2025

Neurology & Neurosurgery UE5 Physician Neuroscientist Training Pipeline (PNTP) Guide

REVISED SEPTEMBER 12, 2025

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PNTP Program Overview

The Washington University UE5 Physician Neuroscientist Training Pipeline (PNTP) offered through the National Institute of Neurological Disorders and Stroke (NINDS) aims to create a seamless pipeline for physician-scientist training in Neurosurgery and Neurology at the Washington University School of Medicine. The major goal is to provide neurosurgery and neurology resident trainees with the skills, education, research experience, and foundations to successfully compete for individual research funding and launch a meaningful independent career as a physician neuroscientist. Importantly, this program provides dedicated mentored research opportunities for residents during and after their residency training, with the expectation of obtaining K award funding (K23, K08 grants).

The program is administered through the Department of Neurosurgery (Gregory Zipfel, co-PI; zipfelg@wustl.edu). Michele Bridges (bmichele@wustl.edu) and Nichole Swanner-Gregory (nichole.s@wustl.edu) are the primary grant staff administrators for the grant.

Neurology residents work with Dr. David Gutmann (co-PI; gutmannd@wustl.edu; Drusilla Jenkins, project manager; nelson_d@wustl.edu).

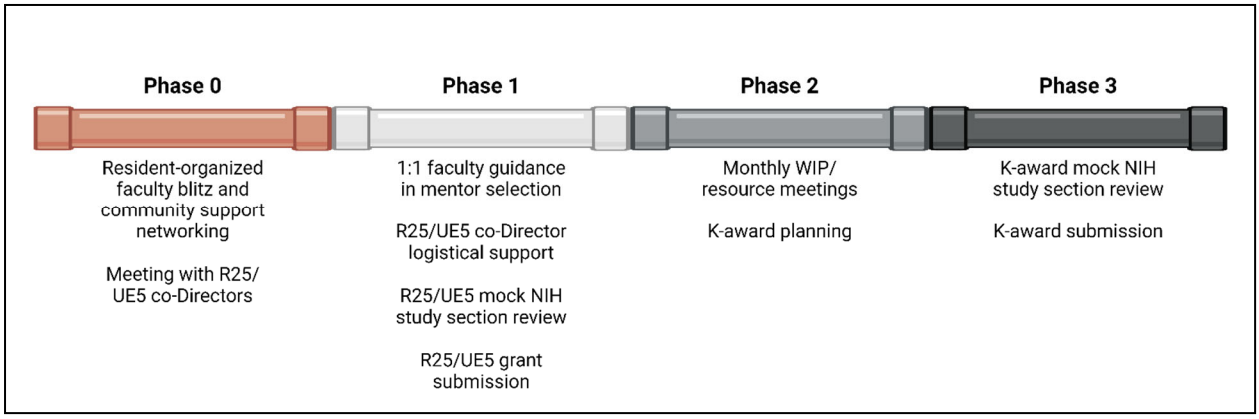

Figure 1. Physician Neuroscientist Training Pipeline (PNTP). While the PNTP pathway is highlighted during residency recruitment and immediately after Match Day, the integrated pipeline starts at the very beginning of residency (Phase 0), in which accepted residents are exposed to the range of research opportunities existing at Washington University through resident-organized faculty blitz sessions are held. These sessions also create a resident research community and facilitate networking with research faculty in an informal setting. Interested residents meet with UE5 co-directors to help chart their formal courses. In Phase 1, one-on-one faculty guidance and UE5 co-director logistical support are provided to assist in the selection of potential mentors and the development of potential projects. All applications are formally reviewed in September by a mock study section, and feedback provided to improve applications prior to submission in October. In Phase 2, funded applicants present annually at monthly resident-led research group meetings that also provide additional resources in the form of workshops on topics, including grant writing, rigor and transparency, K-award planning, and scientific presentation. They also attend the annual NINDS UE5 meeting and present at the annual Neurology/Neurosurgery UE5 Resident Research Day symposium. In Phase 3, trainees prepare their K-award applications for mock study section review prior to submission. Following completion of the pathway training period, there are annual contacts with Neurology/Neurosurgery UE5 leadership to track their continue success as independent physician-scientist investigators.

Phase 0

This initial phase of the PNTP is focused on defining your clinical interests, identifying potential research topics, and finding a mentor. This can be a daunting task, especially when you are immersed in the heavy clinical period of the residency program. Drs. Zipfel (Neurosurgery) and Gutmann (Neurology) are excellent resources to help you navigate this process. You should reach out to them early to establish some regular meetings. Additional resources include Dr. Gregory Wu (Neurology Resident Physician Scientist Training Program) and Dr. Albert Kim (Director of Neurosurgery Research Mentorship Group).

Defining your clinical interests. Some residents have already decided what subspecialty area interests them the most, while others are not committed. A major factor in arriving at a future subspecialty area will derive from your inpatient and outpatient clinical experiences. We encourage all residents to talk with their attendings to learn more about these future career choices.

Identifying potential research topics. While there is no one way to achieve this goal, you might get ideas from attending Neurology and Neurosurgery Grand Rounds, talking with other R25/UE5 PNTP-funded residents, and exploring the Roy and Diana Vagelos Division of Biology and Biomedical Sciences (DBBS) website (https://dbbs.wustl.edu).

Finding a mentor. During this period, you should set up meetings with prospective mentors. You should discuss your individual expectations for postdoctoral training, attend some of their laboratory meetings or social , and sit down with some of their current and past trainees. Some residents may require two mentors (one clinical and one research) or multiple mentors for cross-disciplinary projects. If you are considering more than one mentor, please discuss this with Dr. Gutmann (Neurology) or Dr. Zipfel (Neurosurgery). Factors to consider when choosing a mentor include, but are not limited to, their experience (track record) in training physician scientists, common or overlapping areas of scientific interest (mentor must have grant funding in the topic area), a style of mentoring that meets your needs (see expectations table below), and whether you feel that the laboratory environment suits you best. Your primary research mentor does not have to be within your specialty (neurology or neurosurgery).

Table 1. Example of Mentor/Mentee Expectations

Example Trainee Expectations of Mentor	**Example Mentor Expectations of Trainee**
*I expect my **mentor** to:* meet with me at least every few weeks. be open to my questions and to take time to think about them carefully. actively participate in the preparation of my R25/UE5 grant application be patient with me because I am new to research. initially be directive but eventually let me design and do experiments on my own. challenge and encourage me. teach me basic research techniques/procedures and safety protocols. help me define a project that is doable, yet relevant, and that keeps me busy. help me understand the basic scientific concepts and study design underlying my project. understand when I need to take time away from research to focus on my coursework and allow me to take it. help me network with other researchers in the group and/or discipline. be willing to discuss possible career goals and/or future jobs that will utilize the skills being learned during this research opportunity.	*I expect my **trainee** to:* be present and punctual when we have scheduled meeting times. work hard and give their best effort. manage their time efficiently and effectively when doing research. keep up with course work, but to let me know if they need a break from research to focus on courses. make every effort on their own to understand the research our group does, but to ask questions when they do not understand. listen carefully, take notes, and follow instructions when being taught new techniques. follow all disciplinary ethics and safety procedures. gradually gain independence, but to regularly communicate with me about what they are doing. be able to analyze their experimental data, generate logical conclusions based on that analysis, and propose future experiments, with assistance. work cooperatively, collaboratively, and respectfully with other members of the research team. be attentive, creative and contribute at research group meetings.

Choice of a research project. As you and your chosen mentor narrow down the potential projects, it is important to keep a few general guidelines in mind. First, the project should serve as the foundation for your path to independence. Second, the project should lead to an eventual K-award application. Third, the project should be transportable and provide you with independence. This is an important conversation with your prospective mentor to avoid any misunderstandings down the road.

Phase 1

All interested pediatric and adult Neurology applicants should reach out to Dr. Gutmann no later than the spring of the year before they intend to submit their application (PGY2, Adult Neurology; PGY3, Pediatric Neurology) in order to discuss the logistics of the application process. All interested Neurosurgery applicants should meet with Dr. Zipfel in the spring of their PGY3 year to review submission details and organization. As outlined in **Figures 2 and 3** below, there are specific timelines and deliverables in order to ensure that your application is received and processed internally prior to submission to the National Institute of Neurological Disorders and Stroke (NINDS).

Presubmission grant presentation. Prior to the UE5 grant submission, all applicants should plan to present their proposed grant at the UE5 research group meeting in August (prior to internal grant review and submission). Michele Bridges will be contact you regarding the specific details of this meeting in July.

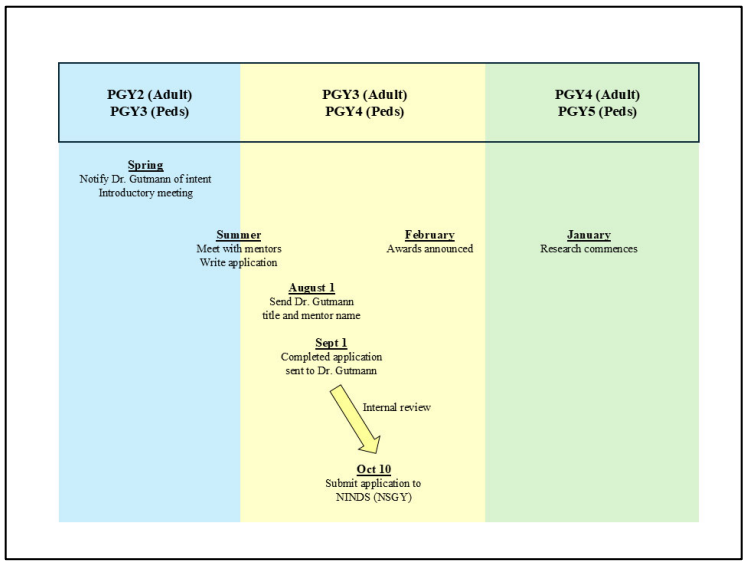

Figure 2. UE5 PNTP application submission cycle for Neurology residents

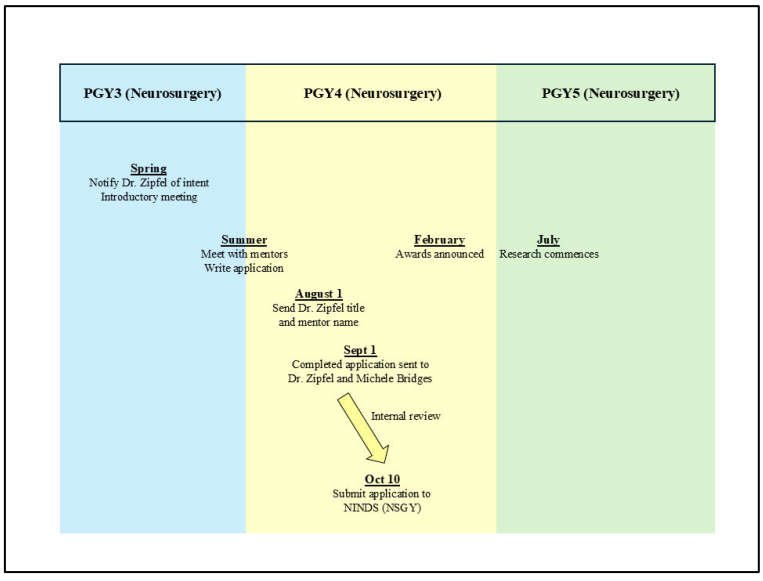

Figure 3. UE5 PNTP application submission cycle for Neurosurgery residents

UE5 Grant Application

Grant applications received will undergo internal review by experienced faculty in Neurosurgery and Neurology. You will receive comments and a brief summary statement, along with a recommendation (not recommended for submission, recommended for submission with substantial revisions, recommended for submission with minor revisions). You and your mentors should take these critiques into consideration when deciding whether to submit an application.

There are several important elements of this application:

1. A four-page (maximum) **description of the research education and research plan** (this should be written by the proposed participant and signed by both the proposed participant and the proposed mentor). This description should make clear the career goals of the participant and how the proposed plan is expected to lead to a future NIH K award or equivalent (i.e., a connection should be made between the proposed research and research education plan and a projected K award project). An example of a successful application is included in this guide.

    i. Title
    ii. Research Training (your background, your mentor(s)' backgrounds, and your long-term career goal
    iii. Research Plan
        1. Background and significance
        2. Experimental plan with aims
        3. Preliminary studies

2. A brief description of the **qualifications of the proposed mentor(s)**, with reference to their experience related to the research education plan and track record in mentoring clinician and non-clinician scientists.

3. A **mentor support statement**, written by the proposed mentor, which describes his/her qualifications to mentor the applicant in the proposed research education plan, the applicant's suitability for the research education plan and the mentoring and overall research education plan for this participant. The mentor should discuss a plan by which the participant will become ready to submit an application for an NIH Career Development (K) award or equivalent, and the expected timeline for this to occur. A vital component of this plan is how the proposed research is expected to lead to a larger project suitable for a K award. In addition, the mentor should describe how the participant's specific research project, whether basic, clinical, or translational, will adhere to the best principles of experimental design and rigor.

4. **Biosketches** for you and your mentors using the most current NIH form

Other attachments:

1. **IACUC Documentation and IRB Documentation**. If applicable, include documentation that the proposed research experience was approved by the Institutional Animal Care and Use Committee

(IACUC) and/or human subjects Institutional Review Board (IRB) at the grantee institution. If you will be using your mentor's protocol; please forward the Approval to Nichole Swanner-Gregory and she will take care of getting a Certification of grant correspondence.

Keys to a successful UE5 application

- Rigor is a key focus
  - Justify sample size
  - Include approach to data analysis and consider statistics
  - Mention blinding and controls
- Hypothesis
  - Recognize potential alternative explanations and pitfalls
  - Aims should not depend on success of prior aims
  - Cite relevant literature
  - Include mechanistic/therapeutic implications
- Document your previous experience in research
  - If no past experience in research, it will be difficult to obtain an UE5
- Demonstrate how this project will lead to a future K award
  - Absolutely key
- Do not need significant preliminary data
  - Many examples of grants funded without it
- Avoid the first-person plural (we).
  - Clearly delineate what the lab has done (e.g., Zipfel lab has previously shown…).
  - Clearly delineate what the applicant has done and will be doing (I performed…).
- Make sure that the scope of work matches the length of proposed UE5 funding
  - If applying for 6 months in a given calendar year but will follow this up with additional research time the following calendar year, make sure to articulate this so the reviewers understand the total amount of time (typically 18 months; potentially 24 months) dedicated to research and how much time the applicant has to complete the proposed experiments.
- Mentorship plan is key
  - Mentor needs to seem engaged and active in the applicant's maturation as a scientist.
  - Detail how often mentor will meet with you
  - Include mentorship plan to get resident to K award
  - If have multiple mentors, explain their roles clearly
  - Do not just say how great the applicant is and how great the mentor is. Needs to say how the mentor will help / guide the applicant.
  - Often a weakness in UE5 applications.
  - Biosketch of mentor – needs to specifically address the applicant and his/her proposal

UE5 PNTP Internal Review. In August of the year of UE5 submission, you will provide Dr. Gutmann (Neurology) or Dr. Zipfel (Neurosurgery) with the title of your grant application and the names of your mentors. This is critical in order for them to identify and assign appropriate subject matter expert reviewers. Following the receipt of completed applications around the first of September, Dr. Gutmann and Dr. Zipfel will ask the assigned reviewers to complete their evaluations, which he will assemble into a Summary Statement.

The grants are rated on five elements (Significance, Trainee, Mentor, Approach, and Career Development Plan) using a scale from 1 (highest) to 9 (lowest). Weaknesses and Strengths are provided as bullet points (**Table 2**).

