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

PRINCIPAL INVESTIGATOR: McIntire, Laura Beth, PhD

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Laura Beth McIntire

E-Mail: lbm2110@cumc.columbia.edu

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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 subcloned 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. **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 IRB as well as Dr. James Noble to obtain approval for use of human derived biospecimens for lipidomic studies.**

Coordinately, using targeted lipidomics we will determine aberrant phospholipid levels associated with age of onset of AD in patient plasma, CSF and brain tissue. 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. Successful completion of this program may identify PIs and PI metabolism as tractable and safe for prophylactic or therapeutic development of these targets as biologics.

15. SUBJECT TERMS
Alzheimer's disease, Lipid metabolism

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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) COMPLETED

Major activities and accomplishments:

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) COMPLETED

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)

Specific objectives and significant results or key outcomes:
In vitro validation of optogeneitc constructs completed.
We have validated of the optogenetic constructs generated, we used viral particles for infection of PC12 (pheochromeoytoma) cell line and will later use mouse embryonic stem cell derived neurons. We have tested the ability of these constructs to modify phosphoinositide content of PC12 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- PLCδ) 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 optimized the co-expression of CIBN-CAAX and catalytic domain constructs PIP5K2A-

CD as well as transient transfection of PI(4,5)P2 sensor PH- PLCδ. The PC12 cells were plated on 8-well chamber slides until 80-90% confluent. Viral particles were used to infect the PC12 cells a MOI of $8.5x10^{12}$ - $8.5x10^{13}$ GC/mL titer. The PH domain of PLCδ (GFP-tagged, green; 3.75 µg plasmid) was delivered via lipofectamine transfection followed by viral transduction to deliver catalytic domain of PIP5K2A (mCherry-tagged, red); and CAAX-CIBN domain (no tag).

