubicin treatment. We currently have all the resources necessary for this project, and believe the goal is attainable over the course of the summer.

5. Investigative Methods.

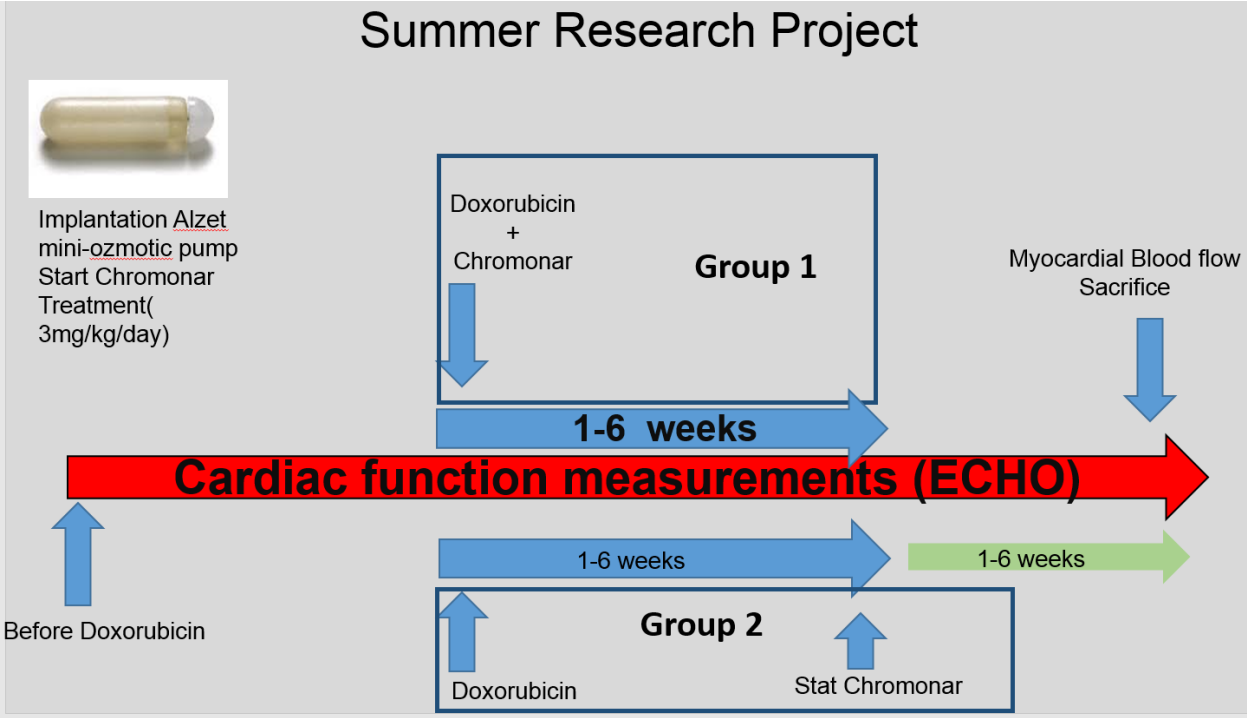
METHODS (*in vivo* studies will be performed in anesthetized [isoflurane] mice)

Model	**Measurement**	**Method**	**Groups**
Wild type mice subjected to i.p. Doxorubicin to induce dilated cardiomyopathy	Hemodynamics: Arterial Pressure, Heart Rate,	Solid state catheter in aorta via femoral artery	Groups 1 WT mice will receive Doxorubicin and Chromonar at the same time Group 2 WT mice will receive doxorubicin 4-6 weeks. After the course we will start Chromonar treatment.
Cardiac function		Echocardiography	
Myocardial blood flow		Contrast echocardiography	

Protocol

The drug induced dilated cardiomyopathy will be approached by injection of Doxorubicin (5mg/kg IP once a week or vehicle for a control (Intraperitoneal bolus 200 ul or, equal volume of sterile saline) for up to 6 weeks. After the development of dilated cardiomyopathy we will start Chromonar treatment in Group 1 animals for 4 weeks. At the end of the Chromonar treatment will do contract and transthoracic echocardiography and sacrifice the animals (4% isoflurane to produce deep anesthesia) and excise the heart.

The second group of animals will receive Doxorubicin and Chromonar the same time. The goal of this treatment is to prevent development of dilated cardiomyopathy. At the end of the drugs treatment we will do contrast and transthoracic echocardiography and sacrifice the animals (4% isoflurane to produce deep anesthesia) and excise the heart. Below figure shows the detail of the project



6. **Proposed method of data analysis.** One-way ANOVA and t-tests with the Bonferroni Inequality to determine intergroup and treatment differences. $P<0.05$ will be accepted for statistical significance.

7. **Significance of anticipated findings.** If our hypothesis is correct (we reject the null hypothesis), we will show that the coronary microvascular dysfunction develops after Doxorubicin treatment, which leads to development of dilated cardiomyopathy. Treatment with Chromonar, which increases myocardial blood flow will prevent and treat development of dilated cardiomyopathy. Our results may lead to a new paradigm for the treatment of this disorder.

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

   a. First, the student will directly interact with Dr. Ohanyan (1:1). Dr. Ohanyan will teach the in vivo experimental procedures, including echocardiographic measurements and will provide instruction for data analysis, construction of the final poster, and writing the final paper.

   Second, the student will interact with Drs. Ohanyan in a 1:1 manner reviewing the data, the protocols, the rationale and the interpretations.

   Third, the student will attend the lab meetings of the Heart and Blood Vessel Disease Group (a combined lab meeting of the faculty with interest in cardiovascular research (Drs. Chilian, Penn, Chen, Raman, Thodeti, Meszaros, Yin, Ohanyan, Dong, Mayorga, and Yun) and will present the 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.

b. All the necessary resources (echocardiographs, Transonic system to measure arterial pressure, anesthesia machines, computer for measuring evaluating echo images, mice, surgical instruments, surgical supplies, ultrasonic contrast) and financial resources for completing the research are available.

c. The research will be completed at NEOMED.

Submit your application to Dr. Charles Thodeti

1. **Project Title:** Regulation of VEGFR2 localization and activation in tumor angiogenesis by a mechanosensitive ion chennel, TRPV4

2. **Abstract of project:**

Solid tumors require angiogenesis for the growth and metastasis. Most of the conventional anti-angiogenic therapies focus on either neutralizing the effect of soluble angiogenic factors such as vascular endothelial growth factor (VEGF) using specific antibodies or inhibiting VEGFR2 (VEGF receptor 2) kinase activity. However, these conventional anti-angiogenic strategies showed only modest success in clinical trials due to the development of resistance (evasive or intrinsic) as tumor endothelial cells became refractory to anti-VEGF therapy over time. Therefore, we have focused on mechanical signaling and, recently demonstrated that a mechanosensitive ion channel TRPV4 regulates tumor angiogenesis as well as tumor growth (Adapala and Thodeti, Oncogene, 201610). Interestingly, we found that tumors from TRPV4KO mice exhibited leaky vessels with increased VEGFR2 expression compared to WT tumors indicating a cross-talk between mechanical (TRPV4) and growth factor (VEGF) signaling in tumor angiogenesis. However, the molecular mechanism by which TRPV4 channel/tumor matrix stiffness modulates VEGF or VEGFR2 signaling is not known. In this project, we proposed to study whether and how tumor matrix stiffness regulate VEGFR2 localization and activation and role of mechanosensitive ion channel TRPV4, using WT and TRPV4KO endothelial cells.

3. **Background and Rationale:**

The growth and progression of tumors are depend on continued blood supply that delivers oxygen and nutrients which is provided by tumor angiogenesis. However, tumor vasculature is structurally and functionally abnormal characterized by tortuous, irregularly patterned and leaky vessels1-3. These abnormal vessels cause impaired oxygen delivery that induce a continuous cycle of abnormal angiogenesis, vascular hyper-permeability and inefficient delivery of anti-cancer agents leading to tumor cell resistance to radio and chemotherapies1-3. The traditional anti-angiogenic strategies focused on VEGF-targeted therapies starve the tumor by inhibiting neo-angiogenesis and destroying existing vessels. But these conventional anti-angiogenic strategies were shown only modest success in the clinical trials due to the insufficient efficacy and the development of resistance. However, new evidence shows that normalization of the tortuous and leaky tumor vessels can improve the delivery and efficacy of anti-cancer drugs and potentiate effects of conventional radiotherapies 3,8. Despite this new line of thought, most anti-angiogenesis strategies still focus on antagonizing VEGF or other growth factor signaling 2,3. In addition to growth factors, mechanical forces are important regulators of angiogenesis, 6,7. Although most studies on angiogenic control focus on soluble stimuli (such as VEGF), local mechanical cues conveyed by extracellular matrix (ECM) due to cyclic deformation of blood vessels, hemodynamic forces or cell-generated traction forces are also potent inducers of directional capillary blood vessel growth and vascular remodeling *in vitro* and *in vivo*6,7. For example, the initial step in neovascularization involves reorientation of a subset of capillary endothelial (CE) cells that spread and migrate perpendicular to the main axis of the pre-existing vessel towards the angiogenic stimulus6,7. Endothelial cells sense mechanical forces associated with tissue distortion through integrin receptors that mediate their adhesion