Table 2. Scoring Table for Research Grant Applications

Degree of Impact	Impact Score	Descriptor	Additional Guidance on Strengths/Weaknesses
High	1	Exceptional	Exceptionally strong with essentially no weaknesses
	2	Outstanding	Extremely strong with negligible weaknesses
	3	Excellent	Very strong with only some minor weaknesses
Moderate	4	Very Good	Strong but with numerous minor weaknesses
	5	Good	Strong but with at least one moderate weakness
	6	Satisfactory	Some strengths but also some moderate weaknesses
Low	7	Fair	Some strengths but with at least one major weakness
	8	Marginal	A few strengths and a few major weaknesses
	9	Poor	Very few strengths and numerous major weaknesses

Definitions:

Minor: easily addressable weakness that does not substantially lessen the impact of the project.
Moderate: weakness that lessens the impact of the project.
Major: weakness that severely limits the impact of the project.

This Summary Statement will be emailed to the applicant and their mentors by the end of September to provide sufficient time for them to modify their application and submit. All applications are packaged together and submitted in mid-October by Nichole Swanner-Gregory in Neurosurgery.

NINDS Review and Outcome. Dr. Zipfel and Dr. Gutmann will be notified by NINDS staff by February of the following year about the success of the application. We do not receive reviews or summary statements, only a "funded/not funded" list. For this reason, we highly recommend that all applicants explore other fellowship funding mechanisms, including T32 and R38 physician-scientist training programs at Washington University. A list of these opportunities, including Department of Defense (https://cdmrp.health.mil/), organization (e.g., American Academy of Neurology), and private foundation grants, can be found on the Institute for Clinical and Translational Sciences (ICTS) website (https://crtc.wustl.edu/otg/washington-university-training-grants/).

After you have received word that you will be funded, you should contact inform your residency program leadership. Neurology trainees should reach out to Dr. Peter Kang (Adult Neurology) or Dr. Soe Mar (Pediatric Neurology) to discuss your final year of residency training. They will work with you and the Chief Residents to front-load your clinical activities and enable you to devote sufficient time to research during the final 6 months of residency training. Neurosurgery residents should contact their Chief Residents and Dr. Joshua Osbun (Program Director, Neurosurgery Residency). All residents will continue to engage in outpatient clinical responsibilities during their research months.

Effects of apoE-dependent glial lipid metabolism on Alzheimer disease pathology

Applicant: Michelle Diane Rudman, M.D., Ph.D.
Mentor: David M. Holtzman, M.D.

Overview

My overarching goal is to become a physician-scientist working in an academic center and pushing forward the boundaries of modern medicine. My stimulus is the understanding of the basic underpinnings of the human mind and how that biology breaks down in disease states. Alzheimer disease (AD) is a neurodegenerative condition that robs people of that which makes us human – our experiences, our personality, and our memories – and impacts almost every person due to its high prevalence, my family included. This is what has made AD such a compelling focus of research and why I plan to devote my career to its study. My background, including a Ph.D. in neuroscience and specifically in the mechanisms of cell death and inflammation in CNS injury, as well as my ongoing residency training in neurology, will provide a strong foundation for the proposed studies. In the highly collaborative environment of Washington University and under the guidance of my mentor Dr. David Holtzman (B. Burton & R.M. Morriss III Professor of Neurology, scientific director of the Hope Center for Neurological Disorders, and Associate Director of the Knight ADRC), I will be optimally poised to develop my skills in the study of AD and more broadly as a physician-scientist. Dr. Holtzman's expertise in the study of the underlying mechanisms of AD, including many of the foundational studies on the role of apoE and glial cells in the development of AD pathology, is unparalleled. He has trained over 70 graduate students, post-doctoral fellows, and physician-scientists, many of whom have gone on to successful careers in academia. For my project, I plan to study the contribution of different apoE isoforms to perturbations in lipid metabolism in the setting of AD pathology in mouse models. The goal of this project is to determine the effects of apoE-mediated lipid accumulation in glial cells on AD pathology and whether this may be targeted for therapeutic intervention. Ultimately, I plan for this project to lay the groundwork for my future research endeavors in identifying novel targets for therapeutic intervention in Alzheimer disease.

Background and Significance

Alzheimer disease is the leading cause of dementia and results in progressive cognitive impairment, disability, and death.[1] There are no treatments proven to prevent or slow cognitive decline in AD and the pathology remains incompletely understood. Therefore there is an urgent need for additional research to elucidate the pathologic mechanisms and help identify targets for clinical intervention. The pathologic hallmarks of AD include accumulation of amyloid plaques and neurofibrillary tangles with synapse loss, neuronal death, and brain atrophy.[2, 3] The strongest genetic risk factor for late-onset AD is the apolipoprotein E (*APOE*) gene which has three major alleles – *APOE2*, *3*, and *4* – that confer variable risk for acquiring AD. The *APOE3* allele is the most prevalent form in the United States and relative to this allele, *APOE2* decreases while *APOE4* increases the risk of developing AD.[4-8] This has led to intense investigation into the mechanisms by which apoE isoforms may modulate the development and progression of AD pathology.

ApoE is expressed in the brain by astrocytes and to a lesser degree by neurons and microglia, [9-12] and is found in HDL-like particles.[10, 12-14] In AD, apoE has been implicated in promoting amyloid-β (Aβ) aggregation and inhibiting its clearance as well as modulating inflammation.[15-17] ApoE is also involved in regulating lipid metabolism given its presence on the surface of lipoprotein particles but the types of lipid species it helps regulate and the differential effects of its isoforms on this process are not known. In the setting of demyelinating injury, excitotoxicity, or oxidative stress, glia have been shown to accumulate lipid droplets in an apoE-dependent fashion.[9, 18-20] Interestingly,

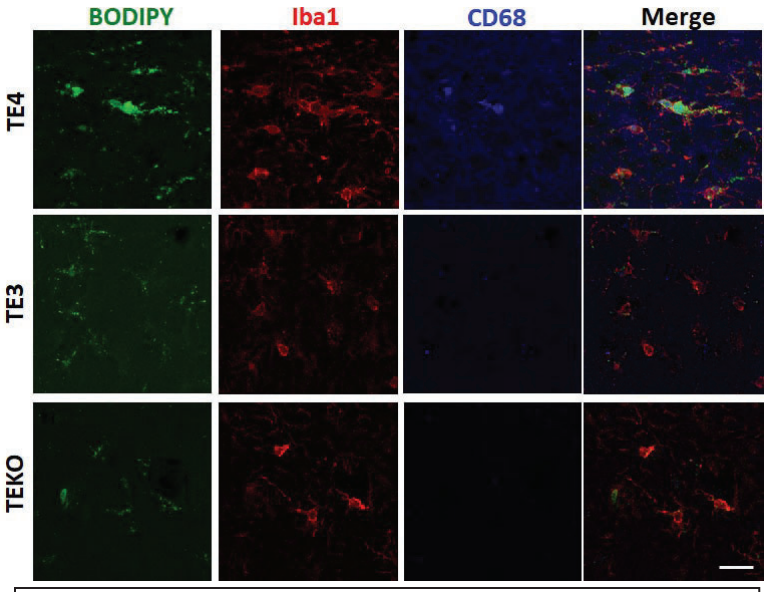

Figure 1. Microglia in 9 month old P301S-Tau;E4 (TE4) mice accumulate many more lipid droplets than TE3 or TEKO mice. Hippocampal staining for BODIPY (lipid droplets, CD68 (activated microglia), and Iba1 (microglia) in 9 month old mice. Scale: 50 μm.

preliminary data from the Holtzman laboratory has demonstrated that microglia accumulate lipid droplets in a mouse model of tauopathy and that this is exacerbated by the apoE4 isoform compared to apoE3 or apoE KO (Figure 1). Furthermore, post-mortem studies have shown that human brains develop similar pathology with intracellular lipid droplet accumulation correlating with AD pathology.[21, 22] The implications of this lipid accumulation for the development of AD pathology is not known. The depletion of apoE via genetic knockout, antisense oligonucleotide knockdown, or overexpression of the apoE receptor low-density lipoprotein receptor (LDLR) is protective in a mouse model of tauopathy.[23-25] The Holtzman laboratory has been exploring pharmacologic methods for lowering apoE in the brain and has recently discovered that certain commonly used cholesterol-lowering drugs known as statins are able to substantially lower apoE levels in the brain when given orally (Figure 2) via increasing brain expression of LDLR. This provides a highly translational experimental paradigm in which to study whether pharmacologic lowering of apoE may be beneficial not only in mouse models of tauopathy but also in combined amyloid-tau pathology models that more closely mimic human pathology in AD.

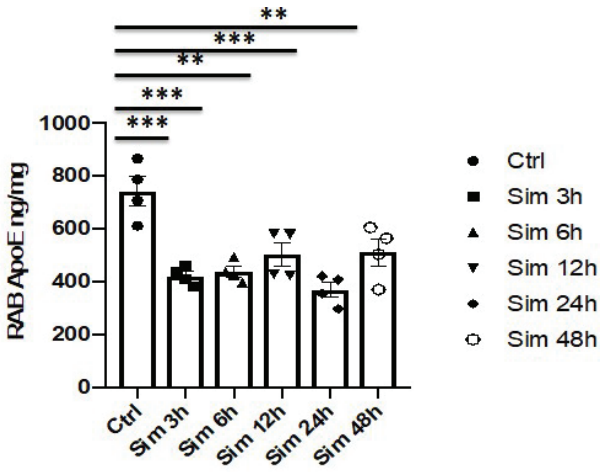

Figure 2. Simvastatin significantly decreased apoE levels in the mouse brain. Oral gavage of 200mg/kg simvastatin-lactone or vehicle with sacrifice at 3, 6, 12, 24, or 48 hrs and measurement of water-soluble apoE protein levels in whole-brain homogenate.

Overall, this project seeks to address the role of apoE in modulating cell-specific lipid metabolism in aging and in the presence of AD pathology and would determine whether pharmacologic lowering of apoE may be beneficial in a combined amyloid-tau model of AD. These results will lay the groundwork for future studies aimed at elucidating the role of apoE-dependent lipid metabolism in the development of AD and may help identify novel targets for intervention.

Approach

For the proposed experiments, I will be using the APP/PS1;P301S-Tau mouse model recently generated in the Holtzman laboratory in combination with already available human apoE3 and apoE4 knock-in (KI) mouse lines. This experimental model allows us to study the effects of different human apoE isoforms with combined amyloid and tau pathology which more closely mimics the pathology observed in AD. Preliminary data from the Holtzman laboratory has demonstrated that this mouse model develops Aβ deposition by 3 months, tau aggregation starting at 5 months, and measurable neurodegeneration by 9 months of age (Figure 3). Using this model, I will study the effects of different apoE isoforms on the development of multiple key aspects of AD pathology including changes in glial lipid metabolism and gene expression, amyloid and tau deposition, inflammation, and neurodegeneration. I will also use this model to assess pharmacologic interventions for modulating apoE with the goal of ameliorating AD pathology and cognitive decline. Ultimately, this work will contribute to our understanding of the role of apoE in glial lipid metabolism and shed light on how aberrations in lipid metabolism may be targeted to alleviate pathology in Alzheimer disease.

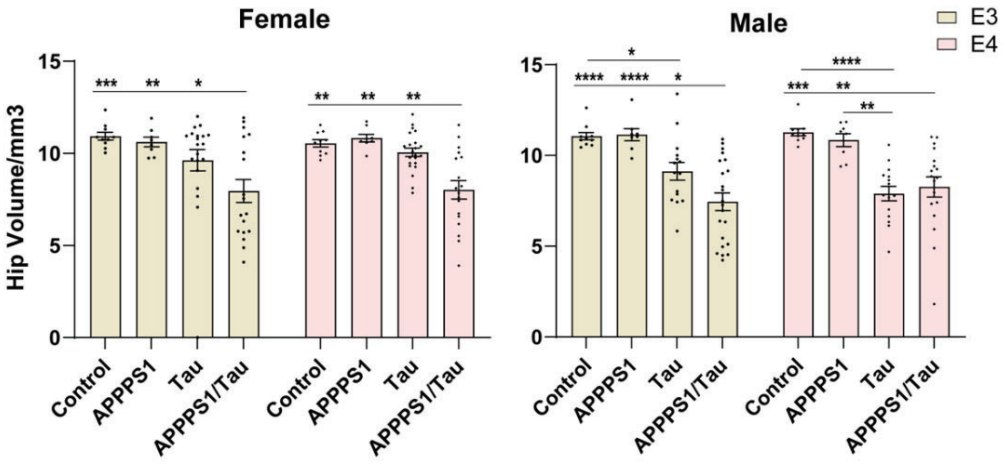

Figure 3. Hippocampal volume quantification of 9-month old female and male Control, APPPS1, P301S, and APPPS1/P301S mice that express either human apoE3 or apoE4 (human apoE knockin) in place of murine apoE. Data expressed as mean ± SEM, Two-way ANOVA with Tukey's post hoc test. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Aim 1: Effect of apoE isoforms on cell-specific lipidomes and transcriptomes and on the development of amyloid and tau pathology in the APP/PS1;P301S-Tau mouse model of Alzheimer disease.

Preliminary data from the Holtzman laboratory has demonstrated that microglia accumulate lipid droplets in the P301S tauopathy mouse model and that this lipid accumulation is exacerbated by the apoE4 isoform (Figure 1). Other recent studies also demonstrate apoE-dependent glial lipid droplet accumulation and inflammatory activation in the setting of demyelination, excitotoxicity, or oxidative stress.[9, 18-20] These findings suggest that changes in glial lipid metabolism occur in various disease states in the brain in an apoE-dependent manner. *I hypothesize that astrocytes and microglia will develop altered lipid metabolism with lipid accumulation in normal aging but to a greater extent in the setting of AD pathology. The apoE4 isoform will have impaired lipid metabolism relative to apoE3, resulting in exacerbated inflammation and AD pathology.*

Aim 1.1: Assess the differential effects of apoE3 and apoE4 on cell-specific lipidomes and transcriptomes in the setting of AD pathology. Using the APP/PS1;P301S-Tau mouse model of AD, I plan to compare the effects of different apoE isoforms on cell-specific lipidomes and transcriptomes at 3 months before the development of pathology and at 7 and 9 months after accumulation of amyloid and tau pathology. APP/PS1;P301S-Tau;E3 or E4 KI mice will be compared to E3 or E4 KI mice. At each time point, there will be N = 10 mice for each sex for a total of N = 20 mice. One half of the brain will be used to prepare a single cell suspension for sorting CNS cells according to the manufacturers' protocol using the adult brain dissociation kit (Miltenyi Biotec 130-107-677). Cells will be Fc blocked and stained for flow cytometric analysis as previously described.[19] CD11b+/CD45low microglia and ACSA-2+ astrocytes will be sorted on a FACS Aria III (BD Biosciences). Sorted cells will be processed for downstream lipidomic analysis as previously described[19] or submitted to 10X Genomics workflow at the McDonnell Genome Institute at Washington University for RNAseq analysis. Bioinformatic analysis for differential lipid presence as well as RNA seq analysis will be performed as described.[23, 26]

Aim 1.2: Assess the differential effects of apoE3 and apoE4 on the development of AD pathology, lipid accumulation, and cognitive function. Using the same mouse groups and time points as described above, I will assess the nesting behavior of mice a day prior to sacrifice as described.[26] This test has previously been shown to be abnormal in P301S;E4 mice and improved with apoE depletion. I will then use the remaining hemi-brain for histopathology including immunohistochemistry and volumetric analysis. Specifically, I will stain sections and perform histological quantification for pathologic markers including Aβ, tau, p-tau, lipid droplets, reactive astrocytes and microglia, neuronal number, and synapse density. For the volumetric analyses, I will measure the volume of the piriform/entorhinal cortex, hippocampus, and posterior lateral ventricles, and measure the thickness of the granule cell layer of the dentate gyrus and CA3/CA1 layers.