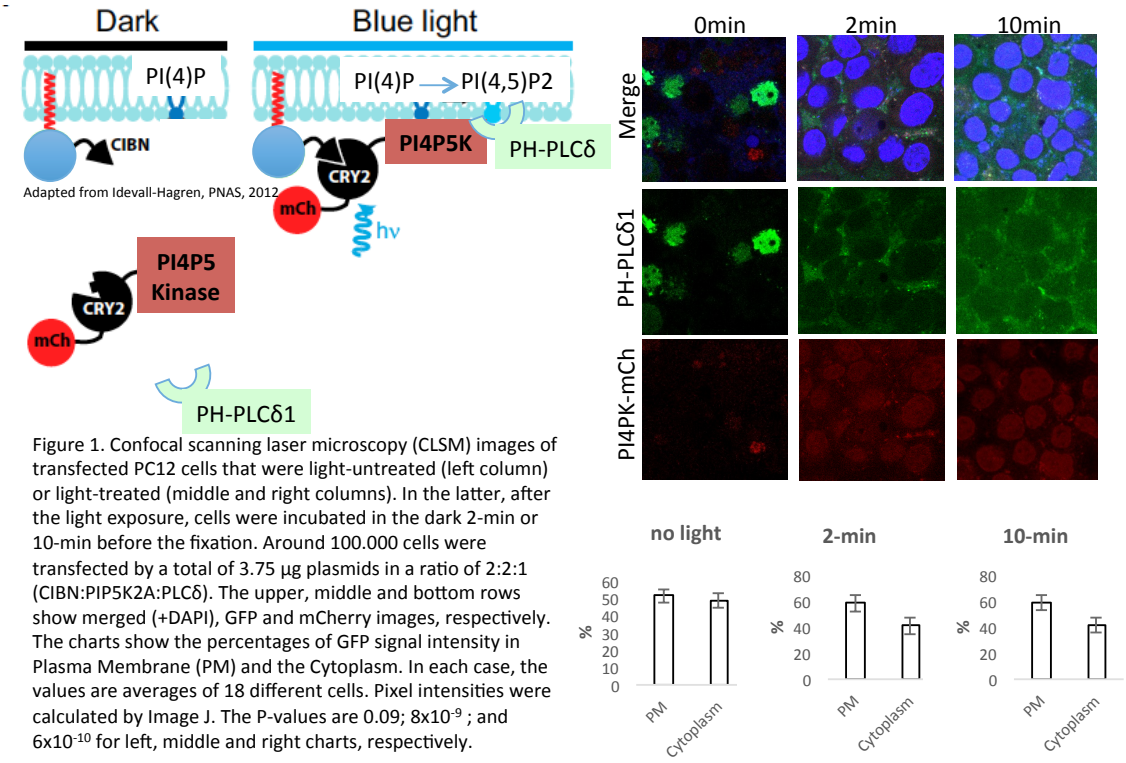

Figure 1. Confocal scanning laser microscopy (CLSM) images of transfected PC12 cells that were light-untreated (left column) or light-treated (middle and right columns). In the latter, after the light exposure, cells were incubated in the dark 2-min or 10-min before the fixation. Around 100.000 cells were transfected by a total of 3.75 µg plasmids in a ratio of 2:2:1 (CIBN:PIP5K2A:PLCδ). The upper, middle and bottom rows show merged (+DAPI), GFP and mCherry images, respectively. The charts show the percentages of GFP signal intensity in Plasma Membrane (PM) and the Cytoplasm. In each case, the values are averages of 18 different cells. Pixel intensities were calculated by Image J. The P-values are 0.09; $8x10^{-9}$; and $6x10^{-10}$ for left, middle and right charts, respectively.

PIP5K2A (mCherry-tagged was delivered at 1 µL ($8.5x10^{12}$ GC/mL titer, 10 µL ($8.5x10^{13}$ GC/mL titer and CAAX-CIBN was delivered at 1 µL ($2.8x10^{13}$ GC/mL titer) or 10 µL $2.8x10^{14}$ GC/mL titer). Both viruses had a *CamkIIa* promoter allowing expression in neurons and neuronal cell lines such as PC12 cells. After the transduction, cells were incubated with virus culture medium overnight (37°C, protected from light). After 24 hours later the viral medium was replaced by a regular, virus-free medium, and the cells continued to grow 24 -48 hours prior to light stimulation and fixation. Cells were kept in the dark until illumination by blue light (λ=470 nm) using an optic fiber and a LED driver from Thorlabs (DC2100). The conditions were as follows: Mode: PWM (Pulse Width Modulation); Current: 1000 mA; Frequency: 3Hz; Duty Cycle: 70%; Counts: 20x. We determined that there was a increase in localization of the PH-PLCδ to the plasma membrane indicating PI(4,5)P2 accumulation (Idevall-Hagren et al., PNAS USA. 2012, Aug 28, 109(35):E2316-E2323). Initial results indicate that in cells that are not stimulated (0 minutes), the PH-PLCδ remained cytosolic. However after 2-10 minutes of blue light (λ=470 nm), cytosolic labeling was largely lost and plasma membrane (PM) labeling was observed. This is consistent with translocation from the cytosol to the plasma membrane of the PI(4,5)P2 sensor PH-PLCδ. The signal intensity was quantified using ImageJ line tool (Figure 1). Studies are currently underway with a second PH-PLCδ-CFP to confirm PM PI(4,5)P2 localization.

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.

We have used a protocol developed by Dr. Wu and Dr. Hussaini (Wu et al. Neuronal activity enhances tau propagation and tau pathology in vivo, Nat. Neurosci, 2016 Aug; 19(8):1085-1092). AAV9 virus particles were injected into the hippocampus and cannulae have been implanted above the injection site. We have completed the viral

injection and cannulae implantation and will determine the extent of AAV9 viral particle infection and induction of PIP5K2A (mCherry).

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). The animal protocols covering these procedures have been approved by the Columbia University IACUC and approved by ACURO. Surgical procedures have been optimized and all canunulae and necessary resources have been procured. Initial experiment for injection of AAV9 virus particles into the hippocampus and cannulae implantation has been accomplished. We will determine the optimal time after injection for virally mediated expression of PI4P5K2A-mCherry. Animals recover for 10 days after injection and cannulae implantation prior to stimulation.