to surrounding ECM6,7. Unlike normal ECM, the stiffness of tumor ECM increases as a result of continuous remodeling of matrix components by stromal fibroblasts and because of its higher stiffness, the tumor ECM can resist the increase in mechanical forces that results from an expanding tumor mass. The tumor vasculature is hyper-permeable and releases plasma components into surrounding extracellular space resulting in the formation of perivascular fibrin gels which further raises interstitial pressure, and thus ECM stiffness, which may feedback to enhance integrin-mediated Rho/ROCK (Rho-associated kinase) activities or contraction of tumor endothelial cells similar to tumor epithelial cells9. Additionally, tumor endothelial cells may encounter abnormal mechanical environment because tumor vasculature has discontinuous basement membrane and perivascular cell coverage1-3. Moreover, due to irregular shape and size, blood flow patterns in tumor vasculature are turbulent and can impose erratic cyclic strain and shear stress on tumor EC. Thus, the physicality of the tumor ECM/BM play a critical role in regulating endothelial cell function and angiogenesis. We have recently show that TRPV4 channels mediate endothelial mechanosensing (towards ECM stiffness, stretch and shear) regulate angiogenesis in vitro and in vivo (tumor)8,10. Our proposal, thus, aims at targeting a mechanosensitive ion channel, which may lead to the development of **a new anti-angiogenesis therapy for treating cancer**. Moreover, the goals are driven towards understanding the mechanisms by which these channels integrate mechanical (TRPV4) and soluble (VEGFR2) signaling, which may also be targets for angiogenesis inhibitors. **This knowledge will greatly facilitate our understanding of the role of mechanotransduction in tumor angiogenesis and should provide insight into conditions with excessive angiogenesis (tumor growth and proliferative retinopathy).**

4. Goal and objectives:

The goal will be to determine the molecular mechanism by which TRPV4 channels regulate VEGFR2 localization and activation in response to tumor matrix stiffness. To achieve this, we will use cultured normal and TRPV4 null endothelial cells (human and mouse) and specific activators/inhibitors of TRPV4 and VEGF/VEGFR2.

The first objective will be to instruct he/she on endothelial cell culture techniques necessary to permit unassisted cell culture. **The second** will be to perform an immunoflurorescence analysis of the endothelial cells cultured on varying stiffness gels (mimic tumor stiffness) using fluorescently labeled antibodies with imaging of cellular/subcellular (cytosol/golgi/nuclear) structures involved in VEGFR2 on a fluorescence microscope. **The third** will be to teach Western blot methods to measure VEGFR2 phosphorylation. **The fourth** will be to do real-time PCR to quantify gene expression. The **fifth** will be to perform siRNA knockdown of proteins of interest (TRPV4) and measure VEGFR2 localization. **The sixth** will be to analyze data and images acquired and perform statistical analysis.

The feasibility of performing these experiments in my laboratory is shown as preliminary results as an appendix at the end.

5. Investigative methods to be used:

Cell Culture: Human (HMEC-1 and HUVEC) and Mouse vascular endothelial cells (NEC, TRPV4Ko EC) will be cultured on gelatin-coated tissue culture dishes and grown in a defined medium composed of low glucose DMEM, 10% fetal bovine serum, 10% Nu

Serum IV, basic fibroblast growth factor (6 ng/ml), heparin salt (0.1 mg/ml), 1% insulin-transferrin-selenium and antibiotic/mycotic mix as described previously10. Cells will be kept in a 37oC, 5%CO2 incubator, split at ~90-95% confluence, and used between passages 11-22. In some experiments endothelial cells will be cultured on gels that mimic tumor stiffness (0.3-50 kPa).

Immunofluorescence staining and microscopy: Cell will be rinsed with phosphate-buffered saline (X 3) and fixed for 20 min at room temperature in PBS containing 4% paraformaldehyde. Cells will then be rinsed and permeabilized with PBS containing 0.25% Triton-X100, washed with PBS and blocked with serum containing media for 20 min. After, cells will be incubated with the primary antibodies (against Total VEGFR2, phospho-VEGFR2 and Golgi) at room temperature for 1 hour. The cells will be then washed with PBS and incubated with Alexa Fluor-conjugated secondary antibodies (1:500). Cells will be mounted on glass slides using fluoromount containing DAPI (Vector labs). Image acquisition will be performed on IX-81 Olympus fluorescence microscope using Meta Morph software and acquired images will be processed using Image J (NIH) software.

SDS-PAGE and Western blot analysis: Cells will be lysed in TritonX-100 with protease and phosphatase inhibitor cocktail (Boston Bioproducts). Cell lysates will be separated by electrophoresis on 8% SDS- polyacrylamide gels and transferred to Immobilon ® polyvinylidene difluoride membrane. The membrane will then be blocked in 5% milk in TBS with 0.1% Tween-20 (TBS-Tw) for 1 h. The blot will be incubated with the following primary antibodies anti-VEGFR2 (1:1000), anti-phospho-VEGFR2 (Y1175) and anti-tubulin/GAPDH (1:1000). The ECL (Pierce West Pico) method will be used with anti-rabbit (Jackson Laboratories) at a dilution of 1:10,000 and developed using Protein Simple. Results will be quantified using Image J software.

siRNA knockdown: Cells will be transfected with 10 nM of TRPV4 specific smartpool siRNAs (Dharmacon) using siLentFect reagent (Biorad). Two days later, knockdown of the proteins will be assessed by Western blot and RT-PCR analysis.

Real-time Quantitative PCR: The expressions of TRPV4 and VEGFR2 transcripts will be determined with real-time PCR performed on ABI 7500 (Applied Biosystems). Cells will be cultured and treated as described above, and total RNA will be isolated with an RNAeasy minikit (Qiagen) according to manufacturer's instructions. RNA concentration will be determined using a NanoDrop 2000 UV-Vis Spectrophotometer. 1µg of total RNA will be used for reverse transcription using cDNA synthesis kit (qscript cDNA SuperMix) from Quanta Biosciences, containing MgCl2, dNTPs, recombinant RNAse inhibitor protein, qScript Reverse Transcriptase, random primers, oligo (dT) primers and stabilizers. Gene expression will be assayed by q-PCR analysis was performed using Fast SYBR green master mix (Applied Biosystems) method on the Fast Real-Time PCR system (Applied Biosystems). Primers for q-PCR were obtained from IDT technologies.

6. Proposed method of data analysis:

The experiments will be repeated 3-4 times to generate sufficient data for statistical analysis. We will obtain multiple images from the each sample which should be sufficient to visualize the changes. The data will be presented mean + SEM. The

significance of the results will be tested using the student's t-test and one-way ANOVA followed by post-hoc analysis.

7. Significance of anticipated findings:

Angiogenesis is a critical component of cancer and many cardiovascular diseases such as atherosclerosis, myocardial infarction and diabetic retionopathy. Most of the work on angiogenesis focus on VEGF signaling but anti-VEGF therapies are met with limited success in clinics. Mechanical forces also critically regulate endothelial function during angiogenesis and could be target for anti-angiogenic therapy. We have recently shown that TRPV4-dependent mechanical signaling is compromised in tumor angiogenesis and restoring TRPV4 function normalized tumor vasculature and improved cancer therapy. Intriguingly, we found that TRPV4 regulates VEGFR2 expression during tumor angiogenesis. However, the cross-talk between TRPV4 and VEGF/VEGFR2 signaling is not known. Therefore, deciphering the mechanism by which TRPV4 regulates VEGF/VEGFR2 signaling could lead to new targets for therapeutic intervention in cancer and various cardiovascular diseases.

8. Student Training/Mentoring Plan:

First, fellow will be introduced to the department, lab, and project. We will discuss how the project was developed, the potential pitfalls and possible outcomes. Fellow will be provided access to background literature and material to assist in project understanding. We will then define the project, expectations, and lab schedule. The PI and/or laboratory graduate students will give the initial experimental training in cell culture, western blot, q-PCR and microscopy methods. Once the fellow successfully completes the first round of experiments, fellow will perform all the experiments on his/her own in the rest of the fellowship period albeit under the supervision of the PI/technician/graduate student.

The PI will be in regular contact with the Fellow in addition to having weekly lab meetings to discuss the results and keep the Fellow on track for project success. The Fellow will also be asked to join weekly departmental meeting to enhance research and presentation skills.

