Award Number: W81XWH-19-1-0817

TITLE: Optogenetic Regulation of Phosphoinositide Metabolism in Susceptibility, Resistance, and Resiliency to Alzheimer's Disease-Associated Deficits and Pathology

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CONTRACTING ORGANIZATION:
The Trustees of Columbia University in the City of New York
New York, NY

REPORT DATE: October 2020

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command

Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
October 2020	Annual	15Sep2019-14Sep2020

4. TITLE AND SUBTITLE

Optogenetic Regulation of Phosphoinositide Metabolism in Susceptibility, Resistance, and Resiliency to Alzheimer's Disease-Associated Deficits and Pathology

5a. CONTRACT NUMBER

5b. GRANT NUMBER

W81XWH-19-1-0817

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Columbia University Medical Center
630 West 168th Street
New York, NY 10032

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Lipid dyshomeostasis in Alzheimer's disease (AD) has been reported for over 30 years, but recent advances in the sensitivity and quantitative accuracy of system level lipidomics have allowed for broader interpretation of dysregulated lipid metabolism. Our lab has demonstrated that a phosphoinositide (PI) signaling lipid, phosphatidylinositol 4,5-bisphosphate [$PI(4,5)P_2$] is depleted in human AD affected brain as well as in animal models of the disease. Genetic disruption of a major $PI(4,5)P_2$ degrading enzyme, Synaptojanin1, ameliorated lipid imbalance and rescued AD-associated deficits in cognition and amyloid beta-peptide (A-beta) induced synapses loss in a mouse model. Single nucleotide polymorphoisms in Synj1 have been shown to be associated with age of onset of AD. We hypothesize that a temporally and spatially specific change in $PI(4,5)P_2$, representing a more physiologically and therapeutically relevant paradigm, will restore cognitive and synaptic function and validate phosphoinositide (PI) metabolism as a necessary and sufficient determinant for susceptibility to AD behavioral and synaptic deficits. Optogenetic tools for enriching or depleting $PI(4,5)P_2$ have been described in cell lines *in vitro*, but have not yet been demonstrated *in vivo*. **Specific Aim 1: We will test the hypothesis that optogenetically mediated enrichment of phosphoinositide levels in mouse brain will ameliorate AD associated behavioral deficits in chronic and acute mouse models of AD-associated cognitive and synaptic deficits. We have successfully sub-cloned the catalytic domain of optogenetically activated PI kinases. We will administer the viral vector into mouse hippocampi to enrich $PI(4,5)P_2$ and test for amelioration of behavioral and synaptic deficits associated with AD. We will use a genetic mouse model overexpressing the amyloid precursor protein with the Swedish mutation (Tg2576) as well as an acute model of A-beta-infusion directly into the hippocampi of freely behaving animals. We have received approval from Columbia University IACUC for the proposed experiments and submitted them to ACRO for approval. Specific Aim 2: We will determine if there is a correlation between phosphoinositide levels in human brain, plasma and CSF with AD age of onset (susceptibility) leading to potential identification of a novel biomarker for AD susceptibility. We have been working closely with the Columbia University IRB as well as Dr. James Noble to obtain approval for use of human derived biospecimens from TBI patients for lipidomics studies.**

15. SUBJECT TERMS
Alzheimer's disease, Lipid metabolism

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
					USAMRMC
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
U	U	U	UU	18	

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1. INTRODUCTION:

Alzheimer's disease (AD) is defined pathologically by the accumulation of neuritic plaques that are primarily composed of amyloid-β peptide (Aβ). Our lab has shown that treatment of neurons with Aβ oligomers, depletes levels of the important signaling lipid phosphotidylinositol-4,5-bisphosphate [PI(4,5)P2]. PI(4,5)P_2 is also depleted in human brain from AD affected patients. However, mice harboring hemizygous deletion of the major PI(4,5)P2 phosphatase in the brain, synaptojanin 1 (*Synj1*+/-) do not show depleted PI(4,5)P_2 and are insensitive to Aβ oligomer-induced synaptic deficits in LTP and changes in dendritic spine morphology and density. Crossing *Synj1*+/- with a mouse model of AD, Tg2576, resulted in amelioration of deficits in learning and memory in multiple behavioral tests. These data suggest that PI(4,5)P2 homeostasis is critical for Aβ-induced defects and could be harnessed at the systems level for amelioration of AD potentially in the context of traumatic brain injury (TBI). We hypothesize that distinct pools of PI(4,5)P2 contribute to the regulatory mechanisms behind the synaptic disruption caused by Aβ oligomers or other synaptotoxic Aβ species. Since there is extensive cross talk among lipid modifying enzymes, and regulation of lipid metabolism forms a network of interconnected modifiers of synaptic function, we hypothesize that PI kinases responsible for PI(4,5)P_2 synthesis may be targeted optogenetically. Specifically, we will enhance PI(4,5)P_2 at the plasma membrane by viral expression of phosphoinositide (PI) kinases, PI4P-5 Kinase and PI4 Kinase IIα fused to light inducible elements allowing recruitment to the plasma membrane after blue light stimulation. With enhanced PI(4,5)P_2 we expect amelioration of AD associated deficits in behavior. We will test the hypothesis that optogenetically mediated enrichment of phosphoinositide levels in mouse brain will ameliorate AD associated behavioral deficits in genetic and acute mouse models of AD-associated cognitive and synaptic deficits. PI(4,5)P_2 levels will be enriched in hippocampus of mice using adeno-associated virus (AAV) delivery of optogenetically controlled PI kinases. To optically stimulate PI kinase activity in a chronic model of AD, after viral infection, we will implant optical fibers in Tg2576 mouse model of AD harboring the APPsw transgene. In an acute model of AD, after viral infection, the same optogenetic paradigm will be used to stimulate PI(4,5)P_2 synthesis prior to injection of Aβ-oligomers in wild-type mice. Behavioral deficits will be assessed using contextual fear conditioning and novel object recognition. After behavioral testing, brains will be analyzed for amyloid pathology, synapse number and lipid content using targeted lipidomics. We expect that increasing PI(4,5)P_2 in the hippocampus of the genetic mouse model (Tg2576) will lead to resilience and amelioration of AD associated behavioral deficits and pathologies. Concordantly, we expect that enriching PI(4,5)P_2 prior to acute Aβ injection, which has been shown to lead to cognitive deficits, will facilitate resistance to Aβ insult. The use of optogenetically controlled PI phosphatases which are expected to worsen phenotypes will also be considered. This paradigm can be used in future studies of traumatic brain injury (TBI) in mouse models to test the hypothesis that enrichment of PI(4,5)P_2 can mitigate progression to AD. We will determine if there is a correlation between phosphoinositide levels in human brain, plasma and CSF with AD age of onset (susceptibility) leading to potential identification of a novel biomarker for AD susceptibility. Anonymized human samples with clinical data for age of onset will be obtained from the Columbia Brain Bank and Alzheimer's Disease Research Center at Columbia University. We will also obtain previously collected (non-recruitment) plasma samples from TBI patients. Biofluids and tissues will be analyzed for lipid content using targeted lipidomics as well as amyloid and tau pathologies. We will request clinical diagnoses, ApoE genotype as well as history of TBI. We expect that enrichment in PI lipids in plasma, CSF and brain will correlate with increased age of onset of AD in the general population as well as case histories of TBI. These studies will evaluate the potential for PIs as biomarkers in plasma or CSF for AD susceptibility in the general population as well as in the context of TBI.