Protocols and procedures were approved by the Committee on the Ethics of Animal Experiments of Columbia University and according to Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. Subjects in this study were Tg2576 mice and WT control siblings. Both male and female mice were used for each in vivo experiment. Surgical procedures are performed according to NIH guidelines in accordance with IACUC approved protocols. Mice are anesthetized with Avertin (500 mg/mL). AAV9 virus particles are injected into the hippocampus (Posterior = 2.46 mm, lateral=1.50 mm to a depth of 1.30 mm) and cannula are implanted above the injection site (Fiber Optic Cannula, Ø1.25 x 6.4 mm Ceramic Ferrule, Ø200 µm Core, 0.39 NA, L=2 mm; ThorLabs). However, many of the cannulae were not secured effectively and were lost after clipping the stimulating optical fiber to the cannulae. We will use these samples to determine if viral infection and tissue distribution within the brain is improved after 10, 17, 24 days. After gaining additional training and surgical technique instruction from Dr. Hussaini, and using a stronger resin acrylic to secure the cannulae, we will use two animals to test the new procedure for securing the cannulae. Mice at 6-8 months available for surgeries. Ten days after viral transduction, the hippocampi from both hemispheres were stimulated with four pulses 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.

After stimulation protocol, behavioral paradigms including novel object recognition (NOR) and Contextual Fear Conditioning (CFC) brain were completed. Brains were harvested and hippocampi were be dissected and will be subjected to lipid extraction, de-acylation and HPLC suppressed conductivity detection of anionic lipids. We will determine the phosphoinositide content using HPLC suppressed conductivity. We are in the process of acquiring a new HPLC instrument that will be able to detect phosphoinositides and anionic lipid species using optimized protocols. We have applied for a DOD DURIP to support acquisition of the new instrument. However, if the new instrument is not funded, we will collaborate with other labs who have vetted experience with phosphoinositide detection. Additionally imaging mass spectrometry will be used to determine regional distribution of changes of lipids, specifically phosphoinositides to determine the regional range of phosphoinositide modulation in brain tissue.