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APPENDIX

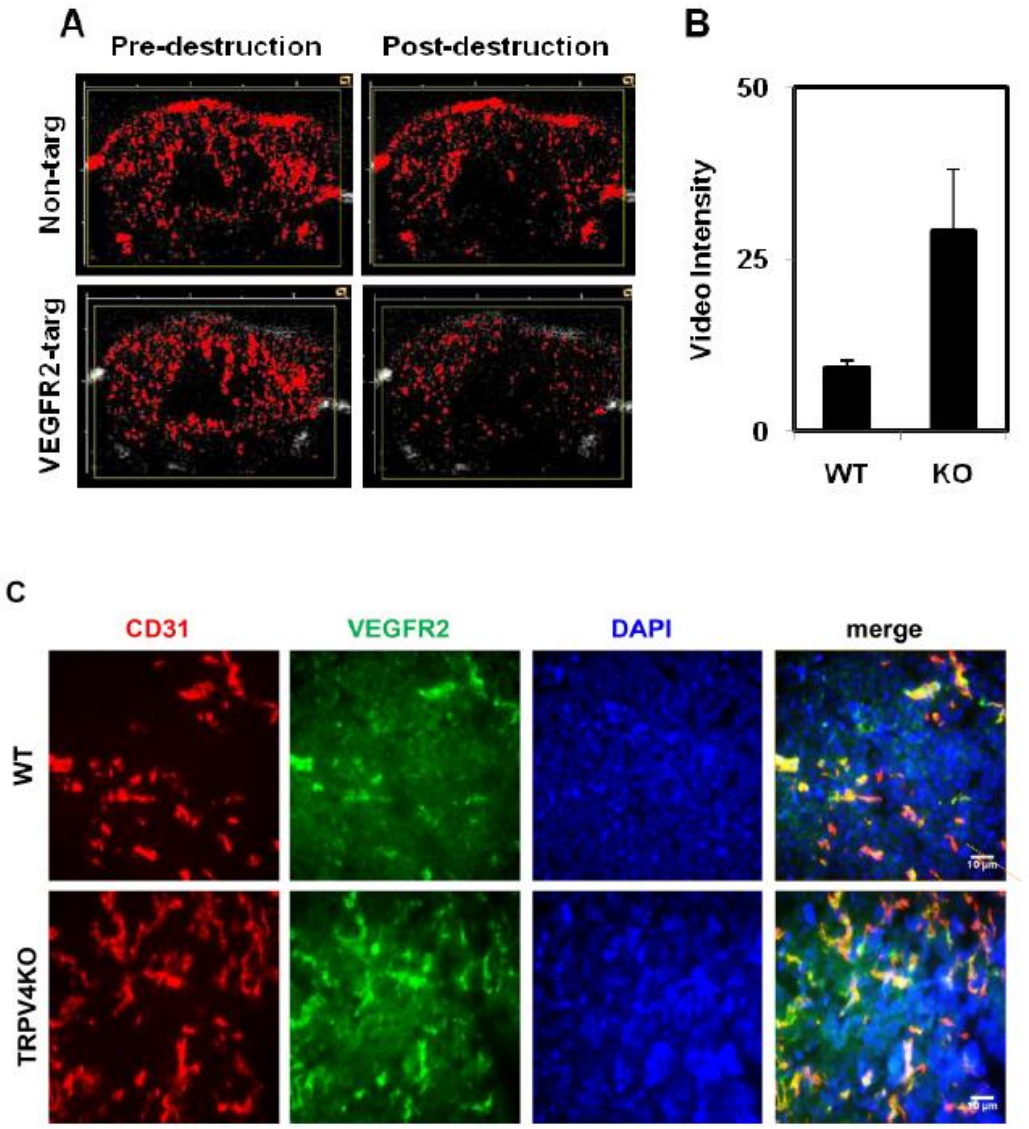

Fig.1. VEGFR2 expression is increased inTRPV4KO tumor vasculature. Contrast ultrasound images showing the video intensity of non-targeted and VEGFR2- (vascular endothelial growth factor receptor 2) targeted contrast agent before or after the destruction of the contrast agent (A) in subcutaneous tumors. B) Quantitative analysis of video intensity of VEGFR2-targeted contrast agent from WT and TRPV4 KO tumors showing increased expression of VEGFR2 in TRPV4 KO tumors. C) Representative images (20X) of the tumor tissue stained with CD31 (red), VEGFR2 (green), and DAPI (nuclei) were used to quantify the VEGFR2-positive vessels.

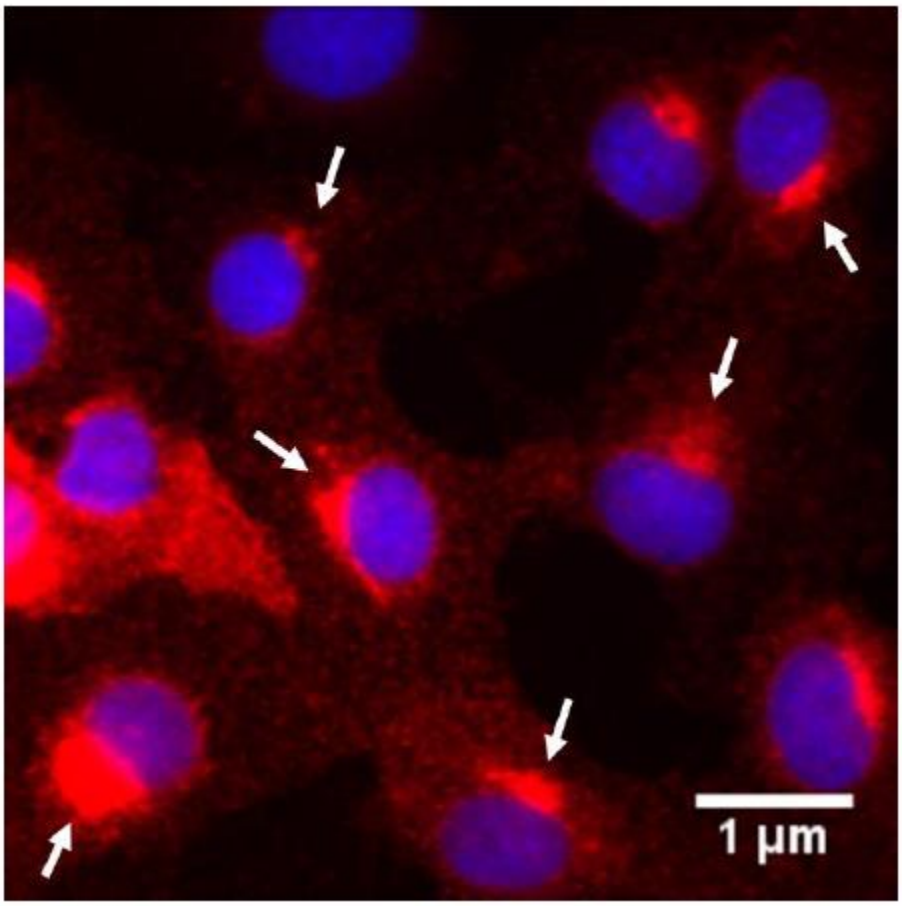

Fig.2. VEGFR2 localization in normal human endothelial cells. Immunofluorescence images showing perinuclear (Golgi; arrows) localization of total VEGFR2 (red) in human endothelial cells. Nuclei were stained with DAPI (blue).

Submit your application to Dr. Liya Yin

1. **Title: The regulation of mouse coronary collateral growth**

2. **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 by lineage tracing. 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.

3. **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.

4. **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.

5. **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 echocardiography. 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 also use the microfil to perfuse the heart for micro-CT to analyze the coronary collateral growth.

6. **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.

7. **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.

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

   a. 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.

   b. All the necessary resources (echocardiographs, anesthesia machines, computer for measuring evaluating 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.

   c. The research will be completed at NEOMED.

Submit your application to Dr. Christine Crish

1. **Project Title:** **Pathological changes to the visual system in Alzheimer's Disease**

2. **Abstract:** A major goal of our laboratory is to understand the mechanisms, timing, and progression of Alzheimer's pathology in the visual system and use this information to develop early interventions to slow, stop, or reverse neurodegeneration in visual or other neural pathways. Disturbances in vision such as decreased visual acuity or inability to detect differences in visual contrast may be some of the earliest symptoms in Alzheimer's disease (AD)--even preceding symptoms of cognitive decline. An increasing amount of research is showing that accumulation of retinal amyloid pathology precedes/predicts the presence of AD pathology in the brain, providing a possible biomarker of risk in human patients. The proposed project will involve identifying pathological changes in retina, optic nerve, and visual brain regions of transgenic AD model mice selective for specific pathological features of the disease, and determining whether these mice experience deficiencies in vision. Students involved in this project will assess visual abilities of mice using an optomotor response behavioral test to determine the time course of visual deficits in htau and 3xtg AD model mice. Additionally, students will use histological assays to determine the presence/timing of AD pathology in the mouse retina and visual brain regions and compare how different types of AD pathology affect the visual system.

3. **Significance:**

Clinical trials for Alzheimer's disease (AD)-modifying therapies have consistently failed to stop the juggernaut of pathological progression in this devastating neurodegenerative disorder. The problem occurs because therapies are often deployed too late in disease progression; yet there are currently no valid, reliable biomarkers for early diagnosis or detection of emerging AD neuropathology.

