Research Alignment: One key aspect of Dr. Andreasson's research is to understand how the modulation of the inflammatory PGE2 pathway through reprogramming cellular metabolism in aging microglia influences aging and Alzheimer's disease using mouse models. Dr. Andreaason's funded project titled "Reprogramming myeloid cell metabolism to prevent cognitive aging and Alzeimer's disease" is of extreme interest to me. This work provides an innovative approach to delineate how microglia and a specific immune/inflammatory response pathway can alter the onset and continuation of pathologies of aging and even neurodegenerative disease especially Alzheimer's Disease. In addition, the recent findings that the knockdown of myeloid EP2 signaling was able to restore phagocytic capability to the levels of young mice and cognition in aged transgenic mice. This work aligns directly with my research interests and small components of numerous projects I am working on as a graduate trainee. For example, my primary research interests is to delineate how altering inflamm-aging modulates neurodegeneration and cognitive decline. My research focuses on how neuroendocrine modulation influences cognition and stroke recovery. The neuroendocrine system has an intricate influence on the immune response of the body and brain; thus, I am extremely interested how the immune system and its cells influences aging and cognitive decline. I am currently working on this passion by determining if human milk oligosaccharides are neuroprotective through microglia responses. Overall, Dr. Andreasson's research encompasses numerous aspects of my research interests, and I believe I have a foundation to contribute to the research.

Preparedness. My expertise in designing transgenic mouse models to study cognition and ischemic stroke will be useful for Dr. Andreasson's laboratory. Moreover, I have an extensive background in performing pharmacological in vitro studies using neurons, astrocytes, endothelial cells, and microglia. Techniques in which I have experience in is transgenic mouse models, aging rodent study design, rodent behavioral tasks (radial arm water maze, Barnes maze, novel object relocation, open field, rotor-rod, balance beam, cylinder, pole-test, and grip-strength), stereotaxic surgery, microsurgery, western blot, qPCR, multiplex array, primary cell culture, IHC, and microscopy. The aforementioned techniques and my research interest are aligned with Dr. Andreasson's ongoing funded projects.

Research Alignment: Dr. Wyss-Coray innovative approaches to answer key aging questions have garnered not only my attention but the entire aging and neuroscience field. The phenotypic changes observed in parabiosis studies were paradigm-shifting and opened the field of geroscience to the possibility that young blood can modulate aging. Dr. Wyss-Coray's research team specifically studies brain aging and neurodegeneration and aims to delineate how circulatory blood factors modulate brain structure and function. The overall goal and research has the potential to define the cellular and molecular rejuvenation aspects of young blood transference. This work is directly aligned with my research interests of discovering novel ways to target senescence and aging. As a trainee, I have numerous projects with small components of my research interests, but I think Dr. Wyss-Coray's innovative approaches and research goals can provide a unique opportunity for me to pursue my research interests and build a career. The projects ability to employ a multi-discipline approach (genetics, cell biology, proteomics, and behavioral paradigms) to extend healthspan and the longevity of quality life are all things I would like to learn. Your 2020 findings that microglia accumulate lipid droplets and LPS increases the accumulation of these droplets align with one of my current projects to assess the neuroprotective properties of human milk oligosaccharides and LPS and HIV-induced neuroinflammation. Thus, I believe my research foundation can aid the goal of your research laboratory.

Preparedness: I have experience in performing pharmacological in vitro studies using neurons, astrocytes, endothelial cells, and microglia. I also have a background in designing transgenic mouse models to study cognition and ischemic stroke. Other techniques include designing an aging rodent study to assess advanced glycation end product signaling influence on aging, rodent behavioral tasks (radial arm water maze, Barnes maze, novel object relocation, open field, rotor-rod, balance beam, cylinder, pole-test, and grip-strength), stereotaxic surgery, microsurgery, western blot, qPCR, multiplex array, primary cell culture, IHC, and microscopy. As a leading aging researcher, I think Dr. Wyss-Coray's research training program and projects can significantly contribute to my future as an independent researcher with the goal of extending healthspan.