2. KEYWORDS:

Alzheimer's disease
Traumatic Brain Injury
lipidomics
lipid metabolism
mouse model
optogenetics
phosphoinositides
phospholipids

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Specific Aim 1: We will test the hypothesis that optogenetically mediated enrichment of phosphoinositide levels in mouse brain will ameliorate AD associated behavioral deficits in genetic and acute mouse models of AD-associated cognitive and synaptic deficits.
Major Task 1: Construct development of optogenetic constructs and viral infection in cell lines and mouse embryonic stem cell derived neurons. (Months 1-4)
Major Task 2: Optimization and validation of expression of optogenetic constructs in vivo using AAV injection into mouse hippocampi as previously described (Wu et al., 2016) and optimization of stimulation paradigm for $PI(4,5)P_2$ enrichment. (Months 4-10)
Major Task 3: Experimental testing of contribution of phosphoinositide metabolism to behavioral outcome in AD mouse models. Express optogeneic constructs under control of optimized stimulation paradigm followed by behavioral analyses. (Months 10-24)
Milestone #1: manuscript describing generation of in vivo tools for PI manipulation and potential amerliorative effects on AD associated behavior deficits (Months 22-24)

Specific Aim 2: We will determine if there is a correlation between phosphoinositide levels in human brain, plasma and CSF with AD age of onset (susceptibility) leading to potential identification of a novel biomarker for AD susceptibility.
Major Task 1: LC/MS-MS targeted lipidomics of brain (cortex and hippocampus), CSF and plasma in human context of AD and TBI (Months 1-36)
Milestone #2: Manuscript describing generation of in vivo tools for PI manipulation and potential amerliorative effects on AD associated behavior deficits

What was accomplished under these goals?

Specific Aim 1: Major Task 1: Construct development of optogenetic constructs and viral infection in cell lines and mouse embryonic stem cell derived neurons. (Months 1-4)

Major activities and accomplishments:
To enhance $PI(4,5)P_2$ at the plasma membrane we will virally express light inducible elements of phosphoinositide kinases, PI4P-5 Kinase and PI4 Kinase IIα which will be stimulated by blue light for recruitment to the plasma membrane (Idevall-Hagren et a., 2012; Xie et al., 2016). This is based on cryptochrome2 (CRY2) and the trascriptoin factor CIB1. A conformational change in CRY2 after absorbtion of a photon, promotes binding to the N-terminal portion of CIB1 (CIBN). CRY2-CIBN dimerization has been shown to recruit phosphatidylinositol 5-phosphatase to the membrane (Idevall-Hagren et al,.2012). In order to carry out optogenetic experiments with the enzymes PIP5K1A and PIP5K2A, the catalytic domain (CD) of the corresponding genes were successfully cloned to express fusion proteins which bear CRY2(1-498) and mCherry.

Specific objectives and significant results or key outcomes:
The CDs of PIP5K1A and PIP5K2A genes were incorporated into the plasmid pCRY2PHR-mCherryN1 (Addgene #26866). However, this original plasmid was not suitable for immediate cloning due to its nucleotide sequence. The cloning would result in 'fusion genes' bearing stop codon in the linker region. Therefore, the plasmid was subjected to site-directed mutagenesis (nucleotide deletion) to adjust the ORFs (Open Reading Frame). Particularly, one nucleotide (position 2851) was deleted from the plasmid pCRY2PHR-mCherryN1. By producing the fusion protein, a classical restriction-cloning approach was applied using restriction enzyme NotI.

The Catalytic Domains (CD) of the genes were defined based on NCBI database. Particularly, a 1026bp fragment was chosen as PIP5K1A-CD (position 286-1311 of the PIP5K1A gene) and a 1131bp fragment was chosen as PIP5K2A-CD (position 79-1209 of the PIP5K2A gene). For the amplification of PIP5K1A-CD and PIP5K2A-CD, p15aC-1D-5 (Addgene #107865) and pDONR223-PIP5K2A (Addgene #23744) plasmids were used, respectively. During the PCR amplification, the primers were designed to introduce NotI restriction sites.

Final constructs were used to transform E. coli cells. The transformations produced several clones for both PIP5K1A-CD and PIP5K2A-CD. To check the presence of the inserts within the vectors, the plasmids were NotI digested and run in agarose gel. Among the insert-positive clones, one clone was selected in each case (clone #3) for further applications. These clones were additionally Sanger-sequenced and had the exact nucleotide sequences as shown in the databases.

We have completed the subcloning of the full length PIP5K1A and PIP5K2A and have additionally subcloned the catalytic domain (CD). The sub-cloned catalytic domains as well as counterpart CIBN-CAAX vector (Addgene #79574), have successfully been packaged into Adeno-associated virus (AAV9) by VectorBioLabs. Vector Biolabs has produced final viral particles for transduction into mouse brain.

For validation of the optogenetic constructs generated, we will infect PC12 (pheochromeoytoma) cell line and mouse embryonic stem cell derived neurons. We will test the ability of these constructs to modify phosphoinositide content of cells in vitro using a PC12 cell line and the expression of the PI(4,5)P2 sensor, plextrin homology domain of phospholipase C delta (PH- PLCd) as previously described (Berman DE, Dall'Armi C, Zhang H, Moore AZ, Voronov SA, McIntire LB, Cremona O, Arancio O, Kim T-W, Di Paolo G. (2008) Oligomeric amyloid-beta peptide disrupts phosphatidylinositol-4,5-bisphosphate metabolism. Nature Neuroscience. May;11(5):547-554.) We have started culturing the PC12 cells and prepared DNA for PH-PLCdelta. When viral particles are obtained from VectorBiolabs, these experiments will preceed in vivo experiments.