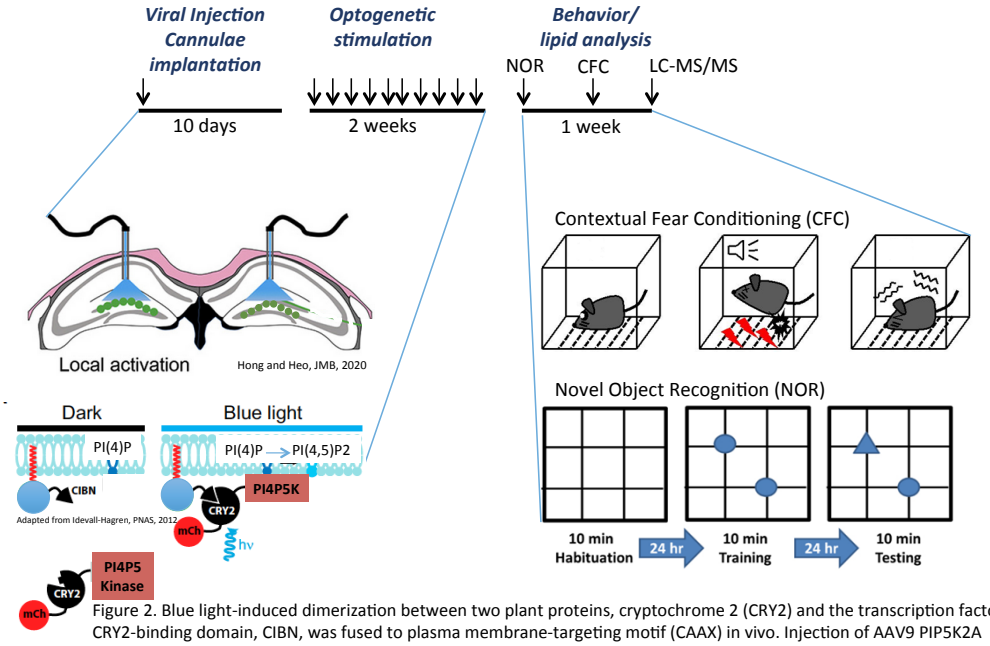

Figure 2. Blue light-induced dimerization between two plant proteins, cryptochrome 2 (CRY2) and the transcription factor CRY2-binding domain, CIBN, was fused to plasma membrane-targeting motif (CAAX) in vivo. Injection of AAV9 PIP5K2A (mCherry-tagged and CAAX-CIBN and cannulae implantation at day 1. After 10 days, illumination by blue light (λ=470 nm) using an optic fiber and a LED driver from Thorlabs (DC2100). The conditions were as follows: Mode: PWM (Pulse Width Modulation); Current: 1000 mA; Frequency: 3Hz; Duty Cycle: 70%; Counts: 20x for 2 weeks, 5 times/week, 3 times/day. Mice were then subjected to contextual fear conditioning and novel object recognition.

Our preliminary data indicate that optogenetic stimulation of PI(4,5)P2 accumulation at the PM is able to rescue behavioral deficits in the mouse model of AD overexpression the Amyloid Precursor Protein (APP) with the Swedish mutation (APP-Tg). This mouse model displays behavioral deficits by age 6-8 months, the time at which amyloid beta begins to accumulate largely as oligomers. We have tested the optogenetic stimulation of PI(4,5)P2 in this age and determined behavior in contextual fear conditioning and novel object recognition tasks which are typically used to determine behavioral deficits in this animal model. Novel Object Recognition discrimination index (NOD) is significantly reduced for Tg-control stimulated with yellow light (Tg-Cntl). No other groups are significantly different from control group indicating rescue of Tg-Opto through optogenetic stimulation (Figure 3). A trend is seen in Contextual fear conditioning for decreased freezing (s) in the Tg-Control animals as expected due to the behavioral deficits in Tg animals at 6-8 months of age. However, a trend is seen in the Tg-Opto condition which suggests that optogenetic stimulation of PI(4,5)P2 synthesis is able to rescue the behavioral deficit.

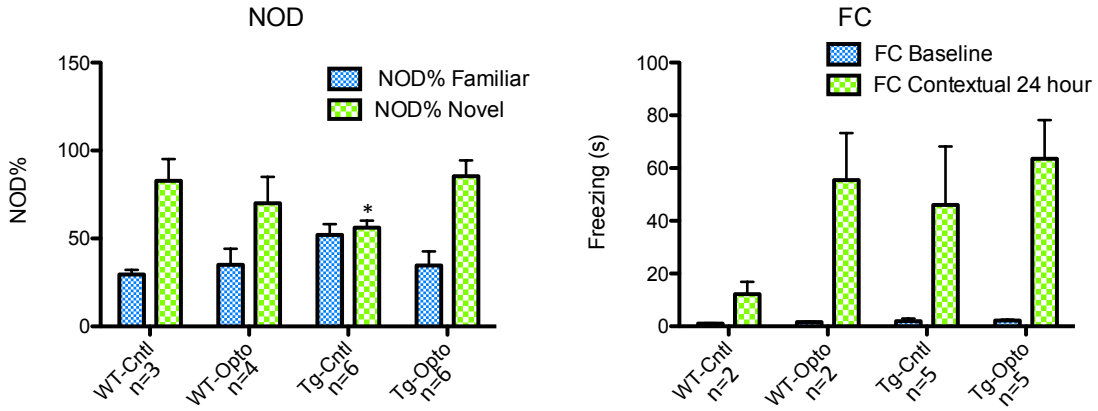

Figure 3. A. Novel Object Recognition discrimination index (NOD) is significantly reduced for Tg-control stimulated with yellow light. No other groups are significantly different from control group indicating rescue of Tg-Opto through optogenetic stimulation. B. A trend is seen in Contextual fear conditioning for decreased freezing (s) in the Tg-Control animals as expected due to the behavioral deficits in Tg animals at 6-8 months of age. However, a trend is seen in the Tg-Opto condition which suggests that optogenetic stimulation of PI(4,5)P2 synthesis is able to rescue the behavioral deficit. For the FC studies, the n should be increased to 8-10 animals per each group.