Aim 2: Effects of APOE KO and pharmacologic lowering of apoE on the development of pathology in the APP/PS1;P301S-Tau mouse model of Alzheimer disease

In mouse models of Aβ deposition and tauopathy, apoE4 exacerbates pathology while depletion of apoE via genetic knockout or antisense oligonucleotide knockdown ameliorates pathology.[24, 25, 27] Similarly, decreasing apoE levels via overexpression of the apoE receptor LDLR was also shown to attenuate Aβ deposition and tau-mediated neurodegeneration.[23, 28] The mechanisms by which decreasing apoE leads to improved outcomes in models of either Aβ deposition or tauopathy are still being elucidated and whether this effect will also be observed in models of combined amyloid-tau pathology is not known. The Holtzman laboratory has also been exploring pharmacologic methods for lowering apoE in the brain and has recently discovered that the commonly used cholesterol-lowering drugs in the statin class, and in particular simvastatin, are able to substantially lower apoE levels in the brain when given orally (Figure 2). This therefore presents an opportunity to attempt to modulate apoE levels *in vivo* in a highly translational paradigm using well-tolerated drugs. *I hypothesize that APOE KO or simvastatin-mediated decreases in apoE in APP/PS1;P301S mice will attenuate the development of AD pathology and this will correlate with improved glial lipid metabolism.*

Aim 2.1: Determine the best pharmacokinetic/pharmacodynamic for simvastatin to lower brain apoE in apoE4 knock-in mice. Preliminary data from the Holtzman laboratory has demonstrated that simvastatin reaches effective brain levels for several hours following a single oral dose and that for at least 48 hours after administration simvastatin results in about a 50% decrease in levels of apoE in the cortex (Figure 2). This is comparable to the depletion observed with apoE knockdown using antisense oligonucleotides.[25] Based on these results I plan to assess the effects of 3 different doses at 20, 60, or 200 mg/kg of simvastatin vs vehicle following a single oral gavage at 6, 24, 48, 72, and 96 hours on soluble brain apoE levels in apoE4 KI mice. I will determine the lowest dose of drug that decreases apoE4 levels by at least 30% as well as the frequency of dosing needed to maintain this effect.

Aim 2.2: Determine the effect of APOE KO or simvastatin treatment on the levels of apoE and apoE receptors, glial lipids, and the development of pathology in the APP/PS1;P301S;E4 KI mouse model of AD. To determine whether APOE KO or pharmacologically decreasing brain levels of apoE can attenuate the development of pathology in a combined amyloid-tau mouse model of AD, APP/PS1;P301S mice will be crossed with APOE KO or human APOE4 KI mice. The APP/PS1;P301S-Tau;E4 KI mice will be treated via oral gavage with one of 2 doses of simvastatin vs vehicle control beginning at 3 months. The doses and frequency of dosing of simvastatin will be determined as per Aim 2.1 with one dose likely being 200 mg/kg and the lower dose being the dose determined to lower brain apoE levels by at least 30%. Just prior to sacrifice at 9 months, mice will undergo behavioral assessment as in Aim 1. Following sacrifice, half of the brain will be processed for histology to assess for the presence of amyloid and tau deposits, activated astrocytes and microglia, neuronal and synaptic density, and volumetrics. The other half of the brain will be processed for FACS cell-based sorting for downstream qPCR quantification of apoE and apoE receptors including LDLR, unbiased RNAseq, and lipidomics as in Aim 1.

Pitfalls, Interpretations, and Future Directions: The mice for these studies are currently available and breeding in the Holtzman laboratory. The protocols for FACS-based cell-specific lipidomic and transcriptomic analyses are also already well-established. Based on unpublished results in a tauopathy mouse model, I anticipate the lipidomic analyses will demonstrate apoE isoform-dependent shifts in cellular cholesterol and lipid species. These results will be the basis for future studies to determine whether these cellular shifts have an impact on the development of AD pathology and the mechanisms by which lipid signaling pathways may impact AD.

Regarding Aim 2, pilot studies have already demonstrated that oral administration of simvastatin lowers brain apoE levels significantly (Figure 2) and we will also include genetic deletion of apoE as a control. Other approaches to decreasing apoE could include antisense oligonucleotide knockdown of apoE or use of small molecules that increase glial lipid efflux such as LXR agonists to assess whether apoE/lipid efflux is necessary/involved in the development of pathology in a mixed amyloid-tau mouse model of AD. The tools and protocols to perform these studies are also already well-established in the Holtzman laboratory. Alternatively, we could study the effects of simvastatin administration in mouse models with isolated amyloid or tau pathology to gain a better understanding of whether statin-mediated lowering of brain apoE affects each of these components individually. This study has important translational implications for not only AD but many other neurodegenerative conditions for which APOE4 has been shown to be a risk factor. The results of these experiments could therefore also be used to guide similar experiments in other models of neurodegenerative disease.

Statistical analysis: Lipidomic data will be analyzed via ANOVA on log transformed data. APP/PS1;P301S-Tau;E3 or $E4^{flox/flox}$ and E3 or $E4^{flox/flox}$ mice will be compared using two-way ANOVA with Tukey posthoc test. For histologic studies, similar prior studies from the Holtzman laboratory analyzing histologic outcomes have shown that treatment groups of N = 10-15 mice per sex generally achieve a power of at least 80% assuming a significance level of 0.05 with a two-tailed equal variance T-test. Group effects will be compared using one- or two-way ANOVA with appropriate post-hoc testing.

Research Training and Career Development

In collaboration with my mentor, I have devised a career development plan that will foster my growth as a physician scientist and clinical neurologist. The proposed studies will build on my prior experience using primary cell cultures and mouse lines to study genetic and pharmacologic interventions to target neuroinflammation. I will gain new skills including biochemical techniques such as RNAseq and lipidomic analysis as well as histologic techniques such as volumetric analyses and train in new behavioral assays specific for cognitive impairment. I plan to dedicate 6 months of my PGY4 and the first year of fellowship training to completing the proposed experiments. I plan to complete my fellowship training in dementia to complement my scientific interests in Alzheimer disease. The clinical fellowship training will be done in coordination with John Morris, MD, Professor of Neurology, head of the Aging & Dementia section, and director of the Knight ADRC. The studies proposed in this grant will provide the preliminary studies to apply for a future NIH career development award in addition to foundation grants. Ultimately the experience gained through this grant and through my clinical training in neurology and dementia will position me for a successful career as a physician scientist at an academic institution studying the basic mechanisms of Alzheimer disease pathogenesis and translational approaches to developing novel therapeutic interventions.

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Effect of astrocyte and microglial ApoE on TBI-mediated neuroinflammation and neurodegeneration

Applicant: Enmanuel J. Perez, MD, PhD;
Mentor: David M. Holtzman, MD, Co-Mentor: Terrance T. Kummer, MD, PhD

Overview

My foremost goal is to become a physician-scientist in an academic setting, as the head of a research group, and a practicing neurologist providing cutting-edge, evidence-based patient care. Previous research experience taught me how to think like a scientist, formulate a strong hypothesis and research plan, and think critically and creatively about my work and the work being done in my field. Clinical and personal experience with traumatic brain injuries (TBIs) have strengthened my desire to pursue a career as a neurologist and a clinician-scientist studying the pathological mechanisms of TBI and developing strategies to promote recovery and prevent neurodegeneration after injury. For the proposed research, I will gain invaluable insight by the mentoring of Dr. David M. Holtzman (Andrew B. and Gretchen P. Jones Professor and Chairman of Neurology) and co-mentoring of Dr. Terrance T. Kummer (Assistant Professor of Neurology). Dr. Holtzman's expertise in the pathobiology of neurodegenerative diseases and Alzheimer's disease (AD), including the contributions of apolipoprotein E (ApoE) to both Aβ metabolism and to tau-mediated neurodegeneration using genetically-modified mouse models, as well as his previous work in the field of TBI, will provide an unparalleled mentoring opportunity. Dr. Kummer's forward-thinking expertise in the field of TBI, with development of both injury models and methodological techniques to study TBI and its connections to AD, will serve as an excellent mentoring complement for the proposed studies. My project will focus on examining the contribution of ApoE isoforms derived from astrocytes and/or microglia to synaptic damage, neurodegeneration, and behavioral deficits after TBI in the presence or absence of tauopathy, as well as neuroprotective strategies to mitigate the detrimental effects of pathogenic ApoE isoforms (ApoE4). This project will contribute to the understanding of the long-term influences of glial subtypes on neurodegenerative cascades in the injured brain, and provide an opportunity to develop neuroprotective therapies. Ultimately, the goal of this project is to generate preliminary data for longer-term efforts, which could be translated to patients at high risk of TBI and subsequent neurodegenerative disease, such as professional athletes, military personnel, and the elderly.

Background and Significance

TBI is a major contributor to mortality in the US, contributes to significant economic burden, and is highly associated with long-term disability. TBI encompasses a heterogeneous collection of pathologies resulting from mechanical insults to the head. While the onset and initial disruption of brain function occur acutely, many effects are long-lasting. The chronic pathophysiology of TBI shares many features with the chronic neurodegeneration and neuroinflammation that characterizes neurodegenerative disorders. In fact, chronic traumatic encephalopathy (CTE), a condition associated with repeated head trauma, is defined pathologically as a degenerative tauopathy, with similarities to the tauopathies seen in AD, frontotemporal dementia (FTD), and progressive supranuclear palsy (PSP), albeit with some differences in distribution[1,2]. However, the mechanisms leading to neurodegeneration after TBI are still poorly understood. ApoE, a major lipid carrier in the brain, is upregulated after brain injury[3]. Different ApoE isoforms (E2, E3, and E4) have been associated with differential risk and disparate outcomes in neurodegenerative disorders, including following TBI[4]. ApoE4 is associated with increased risk of AD and vulnerability after TBI, as well as with increased tau-mediated neurodegeneration in animal models and in humans with AD and primary tauopathies[5–10]. In the brain, astrocytes are the main source of ApoE under normal conditions, but activated microglia contribute substantially to the ApoE pool in the setting of brain disease. Both ApoE and microglia contribute to inflammation and neurodegeneration in mouse models of tauopathy and AD[8,9,11]. Similarly, pathological activation of ApoE4-expressing astrocytes contributes to microglial activation, neurodegeneration and increased AD risk[8,9,12]. However, the specific contribution of ApoE produced by each glial-type to the neuro-inflammatory response and subsequent chronic neurodegeneration following TBI is not yet clear.

Approach

The objective of this proposal is to determine the role of glial-derived ApoE4 on TBI-induced neuroinflammation and neurodegeneration in a tauopathy mouse model. I will make use of multiple TBI models to examine this effect (Aim 1- gain of function studies) and utilize novel ApoE KI mice in which ApoE can be removed in a cell-specific fashion to improve mechanistic understanding (Aim 2- loss of function studies). For my futures plans, I would like to continue to explore possible therapeutic interventions to ameliorate the effects of TBI on tau/ApoE-mediated neurodegeneration. My overarching hypothesis is that astrocytic-ApoE, in the setting of tauopathy and post-TBI, leads to increased microglial-mediated synaptic phagocytosis and that microglial-

derived ApoE leads to enhanced microglial activation and further injury. Thus, blockade of each source will decrease a specific aspect of neurodegeneration in the setting of TBI and tauopathy. To this end, I will take a comprehensive approach (using biochemical, immuno-histochemical, histopathologic, and genetic/transcriptomic methodologies), make use of cutting-edge techniques, and use novel mouse models. This work will fill critical knowledge-gaps in TBI-mediated neurodegeneration and form the ground-work for my future studies in TBI.

Aim 1: TBI effect on ApoE-dependent neurodegeneration in a tauopathy mouse model

Experimental TBI represents a unique model to study the mechanisms of chronic neurodegeneration for multiple reasons. It allows us to establish a starting point for these neuroinflammatory cascades, unlike other neurodegenerative pathologies, where a protracted, poorly-defined preclinical phase predominates. Additionally, it seems to not only increase risk for neurodegenerative disease, but also accelerate these disease processes[13–21]. Impairment after TBI correlates with expression of genes encoding proteins related to Aβ clearance, including Abca1 and ApoE[22]. Unique and distinct gene networks driven by ApoE isoforms are upregulated after TBI[23]. I hypothesize that TBI will accelerate neurodegeneration, neuroinflammation, and cognitive decline in a tauopathy mouse model in an ApoE isoform-dependent manner.

Aim 1.1: Determine the effect of TBI on neurodegeneration, Tau pathology, and synaptic connectivity in a tauopathy mouse model

I will use both a well-established controlled-cortical impact (CCI) injury model and a more novel, closed head (modCHIMERA) injury model to investigate the role of ApoE in neurodegeneration after TBI[24–29]. To examine the effect of different ApoE isoforms, I will make use of transgenic mice available at the Holtzman Lab, which overexpress human tau and consistently express a neurodegenerative phenotype by 9 months of age. Additionally, these mice express different human isoforms of ApoE (ApoE3 vs ApoE4), which will be used for these gain-of-function studies. Mice will undergo TBI at 3 months of age and be assessed at 1 month and 3 months post-TBI, prior to a critical time window where neurodegeneration occurs in this mouse model (i.e. between 6-9 months of age). Specifically, I will assess neurodegeneration by measuring volume of piriform/entorhinal cortex, hippocampus, and posterior lateral ventricles, examine thickness of granule cell layer of dentate gyrus and CA3/CA1 layers, including hippocampal shape analysis, and correlate hippocampal layer thickness with volumes. To assess tau pathology, I will make use of previously established methods to examine overall levels of Tau/pTau (ELISA-based and WB analysis), perform histological quantification of pTau (using % area of AT8 in hippocampus), and examine different pTau staining patterns previously correlated with level of brain atrophy[9]. Finally, I will make use of novel, super-resolution imaging and analysis (SEQUIN) developed by Dr. Kummer, which allows for mesoscale quantification of synaptic structures, to examine the effects on synaptic connectivity[29]. I hypothesize that neurodegeneration, including disrupted connectivity, will be accelerated post-TBI and most pronounced in mice expressing ApoE4. ApoEE3 mice will be used as controls, as well as litter-mate sham controls.

Aim 1.2: Determine the differential effect of TBI on glial (microglial and astrocytic) activation in a tauopathy mouse model

As previously described, neurodegenerative disease and TBI lead to alterations in different glial types, subsequently involved in activation of a wide array of different pathophysiologic cascades contributing to chronic neurodegeneration. However, how the contribution of different ApoE isoforms on glial activation after TBI is not yet clear. I hypothesize that ApoE4 affects glial activation following TBI, specifically it leads to up-regulation of disease-associated astrocytic and microglial genes and activation of neuroinflammatory cascades. To assess general glial activation, I will use immunohistochemical methods to quantify area of microglial activation (CD68+ and Iba1+) and astrocytic activation (GFAP+). To better understand the neuroinflammatory and neurodegenerative cascades associated with these changes, I will make use of single-nucleus (sn) transciptomics, sn RNA seq[30], to measure gene-expression changes within specific glial sub-populations after TBI. Sn RNA seq is a powerful tool for studying cell-type expression changes at the single-cell level, which will be used to analyze the specific effects of neurons, astrocytes, and microglia in our experimental model.