The recent emergence of retinal amyloid imaging holds promise as a diagnostic biomarker of Alzheimer's disease (AD) with maximal potential as a non-invasive, widely deployable method of detecting disease in its most incipient stages. Amyloid-beta (Aß) proteins and plaques—hallmark pathology of AD, are shown to accumulate in the retina of the eye and correlate with amyloid plaque load in the brain. Evidence suggests that retinal amyloid emerges before appearance of brain pathology, lending credence to its potential as an early biomarker.

Retinal Aß likely plays a role in producing visual deficits that frequently emerge in the early stages of AD, and a number of pathological events are associated with amyloid's presence in the eye. In the retina, amyloid is associated with damage and death of retinal ganglion cells (RGCs), thinning of the nerve fiber layer, inflammation, and impairment in retinal blood flow. As diagnostic imaging of the retina moves to the forefront of clinical AD diagnosis, there is a critical need to fill the gap in our understanding of how retinal Aß as well as other AD pathologies such as hyperphosphorylate tau (ptau) affect the overall visual system. Using a number of different transgenic strains and AD mouse models, our lab is currently investigating how amyloid and ptau pathology a) affect the structure and function of the primary visual projection from retina to brain, and b) alter vision capabilities. We are proposing this second objective (b) as the goal of a Summer Research Fellowship.

4. Goals & objectives:

Research goal: Determine whether tau pathology contributes to visual deficits by assessing visual acuity in an AD mouse model selective for tauopathy.

Student goal: Learn how to conduct behavioral tests to assess visual acuity and contrast sensitivity in htau and control mice using an Optomotor Response (OMR) paradigm. Learn how to operate the Phenosys qOMR testing apparatus and software to measure a mouse's reflexive head movements in response to changing visual stimuli.

Research goal: Determine the time course and any sex differences in visual deficits of tauopathy AD mouse mice and contrast with known time-course and deficits in 3xtg mice--a combined amyloid/ptau mouse model.

Student goal: Measure OMR in male and female htau mice across ages that represent presymptomatic, emerging and progressing brain pathology. Using graphing and statistical methods, the student will compare differences in visual ability across htau age and sex and then contrast these findings with existing data our lab has on age and sex-matched 3xtg mice.

Research goal: Measure presence of pathology in htau and 3xtg in central visual structures.

Student goal: Conduct histological and/or immunofluorescent staining on tissue from htau, 3xtg, and control mice to identify AD pathology in retina, optic nerve, lateral geniculate nucleus of the thalamus, and superior colliculus.

5. Research methods

qOMR visual acuity testing

The student will be trained to test vision in mice using an OMR paradigm that is executed and measured via commercially available, non-invasive fully-automated Phenosys (Berlin, Germany) qOMR system. OMR is a reflexive head movement in response to a series of rotating striped patterns. The qOMR system consists of enclosed on an elevated platform within the box where overhead camera tracks its head movements in response to the stimuli appearing on the screens. The resulting head movements are evaluated in relation to the presented stimulus in order to determine thresholds of visual recognition. Head-movements that are correlated with visual stimuli at specific spatial frequencies are quantified to determine visual thresholds, thus providing an index of a mouse's visual acuity. Contrast sensitivity can also be measured using this paradigm by varying the intensity of visual stimuli at each spatial frequency. The student will learn to calibrate the tracking system for each mouse and collect visual acuity/contrast data across several sessions. They will also learn how to analyze the data to determine impairments in mouse visual ability.

Histology of retina and brain tissue

The student will be trained to use Thioflavin-S, Amylo-Glo, and multicolor immunofluorescence assays to identify AD pathology in retina and visual brain regions of mice. We will compare tissue from two different AD mouse strains: htau mice - which

selectively express hyperphosphorylated tau in the absence of amyloid beta pathology - and 3xtg mice that aggressively overexpress both ptau and amyloid beta in forebrain and hippocampus. Nothing is known about visual system pathology and visual ability in htau mice; 3xtg have demonstrated evidence of visual system pathology in retina and brain with preliminary evidence that these changes affect visual ability. Thioflavin-S and Amylo-Glo are high throughput methods for detecting amyloid plaques in tissue, and we will use immunohistochemical techniques standard to our laboratory for antibody-based detection of ptau, amyloid-beta, and inflammatory markers in retina and brain regions associated with visual processing.

Microscopy and Image Analysis

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. Using Image Pro Premier, the student will perform semi-quantitative analysis by measuring percent area fraction of label coverage within each structure.

6. Proposed method of data analysis

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

While advanced models of statistical analysis will be employed by the PI to analyze the data (i.e. mixed model ANOVA factoring sex and age over strain, targeting interactions and main effects), students will be taught to run basic ANOVAs and t-tests to compare groups on the quantified fluorescent label data. The student will compare measurements a) between strains and b) within strains between sex and age groups.

7. Outcomes of research findings

Beyond the noble significance of facilitating early AD diagnosis, studies on early pathological changes to the visual system in AD could provide essential information on disease stage progression, and identify new mechanistic targets for intervention or for monitoring responsiveness to therapy. Currently, there are a lot of unknowns in the field as to how the visual system is affected in AD, and this system may represent cell-types, neurotransmitter systems, and brain structures that are selectively vulnerable to the neurodegenerative changes characteristic of AD. The proposed work will help fill in some of these gaps in our knowledge and contribute to the overall goals of the C.Crish lab to identify mechanisms of presymptomatic changes in AD.

8. 2018 Summer Research Fellow Mentorship/Training Plan

Training and site where research will be conducted

The student will perform the research at NEOMED in the C. Crish research lab and ancillary shared lab rooms on the first 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) and animal use courses (CITI program) and 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 using the Phenosys qOMR system to test mouse visual acuity, tissue sectioning on a freezing sliding microtome, neuroanatomical identification of coronal brain slices, tissue mounting, slide coverslipping, and conducting histological/immunofluorescent assays. The student will be allowed to practice techniques on control animal tissue to gain proficiency before attempting to process tissue from experimental mice. For epifluorescence microscopy and image analysis, the PI and/or post-doc Matthew Smith will directly train the student.

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 currently maintains breeding colonies of the two transgenic AD mouse strains described in this proposal. 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 three freezing sliding microtomes, owned by either Dr. Sam Crish or Dr. Jason Richardson—both colleagues in the Neurodegenerative Disease and Aging (NDA) research focus area. The PI has access to the Phenosys qOMR system, which is core equipment of the NDA and available for faculty researchers in this group. Additionally, faculty in the NDA research focus group have committed to sharing resources between investigators. Freezers and other lab equipment purchased by the department are also available for faculty use. Additionally, the PI manages the use of and has financially contributed to Dr. Crish's epifluorescence Zeiss microscopes and Image Pro image analysis software, granting her full and unfettered access to the equipment. The PI also owns statistical analysis software (SPSS) and image processing software (Adobe Creative Suite). 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 data. The student will also attend the weekly Pharmaceutical Sciences departmental seminar series as well as the monthly Neurodegenerative Diseases and Aging data club in which members of the NDA research focus area (roughly 6 labs) discuss new and interesting data. 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

1. Title: Gene-Environment Interactions in Parkinson's Disease

2. 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 P_5-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, 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 (manganese, MPTP and paraquat). 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.

3. 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.

4. 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*.

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

Environmental Exposures. Separate cohorts of wildtype and Atp13a2-deficient mice will be exposed to either manganese, zinc, or the herbicides paraquat and macozeb. Mice will then be behaviorally tested to determine the effect of the exposures on sensorimotor function. In the brain alpha-synuclein accumulation 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). Motor performance and coordination will be measured using the challenging beam traversal test. Animals will be videotaped while traversing the grid-surfaced beam for five trials. Errors, steps, and time to traverse the beam will be analyzed across the five trials. Spontaneous movements of the mice will be measured in a small, transparent cylinder 15.5 cm high and 12.7 cm in diameter. The number of rears, forelimb and hindlimb steps, and time spent grooming over a three-minute period will be measured for each mouse. Locomotor activity will be measured in an open field arena (56×35×19 cm). The floor of the open field bin is marked with equal sized grid squares in order to determine the amount of locomotor movement by each mouse (grid or line crosses) in a 15-minute period. Lastly, animals will be trained to walk through a narrow alley leading into their home-cage. Once trained, paper will be placed along the alley floor and each animal's hindlimbs will be brushed with non-toxic paint. As mice walk down the alley into their home-cage they leave their prints on the paper underneath. Stride length and variability will be determined by measuring the distance between hindlimb prints.

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.

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.

6. 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, zinc, paraquat, or mancozeb). 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.

7. 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 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.

8. 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.