Control animals are stimulated with control light (λ=595). For the FC studies, the n should be increased to 8-10 animals per each group. These results suggest that stimulation of PI(4,5)P2 at the plasma membrane is able to rescue the behavioral deficits in the mouse model of AD. Brains from experimental animals have been harvested, micro-dissected and stored at -80C for analysis of lipid content using mass spectrometry and imaging mass spectrometry. Hippocampi have been dissected from half the brain and the other half brain has been flash frozen in liquid nitrogen for imaging mass spectrometry to determine the lipid content. The hemi-brain that was flash frozen is intact and will also be used to determine the extent of the viral infection which can be determined from the mCherry expression which is fused to the catalytic domain of the PIP5K2A-CD in AAV9.

After behavioral testing, brains were harvested, frozen in liquid nitrogen and stored at -80°C until analysis for lipid content using targeted lipidomic analysis using liquid chromatography paird with tandem mass spectrometry. Lipid content will be determined regionally in brain using Desportion Electrospray Ioniaztion (DESI)-mass spectrometry imaging (MSI). We have optimized the detection of phosphoinositides using DESI-Imaging Mass Spectrometry with the following standards (from Avanti Polar Lipids) on the Waters Synapt G2Si instrument for use in the Chemistry Department Mass Spectrometry facility. For PI(P) standards, we used 7 different lipids, specifically:

1. 18:0-20:4 PI(4)P
2. 16:0-18:1 PI(4)P
3. 18:1-18:1 PI(4,5)P_2
4. 18:0-18:0 PI
5. 18:0-20:4 PI
6. 18:1-18:1 PI
7. 18:0-20:4 PI(4,5)P_2

All seven were reliably detected after spotting on mouse brain tissue, in negative polarity mode mostly as single charged deprotonated parent ions. Some of the standards, such as # 1, 2, 3, 4, and 7, could also be detected as double charged ions. Detected parent ions had the following m/z values:

1. 965.5104
2. 915.4928
3. 1021.478
4. 865.5723
5. 885.6172
6. 861.5368
7. 522.2317 (z=2)

Discussion of stated goals not met:

We are completing a final round of cannulea implantation, optogenetic stimulation and behavioral assessments (Novel Object Recognition and Contextual Fear Conditioning). After final behavioral assessments, we will harvest the brain, flash freeze using liquid nitrogen and store at -80°C until processed for imaging mass spectrometry. The brains are bisected to reserve half the brain for Imaging Mass Spectrometry using targeted panel of phosphoinositides we have developed. The second half of the brain will be processed for liquid-chromatography, tandem mass spectrometry and HPLC-suppressed conductivity detection of anionic lipids. Phosphoinositides will therefore be quantified using bulk targeted lipidomics from homogenate (molar percent) as well as spacially within tissue using imaging mass spectrometry. We can quantify the relative fold change in lipid content by selecting a reference point outside of the region of interest, as a control region. We expect sufficient n to obtain power to detect significant differences in phosphoinositide lipid content.

Milestone #1: manuscript describing generation of in vivo tools for PI manipulation and potential ameliorative effects on AD associated behavior deficits (Months 22-24). Since we have positive results from the behavioral assessment and expect to find significant results from lipid analyses described above, we expect to publish the work describing the novel optogenetic methods, amelioration of behavior and expected change in the phosphoinositide lipid content. Due to the novelty of these methods and the clinical relevance for identifying tractable target for amelioration of AD associated behavioral deficits, we aim to publish our manuscript in Nature Medicine.

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:

I have received IRB Institutional approval (IRB-AAAT8658) for the use of de-identified biospecimens from the Alzheimer's Disease Research Center (IRB-AAAR2387) and the NYC Columbia Brain Bank (IRB-AAAB0192) which allows the distribution of de-identified tissue as not human subject research. We have submitted this information along with 5. ORP_Cadaver_Submission_Form v 3.0 to Brian Garland, BA, Contractor - Ciconix, LLC, **Proposal / Protocol Coordinator**, Human Research Protection Office, Office of Research Protections; Headquarters, U.S. Army Medical Research and Development Command; Fort Detrick, MD Fort Detrick, MD 21702-5000; 301.619.6242 (desk/voicemail); HRPO E-mail:brian.s.garland.ctr@mail.mil