Aim 1.3: Determine the effect of ApoE isoform-dependent neurodegeneration after TBI on cognitive performance. Traumatic brain injuries confer significant chronic morbidity, as seen by development of post-concussive syndrome (associated with neuropsychiatric symptoms, such as depression and anxiety, and cognitive impairment, mainly affecting attention, concentration, and working-memory). To assess the effect of different ApoE isoforms following TBI at the functional level, I will make use of two well-validated neurobehavioral assays, nesting behavior and Morris-water maze. I hypothesize that ApoE4-dependent

neurodegeneration post-TBI will contribute to significant deficits in function, which will be less pronounced in ApoE3 mice. These functional outcomes will be valuable for future studies to assess functional recovery after therapeutic intervention.

Aim 2: Cellular shift in ApoE4 expression, from astrocytes to microglia, contributes to neuroinflammation and neurodegeneration after TBI

Differential expression of ApoE isoforms has been associated with various neurodegenerative disorders, including AD and TBI. This risk is mostly associated with and/or increased by ApoE4, whereby both astroglial and microglial ApoE4 have been associated with neurodegeneration and increased AD risk. ApoE isoforms also play a role in microgliosis (including up-regulation of various genetic networks) after brain injury[22,31]. However, the specific contributions of cell-type derived ApoE4 to the neuropathology of TBI is still unclear.

Aim 2.1: Determine the contribution of astrocytic ApoE4 to neurodegeneration in a tauopathy model after TBI

Astrocytes are the main source of ApoE under normal conditions. ApoE4 is highly associated with increased risk of chronic neurodegenerative disease, including TBI. However, the role of astrocyte-specific ApoE4 folllowing TBI remains to be elucidated. I hypothesize that while astrocytic ApoE4 has a strong influence on tau-mediated neurodegeneration (see preliminary data), TBI will lead to an additional detrimental role of ApoE4 through actions on microglia. I will make use of unique transgenic mice in which ApoE3 or ApoE4 can be selectively knocked out in astrocytes or all brain cells by crossing P301S/apoE3$^{flox/flox}$ and P301S/apoE4$^{flox/flox}$ with Aldh1l1-Cre/ERT2 or CAG-Cre/ERT2 mice, respectively[32,33]. Whereas studies in Aim 1 will focus on intrinsic expression of different ApoE isoforms *(gain-of-function)* and changes that may occur with TBI, Aim 2 will focus on glial-specific knock-down of ApoE to examine cell-specific contributions to the injury environment *(loss-of-function studies)*. Using similar methodologies introduced as in Aim 1- including volumetric analysis, biochemical and histological Tau analysis, quantification of synaptic structures, measurements of glial activation, gene expression and transcriptomic analysis, and behavioral/cognitive assays- I will examine how astrocyte-specific or complete inducible knock-down of ApoE4 contributes to neuroinflammation and neurodegeneration.

Aim 2.2: Determine the contribution of microglial ApoE4 on ApoE-dependent neurodegeneration after TBI

Microglial activation plays an important neuroinflammatory role in AD and after TBI[34–36]. ApoE from different glial types has different properties, such as microglial-secreted ApoE is very lipid poor relative to that of astrocytes, which suggests ApoE-mediated signaling and effects may be cell-dependent[33]. Elimination of microglia leads to neuroprotection and prevention of ApoE4-mediated neurodegeneration in a tauopathy mouse model[9]. I hypothesize that after TBI, microglial activation and upregulation of pathogenic ApoE4 will separately contribute to ApoE-dependent neurodegeneration, in addition to an effect of astrocytic ApoE. Therefore, knockdown of microglial ApoE4 will lead to decreased glial activation and neuroprotection after TBI. I will use the aforementioned methodologies (Aim 2.1), with the addition of transgenic mice in which in which ApoE3/ApoE4 can be selectively knocked out in microglia (P301S/apoE3$^{flox/flox}$ and P301S/apoE4$^{flox/flox}$ crossed with CX3CR1-Cre/ERT2 mice).

Preliminary studies

Unpublished data from the Holtzman lab shows that astrocyte-derived ApoE4 directly contributes to tau-mediated neurodegeneration in P301S/apoE4 KI mice (see figure below).

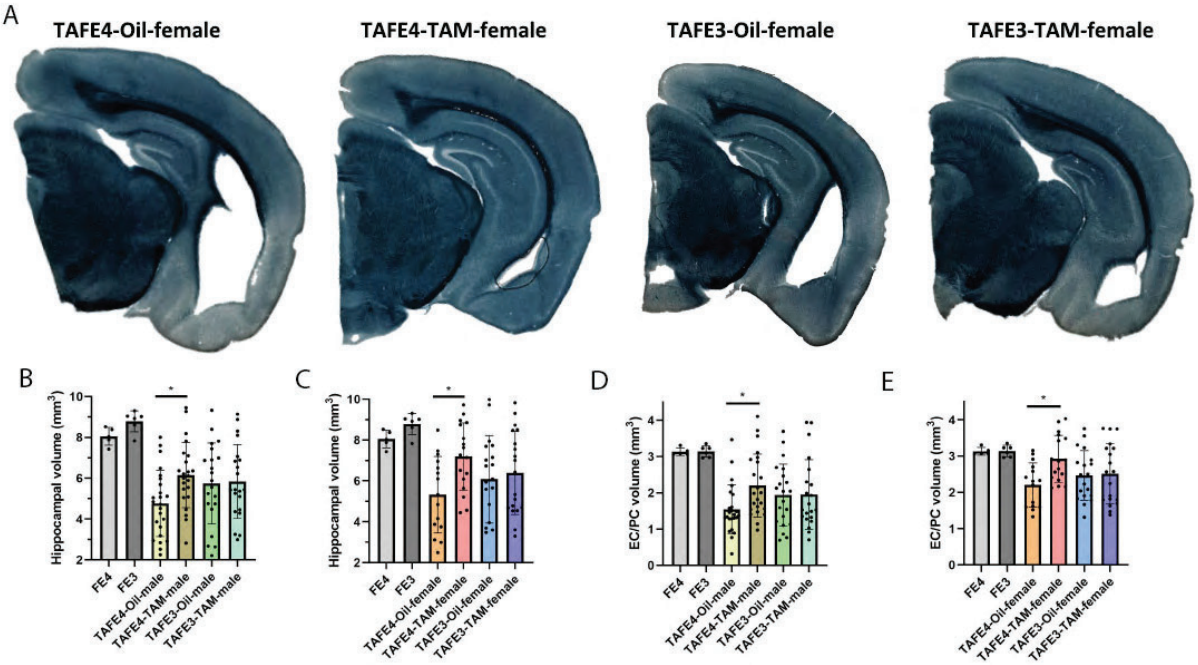

Figure: Knocking down astrocytic apoE4 expression reduces brain atrophy
P301S Tau-ApoE3$^{flox/flox}$ X Aldh1l1-Cre/ERT (TAFE3) and P301S Tau ApoE4$^{flox/flox}$ X Aldh1l1-Cre/ERT (TAFE4) mice were given 100 mg taxomifen (TAM) dissolved in oil or oil alone (vehicle) i.p once per day for one week at 5.5 months of age. Mice were then assessed at 9 months of age (285 days). ApoE3$^{flox/flox}$ (FE3) and ApoE4$^{flox/flox}$ (FE4) mice are also analyzed for comparison. (A) Representative Sudan Black staining images of 9-month-old TAFE mice that received different treatments clearly shows that brain atrophy was considerably reduced after knocking down astrocytic apoE4 expression from 5.5 months of age. (B and C). There were increases in male (B. *p=0.0385) and female (C. *p=0.0414) hippocampal volumes in the TAFE4 mice treated with tamoxifen. (D and E). There were increases in male (D. *p=0.0428) and female (E. *p=0.0265) entorhinal/piriform cortex (EC/PC) volumes after knocking down astrocytic apoE4. All statistics were performed using a one-way ANOVA with multiple comparisons unless otherwise specified.

Pitfalls and Future Directions: A drawback from the proposed study is the significant use of transgenic mice and the age requirements for the mice used (6-9 months). Fortuitously, these mice have been developed in the Holtzman lab, where increased breeding and production of future transgenic mice will be meticulously planned for in order to perform timely experiments. In terms of methodologies, I do not foresee significant barriers; all of the above described methods have previously been well-established and utilized by my mentor, co-mentor, and/or their collaborators. Future directions include targeting multiple pathways involved in ApoE-mediated neurodegeneration after TBI, as well as utilizing different mechanistic approaches (i.e. anti-antibodies, anti-sense oligonucleotides) to block/promote cell-specific ApoE signaling following TBI as possible therapeutic interventions.
Statistical analysis: For the proposed mouse studies, based on prior studies from the Holtzman and Kummer labs, a sample size of N = 15-20 per group (depending on experiment) generally achieves an 80% power to detect an effect size of 25% assuming a significance level of 0.05 using a two-sided two-sample equal variance t-test. Group effects will be compared using One-way or Two-way ANOVA with appropriate post-hoc testing.

Research Training and Career development
In collaboration with my mentors, we have designed a training plan that furthers my development as a physician-scientist and academic neurologist. I will build upon my prior research experiences, continue to learn a diversity of technical skills, which will be necessary to run my own lab. I will also take advantage of instrumental mentoring, from Dr. Holtzman, Dr. Kummer, and many of their collaborators, during this training period. My career research goals are to contribute to the understanding of TBI pathophysiology, with specific aims on glial biology/pathology, and development of novel therapeutic interventions. The experiments proposed in this grant and preliminary data acquired during the NIH R25 training period will form the basis of a future NIH career development award. Additionally, I will apply to other funding agencies with a focus on brain injury and/or neurotrauma, such as with the DOD or NFL.

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Department of Neurology

Mentor Qualifications

Dr. David M. Holtzman is the B. Burton & R.M. Morriss III Professor of Neurology, Professor of Developmental Biology, Scientific Director of the Hope Center for Neurological Disorders, and Associate Director of the Alzheimer Disease Research Center. His clinical responsibilities include serving as the attending on the general neurology ward service and seeing patients for evaluation and treatment of cognitive disorders and dementia in the Memory Diagnostic Center. He also participates in resident education by precepting the weekly Morning Report clinical case presentations. In addition, Dr. Holtzman leads a highly successful basic science and translational research laboratory in the BJC Institute of Health at Washington University. Dr. Holtzman has spent the last 30 years studying the underlying mechanisms of Alzheimer disease (AD) and has published extensively on the neurobiology of apoE including its effects on the innate immune response and the metabolism of amyloid β (Aβ) and tau. His lab has also developed and assessed antibodies against Aβ, apoE, and tau, some of which have demonstrated efficacy in animal models and 2 of which have gone on to clinical trials, one currently in progress.

Dr. Holtzman has a strong record of federal research funding from the National Institutes of Health (NIH) including ongoing support on 2 R01 grants on which he is the Principle Investigator (PI): *Effect of apoE on CNS Neurons: Role of LRP* (9R01NS090934) and *Novel Stretegies and Mechanisms to Target APOE and Alzheimer's Disease* (1R01AG047644). He is also the PI on a program project: *Regional, Synaptic, Cellular Modulation of Abeta Metabolism* (1P01NS074969), co-PI on a U19 grant: *Biology and pathobiology of apoE in aging and Alzheimer's disease* (U19AG069701), and Co-PI on an additional R01 grants: *Sleep and Circadian Rhythms in Alzheimer Disease: Potential Bi-Directional Relationship with Tau* (1R01AG061776). In addition, Dr. Holtzman continues to receive support from private foundations including the JPB Foundation, the Cure Alzheimer's Fund, and the Tau Consortium.

Dr. Holtzman has received numerous honors including being a recipient of a Paul Beeson Physician Faculty Scholar Award in Aging Research, the Potamkin Prize from the American Academy of Neurology (AAN) for research on Alzheimer's Disease, the MetLife Award for Alzheimer's Disease research, a MERIT award from the National Institute on Aging (NIA), election to the National Academy of Medicine of the National Academy of Sciences, election to the National Academy of Inventors, appointed to the National Advisory Council of the NINDS and the NIA, the Chancellor's Award for Innovation and Entrepreneurship from the WUSM STL, and most recent President of the American Neurological Association (ANA). He has trained and mentored over 70 graduate students, post-doctoral fellows, and physician scientists, many of whom have gone on to successful careers in academia and industry, including Anne Fagan, PhD, John Cirrito, PhD, John Fryer, PhD, Randall Bateman, MD, David Brody, MD, PhD, Suzanne Schindler, MD, PhD, Greg Zipfel, MD, Tim Miller, MD, PhD, Jungsu Kim, PhD, Miranda Lim, MD, PhD, Erik Musiek, MD, PhD, Yo-El Ju, MD, Joseph Castellano, PhD, and Gus Davis, MD, PhD all of whom have gone on to successful independent careers in academia and 10 of whom had K-awards with Holtzman as mentor. He was also mentor to Ron DeMattos, PhD, Helen Hu, PhD, Phillip Verghese, PhD, Tim West, PhD, Adam Bero, PhD, Kiran Yanamandra, PhD, Fan Liao, PhD, Jerrah Holth, PhD, and Cheryl Leyns, PhD, who have gone on to successful careers in biotech/industry. These qualifications make Dr. Holtzman an exceptional research and career mentor for Dr. Rudman.

Dr. John C. Morris is the Harvey A. and Dorismae Hacker Friedman Distinguished Professor of Neurology at Washington University School of Medicine, where he is Director and Principal Investigator for the Knight Alzheimer Disease Research Center (ADRC; P50AG05681). He is also Principal Investigator for two program project grants affiliated with the Knight ADRC, Healthy Aging and Senile Dementia (HASD; P01 AG0391) and the Adult Children Study (ACS; P01AG026276). Dr. Morris has a long and successful track record of mentoring clinician-scientists in patient-oriented research. Since 2004, he has served as primary mentor for Chengjie Xiong, PhD (K25 AG025189), Catherine Roe, PhD (K30 RR022251), Susan Stark, PhD (K01 DDD00033301), Nupur Ghoshal, MD, PhD (K12 HD001459), Jason Hassenstab, PhD (K23 0K094982),

Consuelo Wilkins, MD (K23 AG026768), and Gregory S. Day, MD, MSC (K23AG064029). Currently, at Washington University Dr. Xiong is Professor of Biostatistics and Neurology and has been awarded R01 AG034119 and R01 AG053550; Dr. Roe is Associate Professor of Neurology and has been awarded R01 AG043434 and R01 AG056466; Dr. Stark is Professor of Occupational Therapy and has been awarded R01 AG0057680; Dr. Ghoshal is Associate Professor of Neurology and Psychiatry; and Dr. Hassenstab is Associate Professor of Neurology and of Psychological and Brain Sciences and has been awarded R01 AG057840. Dr. Wilkins is Professor of Medicine at Vanderbilt University Medical Center where she is Vice President for Health Equity. In 2020, Dr. Wilkins was elected to the National Academy of Medicine. In addition to these mentees, since 2004 Dr. Morris has served or is serving as a co-mentor for other Washington University clinician-scientists during their career development awards: Randall J. Bateman, MD (K08 AG027091), who also was elected to the National Academy of Medicine in 2020; Erik Musiek, MD, PhD (K08 NS079405); Suzanne Schindler, MD, PhD (K23 AG053426); Eric McDade, DO (K23 AG046363); Brian Gordon, PhD (K01 AG053474); and Justin Long, MD, PhD (K08 AG068611). Drs. Bateman, Musiek, Schindler, and Long were co-mentored with David M. Holtzman, MD, the primary mentor for Dr. Rudman.

Dr. Morris therefore has the experience to successfully co-mentor Dr. Rudman during her Career Development Award.