Discussion of stated goals not met:
Major Task 2: Optimization and validation of expression of optogenetic constructs in vivo using AAV injection into mouse hippocampi and optimization of stimulation paradigm for $PI(4,5)P_2$ enrichment. (Months 4-10)
Major Task 3: Experimental testing of contribution of phosphoinositide metabolism to behavioral outcome in AD mouse models. Express optogeneic constructs under control of optimized stimulation paradigm followed by behavioral analyses. (Months 10-24)

We are currently poised to complete both Major Task 2 and Major Task 3 upon completion of the following key events. We are waiting for the arrival of the viral particles which have been generated from Vector Biolabs. According the an update from Vector Biolabs, viral particles have been produced and can be shipped upon receipt of the purchase order. The purchase order from Columbia University Department of Pathology Purchasing Department is currently in process. We have received institutional approval of the proposed experiments from Colombia Medical Center IACUC. Columbia University AC-AABD8568 Continuation Approved by IACUC 10/09/2020 (expires 10/31/21), status approved. This protocol will all supporting documents was submitted to ACURO 10/9/2020 with all supporting documents, pending approval1) Completed CURRENT VERSION of the ACURO Animal Use Appendix located at: https://mrdc.amedd.army.mil/index.cfm?pageid=Research_Protections.acuro_Animalappendix
2) Signed PI Assurance (Last page of ACURO Appendix)
3) Complete IACUC-approved animal use protocol
4) Documentation of IACUC approval of the protocol
5) Most recent USDA inspection report (can be obtained through your IACUC)

After protocol approval we will continue as planned with the surgery for AAV infection and optogenetic implantation. We have aged Tg2576 mouse model of Alzheimer's disease overexpressing Amyloid Precursor Protein (APP) harboring the Swedish mutation (Hsiao et al., 1996) which are available at the appropriate ages for surgery.

Optimization and validation of expression of optogenetic constructs in vivo using AAV injection into mouse hippocampi as previously described (Wu et al., 2016) and optimization of stimulation paradigm for PI(4,5)P2 enrichment. We will implant cannulae (Dr, Hussaini's lab will train Dr. McIntire's lab in this method) 2 animals and 2 control animals (surgery, no stimulation). This has been delayed to be completed months 12-16 due to the need to wait for animal protocol approval and due to the COVID-19 pandemic mandated research "ramp-

down", see below. The animal protocols covering these procedures have been approved by the Columbia University IACUC and submitted to ACURO.

Upon receipt of viral particles (see Major Task 1), and approval of the animal use protocol, we will design 2 stimulation paradigms likely to enhance PI(4,5)P2 production in brain of wild type mice. Design will be advised by both Dr. McIntire and Dr. Hussaini. We estimated use of 2-3 animals for each stimulation paradigm. AAV9 virus particles will be injected into the hippocampus and cannulae will be implanted above the injection site. Ten days after viral transduction, the right hemisphere hippocampus will be stimulated with four pulsed of LED light (470nm) (Thorlabs) at a frequency of 30 Hz three times with a one-minute interval. Stimulation will occur 3/day, 5/week for 2 weeks, however, duration of stimulation will be determined for different stimulation protocols and optimized for changes in phosphatidylinositol 4,5-bisphosphate under the direction of Dr. Hussaini. (months 5-8 Dr. McIntire and Dr. Hussaini)

Specific Aim 2: We will determine if there is a correlation between phosphoinositide levels in human brain, plasma and CSF with AD age of onset (susceptibility) leading to potential identification of a novel biomarker for AD susceptibility. **Major Task 1:** LC/MS-MS targeted lipidomics of brain (cortex and hippocampus), CSF and plasma in human context of AD and TBI (Months 1-36)

Major activities and accomplishments:
We have developed and are pursuing two IRB protocols. which will allow completion of the major goals of Specific Aim 2. We have met with Dr. Klein, Natalie M CIV USARMY MEDCOM USAMRMC (USA); Dr. Pacifico, Anthony M CIV USARMY MEDCOM CDMRP (USA); Simmons, Ebony S CIV USARMY MEDCOM USAMRAA (USA); Dr. Garland, Brian S CTR USARMY MEDCOM USAMRMC (US) to discuss use of human derived biospecimens and appropriate regulatory requirements especially with use of cadaver tissue.

Specific objectives and significant results or key outcomes:
Prior to any work with human biospecimens we are applying for approval from Columbia University Medical Center Institutional Review Board. A new IRB protocol was submitted by Dr. McIntire and Dr. Noble to Columbia University IRB (AAAT2441 Sports-Related Concussion Study Archive) Title*:* Sports-Related Concussion Study Archive submitted on 10/12/2020. This protocol was based on analyzing biospecimen, plasma, of 44 participants, previously recruited from two previously approved protocols by IRB at Columbia University:
IRB-AAAP7609 Concussion in Columbia Varsity Athletes
IRB-AAAM1909 Concussion in Columbia University Sports Students
We have received notice from the IRB that these protocols must be renewed to continue with the submission process. We are currently in the process of renewing these expired protocols. The protocol had not yet been submitted to HRPO.

A second protocol is also under development for us of biobanked tissue from the Alzheimer's Disease Research Center (ADRC) biobank and the Columbia Brain Bank. Columbia University IRB number AAAS4181. Title*:* BIN1, Synj1 and phosphoinositide homeostasis in AD. This protocol is an attempt to receive Administrative determination of Research eligible for exempt review in accordance with 46.104; Not Human Subjects Research Under 45 CFR 46 designation. IRB-approved protocol and related biobank affiliated with the ADRC (AAAR2387). We are developing the protocol with expectation that not-human subjects research will apply to brain tissue which has been banked by the ADRC and the Columbia University Biobank since 1989. Brain tissue and serum from anonymized or de-identified AD affected and age matched controls is available in collaboration with the Alzheimer's Disease Research Center (ADRC) which has been collecting biospecimines since 1989. Anonymized biospecimens are available by request to the Resource Committee. ORP_Cadaver_Submission _Form under development, not yet submitted. The protocol had not yet been submitted to HRPO.

Dr. McIntire participated in a meeting 07/31/2020 with Dr. Noble, Dr. Klein, Natalie M CIV USARMY MEDCOM USAMRMC (USA); Dr. Pacifico, Anthony M CIV USARMY MEDCOM CDMRP (USA); Simmons, Ebony S CIV USARMY MEDCOM USAMRAA (USA); Dr. Garland, Brian S CTR USARMY MEDCOM USAMRMC (US) to discuss use of human derived biospecimens and appropriate regulatory requirements.

Discussion of stated goals not met:
We are in the process of gaining IRB approvals to move forward with the major goals of Specific Aim 2 to pursue lipidomic studies with di-identified, previously collect human biospecimens.

What opportunities for training and professional development has the project provided?
Nothing to report

How were the results disseminated to communities of interest?