Specific objectives and significant results or key outcomes:

Approval of our protocol for use of biobanked tissue from the Alzheimer's Disease Research Center (ADRC) biobank and the Columbia Brain Bank has been obtained (9/15/2021) (IRB-AAAT8658). IRB-approved

protocol and related biobank affiliated with the ADRC (AAAR2387) and the NYC Columbia Brain Bank (IRB-AAAB0192) which allows the distribution of de-identified tissue as not human subject research. This work will apply to brain tissue which has been banked by the ADRC and the Columbia University Biobank. 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. The 5.ORP_Cadaver_Submission _Form has been submitted to HRPO.

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

Milestone #2: Manuscript describing generation of in vivo tools for PI manipulation and potential ameliorative effects on AD associated behavior deficits. We expect to submit our findings from human tissues after completing analysis of phosphoinositide content.

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

How were the results disseminated to communities of interest?
Nothing to report

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: Completed
Subtask 2: Viral preparation of optognetic constructs: Completed
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.: Completed
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). Completed
Subtask 2: Design 2 stimulation paradigms likely to enhance PI(4,5)P_2 production in brain of wild type mice (B6). Design will be advised by both Dr. McIntire and Dr. Hussaini.

We are completing a final round of cannulea implantation, optogenetic stimulation and behavioral assessments (Novel Object Recognition and Contextual Fear Conditioning). After final behavioral assessments, we will harvest the brain, flash freeze using liquid nitrogen and store at -80°C until processed for imaging mass spectrometry. The brains are bisected to reserve half the brain for Imaging Mass Spectrometry using targeted panel of phosphoinositides we have developed. The second half of the brain will be processed for liquid-chromatography, tandem mass spectrometry and HPLC-suppressed conductivity detection of anionic lipids.

Phosphoinositides will therefore be quantified using bulk targeted lipidomics from homogenate (molar percent) as well as spacially within tissue using imaging mass spectrometry. We can quantify the relative fold change in lipid content by selecting a reference point outside of the region of interest, as a control region. We expect sufficient n to obtain power to detect significant differences in phosphoinositide lipid content.

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. These biospecimens have been identified and we are prepared to acquire the samples after approval of by ORP. 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. 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:

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

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. However, it is no longer functional to replace this instrument, we submitted an application for the Fiscal Year 2021 Defense University Research Instrumentation Program (DURIP) submitted to:
Department of Defense
Army Research Office W911NF-21-S-0004 WS00694151
Air Force Office of Scientific Research FOA-AFRL-AFOSR-2021-0002 WS00694155
Office of Naval Research N00014-21-S-F002 WS000694164

At that time, a replacement for the HPLC-suppressed conductivity, ion chromatography was quoted for [ThermoFisher Scientific, 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.

Meanwhile, we have optimized a protocol for mass spectrometry using Imaging mass spectrometry (Synapt-DESI) which we are currently using in the Chemistry Department, Columbia University, Mass Spectrometry & MS Imaging Core (user run, fee for instrument time). Department of Chemistry, 216 Havemeyer Hall. Dr. McIntire shares 75% of the time available on the instrument among 5 major users. Dr. Lazarian is expert user of Waters Synapt G2-Si QToF with ion-mobility and MS/MS capabilities equipped with DESI source for Imaging Mass Spectrometry (IMS). Instrumentation: Waters Synapt G2-Si QToF with ion-mobility and MS/MS capabilities equipped with (1) a DESI source for MS imaging, or (2) a Waters I-class UPLC and ESI source for LC-MS(/MS) and LC-IMS-MS(/MS) analysis.