Dr. Holtzman and Dr. Morris make an ideal mentorship team for Dr. Rudman due to their overlapping expertise in dementia research and clinical training that is directly relevant to the proposed training plan. They have a long track record of successfully collaborating scientifically with 113 co-authored peer-reviewed manuscripts and have also collaborated to co-mentor multiple early-career physician-scientists, with recent trainees including Randall J. Bateman, MD, Suzanne Schindler, MD, PhD, Erik Musiek, MD, PhD, Justin Long, MD, PhD. Their clinical and research endeavors are highly complementary, and their combined mentorship provides an optimal environment for both exceptional clinical training in the Knight ADRC Fellowship in Dementia and Aging as well as rigorous training in basic and translational research in AD.

Training Plan and Assessment of Applicant

Research in Progress in the Sponsor's Lab

Dr. Holtzman has spent the last 30 years studying the mechanisms underlying neurodegeneration, with a focus on Alzheimer disease (AD). Dr. Holtzman's laboratory takes a highly translational, multipronged approach to studying AD by both addressing the knowledge gaps in the basic pathophysiology of AD as well as targeting known dysregulated pathways in clinical trials to identify novel therapeutics. Dr. Holtzman has published extensively on the neurobiology of apoE including its effects on the innate immune response and the metabolism of amyloid β (Aβ) and tau. His lab has also developed antibodies against Aβ, apoE, and tau which have demonstrated efficacy in animal models of neurodegeneration and of which two have gone on to clinical trials with one trial ongoing and one about to begin.

The laboratory's current basic science efforts are broadly focused on studying the role of the AD risk factor genes APOE and TREM2 and the glial cells that express them in the pathogenesis of AD using biochemistry, cell culture systems, and novel mouse models. One of the areas in which the lab is currently working is the role of apoE in the aggregation and clearance of Aβ. They have found that apoE regulates clearance of Aβ in an isoform-specific manner that is congruent with the effects of apoE isoforms on AD pathogenesis in humans, and that the effects of apoE on Aβ clearance may also be modulated by apoE receptors including the LDL receptor (Kim, *et al.* 2009; Castellano, *et al.* 2011; Huynh, *et al.* 2017; Shi, *et al.* 2017). Other areas in which the Holtzman laboratory has published include the role of synaptic activity and sleep in modulating soluble Aβ release and chronic Aβ deposition (Cirrito, *et al.* 2005; Cirrito, *et al.* 2008; Bero, *et al.* 2011; Kang, *et al.* 2009; Roh, *et al.* 2012; Lucey, *et al.* 2019; Holth, *et al.* 2019). For the last 7 years, the Holtzman laboratory has also been studying the role of the microglial-expressed AD risk factor TREM2 as well as glial expressed apoE in the innate immune response and neurodegeneration in the setting of Aβ and tau pathology (Ulrich, *et al.*2014, Wang, *et al.* 2016, Leyns, *et al.* 2017, Leyns, *et al.* 2019, Shi, *et al.* 2017).

The Holtzman laboratory has also undertaken translational efforts to identify therapeutics to slow the progression of cognitive decline in AD. To this end, they have developed antibodies against the major pathogenic hallmarks off AD – Aβ, apoE, and tau – and demonstrated that in mouse models of Aβ and tau pathology, treatment with these antibodies attenuates the development of pathology and neurodegeneration (DeMattos, *et al.* 2001, DeMattos, *et al.* 2002, Kim, *et al.* 2012, Yanamandra, *et al.* 2013, Liao, *et al.* 2018). Some of these antibodies were further developed for human applications and are now in clinical trials for mild AD (A4 (NCT02008357), DIAN-TU (NCT01760005), and NCT02880956).

Recent work related to this proposal includes the findings that depletion of apoE via genetic knockout, antisense oligonucleotide knockdown, or over-expression of the apoE receptor LDL receptor is protective in mouse models of tauopathy (Shi, *et al.* 2017, Shi, *et al.* 2021, Litvinchuk, *et al.* 2021). In addition, preliminary data from the laboratory has shown that in the setting of tau pathology, microglia accumulate intracellular lipids in an apoE isoform-specific manner. These findings suggest that there are apoE-dependent alterations in glial lipid metabolism that take place in the setting of tau pathology, but it is not yet clear what lipid species are involved and whether similar abnormalities may be observed in the setting of combined Aβ and tau pathology such as in AD.

The research proposed by Dr. Rudman will expand on these studies but with a focus on glial lipid metabolism and how differential regulation of lipid metabolism by apoE isoforms may contribute to the development of AD pathology. This will allow Dr. Rudman to build on the decades of experience and significant resources available for studying AD in the Holtzman laboratory while also establishing a niche from which to branch out and establish an independent research career.

Plan to develop the applicant's research capabilities

This training plan has been designed by Dr. Rudman in collaboration with her sponsor Dr. Holtzman and clinical co-sponsor Dr. Morris. This plan will provide the necessary resources, time, and mentoring support to develop a research program that will be used to apply for a K08 and other early investigator awards. Ultimately, this training plan will provide the foundation of scientific skills and clinical acumen needed to achieve Dr. Rudman's goal of becoming an independent physician scientist studying the pathologic underpinnings of AD.

Research Responsibiliities

Dr. Rudman will be responsible for carrying out all of the experiments proposed in this application. She will have a dedicated workspace in the Holtzman laboratory in the BJC Institute of Health at Washington University School of Medicine. The Holtzman Laboratory already has the supplies, equipment, and protocols in place to complete all of the proposed studies. Specifically, the mouse lines needed for this proposal including APP/PS1;P301S-Tau mouse model of AD as well as the various human apoE knockin and the apoE knockout mouse lines are actively breeding in the mouse colony. In addition, the protocols for FACS-based primary cell purification, RNAseq, lipidomic analysis, behavioral assays, and volumetrics and histopathology for AD pathologic hallmarks are already well-established in the laboratory. Under the guidance of Dr. Holtzman and with the assistance of the numerous other post-doctoral and early career-level physician scientists in the laboratory, Dr. Rudman will learn new biochemical techniques such as RNAseq and lipidomic analysis while continuing to build on her prior experience creating and maintaining mouse lines and using FACS-based primary cell purification, fluorescent microscopy, and histopathologic analyses. This training will add to the skills Dr. Rudman obtained during her predoctoral research and will create a strong foundation on which to build her research moving forward.

Review of the Literature

Dr. Rudman will familiarize herself and continue to stay up to date with literature in the fields of glial lipid metabolism in neurodegeneration and other diseases of the central nervous system through independent literature review. This will be supplemented with attendance at departmental journal clubs and laboratory meetings where relevant new literature will be presented and discussed. She will also co-review a manuscript with Dr. Holtzman about every 3 months on topics related to neurodegeneration. This mentoring experience will provide for insights into experimental design, data assessment, and the process of manuscript review. Any gaps that are identified in Dr. Rudman's knowledge in the field will be addressed with recommended readings suggested by her mentor and colleagues in the Holtzman laboratory. In addition, Dr. Rudman will write a literature review on glial lipid metabolism in neurodegeneration in collaboration with Dr. Holtzman. These activities will provide a strong knowledge base on which to build a research program on glial lipid metabolism in AD.

Meetings, Research Seminars, and Presentations

Dr. Rudman will meet on a weekly basis with Dr. Holtzman to review progress on the proposal, interpret the data, and plan future experiments. She will also attend and participate in the weekly Holtzman laboratory meetings and journal clubs as well as departmental research presentations sponsored by the Office of Neuroscience Research, the Knight Alzheimer Disease Research Center, and the Hope Center for Neurological Disorders which include internal, national, and international invited guest speakers and occur on almost a daily basis.

Dr. Rudman has a history of successful scientific presentations for which she has received multiple awards and honors, and she will continue to develop her public speaking and scientific presentation skills by presenting her own research at laboratory meetings and at departmental seminars including the annual Neurology Resident Research Symposium. Once sufficient preliminary data has been collected, Dr. Rudman will also apply to present at national and international meetings such as the annual meetings of the American Academy of Neurology and the American Neurological Association. Dr. Holtzman will support her attendance at such meetings to disseminate her work, participate in career development activities, and network with other scientists in her field to facilitate future potential collaborations.

Grant Writing

Dr. Holtzman has an extensive track record of successful grant funding and will be an invaluable resource for guidance on crafting a scientifically rigorous grant application. Given Dr. Rudman's goal of becoming an academic neurologist and physician-scientist, this will be a particularly important skill to develop. Dr. Rudman will participate in writing applications for institutional and foundational grants to help fund her work while in the Holtzman laboratory. She will also participate in the NIH Mock Study Section through the Research Development Program at the Institute of Clinical and Translational Sciences at Washington University. Ultimately, the data obtained during the course of this training grant will be used as preliminary data to apply for a future NIH K08 award and other early career development awards.

Overall Development of the Applicant

This training plan provides a multifaceted approach for developing all of the skills necessary to be a successful clinician-scientist. Dr. Rudman will continue to hone the scientific skills she obtained during her pre-doctoral training and expand upon those with new techniques to uniquely address the scientific questions posed in this research proposal. She will also continue to expand her knowledge of the relevant literature with guidance from her mentors as well as through departmental presentations and national and international meetings. She will gain invaluable experience discussing and presenting her work in these settings and will gain further experience presenting her work through writing in submissions for publication and grant applications. Dr. Rudman has previously demonstrated exceptional skill in laboratory benchwork as well as strong scientific curiosity which will provide a strong foundation for the successful completion of the work outlined in this training plan as well as her future scientific endeavors.

Other Related Training and Course Work

Clinical Training

Dr. Rudman has collaborated with her clinical co-mentor Dr. John Morris (Harvey A and Dorismae Hacker Friedman Distinguished Professor of Neurology, Director of the Alzheimer's Disease Research Center (ADRC), Director of the Center for Aging, and Director of the Memory and Aging Project) to craft a clinical training program that will complement her scientific interests in AD and provide a framework from which she will be optimally poised to develop clinically relevant scientific questions. Dr. Rudman plans to pursue a clinical fellowship through the ADRC Fellowship in Aging and Dementia. Clinical duties during this time would include a half-day of dementia clinic per week in the Memory Diagnostic Center as well as a half-day of clinical trials evaluations per week, with total time not to exceed 20% of the covered funding period. Under the supervision of Dr. Morris and other faculty in the MDC, Dr. Rudman will develop expertise in the diagnosis and management of patients with dementia. She will also meet with Dr. Morris at least once monthly to review progress and plan for the next steps in her career development. Overall, the clinical training through the ADRC Fellowship in Aging and Dementia will provide well-rounded training in clinical dementia and exposure to clinical trials work to complement her scientific endeavors in studying the pathologic mechanisms of AD.

Career Development

Throughout this training period, Dr. Rudman plans to stay engaged in clinical teaching and cultivate her skills as a mentor and leader. To this end, she will participate during her PGY4 year in informal medical student teaching with students rotating on the inpatient neurology services as well as more structured resident teaching including Morning Report clinical case presentations, Movement conference, EEG conference, and Journal Club. Dr. Rudman ultimately aims to be an academic leader in the field of neurology and to this end, she will also apply for additional training in leadership skills through programs such as the AAN Enhanced Resident Leadership Program or Emerging Leaders Program.

Relationship of the Research Training Plan to the Applicant's Career Goals

The research and clinical programs proposed in this application will provide the necessary training for Dr. Rudman to achieve her ultimate goal of becoming a clinician-scientist at an academic institution working on cutting edge research in AD. The research proposed will build on her prior experience studying injury and inflammation in the central nervous system in mouse models and will also provide training in new techniques important for her planned research career studying AD. Under the exceptional mentorship of Dr. Holtzman, Dr. Rudman will also obtain invaluable training in core skills for running an independent laboratory including grant writing, effective dissemination of results through publications and research presentations, networking and collaborations, and mentoring and training of junior laboratory members. Dr. Rudman will also continue to hone her clinical skills in dementia and clinical trials enrollment through a uniquely tailored clinical training plan in a dementia fellowship under the mentorship of her clinical co-mentor Dr. Morris. Overall, this training plan provides all the necessary components to ensure Dr. Rudman will be successful in obtaining early career development training though NIH K08 and similar foundational grants, and ultimately establish a career as an independent academic neurologist and clinician-scientist.

Role of the Fellow in the Development of the Research Proposal

Dr. Rudman first approached Dr. Holtzman during the second year of residency training with the goal of developing a project to investigate the basic pathologic mechanisms of AD. She met independently with

multiple members of the Holtzman laboratory and read the relevant primary literature to identify a specific area of interest. Dr. Rudman crafted and subsequently refined the primary hypothesis and specific aims through several rounds of constructive feedback and revisions with Dr. Holtzman to help strengthen the proposal. The specific aims set forth in this research proposal demonstrate the ability of Dr. Rudman to formulate scientifically rigorous hypotheses and experimental designs.

Assessment of the Applicant

Dr. Rudman graduated *Summa Cum Laude* from the University of South Carolina Honors College where she received a Bachelor of Arts and Sciences with a focus in biochemistry and additional coursework in business and Spanish. This degree was a custom bachelor's degree that was designed by Dr. Rudman with the supervision and approval of the Honors Collage and a panel of professors specifically picked by Dr. Rudman based on the expertise needed for the desired degree. During her undergraduate training, Dr. Rudman also sought out research experience and worked in the laboratory of Dr. Paul Housley studying the effects of site-specific phosphorylation on the activity of the glucocorticoid receptor. For this work, she received multiple awards for undergraduate funding including the Science Undergraduate Research Fellowship and the Magellan Scholarship program, each for two consecutive years.

Dr. Rudman then went on to obtain her M.D. from the University of Miami Miller School of Medicine where she also obtained her Ph.D. in neuroscience as part of the combined MD-PhD program. During that training, she was awarded multiple honors including the Lois Pope Fellowship Award (2016), selection for an oral presentation at the National MD/PhD Student Conference (2017), and the distinction of being the first representative from the program to receive sponsorship from the Clinical and Translational Science Institute at the University of Miami to participate in the Summer School in Translational Medicine at the University of Utrecht, Utrecht, Netherlands (2016). Dr. Rudman presented her research at multiple national and international meetings including the International Symposium on Neural Regeneration (2015) and the Society for Neuroscience Annual Meeting (2016), and she was also one of two graduate students selected by her program to attend and present at the National MD/PhD Student Conference in 2017. Ultimately, Dr. Rudman authored 4 peer-reviewed publications during her PhD training including two as first author, and the results of her thesis were published in *Experimental Neurology* (Rudman, *et al.* 2018).

Dr. Rudman has demonstrated productivity and scientific rigor in research, is well-regarded by her clinical training program in Adult Neurology, and has demonstrated drive throughout her career in pursuing a clinician-scientist career path. These qualities make her an excellent applicant for the R25 training grant and portend a successful future career in academic neurology.

Funding

The proposed studies will be completed using mouse lines and protocols already established in the Holtzman laboratory. They do not require the purchase of new equipment and the major expenses will be the maintenance of the necessary mouse colonies, use of core facilities, and biochemical supplies and reagents. These expenses as well as those related to the career development opportunities described above will be supported by funding from the Holtzman laboratory. The Holtzman laboratory currently has funding from multiple grants from the NIH and foundations that will facilitate carrying out the proposed work.

Regulatory Approval

All of the protocols proposed in this application have been approved by Dr. Holtzman's IACUC. Dr. Rudman has previously completed training as required by the IACUC for animal studies and responsible conduct of research and will renew this training through the BJC Learn @ Work portal prior to the initiation of the studies proposed herein.