Appendix:

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Submit your application to Dr. Muhammad Hossain

1. Project Title: Role of ER stress in Neuroinflammation Following Pesticide Exposure

2. Abstract of project

ER stress and neuroinflammation are implicated as significant contributors to neurodegeneration in a variety of diseases, including Alzheimer's disease (Hoozemans et al., 2009; Lindholm et al., 2006; Salminen et al., 2009; Zhang et al., 2013). Many studies reveal that environmental chemicals and pesticides can cause ER stress and neuroinflammation, and deficits in learning and memory in rodents (Falluel-Morel et al., 2007; Mishra et al., 2012). In addition, persistent decline in cognitive function has been observed in people who clinically recovered from acute pesticide poisoning (Starks et al., 2012). Pyrethroids are the most widely used agricultural and household pesticides, accounting for about one fourth of the worldwide insecticide market. Because of the increasing use of these pesticides, attention has focused on the potential human health risks associated with pyrethroid exposure and neurodegeneration. Recently we found that repeated exposure to low levels of deltamethrin causes hippocampal ER stress, neuroinflammation, and cognitive deficits in adult mice. Based on these findings, the goal of this project is to define role of ER stress on microglial activation and neuroinflammation elicited by deltamethrin.

3. Significance

Pyrethroid insecticides are one of the most widely used agricultural and household pesticides, accounting for about 25% of the worldwide insecticide market. The use of pyrethroid insecticides has been increasing over recent years since the use of many organophosphates has been restricted by the US Environmental Protection Agency. Deltamethrin is a potent type II pyrethroid used extensively in agriculture; it also has major uses in residential applications, public health, and commercial usage. Although pyrethroids are rapidly hydrolyzed by serum carboxylesterases (CEs) (Crow et al., 2007), human serum lacks CEs suggesting humans may have a reduced capacity to metabolize pyrethroids. More recently, a physiologically based pharmacokinetic study demonstrated that deltamethrin exposure is predicted to generate a two-fold greater peak brain concentration in humans compared to rats (Godin et al., 2010). In aggregate, these data suggest that deltamethrin may be more slowly metabolized in humans, suggesting increased sensitivity to the toxic effects of pyrethroids such as deltamethrin. Although exposure of the general population to pyrethroids is generally low, significant levels of pyrethroid metabolites, including those of deltamethrin, have been found in human urine and high levels of exposure have been observed in pesticide applicators (Aprea et al., 1997; Berkowitz et al., 2003; Kimata et al., 2009).

Endoplasmic reticulum (ER) stress and neuroinflammation are significant contributors to neurodegeneration and neuronal dysfunction in a variety of diseases, including Alzheimer's disease (AD) and Parkinson's disease (PD) (Hoozemans et al., 2009; Lindholm et al., 2013). ER stress activates apoptotic pathways to remove damaged cells, and persistent activation causes progressive loss of neurons leading to neurodegeneration and cognitive dysfunction in AD (Hotamisligil, 2010; Salminen et al., 2009). Recent studies have demonstrated that ER stress not only activates the apoptotic cell death pathway, but is also directly involved in the induction of inflammation

(McGuckin et al., 2010). A sustained chronic neuroinflammation can lead to neuronal damage and cognitive dysfunction (Block et al., 2007). In addition, environmental chemicals and pesticides can cause ER stress, neuroinflammation, and deficits in learning and memory in rodents (Falluel-Morel et al., 2007; Mishra et al., 2012). Previously, we reported that repeated exposure to low levels of deltamethrin causes hippocampal ER stress Hossain et al., 2015). Recently, we found that repeated exposure to low levels of deltamethrin also causes neuroinflammation in adult mice (Figure 1). However, whether the induction of ER stress by deltamethrin could secondarily initiate inflammatory response is currently unknown. Therefore, the goal of this proposal is to determine whether inhibition of ER stress can reduce microglial activation and neuroinflammation elicited by deltamethrin. Furthermore, the proposed studies will provide a strong experimental framework for future studies to develop potential interventions that can reduce neuroinflammation and neurodegeneration

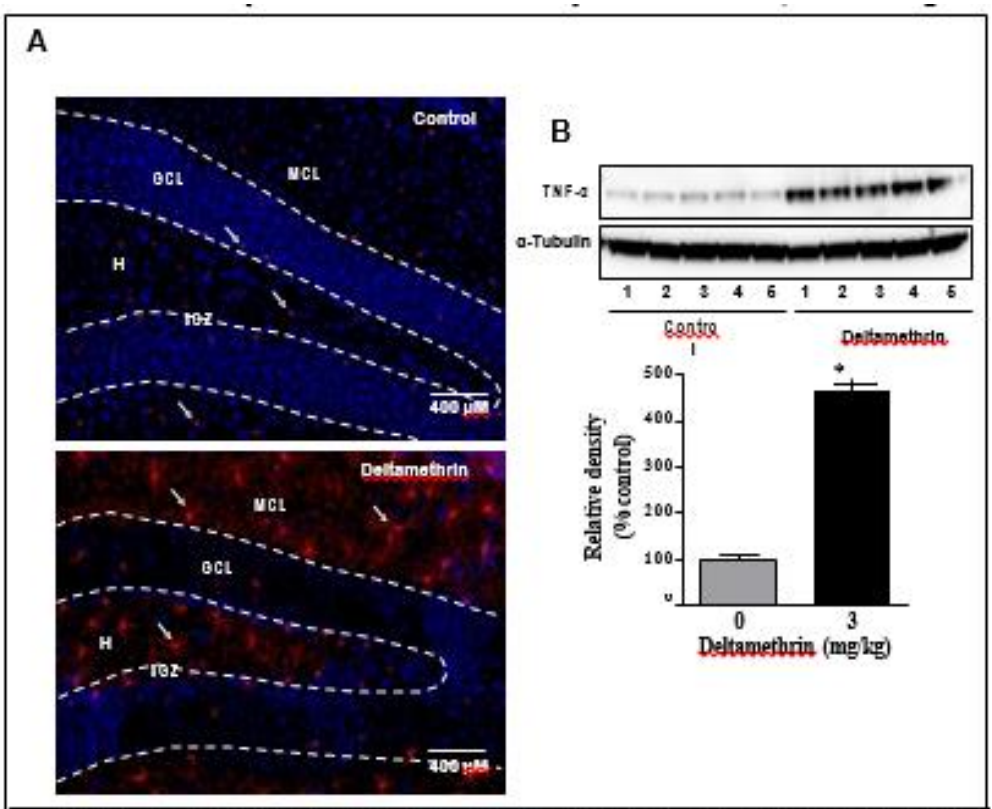

Figure 1. Repeated exposure to deltamethrin (3 mg/kg, every 3 days for 2 months) causes microglial activation and induction of TNF-α in the hippocampus of adult mice. Immunofluorescent staining of microglia (A). Microglia were labeled with anti-Iba1 antibody (red) and Scale bar = 400 µM. (B) Protein levels of TNF-α. All values were expressed as mean ± SEM from 5 animals per group.

4. Goal and Objective

Goal: The goal of this proposal is to determine the role of ER stress in neuroinflammation following deltamethrin exposure.

Objective: To determine whether inhibition of ER stress reduces neuroinflammation elicited by deltamethrin. Here, we will determine whether induction of ER stress by deltamethrin leads to neuroinflammation. We will use a complement of pharmacological, molecular and genetic techniques, including ER

stress inhibition with salubrinal or knockdown of caspase-12 using siRNA transfection to determine the role of ER stress on neuroinflammation.

5. The Research Methods

Experiment *In Vivo*

Drug Administration and Tissue Collection:

Ten week old C57BL/6 male mice will be given two intraperitoneal (i.p.) injections of 1 mg/kg salubrinal at 24 h and 30 min before the administration of deltamethrin. Animals will receive a single dose of deltamethrin (6 mg/kg) via oral gavage. Control animals received the same volume of corn oil as deltamethrin-treated animals. Animals will be sacrificed at 24 and 48 h following deltamethrin administration. From a subset of animals, brains will be removed, and the hippocampus will be dissected on ice and stored at -80 ^{O}C for biochemical assays. The remaining animals will be anesthetized with ketamine/xylazine (100 mg/kg/10 mg/kg, i.p.) mixture, transcardiallly perfused with phosphate buffered saline (PBS) followed by 4% paraformaldehyde in PBS (pH 7.4). Brains will be removed and post-fixed in 4% paraformaldehyde at 4 ^{O}C for overnight, and then transferred into 30% sucrose with 0.1% sodium azide in PBS. Brains will cut at 30 µm coronal sections through the entire hippocampus on a freeze sliding microtome and stored at -20 ^{O}C in cryoprotectant solution (25% sucrose and 25% ethylene glycol inPBS).