Research Alignment: Dr. Mormino has an extensive history in searching for the pathophysiological processes that accompany Alzheimer's disease and attempting to define these specific processes prior to the years before clinical symptoms are present. Dr. Mormino's website informed me that her research program was to focus on utilizing imaging and genetic approaches to determine cognitive trajectory, incorporating novel PET scan to understand human aging, and discovering the critical time window that pathophysiological processes begin prior to the onset of symptoms. For years, neurodegenerative diseases and neuroimaging techniques have always interested me. Your recent work working assessing cerebrospinal fluid biomarkers with hippocampal dependent memory tasks (standardized delayed recall composite and mnemonic discrimination task) are studies in which I would like to participate in designing and executing. Furthermore, one of Dr. Mormino's publication details how F-Flortaucipir is a promising technique to visually see and quantify hall markers of Alzheimer's disease such as tau and amyloid beta. The extensive range of your work using human participants with neuroimaging techniques to divulge biomarkers prior the onset of Alzheimer's Disease symptoms. Your discussion of sex and racial differences that contribute to AD markers are also of interest to me. In regard to human and clinical studies, I have limited experience since my background is in neuropharmacology using preclinical approaches to answer aging and neurodegenerative hypotheses. However, I am a Smith Scholar which is a training program that works with a team of clinical researchers and epidemiologists to analyze previously collected data from the NIH funded Jackson Heart Study, the large single site of cardiovascular disease of African Americans. My current research through this external, supplemental research program is delineating if the association between inflammatory levels (high sensitivity-C Reactive Protein) and stroke incidence in African Americans. Thus, I do have a minute amount of research experience using clinically collected data.

Preparedness. Although I do not have any concrete experience for the ongoing research of Dr. Mormino's laboratory, my research interests and expertise in preclinical study design (in vitro and in vivo) and execution provides a unique perspective as a potential candidate of your laboratory.

Research Strategy

In a broad sense, my time as a trainee in the Ashpole lab at the University of Mississippi has focused on multiple aspects of aging utilizing pharmacological, genetic, in vitro and in vivo approaches to answer questions regarding neurological disorders and aging. Through my six years of laboratory experience as an undergraduate and graduate student, I have been instrumental in development and leading multiple projects which has allowed me to pursue many of my research interests.

As a sophomore undergraduate research, I began my first research project centered around studying the role of neuronal insulin-like growth factor-1 receptor (IGF-1R) in learning and memory. We hypothesized that neuronal IGF-1R in the hippocampus was essential in maintaining learning and memory in adulthood. My initial contributions for this project involved maintaining the animal colony, genotyping transgenic mouse models, and learning stereotaxic surgery to inject cell-specific viral vector targets into the hippocampi of mice. Following stereotaxic surgery, I then carried out behavioral analyses to assess their working memory (radial arm water maze) and spatial recognition memory (novel object relocation). We concluded that neuronal IGF-1R is essential in maintaining learning and memory in sex-dependent manner. To better understand how neuronal IGF-1R reductions influences memory and neurons, we utilized in vitro studies of hippocampal neurons and discovered that when IGFR-1R was inhibited pharmacologically neurite outgrowth was reduced and Rho-Kinase (ROCK) activity increased, but downstream ROCK pathways were not altered significantly. We also found that ROCK levels were also elevated in the brains of knockout IGF-1R mice, and Hydroxyfasudil, a known ROCK inhibitor, restore neurite outgrowth in the presence of IGF-1R inhibitors suggesting a potential downstream target for memory rescue. Based on the structural restoration seen in vitro, we administered Hydroxyfasudil to IGF-1R deficient mice to assess if spatial learning and memory could be restored and found that a 14-day treatment of Hydroxyfasudil was not sufficient to restore spatial learning and memory in IGF-1R deficient male mice. I served as the first-author of this manuscript, and it is currently under the second round of revisions at Hormones and Behavior (preprint: https://doi.org/10.1101/2021.08.08.455596).

In addition to understanding how IGF-1 continues to modulate neurons in adulthood, we also set out to delineate a role for IGF-1 in the regulation of astrocytes. I am first author on an upcoming manuscript describing the behavioral consequences of astrocytic IGF-1R reductions. We utilized in-house developed transgenic mice to pharmacologically reduce astrocytic IGFR in male and female mice. Next, the mice underwent behavioral evaluation for cognition, depression/anxiety, and muscle function. Our results indicate that astrocytic IGFR impacts cognition and anxiety depending upon sex and that the glutamate-glutamine handling machinery was altered. The findings within this project is what led to my idea that astrocytic IGF-1R deficiency impacts glutamate uptake which has a direct effect on the onset and exacerbation of neurodegeneration and associated diseases including stroke. Thus, I proposed to develop a novel laboratory project to assess how cell-specific IGF-1R contributes to stroke damage.

My dissertation research studying the neuroprotective cellular mechanisms of IGF-1 against ischemic stroke recently received an impact factor of 29 (13th percentile) when reviewed for the Ruth L. Kirschstein Predoctoral National Research Service Award (F31), thus, funding is anticipated shortly. Through developing a novel project for my graduate studies, I gained a significant amount of experience in writing a literature review, experimental design, grant writing, and responding to grant reviewers to better the proposal. Considering our research laboratory does not study stroke, I published a literature review comprising the preclinical and clinical benefits of IGF-1 along with how IGF-1 is beneficial to each cell type within the brain regarding a stroke and the continuous damage following the insult [1]. In the second aspect of my dissertation and graduate studies, I hypothesized that IGFR deficiency in the neurogliovascular unit increases susceptibility to stroke-related insults such as glutamate-induced toxicity and oxidative stress. To test this hypothesis, we used pharmacologic interventions to reduce IGF-1R in the presence of glutamate in individual cell cultures and assess cellular toxicity, reactive oxygen species (ROS) production, and mitochondrial dysfunction. Our results concluded that neurons are highly susceptible to excitotoxicity