TRAINING PLAN & ASSESSMENT OF APPLICANT

1. Research in progress in the sponsor's lab

Dr. Holtzman's lab focuses on understanding the mechanisms underlying neurodegeneration, particularly as they are relevant to Alzheimer's disease (AD). The Holtzman lab has published extensively on the neurobiology of ApoE and its receptors, ApoE's effects on the innate immune system, how ApoE, Aβ-binding molecules, and other factors such as neuronal activity and sleep influence Aβ and tau metabolism, their accumulation, and their effects. Current work focuses on 1) how microglia and specific microglial genes such as TREM2 and ApoE (produced by astrocytes and microglia) influence neurodegeneration in the setting of Aβ and tau pathology, as well as Aβ-induced tau spreading, and 2) advancement of therapeutic interventions by development of antibodies targeting different molecular players in the neurodegenerative cascade, including anti-Aβ, anti-tau, anti-ApoE antibodies. Relevant work to this proposal includes a recent *Nature* publication by Shi et al. (2017) showing ApoE4 has strong contributions to neurodegeneration in a tauopathy mouse model, which was followed by a more recent study demonstrating microglia drive ApoE-dependent neurodegeneration in this model. In addition, Dr. Holtzman's lab generated and has been utilizing a novel conditional human *APOE* knockin mouse Huynh et al. (2019) *Molecular Neurodegeneration*, that will be utilized in these studies. Dr. Holtzman's expertise in studying the pathogenesis of neurodegenerative diseases using different genetically-modified mouse models combined with translational studies in humans make him an exceptional primary mentor for Dr. Perez.

Co-mentor Dr. Terrance Kummer's lab focuses on long-term neurodegeneration in traumatic brain injury (TBI) and its connection to AD, mechanisms of acute TBI, and development of tools to study how disruptions in neural networks lead to neurologic deficits. Dr. Kummer has developed multiple tools to aid in the study of neurodegeneration after TBI, including a novel model of brain injury with parallels to human brain injury. More recently, the Kummer Lab published a pioneering technique for large scale and high-speed quantification of synaptic structures, which are altered in an injury model, as well as in neurodegenerative diseases. Dr. Kummer's forward-thinking expertise in the field of TBI will serve as an excellent mentoring complement for the proposed studies. The research proposed by Dr. Perez will make use of the strengths of both of his mentors, while at the same answering questions and performing experiments not proposed by either. This innovative approach will allow him to not only build on current work, but also branch out in his future studies to establish research independence.

2. Plan to develop the applicant's research capabilities

As mentors, Dr. Holtzman and Dr. Kummer are fully committed to helping Dr. Perez achieve both the goals proposed in this post-doctoral fellowship and in his overall career plan. This training fellowship will provide Dr. Perez an opportunity to continue to advance his research skills, as well as the requisite time and resources to ultimately develop a K08 grant proposal. Dr. Perez has worked with Dr. Holtzman and Dr. Kummer to devise the following training components:

<u>Research responsibilities:</u> Enmanuel will be responsible for carrying out all of the experiments proposed in this grant. He has a dedicated work-space in the Holtzman Laboratory (~3000 square foot lab) in the BJC Institute of Health building at Washington University School of Medicine. Additionally, Dr. Holtzman and Dr. Kummer have the equipment and well-developed techniques necessary for Enmanuel to complete his proposal. Specifically, novel P301S/human apoE$^{flox/flox}$ mice have been developed and crossed with Aldh1l1-Cre/ERT2 and CX3CR1-Cre/ERT2 mice, for astrocyte and microglial-specific inducible knockdown studies. Both mentors have previously and continue to be collaborators, including recent publications. Dr. Holtzman and Dr. Kummer have worked with different models of TBI (CCI injury and modCHIMERA) and have the infrastructure necessary to continue with these studies. Under their supervision, as well as with hands on training from senior graduate students, post-docs, and research technicians, Enmanuel will continue to master technical skills at the bench-top in diverse methodologies, including histopathologic volumetric analysis, ELISA-based and WB analysis, immunohistochemistry, super-resolution imaging and analysis (SEQUIN), genetic & transcriptomic analysis, learning and behavioral assays, and multiple experimental injury paradigms. Additionally, he will continue to improve in his skills utilizing cell-specific inducible transgenic mice, which was at the core of his doctoral thesis, and will continue to play a large role in his ongoing and future studies. The collaborative environment established by Enmanuel's mentors is truly ideal for fostering young scientific minds and promoting future successes.

Review of literature: Dr. Perez will continue to expand his knowledge of neurodegeneration, TBI, and glial biology. He will stay up-to-date with the most current peer-reviewed literature through independent reading. His mentors have already provided Enmanuel with a reading list of background pertinent publications and will continue to point out future high impact publications. Enmanuel will continue to review advances in the field of neurodegeneration and TBI, both as these pertain to his studies and for overall knowledge. In addition to his ongoing literature review, Enmanuel will have ongoing discussions about possible knowledge gaps in the field with opportunities for development of future research projects. In collaboration with Dr. Holtzman and Dr. Kummer, Enmanuel will work on a review of glial biology in TBI.

Meetings and presentations: Dr. Perez will meet on a weekly basis with Dr. Holtzman and bi-monthly with Dr. Kummer to discuss current progress on the proposal, including ongoing experiments, review of raw data, data analysis, and future experiments. Discussion of good ethics and practices (i.e. Responsible Conduct of Research) will also be held during these meetings. Both mentors understand Enmanuel may have other clinical responsibilities and have made themselves available outside of “normal hours” for both formal and informal meetings and discussions. As part of his development, Enmanuel will continue to cultivate his oral presentation skills. He will do so by active participation in weekly Holtzman lab meetings and journal clubs, Neurology residency Summer Stock lecture series, and weekly seminar series hosted by the Hope Center for Neurologic Disorders (HCND). As the project progresses, Dr. Perez will plan on presenting his data at both local and national venues (~ 2 per year). These will include, but are not limited to, the Neurology Resident Research Symposium, the National Neurotrauma Society Meeting, the American Academy of Neurology (AAN) Annual Meeting and/or AAN’s Sports Concussion Conference, and the American Neurological Association (ANA) Annual Meeting. During these meetings, Enmanuel will also be provided and make diligent use of networking opportunities, which will hopefully lead to meeting future mentors and collaborators. As one of his long-term goals is to succeed as a scientific lecturer, the opportunities to perform and present basic science research afforded by the time allowed by this training grant will be instrumental in his future successful career.

Research Seminars: Washington University School of Medicine in St. Louis (WUSM-STL) has near-daily seminars from leaders in the fields of biology, physiology, biomedical engineering, neuroimaging, neurosurgery, and neuroscience. Dr. Perez will attend at least one of these campus-wide seminars on a weekly basis. Specifically, Dr. Perez will attend weekly Knight Alzheimer Disease Research Center and Hope Center seminars, weekly Holtzman and Kummer Labs Journal club, and monthly Hope Center for Neurological Disorders (HCND) Group meetings. HCND Group meetings consist of a monthly meeting where Hope Center researchers with specific interests attend a presentation/discussion. The specific research groups Enmanuel will be participating in will be: Glia, Axon Injury & Repair, and NeuroRestorative Therapy.

Grant Writing: Grant writing is a critical skill for an academic neurologist. In addition to developing preliminary data that will support his NIH K08 application, Dr. Perez will be involved in writing applications for institutional and foundational funding for his work. Dr. Holtzman and Dr. Kummer have had extensive success at grant writing. As a graduate student, Enmanuel also had a successful application for a training grant (F31) that lead to two first author publications and successful application of an R01 (from Graduate PI, Dr. Liebl) based on his studies. Both Dr. Holtzman and Dr. Kummer are passionately dedicated in mentoring Dr. Perez through all the stages of the grant writing process.

Overall development of the applicant: Dr. Perez will gain significant experience and expertise during the proposed training time, which will span from learning/perfecting research methodologies to networking with future collaborators. Enmanuel is expected to excel in his ability to design, perform, analyze, interpret, and present his research. The end goal of the fellowship will be for the proposed work to form the basis of future career development grants such as a National Institutes of Health (NIH K08), Department of Defense (DoD), AAN, and/or other foundational grants. Based on Enmanuel’s previous achievements, talented bench-top skills, strong intellectual independence, well-versed scientific curiosity, and overall goal-driven attitude, he will be positioned to successfully compete for the aforementioned awards after completion of this fellowship.

3. Other related training or course work

Clinical training: Dr. Perez is currently collaborating with members of multiple departments to create a clinical prospectus based on his goals as a clinician. In partnership with Dr. Varadhachary, Associate Professor of Neurology and Head of the Neurohospitalist Section, and the Sports Medicine division of the Department of

Orthopedics, Dr. Perez is crafting his clinical syllabus and schedule. It will consist of working as a Neurology Consultant at most for 1 weeks every other month, in addition to a half-day of Sports Medicine clinic, focusing on TBIs, spinal cord injuries, and nervous system injuries in athletes. These clinical duties will consist of no more than 20% of his dedicated time.

Career development: Dr. Perez aims to become a leader in the field of Neurology. As an academic neurologist will be engaged in the teaching and training of medical students and Neurology residents. He hopes to promote Neurology as a future career option for medical students and provide students with opportunities for research, clinical, and service activities in this field. He anticipates being intimately involved with the Neurology resident program, leading the "Clinical Case Morning Report", running the "CNS Lecture" series, providing "LP workshops", and mentoring residents, with particular interest in those of under-represented minority backgrounds. To be able to achieve his full potential, Enmanuel will apply to leadership programs, which provide a foundation and learning experience for future Neurology leaders, such AAN programs include: Enhanced Resident Leadership Program, Emerging Leaders Program, and Diversity Leadership Program.

4. Relationship of the research training plan to the applicant's career goals

Dr. Perez has created a unique path for his ongoing career goals. His ultimate goal is to be a physician-scientist at a large academic center, where he can practice as an academic neurologist with a specialized clinic in Sports Neurology while running a basic science/translational research laboratory focused on TBI. This fellowship will allow him the opportunity to establish a personalized clinical fellowship, combining his ambitions at becoming an academic neurohospitalist and Sports neurologist. Most importantly, it will aid in launching his research career in multiple ways. He will improve his technical skills at the bench, aside from learning new cutting-edge techniques. He will also learn from his mentors the ins-and-outs of operating an independent laboratory, including budgeting, grant writing, personnel management, responsible conduct in research, and regulatory approval. At the end of the fellowship, Dr. Perez will be one step closer in reaching his career goals and will be applying to multiple career development awards, including a mentored NIH K08 award.

5. Role of the fellow in the development of the research proposal

Dr. Perez approached both Dr. Holtzman and Dr. Kummer during his second year of residency with the idea of a combined mentorship based on his overarching research proposal. After multiple meetings, he independently explored specific scientific questions and knowledge gaps, established a central hypothesis for his work, developed a research plan and detailed proposal, and presented it to his mentors. With assistance from them, this research proposal was fine-tuned. Dr. Holtzman and Dr. Kummer are both very pleased with Enmanuel's advanced ability to operate independently as highlighted by the creation of this proposal.

6. Assessment of the applicant

Dr. Perez graduated Summa Cum Laude from a BS in General Sciences at the University of Puerto Rico Rio Piedras in 2010. As an undergraduate, he was heavily engaged in neuroscience research, participating as a Leadership Alliance Summer Research Early Identification Program Fellow (2008), Minority Access to Research Careers Fellow (2008-2010), and Stanford Summer Research Program Fellow (2009). All of these opportunities, provided an amazing foundation for his research skills and led to presenting his work at multiple national meetings. Dr. Perez then completed his MD and PhD at the University of Miami Miller School of Medicine (UMMSM) MD-PhD Program. He completed his PhD training with Dr. Daniel Liebl, PhD (Neuroscience). Dr. Perez is currently a third-year Neurology resident at Washington University School of Medicine in St. Louis – Barnes-Jewish Hospital.

During his PhD, he successfully applied for an NIH NINDS F31 (NRSA Individual Predoctoral MD/PhD or Other Dual-Doctoral Degree Fellowship) for his graduate research proposal. His thesis work led to the publication of two first author papers in high impact journals, including the *Journal of Clinical Investigation*, aside from presenting his work at national and international meetings. He has won several awards for his research including UMMSM Lois Pope LIFE Foundation Development Award, Melvin Denis Travel Award Annual National MD/PhD Student Conference, and AAN Medical Student Scholarship to the Annual Meeting. Additionally, he has won multiple awards given his aspirations at becoming a leader in the field of Neurology including AAN Medical Student Diversity Scholar, SfN Neuroscience Scholars Program Associate, and SfN Preparing the Next Generation of Neuroscience Leaders Scholar.

Dr. Perez has a history of productive research and academic output, is well-regarded among faculty for his clinical acumen, and has demonstrated forward-thinking leadership qualities throughout his training. All of

the above make Dr. Perez a superb recipient of this fellowship, which will contribute to his successful future as a clinician-scientist.

7. Funding

The proposed research project does not require purchase of new equipment for any of the proposed methodologies. The major expenses consist of animal costs, imaging costs, and protein/genetic reagents. This and other project expenses for the current proposal will be supported by funds from the laboratory of Dr. Holtzman. His lab is well funded with grants, sponsored projects, and other funds to produce, breed, and maintain transgenic mouse colonies proposed for this research project. Dr. Perez will apply for supplementary funding available through Departmental funds, if the need arises. Overall, no shortage of funds for the experiments in research plan outlined by Dr. Perez is anticipated.

8. Regulatory Approval

IACUC and IRB documentation- All of the procedures outlined in research proposal have been approved by mentors IACUC/IRB. Addendums to these protocols are in the process of being submitted based experiments from research proposal. Dr. Perez has previously completed the required IACUC training for animal studies and is in the process of updating his credentials at BJC through Learn @ Work.

BIOGRAPHICAL SKETCH

NAME OF APPLICANT: Michelle Diane Rudman

POSITION TITLE: Resident Physician

EDUCATION/TRAINING *(Most applicants will begin with baccalaureate or other initial professional education, such as nursing. Include postdoctoral training and residency training if applicable. High school students should list their current institution and associated information. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	START DATE MM/YYYY	END DATE *(or expected end date)* MM/YYYY	FIELD OF STUDY
University of South Carolina Honors College, Columbia, SC	B.S./B.A.	08/2007	05/2011	Biochemistry
University of Miami Miller School of Medicine, Miami, FL	M.D./Ph.D.	08/2011	05/2019	Medicine/ Neuroscience
Washington University School of Medicine, Saint Louis, MO		07/2019	06/2023	Neurology

A. Personal Statement

My overarching goal is to become a physician-scientist working in an academic center and pushing forward the boundaries of modern medicine. My stimulus is the understanding of the basic underpinnings of the human mind and how that biology breaks down in disease states. Alzheimer disease (AD) is a neurodegenerative condition that robs people of that which makes us human – our experiences, our personality, and our memories – and impacts almost every person due to its high prevalence, my family included. This is what has made AD such a compelling focus of research and why I plan to devote my career to its study. My background, including a Ph.D. in neuroscience and specifically in the mechanisms of cell death and inflammation in CNS injury, as well as my ongoing residency training in neurology, will provide a strong foundation for my planned career as an academic neurologist and physician scientist studying the pathologic mechanisms of AD with the ultimate goal of identifying novel targets for therapeutic intervention.

As an undergraduate at the University of South Carolina Honors College I began my research career studying the modulation of glucocorticoid signaling pathways under the mentorship of Dr. Paul R. Housley. During these two years, I gained experience in many key biochemical and cell culture techniques, and I funded my work with undergraduate research grants including the Science Undergraduate Research Fellowship and the Magellan Scholarship Program. I then built upon this experience during my Ph.D. training in the University of Miami Miller School of Medicine MD-PhD Program where I studied epigenetic mechanisms of innate inflammation in neurotrauma using mouse models of spinal cord injury under the joint mentorship of Dr. Jae K. Lee and Dr. Nagi G. Ayad. I received multiple awards and honors for this work including the Lois Pope Fellowship and sponsorship to attend the National MD/PhD Student Conference, and successfully published multiple peer-reviewed manuscripts. Throughout this training I continually demonstrated dedication to pursuing a career as a physician scientist and have participated in networking and leadership training including as the first attendee sponsored by the Clinical and Translational Science Institute to attend the Summer School in Translational Medicine at the University of Utrecht, Netherlands as well as the Combining Clinical Careers in Neuroscience symposium and networking event at the NIH in Bethesda, MD.