Protein Assay:

Hippocampal tissues will be homogenized in tissue homogenization buffer (0.32 M sucrose, 5 mM HEPES; pH 7.4) supplemented with 0.1% protease inhibitor cocktail. For tissue, samples will be centrifuged at 3500 rpm g for 5 min at 4 ^{0}C. Supernatants will be collected and re-centrifuged at 14000 rpm for 45 min at 4^{0}C. Resulting pellets will be re-suspended in 100 µl of homogenization buffer supplemented with 0.1% protease inhibitor, as described previously (Schuh et al., 2009). Protein concentrations will be quantified using the bicinchoninic acid (BCA) assay kit ()

Western Blot Analysis:

Protein extraction and western blot analysis will be performed as described previously (Hossain, DiCicco- Bloom and Richardson, 2015). Twenty micrograms of protein from each sample will be separated on 4-12% Bis-Tris Midi gels (Invitrogen, Carlsbad, CA) and separated protein bands will be then transferred onto PVDF membranes. The membranes will be blocked with 7.5% non-fat milk containing in Tris buffered saline (TTBS) for 1 h at room temperature, followed by overnight incubation with anti-TNF-α (cat #AB1793, Abcam, Cambridge, MA), anti-gp91phox (cat # sc-5827 Santa Cruz, CA), anti-GRP-78 (1:1000, cat #3177, Cell Signaling), and anti-CHOP (cat #sc575, Santa Cruz, CA) primary antibodies. Membranes will be washed and incubated with a secondary antibody conjugated to horseradish peroxidase. Immunoreactive bands will be visualized by SuperSignal® West Dura Extended Duration Substrate (Thermo Scientific, Rockford, IL) and images will be captured using Alpha Innotech

Fluorochem (San Leandro, CA). Membranes will be stripped and re-probed with an anti-tubulin antibody to confirm that equal amounts of protein are loaded.

Immunofluorescent Staining:

Immunofluorescent staining will be performed as described previously with some modification (Alam et al., 2017). Briefly, free-floating sections will be rinsed, then blocked with 10% goat serum in PBS and 0.3% Triton X-100 for 60 min at room temp. Sections will then incubated overnight at 4 ^{o}C with anti-Iba1 (1:250 dilution, Cat #019-19741, Wako Pure Chemical Industries, Ltd., Osaka, Japan), anti-TNF-α (1:500 dilution, cat #AB1793, Abcam, Cambridge, MA), anti-gp91^{phox} (1:250 dilution, Cat # SC27635, Santa Cruz, CA) with 3% goat serum in PBS containing 0.3% Triton X-100. After being washed three times, sections will be incubated with appropriate secondary antibody conjugated to Alexa Fluor (1:250 dilution, Life Technologies, Grand Island, NY) for 1 hr at room temperature in the dark. Sections will be then rinsed of excess secondary antibody, mounted onto slides, dried, and cover lipped with Prolong Gold containing 4',6-diamidino-2-phenylindole (Life Technologies). Fluorescent images will capture as described above for the human samples. A negative control consisting of incubations without primary antibody will be included to determine specific staining.

Experiment *In Vitro*

For further confirmation of the role of ER stress on the process of neuroinflammation following deltamethrin exposure, we will utilize in vitro cell culture system and employ siRNA technique to knockdown the caspase-12 associated with induction of ER stress.

Cell Culture:

Immortalized mouse (C57Bl/6) microglial cells (BV-2) will be cultured in minimum sodium pyruvate, 1 mM non-essential amino acids, 50 IU penicillin, and 50 µg/ml streptomycin, as described previously (Hossain et al., 2017). The cells will be maintained at 37 °C in a humidified atmosphere of 5% CO2. A stock solution (1 mg/ml) of LPS (γ-irradiated Escherichia coli 0111:B4; Sigma Aldrich, St. Louis, MO) will be prepared in sterile water. Dilutions will be made in MEM medium and added to the cells in the presence or absence of the ER stress antagonist a, salubrinal (1 µM) and the voltage-gated sodium channel antagonist, TTX (1 µM).

siRNA Transfection:

Transfection will be performed according to the manufacturer's (Invitrogen) protocol. Twenty-four hours after culturing in antibiotic free MEM media, cells (50–60% confluence) will be transfected with 15 nM siRNA (Santa Cruz, CA) using a lipid-based transfection reagent (Lipofectamine RNAiMAX, Invitrogen) in order to knockdown caspase-12. A scrambled siRNA sequence will be employed in parallel wells to control for nonspecific effects of transfection. At 24 h post transfection, cells will expose to 5 µM deltamethrin for an additional 24 h and used for Western blot analysis or ELISA

Measurement of TNF –α release:

At the end of deltamethrin treatment, cultured media will be collected and secretion of TNF-α into media will be measured using a commercial enzyme-linked immunosorbent assay (ELISA) kit as per manufacturer's instructions (Invitrogen, CA, USA). The

absorbance at 450 nm will determine using a Spectramax microplate reader (Molecular Devices, Sunnyvale, CA).

6. Data Analysis

Data will be tested for normality and either log-transformed to achieve normality or appropriate non-parametric tests used. Repeated measures ANOVA will be used for behavioral experiments. A repeated measure ANOVA (Statview 5.0.1) and a Mixed Model Analysis (SAS/ STAT 9.2 software) will be used to determine the main effects of treatment, sex and time as well as the interaction between these variables. 1-way or 2-way ANOVA will be used for other tests where appropriate. When warranted, Fisher's LSD post hoc test will be applied except when the number of comparisons requires a Dunnett or Bonferroni correction factor.

7. Anticipated Findings

We expect that both pharmacologic (salubrinal) and genetic inhibition of ER stress will be effective against induction of neuroinflamnmation caused by deltamethrin. We do not anticipate difficulties conducting the proposed assays, as these techniques are routinely conducted in our laboratory. The information gained would be used to design a grant renewal to define the mechanism(s) by which deltamethrin causes behavioral deficits observed following deltamethrin exposure (Hossain et al., 2015) and may open the door for potential new therapeutic interventions to ameliorate cognitive deficits in human.

8. Student Fellow Training/Mentoring Plan

The summer fellow will be trained by the PI. During this training period, student will learn to perform the biochemical (protein assay and western blotting) and immunohistochemical and immunofluorescence staining, image visualization, cell counts and data analysis for preparation of poster and manuscript. A research assistant or postdoc will assist in the oversight of experiments. The summer fellow will have weekly meeting with the PI. Further, the fellow student will be encouraged to come to the PI at any time with research questions or academic matter as PI have open door policy for students. The PI oversees weekly lab meeting attended by lab members, at which every student will present data, ask question, and discuss a problem for solution. The PI will also assign scientific reading to the summer fellow that will form the foundation of understanding and critical thinking in the field of neuroscience and pesticide toxicity research. Based on the current and past information in the literatures, the PI will help the student identify interesting questions and develop them into hypotheses. The student will encourage to consider each hypothesis and then pick up one that is achievable and nicely fit with the proposed research as outlined herewith this proposal. The student will also be encouraged to attend any research-related seminars that take place on campus during the fellowship period, as well as our neuroscience journal club. Based on data generated from the project, the summer fellow will write an abstract under the guidance of the PI and submit it for poster presentation. Finally, student fellow will present his results in the poster session at ENOMED in Fall.

Resources will be available to the student include a desktop computer and access to the core facilities and library at NEOMED.

The research will be conducted in the Research and Graduate Education building, in the Department of Pharmaceutical Sciences.

9. Appendix

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Submit your application to Dr. Takhar Kasumov

1. Project title:

The effect of hyperglycemia in HDL composition and dynamics in patients with type 2 diabetes.

2. Abstract:

Cardiovascular disease (CVD) related mortality is the major cause of death among individuals with diabetes. High-density lipoprotein (HDL) inhibits atherosclerosis, the most common cause of CVD, through reverse cholesterol transport (RCT) from peripheral macrophages, a monocyte-derived immune cells involved in arterial inflammation and atherosclerosis, to the liver for the clearance. HDL also possesses anti-oxidative, anti-inflammatory, and endothelium-vaso-protective activities. HDL is mixture of particles with different protein composition and functions. Type 2 diabetes (T2D) is associated with endothelial dysfunction and alterations in HDL functionality. To study the alterations in HDL composition and HDL function we developed ^{2}H-metabolic labeling based mass spectrometry method called "Lipoproteome Dynamics". We applied this technique to assess the effect of hyperglycemia on HDL functionality in diet-controlled patients with T2D and healthy controls (n=9/group).

This project will utilize existing samples from this clinical study to test hypothesis that hyperglycemia induced oxidative stress in T2D causes loss of RCT, and anti-oxidative and anti-inflammatory functions of HDL due to degradation of apoAI and other anti-oxidant proteins, and increased production of pro-inflammatory proteins of HDL.

These studies will lead to the development of new clinical in vivo metrics of HDL function that can be used to test HDL-targeted therapy in T2D and other diseases.

3. The significance of overall research.

Multiple epidemiological studies have shown strong inverse relationship between HDL cholesterol (**HDLc**) and CVD risk[1, 2]. However, recently the validity of the HDLc hypothesis was questioned because efforts to raise HDLc failed to show beneficial cardiovascular outcome[3]. In contrast, high levels of dysfunctional HDL are associated with an increased risk of CVD[4]. These studies indicate that static HDLc levels have limitations as the predictors of HDL function, as they do not reflect HDL turnover and function. Thus, there is an urgent need for HDL functionality studies to assess the CVD risk in patients with T2D[5, 6].