Artur Lazarian, Vilas Menon, PhD and Laura Beth McIntire, PhD Lipid profiling of healthy and Alzheimer's affected mouse brains by using DESI Imaging Mass Spectrometry: how lipid dyshomeostasis can contribute to Alzheimer's disease. Alzheimer's Association International Conference 2020.
7/28/2020 *POSTER PRESENTATION*

Ana Paula Costa, PhD, Vilas Menon, PhD and Laura Beth McIntire, PhD Identification of dysregulated lipid metabolic pathways in mouse embryonic derived neurons and in a mouse model of Alzheimer's disease Alzheimer's Association International Conference 2020.
7/27/2020 *POSTER PRESENTATION*

Artur Lazarian, PhD, Vilas Menon, PhD, Brandon Fowler, PhD, Laura Beth McIntire, PhD. Lipidomic profiling of healthy and Alzheimer's disease mouse brains by using DESI Imaging Mass Spectrometry (IMS): regional lipid dyshomeostasis in Alzheimer's disease. IGM Symposium on Spatial "Omics" The UC Diego Institute for Genomic Medicine is holding a virtual symposium on Monday, October 26, 2020 in conjunction with the virtual American Society of Human Genetics meeting occurring on October 27th through October 30th, 2020.virtually
10/26/2020 *POSTER PRESENTATION*

What do you plan to do during the next reporting period to accomplish the goals?

Specific Aim 1: Test the hypothesis that optogenetically mediated enrichment of phosphoinositide levels in mouse brain will ameliorate AD associated behavioral deficits in genetic and acute mouse models of AD-associated cognitive and synaptic deficits.
Subtask 1: Optogeneitc construct acquisition and generation
We will receive the viral particles encoding optogenetic constructs for infection of PC12 cells and embryonic stem cell derived neurons for validation prior to infection of mouse hippocampi.
Subtask 2: Viral preparation of optognetic constructs
We will continue with subtask 2: Construct have been successfully subcloneed into the viral vectors and viral particles have been produced. Once obtained, we will validate optogenetic constructs and viral infection first in cell lines such as PC12 cells and mouse embryonic stem cell derived neurons. We will test the ability of these constructs to modify phosphoinositide content of cells in vitro using a PC12 cell line and the expression of the PI(4,5)P2 sensor, plextrin homology domain of phospholipase C delta (PLC-delta) as previously described (Berman DE, Dall'Armi C, Zhang H, Moore AZ, Voronov SA, McIntire LB, Cremona O, Arancio O, Kim T-W, Di Paolo G. (2008) Oligomeric amyloid-beta peptide disrupts phosphatidylinositol-4,5-bisphosphate metabolism. Nature Neuroscience. May;11(5):547-554.)
Major Task 2: Optimization and validation of expression of optogenetic constructs in vivo using AAV injection into mouse hippocampi as previously described (Wu et al., 2016) and optimization of stimulation paradigm for PI(4,5)P2 enrichment.
Subtask 1: Implant cannulae (Dr, Hussaini's lab will train Dr. McIntire's lab in this method) 2 animals and 2 control animals (surgery, no stimulation). (months 4-5 Dr. McIntire and Dr. Hussaini). This has been delayed to be completed months 11-12 due to the COVID pandemic, see below. The animal protocols covering these procedures have been approved by the Columbia University IACUC and submitted to ACURO. Once these constructs are validated and an optogeneic protocol has been validated for changes in PI(4,5)P2 levels, we are planning to submit a manuscript (Months 22-24) as a description of the methods we have employed since we have generated new constructs which have not yet been described.
Subtask 2: Design 2 stimulation paradigms likely to enhance PI(4,5)P2 production in brain of wild type mice (B6). Design will be advised by both Dr. McIntire and Dr. Hussaini. We estimated use of 2-3 animals for each

stimulation paradigm. (months 5-8 Dr. McIntire and Dr. Hussaini). AAV9 virus particles will be injected into the hippocampus and cannulae will be implanted above the injection site. Ten days after viral transduction, the right hemisphere hippocampus will be stimulated with four pulsed of LED light (470nm) (Thorlabs) at a frequency of 30 Hz three times with a one-minute interval. Stimulation will occur 3/day, 5/week for 2 weeks, however, duration of stimulation will be determined for different stimulation protocols and optimized for changes in phosphatidylinositol 4,5-bisphosphate under the direction of Dr. Hussaini. (months 5-8 Dr. McIntire and Dr. Hussaini)

Specific Aim 2: We will determine if there is a correlation between phosphoinositide levels in human brain, plasma and CSF with AD age of onset (susceptibility) leading to potential identification of a novel biomarker for AD susceptibility.
Major Task 1: LC/MS-MS targeted lipidomics of brain (cortex and hippocampus), CSF and plasma in human context of AD and TBI
We will continue to pursue the acquisition of biospecimens from the ADRC and previously collected blood samples from Dr. Nobel. Have submitted a second IRB protocol in collaboration with Dr. Noble which describes the use of biospecimens from the ADRC for lipidomic analyses. The IRB protocol will also describe the use of previously collected biospecimens by Dr. Nobel for lipidomic analysis. We will extract the previously collected blood samples from Dr. Noble's pilot experiment and will submit them for targeted lipidomic analysis by the Biomarker's Core run by Dr. Nandakumar. The protocol was submitted 10/12/2020. All biospecimens and data will be de-identified. Experimental groups will be age matched control groups 2 day, 2 weeks, 2 months after concussion (n=3-5/group). Samples to be stored at -80C until lipid extraction and targeted lipidomics in collaboratin with the Irving Insitute Biomarker's core facility.

4. IMPACT: Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?

The critical nature of the function of phosphoinositide lipids in brain has been well established by our lab and work over several decades. Our lab has recently determined that AD associated deficits are sensitive to levels of phosphoinositides (PI). In fact, maintenance or enhancement of PI in the brain is a validated target for development of therapeutics and as a biomarker of AD. Completion of our proposed research will lead to generation of critical cellular tools which can uniquely manipulate the level of PI in live animals in a temporally and spatially specific way. This tight control is an improvement on currently used genetic models used for study of PI metabolism, in which expression level is changed in a whole animal throughout life. Our proposed model is the first in vivo manipulation of PI and will serve to validate our strategy which can be applied to multiple PI and lipid metabolic enzymes in future work. Our optogenetic tools will serve the research community in basic cell biology, neurodegenerative disease as well as have potential for oncology research. Our studies have potential to validate PI levels as clinical targets for AD as well as TBI. Our studies will assess both a genetic (chronic) model of AD and an acute model (Aβ-infusion) of AD deficits leading to results which can be applicable to amelioration of the disease, but also as preventative in the acute model. Altering PI accumulation prior to Aβ-insult may be a novel intervention for TBI, reducing likelihood of conversion to AD. This is a novel strategy with great promise in the short term for creation of valuable research tools and in the long term for development of a novel strategy which could lead to ameliorative therapeutics for AD, but also lead to preventative measures for AD in the general population and for TBI. There are currently very few (if any) strategies under investigation for prophylactic interventions in AD and TBI, making this work of critical importance. If successful, this work will lead the way for prophylactic interventions changing the outlook for patients, caregivers and their families.