In the highly collaborative environment of Washington University and under the guidance of my mentor Dr. David Holtzman (B. Burton & R.M. Morriss III Professor of Neurology, scientific director of the Hope Center for Neurological Disorders, and Associate Director of the Knight ADRC), I will be optimally poised to develop my

skills as a physician-scientist. Dr. Holtzman's expertise in the study of the underlying mechanisms of AD is unparalleled, and he has trained over 70 graduate students, post-doctoral fellows, and physician-scientists, many of whom have gone on to successful careers in academia. For my project, I plan to study the contribution of different apoE isoforms to perturbations in lipid metabolism in the setting of AD pathology in mouse models. The goal of this project is to determine the effects of apoE-mediated lipid accumulation in glial cells on AD pathology and whether this may be targeted for therapeutic intervention. Ultimately, I plan for this project to lay the groundwork for my future research endeavors in identifying novel targets for therapeutic intervention in Alzheimer disease.

B. Positions, Scientific Appointments, and Honors

ACTIVITY/ OCCUPATION	START DATE (mm/yy)	ENDING DATE (mm/yy)	FIELD	INSTITUTION/ COMPANY	SUPERVISOR/ EMPLOYER
Student Research Assistant	05/08	08/08	Physics	University of South Carolina	Dr. Scott Crittenden
Student Research Assistant	06/09	06/11	Cell Biology	University of South Carolina School of Medicine	Dr. Paul R. Housley
Student Interest Group in Neurology, Vice President	08/12	05/13	Neurology	University of Miami Miller School of Medicine	Dr. Richard Isaacson
MD/PhD Student Research Symposium Committee, Member	08/13	08/14	MD/PhD Program	University of Miami Miller School of Medicine	Dr. Sandra Lemmon
American Physician Scientist Association Student Chapter, International Liaison	04/17	04/18	Medicine	University of Miami Miller School of Medicine	Dr. Emmanuel Thomas
Student Interest Group in Neurology, Research Liaison	04/17	04/18	Medicine	University of Miami Miller School of Medicine	Dr. Yolanda Reyes-Iglesias
Class Representative	10/20	Present	Neurology	Washington University Adult Neurology Program	Dr. B. Joy Snider, Program Director

Academic and Professional Honors

National Merit Finalist	2007
National Merit Scholarship	2007-2011
Palmetto Fellows Scholarship Recipient, University of South Carolina	2007 - 2011
Trustees' Endowment Scholarship Recipient, University of South Carolina	2007 - 2011
Science Undergraduate Research Fellowship (SURF), University of South Carolina	2009 and 2010
Magellan Scholarship, University of South Carolina	2009 and 2010
B.S./B.A. awarded with high honors, University of South Carolina	2011
Summer School in Translational Medicine, Travel Award Recipient	2016
Lois Pope Fellowship Winner	2016
National MG/PhD Student Conference, Sponsorship and Selection for Oral Presentation	2017
Combining Clinical and Research Careers in Neuroscience, Selection for Attendance and Sponsorship	2018

Memberships in Professional Societies

American Academy of Neurology	2017-present
American Neurological Association	2017-2019

Society for Neuroscience	2016-2018
American Physician Scientist Association	2016-2019
American Medical Association	2007-2019

C. Contributions to Science

I. Characterization of Myeloid Cell labeling in the CNS of LysM-Cre Mouse Line.
During my second year of medical school, I volunteered in the lab of Dr. Jae Lee to study the cell-specificity of the LysM-Cre mouse line. I characterized cell-specific Cre recombinase expression in LysM-Cre/ROSA26-TdT mice with conditional knock-in of the TdTomato red fluorescence protein. I found that, although this mouse line was believed to specifically label myeloid cells, approximately 34% of labeled cells were neurons. This finding is an important consideration in any studies proposing to use this mouse line to target myeloid cells in the CNS. The results of this study were published in conjunction with additional studies using this mouse line in spinal cord injury and stroke.

Publications:
Zhu Y, Soderblom C, **Trojanowsky M**, Lee DH, Lee JK. "Fibronectin Matrix Assembly after Spinal Cord Injury." *J Neurotrauma* 2015 Aug 1;32(15): 1158-67.

Clausen BH, Degn M, Sivasaravanaparan M, Fogtmann T, Andersen MG, **Trojanowsky MD**, Gao H, Hvidsten S, Baun C, Deierborg T, Finsen B, Kristensen BW, Bak ST, Meyer M, Lee J, Nedospasov SA, Brambilla R, Lambertsen KL. Conditional ablation of myeloid TNF increases lesion volume after experimental stroke in mice, possibly via altered ERK1/2 signaling. *Sci Rep* 2016 Jul 7;6:29291.

II. Kinase Signaling Networks Regulating Cell Cycle Protein Turnover
In 2013, I rotated in the lab of Dr. Nagi Ayad at the Center for Therapeutic Innovation at the University of Miami Miller School of Medicine. The goal of my project was to discover novel kinase signaling networks that may regulate the turnover of cell cycle proteins, especially the cyclin-dependent kinase inhibitors $p27^{Kip1}$ and $p21^{Cip1}$. I screened the Library of Pharmacologically Active Compounds in HeLa cells transfected with plasmids encoding luciferase fusion cell cycle proteins and collaborated with the lab of Dr. Stephan Schurer to perform parallel *in silico* kinome profiling to determine the probability that each compound in the library would inhibit various enzymes involved in regulating the cell cycle. These methods successfully uncovered known regulators of $p27^{Kip1}$ protein turnover, indicating that similar methods may be used to uncover signaling networks for other proteins regulated by proteasomal degradation.

Publications:
Trojanowsky M, Vidovic D, Simanski S, Penas C, Schurer S, Ayad NG. "Screening of Cell Cycle Fusion Proteins to Identify Kinase Signaling Networks" *Cell Cycle* 2015; 14(8): 1274-81.

III. The Role of Bromodomain and Extraterminal Domain-Containing Proteins in Inflammation after Spinal Cord Injury
For the past three years, I have been working under the mentorship of Drs. Jae Lee and Nagi Ayad studying the role of bromodomain and extraterminal domain-containing proteins (BETs) in inflammation after spinal cord injury (SCI). BETs are known to play an important role in the inflammatory response in many peripheral tissues but have not yet been studied in the central nervous system (CNS). I have discovered that CNS cells not only express BETs but that inhibition of BET activity attenuates expression of proinflammatory cytokines and chemokines. Excitingly, BET inhibition after experimental SCI decreases the expression of many proinflammatory cytokines and chemokines and may improve lesion pathology and promote locomotor recovery.

Publications:
Rudman MD, Choi JS, Lee HE, Tan SK, Ayad NG, and Lee JK. Bromodomain and Extraterminal Domain-Containing Protein Inhibition Attenuates Acute Inflammation after Spinal Cord Injury. Exp Neurol 2018 Aug 19;309: 181-192. PMID: 30134146.

Abstracts:

- **Rudman MD**, Ayad NG, and Lee JK. Inhibition of an Epigenetic Reader Decreases Inflammation after Spinal Cord Injury. National MD/PhD Student Conference, July 2017, Keystone, CO.
- **Rudman MD**, Ayad NG, and Lee JK. Bromodomain and Extraterminal Domain-Containing Protein Inhibition Decreases Inflammation After Spinal Cord Injury. The Annual MD/PhD Student Research Symposium, April 2017, Miami, FL.
- **Trojanowsky MD**, Ayad NG, and Lee JK. Inhibition of an Epigenetic Reader Decreases Inflammation After Spinal Cord Injury. Distinguished Lecture Series, Feb 2017, Miami, FL.
- **Trojanowsky MD**, Ayad NG, and Lee JK. Inhibition of an Epigenetic Reader Decreases Inflammation After Spinal Cord Injury. Neuroscience Research Day, Nov 2016, Miami, FL.
- **Trojanowsky MD**, Ayad NG, and Lee JK. Bromodomain and Extraterminal Domain-Containing Proteins Modulate the Inflammatory Response After Spinal Cord Injury. Society for Neuroscience Annual Meeting, Nov 2016, San Diego, CA.
- **Trojanowsky MD**, Ayad NG, and Lee JK. Bromodomain and Extraterminal Domain-Containing Proteins Modulate the Inflammatory Response After Spinal Cord Injury. MD/PhD Program Annual Research Symposium, April 2016, The University of Miami Miller School of Medicine, Miami, FL.
- **Trojanowsky MD**, Ayad NG, and Lee JK. Bromodomain and Extraterminal Domain-Containing Proteins Modulate Inflammatory Signaling After Spinal Cord Injury. Miami Winter Symposium, Jan 2016, Miami, FL.
- **Trojanowsky MD**, Ayad NG, and Lee JK. Bromodomain and Extraterminal Domain-Containing Proteins Modulate Inflammatory Signaling After Spinal Cord Injury. International Symposium on Neural Regeneration, Nov 2015, Pacific Grove, CA.
- **Trojanowsky MD**, Ayad NG, and Lee JK. Bromodomain and Extraterminal Domain-Containing Proteins Modulate Inflammatory Signaling in Astrocytes. Neuroscience Research Day, Nov 2015, University of Miami Miller School of Medicine, Miami, FL.
- **Trojanowsky MD**, Penas C, Soderblom C, Lee DH, Ayad NG, and Lee JK. BET Proteins Modulate the Inflammatory Response After Spinal Cord Injury. The Annual MD/PhD Student Research Symposium, April 2015, The University of Miami Miller School of Medicine, Miami, FL.
- **Trojanowsky MD**, Penas C, Zhu Y, Stathias V, Ayad NG, and Lee JK. Epigenetic Modulation of Inflammation in Spinal Cord Injury. Neuroscience Research Day, Nov 2014, The University of Miami Miller School of Medicine, Miami, FL.

Fellowship Applications:
F30 Predoctoral Application, submitted Aug 2015

Complete List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/sites/myncbi/1HofjiJiMiYQH/bibliography/41507253/public/?sort=date&direction=ascending

D. Scholastic Performance

YEAR	**University of South Carolina** SCIENCE COURSE TITLE	GRADE	YEAR	**University of South Carolina** OTHER COURSE TITLE	GRADE
2008	General Chemistry I	A	2007	Principles of Microeconomics	A
2008	Essentials of Physics I	A	2007	Composition	A
2008	Nuclear Reactor Physics	B	2007	Calculus II	A
2008	Biological Principles I	A	2007	Introduction to Music	A
2008	General Chemistry II	A	2007	Intermediate Spanish I	A
2009	Biological Principles II	A	2008	Composition & Literature	A
2009	Quantitative Analysis, Chemistry	A	2008	Vector Calculus	A
2009	Cell & Molecular Biology	A	2008	Intermediate Spanish II	A
2009	Organic Chemistry I	A	2008	Survey of Accounting	A
2010	General Physiology	A	2008	Advanced Spanish Language I	A

YEAR	**University of South Carolina** SCIENCE COURSE TITLE	GRADE
2010	Organic Chemistry II	A
2010	General Physics II	A
2010	Principles of Biochemistry	A
2010	Immunobiology	A
2010	Algorithmic Design I	A
2010	Statistical Methods I	A
2011	Fundamentals of Genetics	A
2011	Senior Thesis/Project	A

YEAR	**University of South Carolina** OTHER COURSE TITLE	GRADE
2009	Principles of Macroeconomics	A
2009	Classical Origins of Western Medical Ethics	A
2009	Advanced Spanish Language II	A
2009	Spanish for Healthcare Professionals	A
2009	Introduction to Finance	A
2009	Principles of Marketing	A
2010	Business and Professional Speaking	A
2010	Contemporary Dance Technique I	A
2011	Entrepreneurship and Small Business	A
2011	Independent Study: Contemporary and West African Dance	A

YEAR	**University of Miami Miller School of Medicine**	GRADE
2011	Human Structure (embryology, histology, cellular biology, gross anatomy)	89
2011	Molecular Basis of Life (medical genetics, biochemistry)	88
2011	Host Defenses, Pathogens & Pathology	84
2012	Cellular Function and Regulation	90
2012	Neuroscience and Behavioral Science	88
2012	Cardiovascular System	95
2012	Epidemiology	97
2012	Respiratory System	87
2012	Renal System	89
2012	Gastroenterology and Nutrition	83
2012	Doctoring I	98
2012	Doctoring II	98
2012	Doctoring III	93
2013	Doctoring IV	94
2013	Problem-Based Learning	P
2013	Case-Based Infection & Inflammation	85
2013	Hematology and Oncology	96
2013	Endocrine and Reproductive Systems	89
2013	Ophthalmology and Dermatology	86
2010	MCAT	34

YEAR	**University of Miami Program in Biomedical Sciences**	GRADE
2013	PIBS 602: Scientific Reasoning	S
2013	PIBS 600: Journal Club	S
2013	PIBS 680: Research Ethics	S
2013	PIBS 682: Survival Skills I & II	S
2013	Neuroscience II	A
2013	Developmental Neuroscience	A
2014	Neuroscience I	B+
2014	Principles of Membrane Physiology and Biophysics II	A+
2014	PIBS Bioinformatics Workshop	S
2014	NEU 600: Neuroscience Seminar	A
2014	Principles of Membrane Physiology and Biophysics I	A+
2015	NEU 600: Neuroscience Seminar	A-
2016	NEU 700: Neuroscience Seminar	A
2016	NEU 731: Advanced Topics in Neuroscience	A
2013	USMLE Step 1	245

P = pass, S = satisfactory, 1-100: 70 is passing, A-F: C is passing.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Enmanuel J. Perez

eRA COMMONS USER NAME (credential, e.g., agency login): EPEREZMARTINEZ

POSITION TITLE: Resident Physician

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Puerto Rico – Rio Piedras, PR	BS	05/2010	General Sciences
University of Miami Miller School of Medicine – Miami, FL	MD	05/2018	Medicine
University of Miami Miller School of Medicine – Miami, FL	PhD	05/2018	Neuroscience
Washington University in St. Louis-Barnes Jewish Hospital - Saint Louis, MO	Resident	06/2018-Present	Neurology Residency

A. Personal Statement

I aim to be a physician-scientist in an academic setting, as the head of a research group and a practicing physician providing advanced, cutting-edge, and research-based patient care. I have been engaged in a diversity of neuroscientific research throughout my years of training, ranging from examining the neurobiology of drug abuse in a rodent model to studying the functional connectivity of pain using fMRI in human subjects. These experiences lead me to my current research interests, mainly examining the pathophysiology of concussions and traumatic brain injury. Similarly, my clinical interests are focused on Sports Neurology, including brain and spine trauma, as well as complications of major nervous system injury.

I am a third year Neurology resident, currently designing a path towards my ultimate career goals. Following residency, my plan is to create and/or join a Sports Neurology training curriculum as an inter-departmental collaboration with Orthopedic Surgery and Neurosurgery. My fundamental goal will be working as an academic neurologist, while also running a collaborative Sports Neurology practice as an independent clinician.