HDL displays multiple functions, including preventing inflammation, oxidation, platelet activation and promoting endothelial health. In metabolic diseases, including T2D, HDL may lose these protective functions and become dysfunctional[7]. The reasons for these changes are not fully understood and may be attributed to the alterations of HDL particle composition and modifications of HDL proteins. In addition to apoAI and apoAII, recently more than 80 less abundant HDL proteins have been identified[8-11].
These HDL proteins are involved in lipid metabolism, acute-phase response, innate immunity, protease inhibition, and regulation of endothelial cell apoptosis that determine HDL's anti-inflammatory, anti-atherogenic, and cell survival properties. Although several *in vitro* assays measure different aspects of HDL functionality, currently no *in vivo* methods are available to measure HDL functions in humans. It is also

unknown how hypoglycemia and hyperlipidemia in T2D impacts the HDL composition and function. This limits the treatment options in T2D associated CVD.

4. The goals and objectives of the project:

Recently we applied *lipoproteome dynamics* approach to measure HDL functionality in mice[12]. In this project we will use this approach to d*etermine the effects of hyperglycemia on HDL function measured via HDL proteome composition and dynamics in patients with* T2D. We have completed the study (n=10 T2D and n=9 healthy controls) and collected samples. This summer research project will take advantage of the existing samples to achieve the following objectives:

- isolate the HDL from human plasma
- quantify the composition of HDL proteins
- quantify the kinetics of HDL proteins
- quantify HDL's anti-oxidant, anti-inflammatory functions using enzymatic assays

5. The research methods:

We have established D_2O as a safe, non-radioactive tracer to study the synthesis rates of individual proteins *in vivo*. Our rationale is based on the fact that in the presence of D_2O, cells generate ^2H-labeled amino acid via transamination and/or *de novo* synthesis. We validated the underlying assumptions that tissue amino acids are rapidly labeled (~20 min) and attain a steady state indefinitely and we proved that there is no post-synthetic hydrogen/deuterium exchange in plasma after lipoproteins are excreted. We also confirmed that during the kinetic measurements the levels of analyzed proteins are at a steady state. We recently refined this approach by combining advanced high resolution LC-MS/MS proteomics and studied a global plasma[13] and mitochondrial[14] proteome dynamics in rodents. The experimental approach is as follows: D_2O is administered in the drinking water to maintain a constant steady state enrichment of D_2O in body water. Body water, proteogenic (amino acids) substrates are rapidly labeled and attain a steady state. At specific time points plasma is obtained and lipoproteins are isolated and proteins are analyzed by MS[15]. The rates of synthesis of individual proteins are calculated based on ^2H-incorporation using algorithms established by our group[16]. Here we will use this approach to assess the mechanisms of HDL dysfunction in T2D.

Vascular function will be assessed based on plasma concentrations of endothelin-1 and omentin-1 using ELISA kits (Enzo Life Sciences and EMD Millipore, respectively). Other in vitro measures of HDL functionality, including *cholesterol efflux, anti-inflammatory and anti-oxidative functions of HDL* will be measured.

HDL Isolation for HDL proteome Dynamics Study: Since high ionic strength and centrifugal shear-stress may alter the pattern of exchangeable HDL proteins, instead of density-based ultracentrifugation, we will isolate HDL with anti-HDL IgY spin columns according to the manufacturer's instructions (GenWay Biotech). The purity of HDL isolation will be confirmed using SDS PAGE. After delipidation HDL proteins will be digested and analyzed by high-resolution MS[17]. Plasma levels of selected HDL proteins will be measured with commercially available ELISA kits and the isotope dilution method by mass spectrometry.

Calculations and Data Analysis: Kinetic parameters will be performed based on a single compartmental model using existing bioinformatics tools and PRIZMA software. At steady state the rate constant represents both the fractional synthesis rate (FSR) and the fractional catabolic rate (FCR). The production rate (PR) of each protein will be calculated as the product of FSR and their respective pool sizes.
Correlation analysis will be used to assess associations between the rates of HDL kinetics with insulin resistance and in vitro measures of HDL functionality.

6. **Expected results and overall contribution to the success of the overall research.**
This work proposed here will allow to fully explore the dynamics of HDL proteome. This study will determine the specific mechanisms responsible for changes in HDL proteome that are relevant to HDL's anti-oxidant, anti-inflammatory, vaso-protective activities which are compromised in T2D. The successful completion of this project will provide a major advance in the understanding of HDL dysfunction in T2D and other diseases.

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7. 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.

The training will take place in the PI's laboratory located in F-1. This laboratory has all necessary resources to conduct this research project. Particularly, our laboratory and adjacent mass spectrometry facility have mass spectrometers, bioinformatic tools, spectrophotometers, mixers, centrifuges, protein assay kits and reagents and supplies needed for this research project.

Submit your application to Dr. Natalie Bonfine

1. Project title

"Characteristics of People with Mental Health Needs Who are Experiencing First Episode Incarceration"

2. Abstract of project

The over-representation of people with mental illness in jails and prisons is well documented. Researchers, practitioners and policy makers are recognizing the importance of intervening early in the trajectory of lifetime criminal justice involvement, especially for this specialized group of offenders. This project explores the needs of people with mental health needs who are experiencing a first episode of incarceration, meaning those individuals who have their initial arrest and incarceration. Despite the importance of intervening early to mitigate the harmful effect of justice involvement, we know little about the characteristics or the criminogenic risks of this vulnerable population, and therefore know little about how to best meet their needs.

3. Significance of the overall research

People with mental illness are over-represented in jails and prisons. Approximately 14-17% of individuals entering jail (15% of men and 31% of women) meet criteria for serious mental illness- prevalence rates that are about three times higher than the general population.[1-3] People with mental illness who have justice involvement have a recidivism rate of about 45%.[4-5]

Perhaps even more troubling than the prevalence of mental illness in jail is the differential effect of incarceration on these individuals. People with mental illness have more difficulties while incarcerated than those without, are incarcerated for longer, and are more likely to be viewed as noncompliant[1]. People with mental illness in jail are likely also have a history of non-adherence to medications, lack insight into their illness, and have a history of current substance use issues.[6] They are more likely to be victimized while incarcerated compared to the general population[7], contributing to increased trauma and psychological toll of incarceration.

Research has shown that even brief exposure to the criminal justice system can be a disruptive and traumatic experience.[1, 6-7] Programming such as the "3DaysCount" campaign is starting to recognize and address this.[8] Over the long term, criminal justice involvement may lead to additional instances of arrest and incarceration, increasing social and economic costs of responding to people who have repeated contact with the justice system.

Pope and colleagues[9] (2016) developed a "first episode incarceration framework" to divert people with mental illness from the justice system. This approach is modeled after first episode interventions in the treatment of psychosis, which recognizes that psychosis is damaging to people, often in young adulthood, interrupting formative social, educational and vocational development. This disruption leads to cumulative disadvantage and social marginalization.[9-11] A similar process can occur for people with mental illness who become involved in the criminal justice system. The first episode incarceration framework recognizes the need for intervention and connections made

early in the trajectory of lifetime criminal justice involvement to minimize the disruption and negative impact of incarceration, and perhaps prevent against future incarcerations.

Much like programming that seeks to interrupt the harmful trajectory of psychosis, the first episode incarceration framework is rooted in prevention, early intervention and recovery-oriented practices.[9] Current approaches to addressing the needs of people with mental illness who are in the criminal justice system focus on those who are in jails or prisons or on the re-entry phase. Emphasizing such "late-stage" interventions is necessary work,[9] but comes at the expense of diverting people early on in their justice involvement or preventing a trajectory of justice involvement.

Adopting Pope and colleagues' (2016) first episode incarceration approach would require a fundamental reframing in how to address people with mental illness in the justice system. A critical first step toward adapting programming to meet the needs of the target population, meaning those individuals who enter jail for the first time who are assessed as having high clinical mental health needs and/or high substance use needs, is to identify sociodemographic, criminal and clinical characteristics of this group. This research project will do so while also comparing characteristics of these individuals to other groups of offenders with and without mental illness, substance use, and criminal justice involvement.

This project is an exploratory study that will examine the characteristics of people who may benefit from a first episode incarceration framework. The study uses secondary data analysis of existing administrative data. Findings from this study will lead to better understanding of the characteristics of people who may be appropriate candidates for early intervention and diversion. Such information could help program administrators develop new or tailor existing diversion programming to the meet the target population.

4. Goals and objectives

The aim of this study is to complete secondary data analysis to better understand the characteristics of the target population: individuals with mental health needs who are new to the criminal justice system. This research will consist of descriptive and comparative analyses. While the student Fellow will work with the PI to develop and define specific research questions for the analysis, the general, guiding research objectives for this study are to:

1) Assess sociodemographic factors, types of offenses, and level of criminogenic and clinical needs of the target group
2) Explore differences in sociodemographic factors, types of offenses, and level of criminogenic and clinical needs between the target group and people who have mental illness and substance use needs but who have prior incarcerations, and
3) Compare characteristics of new offenders with mental illness and/or substance use needs with characteristics of new offenders who do not have mental health and/or substance use needs in length of initial incarceration, type of offense and criminogenic risk.