What was the impact on other disciplines?

Our methods to optogenetically manipulate phosphoinositides will be able to impact the field of basic Cell Biology. We have been attending virtual lipid seminars which display a marked interest in phosphoinositide regulation at different intracellular compartments. Our technology will be of value to multiple fields.

What was the impact on technology transfer?

Once validated our methods to optogenetically manipulate phosphoinositides may be subject to patent protection. Further, we may pursue therapeutic applications of our technology. The PI has worked closely with the Columbia University Technology Venture Office (CTV) and is familiar with patent filing.

What was the impact on society beyond science and technology?

Nothing to Report

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them

During the COVID-19 pandemic, Columbia University "ramped-down" research beginning 3/20/2020. This prevented faculty and staff from working on campus that were not conducting essential duties such as animal husbandry and maintaining essential lab functions such as equipment. During this time, Dr. McIntire and Dr. Lazarian worked remotely to prepare grants and manuscripts as well as analyze data. Dr. Lazarian also worked remotely with the Mass Spectrometry Core facility to determine lipid content of mouse brain from wild type and Alzheimer's disease mouse model using desorption electrospray ionization. Dr. Lazarian will become familiar with this type of mass spectrometry for lipid detection. Dr. Lazarian also prepared analysis of the phylogenic comparison of lipid modifying enzymes with AD risk including [CLU (Clusterin; UniProt ID: P10909, NCBI Gene ID: 1191), ABCA7 (ATP-binding cassette (ABC) transporters; UniProt ID: Q8IZY2, NCBI Gene ID: 10347), PLCG2 (1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase gamma-2; UniProt ID: P16885, NCBI Gene ID: 5336) and CETP (Cholesteryl ester transfer protein; UniProt ID: P11597, NCBI Gene ID: 1071)]. According to GWAS studies, these genes or their specific SNPs are known as risk factors for AD. This work could be done remotely. Dr. Lazarian also completed a certificate issuing online course offered by the New York Academy of Science: Schrödinger's Maestro LiveDesign online course, Introduction to Molecular Modeling in Drug Discovery, Monday, May 4, 2020 - Wednesday, June 10, 2020. The McIntire lab received "essential research" designation and was allowed to ramp up May 18th, 2020. This was due to impending deadlines for the DOD reporting and submission of NIH and other grants by Dr. McIntire. All lab activities were able to resume May 18th 2020 and continue currently.

In order to quantify poly-phosphorylated species of phosphoinositides, we de-acylate the phosphoinositide lipids and then separate and detect the glycerol-head group from anionic lipids using HPLC with a Dionex IonPac AS11-HC RFIC column with suppressed conductivity detection. Our HPLC- suppressed conductivity Ion Chromatography system is quite old (2005) and has recently been inactive because the breakdown of the computer running the controlling software, Chromeleon. We have not been able to locate or obtain a replacement computer with Windows95, the required operating system for the previous version of Chromeleon. Our system was running on Chromeleon version 6.8 when the computer stopped working. We attempted installing it on a replacement computer with WindowsXP (which required installation of NetBeui) but we were unable to re-gain full function as there was a persistent problem with the autosampler. The Chromeleon program recognized that the samples had already been run and would not overwrite them. We tried three different computers with WindowsXP, but were unable to re-gain function. This HPLC is no longer covered by service agreement and the software is out-of-date and is no longer supported by Chromeleon customer service. In order to address this issue, we submitted an application for a new instrument as part of an application to

Fiscal Year 2021 Defense University Instrumentation Program (DURIP) for funding. Submitted 05/15/2020
Department of Defense: Defense University Research Instrumentation Program (DURIP)
Army Research Office GRANT13107956 W911NF-20-S-0006 McIntire DoD Instrumentation
Air Force Office of Scientific Research GRANT13107961 FOA-AFRL-AFOSR-2020-0001 McIntire Air Force Office of Scientific Research DoD Instrumentation
Office of Naval Research GRANT13107965 N00014-20-S-F004 McIntire ONR DoD Instrumentation

Title: Lipid discovery platform for basic and translational science in Traumatic Brain Injury, Alzheimer's disease and COVID-19.
Role: McIntire PI, Tal Nuriel Co-I, Ajit Muley Co-I
1) Dionex HPLC-Suppressed Ion Conductivity Detection [phosphoinositide detection]
2) Synapt XS – Desoprtion Electro Spray Ionization (DESI) [lipidomoics and imaging mass spectrometry]
3) Open Surface Plasmon Resonance (SPR)-XT [mid-throughput lipid binding OPEN SPR]
0% PI effort
At that time, a replacement for the HPLC-suppressed conductivity, ion chromatography was quoted for $50,754.79 [ThermoFisher Scientific, Quote CPQ-00261160, point of contact Lincoln Tucker, tucker.lincoln@thermofisher.com] which is not covered by the current budget for W81XWH-19-1-0817. Consumable chromatography supplies are included in the current budget, but will not be enough to replace the HPLC at this time.
If the DURIP grant applications are not funded, we would like to request supplemental funding to replace the HPLC-Ion Chromatography system. This is a unique system and is required for detection of poly-phosphorylated phosphoinositide lipid species. Otherwise, we would need to optimize a protocol for mass spectrometry which is currently unavailable which would be possible through collaboration.

Changes that had a significant impact on expenditures
There has been an increase of McIntire-PI effort dedicated to the grant from 22% to 80% effort. This is expected to be a temporary effort increase since funding from NIH and the BrighFoucs Foundation have been applied for to offset the PI's effort as well as effort from collaborators' grants, if funded. Effort will be reduced based on funding success. His effort has been reduced to 70% on this grant due to obtaining additional funding from other sources. However, he is still dedicated to completion of the program. This is appropriate since now that subcloning and viral particle production have been accomplished, animal surgeries and optogenetic experiments will be assisted by Technician Mathieu Herman. However, Dr. Lazarian will still maintain an active leading roll for lipidomics and cell biology experiments as well as data analysis for lipidomics studies.