During my doctoral thesis work (detailed below), I focused on examining the contribution of gliotransmission to synaptic stability and dysfunction after brain injury using a mouse model of TBI. These years were transformative for my career. I acquired and developed a myriad of technical skills, from basic biochemical methodologies, to *in vitro* and *in vivo* eletrophysiological recordings, to generating and utilizing cell-specific transgenic mice (for gain- and loss-of-function studies), to multiple behavioral approaches. I also learned about project/personnel management, grant writing, manuscript publication, and public speaking, all of which are necessary as I progress towards a more independent scientific career track.

My current research proposal will focus on examining the contributions of glial-specific ApoE isoforms on neurodegeneration in after TBI. The experiments proposed in this training grant will be performed under the guidance of Dr. Holtzman and Dr. Kummer, who are well-established investigators with expertise in glial contributions to neurodegeneration in AD models and neurodegeneration in TBI, respectively. My previous research experiences and training, along with mentorship provided by Dr. Holtzman and Dr. Kummer, should lead to a successful post-doctoral fellowship and will form the base of my future career as a neurologist-scientist.

B. Positions and Honors

Academic Positions

2008	Leadership Alliance Summer Research Early Identification Program Undergraduate Research Fellow, University of Miami Miller School of Medicine
2008 – 2010	Minority Access to Research Careers (MARC) Undergraduate Research Fellow, University of Puerto Rico Rio Piedras
2009	Amgen Scholars – Stanford Summer Research Program Undergraduate Research Fellow, Stanford University
2010 – 2018	MD-PhD Student, University of Miami Miller School of Medicine
2018 – Present	Neurology Resident, Washington University School of Medicine in St. Louis – Barnes Jewish Hospital

Honors

2006 – 2010	President's Honor Roll and Dean's List, University of Puerto Rico Rio Piedras
2006 – 2010	Academic Excellence Scholarship, University of Puerto Rico Rio Piedras
2006 – 2010	*Summa Cum Laude*, Distinguished Honors Graduate in Natural Sciences, University of Puerto Rico Rio Piedras
2013 – 2015	Lois Pope LIFE Foundation Development Award, University of Miami Miller School of Medicine
2015	American Academy of Neurology (AAN) Medical Student Diversity Scholar
2015	Society for Neuroscience (SfN) Neuroscience Scholars Program Associate
2016	Melvin Denis Travel Award, Annual National MD/PhD Student Conference
2016	Diversity Travel Award, Annual National MD/PhD Student Conference
2017	Society for Neuroscience (SfN) Preparing the Next Generation of Neuroscience Leaders Scholar
2018	American Academy of Neurology (AAN) Medical Student Scholarship to the Annual Meeting

Professional Societies

2008 - Member	Society for Neuroscience (SfN)
2010 - Member	American Academy of Neurology (AAN)
2014 - Member	American Physician Scientists Association (APSA)
2019 - Member	American Neurological Association (ANA)

C. Contributions to Science

1. Modulating Post-Traumatic Brain Injury Gliotransmitter Levels Leads to Improved Synaptic Function

Synaptic dysfunction and impaired plasticity can have a significant impact on motor, sensory and cognitive functions in traumatic brain injury (TBI) patients. Neuronal death is a predominant underlying cause for synaptic loss in regions of tissue damage. However, synaptic dysfunction can also be present in more distal brain regions where neuronal loss is not observed. To examine synaptic dysfunction within the complex and evolving TBI environment, I utilized a moderate controlled cortical impact (CCI) injury model that leads to hippocampal synaptic damage, and deficits in hippocampal synaptic plasticity and learning without neuronal losses. Proliferation and hypertrophy of astrocytes (i.e. reactive astrocytosis) is a common pathological feature of TBI. However, how reactive astrocytes participate in changes in tripartite synaptic function is not well understood. Therefore, I examined the role of gliotransmission in synaptic function and plasticity following a well-established mild CCI injury model. This CCI injury model enabled me to investigate mechanisms of progressive synaptic damage by eliminating serine racemase (SR), an essential enzyme that converts L-serine to D-serine, in cell-specific inducible SR knockout mice (i.e. cSRKO). I showed that astrocytic and neuronal D-serine play unique roles in the injured and non-injured CA1 hippocampus, respectively. In non-injured conditions SR is expressed by pyramidal neurons, where D-serine functions to regulate long-term potentiation (LTP). After CCI injury, SR expression switches from neurons to astrocytes, where astrocyte-derived D-serine lead to CCI injury-induced synaptic damage that results in LTP and learning deficits.

A family of receptor tyrosine kinases, Eph receptors, and their coganate ligands, ephrins, have been associated with pre-and post-synaptic membranes as well as glial cells in the hippocampus. In particular, EphB3 receptors contribute to regulation of excitatory synaptic numbers, synaptic plasticity and gliotransmission of D-serine; however, the contribution of EphB3 to regulating D-serine levels and synaptic

function after TBI is yet to be elucidated. In the CCI injured hippocampus, I found that EphB3-/- mice showed no decrease in synaptic numbers compared to sham controls, and improved synaptic plasticity and learning as compared with wild-type (WT) CCI injured mice. Comparison of key synaptic proteins essential for excitatory synaptic transmission showed no differences between WT and EphB3-/- mice; however, increases in the NMDAR co-agonist D-serine following CCI injury were significantly reduced in the absence of EphB3. Furthermore, rescuing D-serine levels in EphB3-/- mice restored the synaptic deficits observed in WT CCI injured mice. These findings support previous conclusions, where elevated D-serine levels in the hippocampus may play deleterious roles by destabilizing synapses after TBI, and suggest ephrin-EphB3 signaling regulates these processes.

A. **Perez, Enmanuel J.**, Tapanes, SA, Loris, ZB, Balu, DT, Sick, TJ, Coyle, JT, Liebl., DJ. (2017) Enhanced astrocytic D-serine underlies synaptic damage after traumatic brain injury. **J Clinical Investigation**, 127(8):3114-3125. PMID: 28714867
B. **Perez, Enmanuel J.,** Cepero, Maria L., Perez, Sebastian U., Coyle, Joseph T., Sick, Thomas J., Liebl, Daniel J. (2016). EphB3 signaling propagates synaptic dysfunction in the traumatic injured brain. **Neurobiology of Disease**, 94:73-84. PMID: 27317833

2. Subclinical cerebrovascular disease and risk of stroke in a race-ethnically diverse population
Evidence of white matter disease and subclinical brain infarcts on imaging is associated with increased risk of incident stroke and mortality, however the association of these factors as predictors of outcomes is unclear, as well as the association with increased risk of specific ischemic stroke subtypes. We used observational, prospective data from the Northern Manhattan study to show that increased subclinical cerebrovascular disease was associated with increased risk of stroke in a culturally diverse population and varied by race-ethnicity and lesion type.

- Wright, C.; Dong, C., **Perez, Enmanuel J.**, De Rosa, J., Yoshita, M., Rundek, T., DeCarli, C.; Elkind, M., and Sacco, R. (2017). Subclinical cerebrovascular disease increases the risk of incident stroke and mortality: The Northern Manhattan Study. **Journal of the American Heart Association**, 6(9). PMID: 28847914

3. Disparities in Door-to-Needle times in a race-ethnically diverse population
Significant advances in acute stroke care has led to development of national guidelines and quality improvement programs aimed at reducing morbidity and mortality. Among these, door-to-needle (DTN) time (i.e. arrival to administration of thrombolytics) has been associated with overall better outcomes. However, women and race-ethnic minority groups are less likely to achieve these metrics. We used data from the Florida-Puerto Rico Collaboration to Reduce Stroke Disparities to examine sex and race-ethnic disparities, as well as predictors of achieving favorable DTN times. We showed that women and African-Americans were less likely to achieve these outcomes and that these differences may be regional-dependent.

- Oluwole, S., Wang, K., Dong, C., Ciliberti-Vargas, M., Gutierrez, C., Yi, L., Romano, J., **Perez, Enmanuel J.**, Tyson, B., Ayodele, M., Asdaghi, N., Gardener, H., Rose, D., Garcia, E., Zevallos, J., Foster, D., Robichaux, M., Waddy, S., Sacco, R., Rundek, T., Collaboration to Reduce Stroke Disparities Investigators. (2017). Disparities and Trends in Door-to-Needle Time: The FL-PR CReSD (Florida- Puerto Rico Collaboration to Reduce Stroke Disparities). **Stroke**, 48(8):2192-2197. PMID: 28706119

D. Additional Information: Research Support and/or Scholastic Performance

Completed Research Support

2013 – 2015	Lois Pope LIFE Foundation Development Award, University of Miami Miller School of Medicine
2014 – 2018	F31 NRSA Individual Predoctoral MD/PhD or Other Dual-Doctoral Degree Fellowship NIH/NINDS F31 NS089335

Phase 2

During this phase, you will commence with your research training. While you will have some clinical responsibilities, the primary focus will be on your experiments and investigative studies.

In addition, there are several UE5 PNTP program expectations, which are listed below:

Monthly workshop series. To address the needs of our PNTP trainees, we have created a workshop series. They are designed to create a sense of community, allow you to share your research, and provide trainees exposure to subject matter experts in various stages of academic Neurology/Neurosurgery career development. The schedule for these meetings will be sent to you in June each year.

Mentorship Committee. We also strongly suggest that all UE5 fellows assemble a mentorship committee comprised of content experts and additional career mentors in addition to your mentor, which could meet once or twice a year to provide advice and input. This committee will be invaluable in the preparation of your K award.

Annual NINDS UE5 meetings. There is an annual NINDS UE5 meeting organized by Drs. Michael Tennekoon (Scientific Program Manager Office of Training and Workforce Development, NINDS) and Tish Weigand (Director, Office of Training, Career Development and Workforce Development, NINDS). You will receive notification from Dr. Tennekoon about this March meeting. Attendance each year is mandatory.

Annual progress reports. Each fall, you will be required to submit a progress report. This request is to continue support during a fellowship (or second residency) period for a participant who was previously supported (i.e., support for a second or third increment of support). This progress report should be written by the participant and is in addition to the 4-page research plan. For 2nd increment requests, depending on when in residency the participant started in the UE5 program, there may have been little or no progress made at the time of supplement request submission. Nonetheless, a brief progress report should be provided to update information about the candidate's relevant experience since the last submission. Further, a description of the participant's participation in Ethical and Successful Scientific Practices (ESSP) activities and discussions should be provided (note, simply attending the quarterly inter-institution ESSP zoom meetings is not sufficient). Please reach out to Nichole Swanner-Gregory in the summer when your UE5 increment begins to make plans for submitting that fall for funding for the next academic year. In addition to the required documents, you are now required to include personalized information about your training in ethical and successful practices (ESSP). This is different from the Responsible Conduct of Research (RCR) training.

Programmatic Ethical and Successful Scientific Practices (ESSP) Training. The Washington University Physician Neuroscientist Training Pipeline (PNTP) Pathway engages both trainees and faculty in regular discussions on establishing and maintaining a successful and ethical scientific career. This is accomplished in three ways: First, we developed a combined Neurology/Neurosurgery monthly series that provides an open forum to talk about issues that pertain to career development. These meetings include faculty at multiple levels of academic advancement (e.g., Assistant Professor, Associate Professor, Division Chief, etc.) and operate as open discussions that cover topics ranging from "leading a balanced life as a physician scientist", "career development", "the path to independence", and "staying the course". We also have an open discussion during one of these sessions about the grants/funding process and working with

animal and/or human subjects by experts within the Neurology and Neurosurgery departments. Core to all these meetings are issues that pertain to ethical practices in research. Second, based on establishing a community of trainees at different post-graduate levels, peer-based discussions and mentoring occur to promote ethical and successful scientific practices. These are reinforced in each individual laboratory. Third, all mentors spend time in informal regular meetings with their trainees to discuss the design, analysis, and interpretation of experiments, as these are often method- or technology-specific. In addition, each mentor schedules meetings with their trainees to enforce standards for acceptable data manipulation, data storage and sharing, and project ownership. In addition to regular individual laboratory meetings, HOPE Center work-in-progress meetings, and UE5 PNTP annual oral presentations, mentors provide direct training in manuscript and grant writing and oral presentations to different audiences. Lastly, mentors provide opportunities to review manuscripts, respond to reviewer critiques, and navigate the funding landscape specific to each subspecialty or discipline.

Annual Neurology/Neurosurgery PNTP UE5 symposium. Each year, you will be asked to present an update of your UE5 studies at the annual Neurology PNTP UE5 symposium to be held in conjunction with one of the named Neurology lectureships in the spring. Dru Jenkins and Dr. Gutmann will contact you about the logistics and agenda.

Annual surveys. Each summer during and after the UE5 PNTP training period, you will receive a survey to complete. This allows us to obtain critical feedback about our program and make necessary course corrections to improve the pipeline. In addition, you will provide information required by the NINDS about the success of our trainees. We may also contact you about meeting in-person or by Zoom to learn more about your experience in the UE5 PNTP program.

Phase 3

During this phase of the PNTP program, you will be focused on your research project, manuscript, career development activities, and preparing to submit your K award.

Submission of K award. One of the important goals of the program is to submit a K award to the NIH. The timing of this submission will depend upon your experimental progress and subspecialty training plans. We will provide a mock NIH review for all applicants submitting their K awards. Please contact Dr. Gutmann (Neurology) or Nichole Swanner-Gregory (Neurosurgery) at least 4 months prior to your planned submission date, so we can arrange for your application to be reviewed.

An alternative option is to have your application reviewed through the Mock Study Section service offered by the Institute for Clinical and Translational Sciences (https://icts.wustl.edu/research-services/research-development-program/nih-mock-study-section).

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Other Resources

In addition to the workshops provided by the UE5 PNTP program, there are numerous other resources available at Washington University and through the National Institutes of Health.

National Center for Faculty Development & Diversity. Washington University is now an institutional member of the National Center for Faculty Development & Diversity (https://faculty.med.wustl.edu/career-development/national-center-for-faculty-development-diversity/), which provides research productivity, academic mentoring, and professional development resources.

National Institutes of Health Office of Intramural Training and Education. This NIH program offers seminars and workshops designed to help trainees and fellows develop scientific, professional, well-being, and resilience skills. More information can be found on their website (https://www.training.nih.gov/skills/).

Washington University Institute of Clinical and Translational Sciences (ICTS). The ICTS offers a large number of resources, pilot funding opportunities, and training services. More information can be found on their website (https://icts.wustl.edu/). They also house a grants library with examples of successful applications to federal funding agencies, as well as numerous expert consultation and grant editing services.

Washington University Division of Physician Scientists. Led by Dr. Wayne Yokoyama, the Division of Physician-Scientists was established with the goal to enrich the national pipeline of physician-scientists by providing strong institutional support for their training and recruitment. They offer programming and other resources on their website relevant to physician scientist career development (https://physicianscientists.wustl.edu/).

Washington University Bernard Becker Library. The Bernard Becker Medical Library (https://becker.wustl.edu/) houses a large number of critical resources related to medical information (electronic journals and books), health & wellness, health & science communication, health literacy & communication, and federal grant applications.

Writing & Speaking Center. This institutional resource (https://writingcenter.wustl.edu/) offers free one-on-one support for trainees and faculty working on any writing or public speaking project.

Grant Writing & Review Matrix (https://research.wustl.edu/app/uploads/2017/09/WU-Grant-Writing-and-Review-Matrix.pdf) provides a summary of resources available to trainees for grant writing support. Other services, including fee-for-service, resources can be found on the Grant Writing website (https://research.wustl.edu/grant-writing-resources/).

Scientific Editing Service. The Research Development Office offers review and editing services for grant proposals on a first-come, first-serve basis (https://research.wustl.edu/offices/research-development/scientific-editing-service-for-proposals/).

InPrint. InPrint (https://inprintscience.wustl.edu/) is a trainee-run scientific communication network and resource that provides free, confidential editing and presentation consulting services to the Washington University community.

Contact List

Neurology

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