Research questions for the descriptive analysis will examine the sociodemographic characteristics, level of criminogenic risk and clinical need of individuals who have mental health and/or substance use needs who are new to the criminal justice system, the average length of stay in jail for this target population, and nature of the offenses

that bring individuals with first episode incarceration to jail. The comparative analysis will explore differences among the target population and other groups of people in the criminal justice system.

5. Research methods to be used

This exploratory research project will use secondary data analysis of an existing dataset that has been collected as part of the Hancock County (Ohio) Linkages Program and evaluation. The Linkages Program seeks to identify mental health, substance use and criminogenic needs of adults entering the Hancock County Justice Center. Program stakeholders classify individuals based on their level of needs in these three areas using the Shared Framework for Adults with Behavioral Health Issues in the Criminal Justice System developed by Dr. Fred Osher.[1] Once classified, Linkages Program participants are offered services in jail and referrals based on their level and type of need.

6. Proposed method of data analysis

The student Fellow will engage in several aspects of quantitative data analysis, including developing research questions, conducting and interpreting descriptives analyses, means comparisons and multiple ordinary least squares and logistic regression. Data analyses will be completing using SPSS software. The PI will seek IRB approval for this study prior to the start of the project. The student Fellow will also participate in other aspects of the project, including literature review and summary, and developing tables and figures to display 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 expected or required. The PI intends to provide hands-on training and instruction on how to engage in such research in a scientifically rigorous manner.

7. Significance of anticipated findings

This project is an exploratory data analysis project that has the potential to inform practitioners and policy makers. Information gathered about individuals who have mental health and/or substance use needs who enter the justice system can help stakeholders develop or implement new programming, or modify existing programming to better serve these individuals. While there are existing jail diversion options, such as mental health courts and specialized probation, there are no programs that exist that are specifically available for people with mental illness who are early on in criminal justice involvement.[9] This problem represents an emerging area of research and program development that would prioritize prevention and early criminal justice diversion. Ultimately, this research may improve future programming to divert adults with mental health needs who experience an initial episode of incarceration, as appropriate, to an informed system of care in a timely way.

As little is known to date about the characteristics of the population of people who have mental health needs who are entering the criminal justice system for the first time, this study can contribute to the academic literature in a way that will inform future studies that examine program and intervention development, as well as studies focusing on criminal justice outcomes, (i.e. recidivism), and mental health outcomes, such as treatment engagement.

8. Student Fellow Training/Mentoring Plan

The PI will have regular and frequent communication with the summer research Fellow. This Summer Fellowship will be an independent investigation into issues surrounding the characteristics of people who have mental illness and/or substance use needs who are experiencing an initial incarceration. However, given the nature of quantitative data analysis, the PI will work in an instructive and collaborative way with the student Fellow to develop the project and research questions and for analyzing the data. Given the close working relationship that is necessary, weekly meetings will be scheduled between the PI and summer research fellow. The student fellow will have access to work space within the Department of Psychiatry, including access to a work station with a computer and appropriate data and software (e.g. SPSS). As this study involves secondary data analysis, all research will be conducted at NEOMED in the Department of Psychiatry.

In addition to attending weekly meetings with the PI, the student Fellow will also be invited to participate in meetings of the collaboration board for this project (as summer scheduling allows), as well as other general research team meetings with other faculty and programmatic staff in the Department of Psychiatry. Attending these meetings will allow the student fellow to present aspects of this project to the Department Chair, as well as other researchers, while also engaging in discussions around other Departmental research activities.

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Submit your application to Dr. Julia Jones Huyck

1. Project title, Principal Investigator name, title and location

Project: The Role of Attention in the Development of Auditory Processing during Adolescence

Principal Investigator: Julia Jones Huyck Ph.D., Assistant Professor, Speech Pathology and Audiology, Kent State University, and Voluntary Adjunct Assistant Professor, Department of Anatomy and Neurobiology, NEOMED.

Location: Speech Pathology and Audiology Program, Kent State University (1325 Theatre Drive, Kent, OH)

2. Abstract of project

Auditory processing is important for both communication and survival (e.g., hearing traffic while crossing the street). Most of what is known about normal auditory development pertains to infants and children (≤10 years) but recent evidence suggests that adolescents are still developing both in terms of their naïve performance and training-induced learning on some auditory tasks. One potential contributor to this late maturation is attention, which is thought to develop well into adolescence. However, attention is usually indexed using visual rather than auditory stimuli; therefore the role of attention in auditory processing during adolescence is largely unknown. The long-term goal of this research is to establish a developmental trajectory for auditory attention. This summer project will focus on the role of attention in perceptual learning of degraded speech during adolescence.

3. Background and rationale

The acoustic realization of speech and other sounds depends on the environment, method of presentation (e.g., over the phone), emotional state of the talker, and other factors. One key way we adapt to this variability in auditory signals is through perceptual learning. While both adolescents and adults are able to learn on perceptual tasks with experience, the actual requirements for this learning differ with age (Huyck & Wright, 2010, 2013). Previously we reported that young adults were unable to improve their performance on a degraded (noise-vocoded) speech comprehension task when their attention was directed towards a competing auditory or visual task (Huyck & Johnsrude, 2012). Here we are interested in whether adolescents also need to direct their attention to the degraded speech signal in order to learn.

4. Goals and objectives

The goal is to examine whether adolescents need to attend to degraded speech in order to learn to comprehend it. The outcome will provide insight into the maturation of auditory attention.

Learning objectives:

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 become familiar with the medical research environment by actively participating in lab meetings and departmental journal clubs.

5. Investigative methods to be used

Human Subjects: All procedures are approved by the Institutional Review Board at Kent State University (IRB #15-355). 10- to 21-year-olds will be recruited from northeast Ohio through flyers and letters sent home from local schools. Participants will be split into three groups (10-13, 14-17, and 18-21 years). They 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, tubes in the ears, language impairments, learning disabilities, attention deficit/hyperactivity disorder, traumatic brain injury, or other major neurological problems. Participants will be given $10 gift cards or class credit (adults) for the 1-1.5 hour session. Study procedures pose minimal risk.

Auditory Testing: The experiment will be split into three phases. All participants will complete the pre-training and post-training tests during which they will hear degraded speech stimuli and be asked to report what they heard. During training, the speech stimuli will be presented simultaneously with a series of noise bursts. One third of participants will complete the speech task during training, one third will try to detect a target noise, and one-third will not hear anything and will play an unrelated computer game.

Neuropsychological Testing: To assess attention, working memory, verbal reasoning, phonological processing and other cognitive skills that may affect performance on the speech perception task, all subjects will be given a battery of published and research-validated neuropsychological tests, including one or more subtests of the Comprehensive Test of Phonological Awareness-2, Weschler Intelligence Scale for Children-V or Weschler Adult Intelligence Scale-IV, and/or CANTAB battery, which assesses executive functions.

6. Proposed method of data analysis

Auditory Testing: Participants' responses to the degraded speech stimuli will be recorded using a microphone, and will later be transcribed and scored according to the number of correct words per sentence. Participants will indicate their responses on the target-detection task using a button press, and target-detection data will be scored using signal detection theory. Data will be analyzed using parametric statistics.

Neuropsychological Testing: Standardized tests will be scored according to their respective manuals. Correlational analyses and/or mathematical modeling will be used to explore the relationships between performance on the perceptual and cognitive tasks.

7. Significance of anticipated findings

In the United States, ~19.5% of adolescents are affected by hearing loss (Shargorodsky, 2010), and thousands more (actual prevalence unknown) have been diagnosed with auditory processing disorders. Unfortunately, few studies of perception have centered on normally-developing adolescents, therefore there is little basis of comparison for adolescents with communication disorders. Moreover, because little is known about perceptual learning in this age group, it is not currently possible to customize perceptual training programs to their developmental needs. The role of auditory attention during adolescence is particularly important because (1) it may be an area of weakness in adolescents with auditory processing problems (Bellis & Bellis, 2015) and (2) if learning

can occur without attention, as has been reported on some tasks (Best & Dinse, 2013), it may be easier to persuade clinical populations to participate in training (i.e., listening to sounds in the background while going about day-to-day activities). A better understanding of the development of auditory attention could thus improve both the identification and treatment of communication disorders during adolescence.

Summer Research Fellow Training/Mentoring Plan

All research will be conducted in the Perception, Learning, and Individual Differences (PLAID) 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. The laboratory is directed by Dr. Julia Huyck and currently includes one graduate student, one undergraduate research assistant, and several undergraduate volunteers. The fellow will work closely with Dr. Huyck and will be trained to collect behavioral and neuropsychological data from participants, to transcribe and score speech perception data, to apply signal detection theory, and to conduct statistical analyses.

The PLAID lab emphasizes professionalism, enthusiasm, and scientific rigor. The group meets weekly to develop new projects, address technical concerns, and discuss results and related research. Our laboratory has previously mentored undergraduate, doctor of audiology (Au.D.), and Ph.D. students.

The fellow will also attend the weekly journal club of the Hearing Research Group (HRG). The highly interactive HRG is composed of members of nine hearing neuroscience laboratories with a wide range of experimental approaches. The fellow is expected to present a summary of their summer project to this group.

For more information on this project please contact:

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