MCINTIRE APPLICATIONS FOR FUTURE FUNDING
NIH R01, NIA /year 4/01/2021 - 03/31/2026
McIntire (PI) 20% effort submitted Aug 10, 2020
Title: Regional lipid distribution during normal aging and effects of lipid augmentation on brain lipid content, neuronal function and behavior
NIH Application# 1R01AG072794-01
Dr. Jennifer Browning, PhD jenny.browning@nih.gov
2021/01 Chronic Dysfunction and Integrative Neurodegeneration (CDIN) Study Section,
October 22nd-23rd, 2020 CDIN
No scientific or budgetary overlap.

Instrumentation Grant Submitted 06/15/2020
Department of Defense: Defense University Research Instrumentation Program (DURIP)
Air Force Office of Scientific Research
Office of Naval Research
Army Research Office
McIntire (PI)
0% effort
Title: Lipid discovery platform for basic and translational science in Traumatic Brain Injury, Alzheimer's disease and COVID-19.
Role: **McIntire PI**, Tal Nuriel Co-I, Ajit Muley Co-I
Department of Defense: Defense University Research Instrumentation Program (DURIP)
Army Research Office GRANT13107956 W911NF-20-S-0006 McIntire DoD Instrumentation
Air Force Office of Scientific Research GRANT13107961 FOA-AFRL-AFOSR-2020-0001 McIntire Air Force Office of Scientific Research DoD Instrumentation
Office of Naval Research GRANT13107965 N00014-20-S-F004 McIntire ONR DoD Instrumentation
1) Dionex HPLC-Suppressed Ion Conductivity Detection [phosphoinositide detection]
2) Synapt XS – Desoprtion Electro Spray Ionization (DESI) [lipidomoics and imaging mass spectrometry]

3) Open Surface Plasmon Resonance (SPR)-XT [mid-throughput lipid binding OPEN SPR]
Overlap exists in that we will be using this instrumentation to complete the proposed experiments.

Brightfocus Foundation /year 07/01/2021 – 6/30/2024
McIntrire (PI) 25% effort Submitted 11/17/2020
Title: Targeting GWAS Variants Associated with Lipid Dyshomeostasis in Alzheimer's Disease
No scientific or budgetary overlap

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects.
Nothing to Report

Significant changes in use or care of vertebrate animals.
Nothing to Report

Significant changes in use of biohazards and/or select agents
Nothing to Report

6. PRODUCTS:

Journal publications.
Nothing to Report

Books or other non-periodical, one-time publications.
Nothing to Report

Other publications, conference papers, and presentations.

Artur Lazarian, Vilas Menon, PhD and Laura Beth McIntire, PhD Lipid profiling of healthy and Alzheimer's affected mouse brains by using DESI Imaging Mass Spectrometry: how lipid dyshomeostasis can contribute to Alzheimer's disease. Alzheimer's Association International Conference 2020.
7/28/2020 *POSTER PRESENTATION*

Ana Paula Costa, PhD, Vilas Menon, PhD and Laura Beth McIntire, PhD Identification of dysregulated lipid metabolic pathways in mouse embryonic derived neurons and in a mouse model of Alzheimer's disease Alzheimer's Association International Conference 2020.
7/27/2020 *POSTER PRESENTATION*

Artur Lazarian, PhD, Vilas Menon, PhD, Brandon Fowler, PhD, Laura Beth McIntire, PhD. Lipidomic profiling of healthy and Alzheimer's disease mouse brains by using DESI Imaging Mass Spectrometry (IMS): regional lipid dyshomeostasis in Alzheimer's disease. IGM Symposium on Spatial "Omics" The UC Diego Institute for Genomic Medicine is holding a virtual symposium on Monday, October 26, 2020 in conjunction with the virtual American Society of Human Genetics meeting occurring on October 27th through October 30th, 2020.virtually
10/26/2020 *POSTER PRESENTATION*

Website(s) or other Internet site(s)
Nothing to Report

Technologies or techniques
Once phosphoinositide kinase catalytic domain constructs are validated and an optogeneic protocol has been validated for changes in PI(4,5)P2 levels in vivo, we are planning to submit a manuscript (Milestone 1; Months 22-24) as a description of the methods we have employed since we have generated new constructs which have not yet been described.

Inventions, patent applications, and/or licenses
Nothing to Report

Other Products

Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Laura Beth McIntire
Project Role: PI
Researcher Identifier: lbm2110@cumc.columbia.edu
Nearest person month worked: 5 months [9/15/2019 – current]
Contribution to Project: Dr. McIntire has worked on planning the strategy for subcloning optogenetic constructs, procuring currently available genetic constructs and working on regulatory approval for both human and animal IRB protocols. She has consulted with co-PI Dr. Hussani for advice on design of the optogeneic constructs using PennVector, UNC Vector and Stanford Vector.
She has consulted with collaborator Dr. James Noble for future use of human subjects in later Aims of the proposal and for IRB approvals. Dr. McIntire obtained non-human subjects research designation for use of data and biospecimens. Dr. McIntire participated in a meeting 07/31/2020 with Dr. Noble, Dr. Klein, Natalie M CIV USARMY MEDCOM USAMRMC (USA); Dr. Pacifico, Anthony M CIV USARMY MEDCOM CDMRP (USA); Simmons, Ebony S CIV USARMY MEDCOM USAMRAA (USA); Dr. Garland, Brian S CTR USARMY MEDCOM USAMRMC (US) to discuss use of human derived biospecimens and appropriate regulatory requirements. Dr. McIntire successfully received approval for the continuation of animal protocol (IACUC) and a modified protocol for the specific work to be conducted in this project (10/09/2020). Dr. McIntire has submitted the approved animal protocol to ACURO and required documentation (10/09/2020).

Dr. Artur Lazarian
Project Role: Postdoctoral Research Scientist
Research Identifier: al4094@cumc.columbia.edu
Nearest person month worked: 10 months [9/15/2019 – current]
Dr. Lazarian was hired during Q1 2020 and started working 1/21/2020.
Contribution to Project: Dr. Lazarian has helped to plan the strategy for subcloning optogenetic constructs and procuring currently available genetic constructs from Addgene. Dr. Lazarian has procured general laboratory supplies including specific supplies required such as specific restriction enzymes. Dr. Lazarian has devised and executed subcloning strategies for generation of optogenetic constructs for phosphoinositide manipulation at the plasma membrane. He sucessfully generated optogeneic constructs and submitted them to VectorBiolabs for cloning into AAV9-CamK11 viral expression vectors. Dr. Lazarian has initiated experiments with PC12 cells for investigation of the distribution of phosphoinositides due to optogeneitc stimulation in a cell model. Submitted a poster abstract for IGM Symposium on Spatial “Omics” The UC San Diego Institute for Genomic Medicine is holding a virtual symposium on Monday, October 26, 2020 in conjunction with the virtual American Society of Human Genetics meeting occurring on October 27th through October 30th, 2020.virtually [Lipidomic profiling of healthy and Alzheimer’s disease mouse brains by using DESI Imaging Mass Spectrometry (IMS): regional lipid dyshomeostasis in Alzheimer’s disease.] His effort has been reduced to 70% on this grant due to obtaining additional funding from other sources. However, he is still dedicated to completion of the program. This is appropriate since now that subcloning and viral particle production have been accomplished, animal surgeries and optogenetic experiments will be assisted by Technician Mathieu Herman. However, Dr. Lazarian will still maintain an active leading roll for lipidomics and cell biology experiments as well as data analysis for lipidomics.