compared to astrocytes or endothelial cells. A reduction in IGF-1 does not lead to overt toxicity. While astrocytes and endothelial cells are highly resistant to glutamate-induced toxicity, reduced IGF-1R signaling in astrocytes causes increased ROS in the hours following insult. Mitochondrial dysfunction was also seen when IGFR was inhibited in glial cells and subjected to toxic neuronal levels of glutamate. Thus, we conclude that IGF-1 modulates the entire neuro-glio-vascular unit and this entire system may be a target for therapeutic development. The aforementioned project is now published in Frontiers in Neuroscience [2].

My dissertation project studying the neuroprotective cellular mechanisms of IGF-1 has now entered into the in vivo component, which utilizes our established astrocytic IGF-1R transgenic mouse model (igfap-cre/igfr$^{fl/fl}$) and a newly developed neuronal knockout model (icamk2a/igf1$^{fl/fl}$). The inducible transgenic mouse models allow us to selectively reduce IGF-1R in either neurons or astrocytes. I have established cohorts of mice, and have instigated IGF-1R knock-out. I am now awaiting the appropriate timepoints to induce ischemic stroke and assess outcome. I have worked hard to establish ischemic stroke models of middle cerebral artery occlusion and photothrombosis in the Ashpole laboratory. Our intention is to assess stroke outcomes in these transgenic mice with and without exogenous IGF-1 administration, which has been shown to be protective against ischemic stroke previously. Two separate cohorts will be used to assess the immediate (3 days) and long-term (7 days) response that reducing IGF-1 signaling has on infarct, behavioral deficits, and cellular changes that accompany stroke. More specifically, locomotor asymmetry will be examined using pole test, cylinder test, balance beam, and rotarod tests. In the astrocytic knockout mice, we are focusing on histological changes in astrocyte size/number, glial activation, and neuro-glio-vascular structure will be assessed using immunohistochemistry and confocal microscopy. Moreover, the expression of astrocyte-derived growth factors, cytokines, and the machinery critical for glutamate uptake will be quantified using multiplex bead arrays and/or qPCR. Overall, this project provides an innovative departure from the status quo by altering the endogenous IGF-1 signaling cascades in a cell-specific, inducible manner, and seeks to understand if astrocytic or neuronal IGF-1R signaling is responsible for the beneficial outcomes associated with exogenous IGF-1 treatment following ischemic stroke. The aforementioned project is expected to be completed within the year.

In addition to my dissertation work, I am currently a Smith Scholar at the University of Mississippi Graduate Training Educational Center. As a Smith scholar, I work with a team of clinical researchers and epidemiologist from University of Mississippi Medical Center and John Hopkins to analyze previously collected data from the NIH funded Jackson Heart Study, the large single site of cardiovascular disease of African Americans. My current research through this external, supplemental research program is assessing the association between inflammatory levels (high sensitivity-C Reactive Protein) and stroke incidence in African Americans. As the largest minority health disparity study in the United States, JHS being a Smith scholar allows me to access this data to gain valuable insight on how cardiovascular disorders affect African Americans in the population and experience in analyzing secondary data from a longitudinal epidemiology health disparity study.

A secondary project in which I led for the last year was to examine the effects of advanced glycation end products (AGEs) on change in cognitive function, inflammation, and oxidative stress in the aged brain. We hypothesize that systemic knockout of the receptor for AGE (RAGE) will delay the onset of cognitive and physical impairments, and the accompanying biological hallmarks of aging and senescence. To test the hypothesis, transgenic mice are undergoing tests of learning/memory, depression/anxiety, and muscle function/coordination throughout their lifespan. For this project, I developed the breeding plan to generate cohorts for the long-term lifespan/healthspan studies and have performed an array of cognitive and motor function assessments. I will continue assisting our new graduate student and undergraduate researcher in the demands of a healthspan/lifespan rodent study until the completion of the graduate studies.

The previous projects enhance the feasibility of the proposed aims while positioning me as a unique candidate for a post-doc with backgrounds in both *in vitro and in vivo* studies with backgrounds in

neuroendocrine modulation, learning/memory, aging, glycobiology, and mechanistic stroke research. My technical skillset includes developing transgenic mouse models, various behavioral analysis, and cellular/molecular techniques (western blotting, qPCR, ELISAs, and microscopy). Although my expertise do not directly align with the typical neuroscience techniques, my foundation of developing and using transgenic rodents in cognitive and health span studies along with pharmacological in vitro studies evaluating neuroprotection for mechanisms of neurodegeneration like excitotoxity. My research interests that I hope to seek in a post-doctoral opportunity can be divided into either the neuroendocrine and immune system modulation of neurological diseases and delineating the critical time window in which preventative pharmacological therapies can be applied to defer and prevent the onset of neurodegenerative diseases.