Dr. Abid Hussaini
Project Role: Co-Investigator
Nearest person month worked: 1
Contribution to Project: Dr. Hussaini met with and advised Dr. McIntire on the the animal protocol covering in vivo optogenetic manipulations of phosphoinositides. Dr. Hussaini also aided Dr. McIntire in the IACUC

submission as well as necessary appendices, which was approved 10/09/2020. Dr. Hussaini also recommended using Vector BioLabs for generation of AAV9 viral encoded optogenetic constructs.

Dr. James Noble
Project Role: Co-Investigator
Nearest person month worked: 1
Contribution to Project: Dr. Nobel met with Dr. McIntire to advise on resources and regulations surrounding collection of blood from exiting TBI patients at Columbia University. Dr. Nobel advised Dr. McIntire on needed IRB approvals. Dr. Nobel also advised Dr. McIntire on current research into biomarkers for TBI. Dr. Noble has coordinated the submission of IRB protocols for use of human biospecimens. He participated in a meeting 07/31/2020 with Dr. Noble, Dr. Klein, Natalie M CIV USARMY MEDCOM USAMRMC (USA); Dr. Pacifico, Anthony M CIV USARMY MEDCOM CDMRP (USA); Simmons, Ebony S CIV USARMY MEDCOM USAMRAA (USA); Dr. Garland, Brian S CTR USARMY MEDCOM USAMRMC (US) to discuss use of human derived biospecimens and appropriate regulatory requirements.

Technician Mathieu Herman
Project Role: technician
Nearest person month worked: 3 [9/15/2019 – current]
Contribution to Project: Mr. Herman has advised Dr. McIntire regarding practical issues that are necessary for planning for mouse optogenetic experiments including animal handling and surgery. He is working to prepare for optogenetic surgeries when animal protocol is approved.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

There has been an increase of McIntire-PI effort dedicated to the grant from 22% to 80% effort. This is expected as Dr. McIntire will has been in charge of regulatory aspects as well as in the coming months, leading lipidomics experiments and behavioral experiments. This is primarily due to the near completion of R56 funding, which is now in No-Cost Extension, but funds do not remain for support of Dr. McIntire effort. This is also expected to be a temporary effort increase since funding from NIH have been applied for to offset the PI's effort as well as up to 20% effort from collaborators' grants, if funded. Effort will be reduced based on funding success. Dr. Lararian's effort has been reduced from 100% to 70% on this grant due to obtaining additional funding from other sources. However, he is still dedicated to completion of the program. This is appropriate since now that subcloning and viral particle production have been accomplished, animal surgeries and optogenetic experiments will be assisted by Technician Mathieu Herman. However, Dr. Lazarian will still maintain an active roll for lipidomics and cell biology experiments as well as data analysis.

MCINTIRE OTHER SUPPORT
ACTIVE
1R56AG062271-01A1 0 calendar months/no effort direct cost
06/15/2019 – 05/31/2020 (no cost extension)
Role: PI
a) Title: Contribution of BIN1 and Synj1 to endosomal pathogenesis Alzheimer's Disease and Down Syndrome
b) Funding Agency: NIH/NIA
c) Goals of the project: The major goal of this grant is to characterize the putative interaction between the lipid phosphatase Synaptojanin 1 and the Alzheimer's disease GWAS hits BIN1 and PICALM using iPSC derived neurons
d) Specific Aims/tasks:
Specific Aim 1. To test the hypothesis that Synj1 interacts with BIN1, PICALM in induced pluripotent stem cells (iPSC) derived forebrain neurons from AD to mediate neuronal phenotypes. We will overexpress and knock-down (shRNA) BIN1 and PICALM and Dyrk1a to determine if these proteins functionally modify Synj1 phosphatase activity to establish the mechanism underlying endosomal phenotypes likely to be regulated by their interaction in human neurons. Forebrain neurons derived from iPSC as well as astrocytes and oligodendricytes will be assessed for phenotypes hypothesized to be dependent on Synj1 activity including a) clathrin mediated endocytosis, b) endosomal dysfunction and morphology c) spine morphology and synaptic

function using electrophysiology and d) a Synj1 mediated phenotype, astrogliogenesis. Neurons will be prioritized, however astrocytes and oligodendrocytes will be investigated in future experiments due to expression of Syj1, BIN1 and PICALM in non-neuronal cell types.
e) Start and end date: 06/15/2019 – 05/31/2021
f) Level (%) of effort in the project: 0%
g) Point of contact at the funding agency: Marilyn Miller
OVERLAP
There is no scientific overlap.

ACTIVE
Thompson Family Foundation Pilot Grant 1.2 calendar months direct cost
01/01/2019 – 12/31/2020
Role: PI
a) Title: Identification of Lecithin:Cholesterol Acyltransferase (LCAT) activators for Alzheimer's disease.
b) Funding Agency: Thompson Family Foundation Program for Accelerated Medicines Exploration in Alzheimer's Disease and Related Disorders of the Nervous System (TAME-AD)
c) Goals of the project: The major goal of this grant is to test chemical analogs of a known Lecithin:Cholesterol Acyltransferase (LCAT) activators in stimulation of LCAT activity, reduction of Aβ and synapse loss, BBB penetration and impact on behavior in a mouse model of Alzheimer's disease.
d) Specific Aims/tasks:
SPECIFIC AIM 1. Based on structures of known Lecithin:Cholesterol Acyltransferase (LCAT) activator moieties, we will synthesize analogs which will be tested for LCAT activation in vitro using recombinant LCAT, mouse embryonic stem cells (mESN) and in a mouse model of AD. We will test the hypothesis that LCAT activator moieties and PC or lysoPC containing 22:6 at the sn-2 position will induced cholesterol esterification by LCAT and reduce AD associated synaptic defects and behavioral deficits. Lipid head group modification of PC 22:6 while preserving the LCAT catalytic activity (Freeman, et al., 2017; Davit-Spraul et al., 1999) may allow composition of matter IP to be developed around PC or LPC 22:6 or sulfhydryl-reactive small molecules (Freeman et al., 2017) as a carrier of polyunsaturated fatty acids for incorporation into phospholipid and cholesterol metabolism in the brain.
SPECIFIC AIM 2. We will optimize an LCAT activity assay in mESN for HTS, prioritize LCAT activators based on drug-like properties. Hits will be tested in secondary assays for repression of Aβ-triggered synapse loss and BBB penetration. Lead compounds (1-2) will be tested in an animal model of AD, Tg2576.
e) Est. start and end date: 01/01/2019 – 01/31/2021
f) Level (%) of effort in the project: 10%
g) Point of contact at the funding agency: Jennifer Heredia
OVERLAP
There is no scientific overlap