Overall, my research interests and expertise had led me to pursue post-doctoral position that focus on many aspects of neurodegeneration and associated diseases. More specifically, my background in attempting to understand how neuroendocrine modulation (IGF-1) alters neurodegeneration pathways, cognition and ischemic stroke recovery has led me to accumulate a broad array of knowledge and techniques. In the past I have conducted research mainly focused on cognition and aging, however, now I am seeking opportunities that will directly allow me to enhance the scientific community knowledge of how the neuroendocrine and immune system can modulate neurological diseases. Thus, I seek to use my experience of in vitro and in vivo studies to learn novel and innovative approaches to answer dire questions of cognitive decline and aging. As a collective, my goal is to use my background in transgenic mouse models, rodent surgical procedures, aging long-term studies, and behavioral cognitive assessments to pursue my research interests: biomarkers of aging to extend healthspan, immune/hormone modulation of neurodegeneration, and neuroimaging to define the critical window for therapeutic intervention prior to symptom onset of neurodegenerative diseases.

1. Hayes, C.A., M.N. Valcarcel-Ares, and N.M. Ashpole, *Preclinical and clinical evidence of IGF-1 as a prognostic marker and acute intervention with ischemic stroke.* J Cereb Blood Flow Metab, 2021: p. 271678X211000894.
2. Hayes, C.A., et al., *Insulin-Like Growth Factor-1 Differentially Modulates Glutamate-Induced Toxicity and Stress in Cells of the Neurogliovascular Unit.* Front Aging Neurosci, 2021. **13**: p. 751304.

Diversity Statement

The current status of my career trajectory remains clear; nevertheless, the journey escaping so many systemic and familial cycles to get here remains ambiguous. Ludlow, Mississippi, is where people who live 30 minutes away do not even know it exists. As a small, family-oriented community with about 250 people, I grew up surrounded by the comfortableness and sameness. The comfortableness in Ludlow transcends money and even the apparent segregation of blacks and whites living on different sides of town. Growing up in a rural environment, I spent much of my time farming, tending to animals, and fishing/hunting for wild game. Yet, somehow, I always felt like the persistence of continuing down the same paths previous generations had followed was not for me. Growing up, my father was absent due to gang violence, and my mother had to work constantly to support us. Although I am a product of an environment where lives are stunted by the lack of opportunities in education, healthcare, and employment, the opportunity to explore nature and provide food for my family fostered a budding passion for science.

I chose my profession, academia, and research, due to the mentorship of an amazing advisor and the possibility of achieving a type of success that was not built for me. For years, my advisor, Nicole Ashpole, instilled in me the importance of not only "looking" differing in science but disseminating my thoughts and opinions since they come from a very different upbringing than the "majority." With over a dozen mentors that have helped me become a first-generation college grade and doctoral candidate, they provided me the capacity to learn respect, teach, and cultivate the success of all. For years, diversity has been a goal of institutions and industries whether true actions were put behind their statements or not. Yet, the pandemic served a new development and highlighted the importance of diversity and equality and the aspect of equity. And frankly, without equity, the others are meaningless.

I always title my journey "From First Gen. to First Doctor." I firmly believe that rising above societal conditions and overcoming makes me a unique prospect for entering academia and even more so for science rooted in privilege. For years, I honed my voice and my comfortableness to be able to speak against the importance of having more people that visible "looked" like me. However, I realized that that was meaningless if the environments we are recruited to are not equitable and value us but overall safe for us. Thus, my primary goal is to not only promote the advancement of minorities in STEM, but teach those in charge that I matter, we matter and are more than tokens and checkboxes. Hence, I have been extremely involved in the Science Twitter space, where I have given numerous talks discussing my academic journey and the importance of making science more equitable. Unequivocally, I spent hours a month assisting undergraduate students craft statements and applications for graduate school so that they to can have similar opportunities as myself.

As Maya Angelou said, "in diversity, there is beauty, and there is strength." When I envision diversity within our institutions and furthermore, within the future of our country's education, I equate it with tolerance, for tolerance is the meaning of being open to the differences that exist among us all. It means respecting and learning from others while simultaneously valuing our differences and discovering the various interests, motivations, and desires we share in common. As I have learned through the course of my academic journey, people who are open to differences will not only give birth to a more peaceful community one generation at a time but also serve

themselves and others with better and equal opportunities in life, whether it is through education, career, or friendships. Possessing the virtue of tolerance enhances the ability to connect with those physically and economically different on a more profound level while all the while creating a village that will work endlessly together to achieve the same goal: unity.