ACTIVE
2019-2020 Translational Therapeutics (TRx) Pilot Award *direct cost*
08/01/2020 – 7/31/2021
no effort
Role: PI
a) Title: "Targeting Rare Pediatric Disease Niemann-Pick type C and Alzheimer's Disease with Activators of Lecithin Cholesterol Acyl Transferase"
b) Funding Agency: NIH/NCATS
National Center for Advancing Translational Sciences, National Institutes of Health, through Grant Number UL1TR001873
Columbia Technology Ventures
c) Goals of the project:
Specific Aim 1. Optimization of brain permeable Lecithin Cholesterol Acyl Transferase activator. Based on the structure of known LCAT activators, an analog series of small molecules will be synthesized and tested in 1) LCAT activity assay and 2) N2A neuroblastoma cell line for ability to alter lipid content of cells 3) BBB penetration assay.
Specific Aim 2. Compound administration, pharmacokinetics and lipidomics: Compounds which are able

to activate cholesterol esterification and prevent cholesterol accumulation will be administered to an NPC mouse model (Npc1tm(I1061T)Dso) and Alzheimer's disease mouse model overexpressing APP with the Swedish mutation (Tg2576).
e) Start and end date: 08/01/2020 – 07/31/2021
f) Level (%) of effort in the project: 0% no effort
g) Point of contact at the funding agency: Mirah Rahman
OVERLAP
There is no scientific overlap

LAZARIAN OTHER SUPPORT
ACTIVE
Thompson Family Foundation Pilot Grant 1.2 calendar months direct cost
01/01/2019 – 12/31/2020
Role: Post-doc
a) Title: Identification of Lecithin:Cholesterol Acyltransferase (LCAT) activators for Alzheimer's disease.
b) Funding Agency: Thompson Family Foundation Program for Accelerated Medicines Exploration in Alzheimer's Disease and Related Disorders of the Nervous System (TAME-AD)
c) Goals of the project: The major goal of this grant is to test chemical analogs of a known Lecithin:Cholesterol Acyltransferase (LCAT) activators in stimulation of LCAT activity, reduction of Aβ and synapse loss, BBB penetration and impact on behavior in a mouse model of Alzheimer's disease.
d) Specific Aims/tasks:
SPECIFIC AIM 1. Based on structures of known Lecithin:Cholesterol Acyltransferase (LCAT) activator moieties, we will synthesize analogs which will be tested for LCAT activation in vitro using recombinant LCAT, mouse embryonic stem cells (mESN) and in a mouse model of AD. We will test the hypothesis that LCAT activator moieties and PC or lysoPC containing 22:6 at the sn-2 position will induced cholesterol esterification by LCAT and reduce AD associated synaptic defects and behavioral deficits. Lipid head group modification of PC 22:6 while preserving the LCAT catalytic activity (Freeman, et al., 2017; Davit-Spraul et al., 1999) may allow composition of matter IP to be developed around PC or LPC 22:6 or sulfhydryl-reactive small molecules (Freeman et al., 2017) as a carrier of polyunsaturated fatty acids for incorporation into phospholipid and cholesterol metabolism in the brain.
SPECIFIC AIM 2. We will optimize an LCAT activity assay in mESN for HTS, prioritize LCAT activators based on drug-like properties. Hits will be tested in secondary assays for repression of Aβ-triggered synapse loss and BBB penetration. Lead compounds (1-2) will be tested in an animal model of AD, Tg2576.
e) Est. start and end date: 01/01/2019 – 01/31/2021
f) Level (%) of effort in the project: 10%
g) Point of contact at the funding agency: Jennifer Heredia
OVERLAP
There is no scientific overlap

ACTIVE
2019-2020 Translational Therapeutics (TRx) Pilot Award *direct costs* 08/01/2020 – 7/31/2021
no effort
McIntire (PI)
a) Title: "Targeting Rare Pediatric Disease Niemann-Pick type C and Alzheimer's Disease with Activators of Lecithin Cholesterol Acyl Transferase"
b) Funding Agency: NIH/NCATS
National Center for Advancing Translational Sciences, National Institutes of Health, through Grant Number UL1TR001873
Columbia Technology Ventures
c) Goals of the project:
Specific Aim 1. Optimization of brain permeable Lecithin Cholesterol Acyl Transferase activator. Based on the structure of known LCAT activators, an analog series of small molecules will be synthesized and tested in 1) LCAT activity assay and 2) N2A neuroblastoma cell line for ability to alter lipid content of cells 3) BBB penetration assay.

Specific Aim 2. Compound administration, pharmacokinetics and lipidomics: Compounds which are able to activate cholesterol esterification and prevent cholesterol accumulation will be administered to an NPC mouse model (Npc1tm(I1061T)Dso) and Alzheimer's disease mouse model overexpressing APP with the Swedish mutation (Tg2576).
e) Start and end date: 08/01/2020 – 07/31/2021
f) Level (%) of effort in the project: 20%
g) Point of contact at the funding agency: Mirah Rahman
OVERLAP
There is no scientific overlap

What other organizations were involved as partners?
Nothing to Report

SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: **N/A**

CONCLUSION:
Successful completion of these Aims will lead to proof of concept for the tractability of $PI(4,5)P_2$ for further clinical development for AD in the context of sporadic AD as well as potential for development of AD in the context of Traumatic Brain Injury (TBI). This study also has promise to identify new biomarkers with functional relevance to changes in lipid content in AD brain. Optogenetic control of these (depleted) lipids may validate a novel target for future studies for resistance to TBI induced pathological changes leading to AD susceptibility. Amelioration of behavioral deficits in genetic model indicate $PI(4,5)P_2$ levels confer resilience for neurons while rescue of behavioral deficits in the acute model indicate $PI(4,5)P_2$ levels confer resistance to Aβ insult and may be applicable to future studies of TBI. We have nearly completed the critical groundwork including generation of optogenetic tools and initiation of regulatory requirements that are required for for pursuing major tasks in animals and in human biospecimens. We look forward to moving quickly in the next year toward data generation and an initial methodology manuscript. We expect to be able to report preliminary data outcomes within the next reporting period.

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APPENDICES: N/A