Changes that had a significant impact on expenditures

There has been a decrease of McIntire-PI effort dedicated to the grant from 80% to 60% effort. This is due to the funding of NIH 1R56AG072794-01 (MCINTIRE); 4/01/2021 - 08/31/2022; 2.40 CM
National Institute of Aging; *Acyl chain remodeling and regional lipid dysregulation in Alzheimer's disease and subsequent funding of resubmission* 1R01AG072794-01 (MCINTIRE); 9/01/2021 - 08/31/2026; 2.40 CM
National Institute of Aging; *Acyl chain remodeling and regional lipid dysregulation in Alzheimer's disease.*

The effort for Artur Lazarian has been reduced to 50% on this grant due to obtaining additional funding from other sources which do not overlap scientifically. 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:

ADRC Applied 2/1/2021
Alzheimer's Disease Research Center Start date 5/1/2021
Development Project Grants for Alzheimer's Disease Research:
Association of cognitive decline with regional lipid dyshomeostasis in AD brain using imaging mass spectrometry
McIntire (PI) 20%
Lazarian 33.3%

Multi-PI Planning Grant Irving Institute submitted 12/04/2020
Prognostic lipidomic and small RNA signatures in exosomes for COVID-19clinical course and outcome
Lazarian (50%)
Co-PI Ismael Santa-Perez, Alex Rai
McIntire no salary

Joint Pilot Precision Medicine Grant submitted 12/07/2020
Co-IP Myrna Weismann
Lazarian (50%)
Columbia Precision Medicine Joint Pilot Grants Program
Roy and Diana Vagelos Precision Medicine Basic Science Award
Irving Institute for Clinical and Translational Research Precision Medicine Award
The Herbert Irving Comprehensive Cancer Center Precision Medicine Award
Identification of Lipid Metabolic Networks in Risk for Major Depressive Disorder

American Federation for Aging Research 01/25/2021
AFAR Glenn Foundation for Medical Research Postdoctoral Fellowship in Aging Research
Title: Lipid profiling of healthy and Alzheimer's affected brains of mice of various ages by using DESI Imaging Mass Spectrometry: how lipid dyshomeostasis can contribute to Alzheimer's disease
Lazarian (78%)

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.

Under Review

Nuria Martinez-Lopez, Pamela Mattar-Aranguiz, Miriam Toledo, Henrietta Bains, Manu Kalyani, Marie Louise Aoun, **Laura Beth J. McIntire**, Leslie Gunther-Cummins, Frank P. Macaluso, Jennifer T Aguilan, Simon Sidoli, Mathieu Bourdenx, Rajat Singh. (2021) mTORC2 couples fasting to mitochondrial fission Nature

Under Review

Andre Miguel Miranda; Archana Ashok; Robin Barry Chan; Bowen Zhou; Yimeng Xu; **Laura Beth McIntire**; Estela Area-Gomez; Gilbert Di Paolo; Karen E. Duff; Tiago Gil Oliveira; Tal Nuriel. (2021) *Effects of APOE4 allelic dosage on lipidomic signatures in the entorhinal cortex of aged mice* Molecular Neurodegeneration

Under Review

Mikhail Melnik, S. Whitaker Cohn, Calvin Huang, **Laura Beth McIntire**, Varghese John, Karen Gylys, and Tina Bilousova (2021) *Multi-omics analysis of microglial extracellular vesicles from human Alzheimer's Disease brain reveals pro-inflammatory disease-associated signature* Special Issue Frontiers in Pharmacology: Exosomes: Message in a Vesicle

Under Review

Dyakin, V., **Mcintire L.B.,** Dyakina-Fagnano N.V. Uversky V.N. Fundamental Clock of Biological Aging: Convergence of Molecular, Neurodegenerative, Cognitive, and Psychiatric Pathways Non-Equilibrium Thermodynamics Meet Psychology Psychological Bulletin

Books or other non-periodical, one-time publications.

Nothing to Report

Other publications, conference papers, and presentations.

Ana Paula Costa; Milenna T. van Dijk; Irina Pokhvisneva; Patricia Pelufo Silveira; Ardesheer Talati; Jonathan Posner; Michael J. Meaney; Myrna M. Weissman; **Laura Beth McIntire**. Mechanisms Underlying Lipidomic Changes in Major Depressive Disorder. Translational Science 2021 – Association For Clinical and Translational Science Mar 31 – April 2, 2021

Ajit Muley, Anna P.Costa, Hong-Jian Wei, Joseph McCarron, Noa Shapiro-Franklin, Guy Garty, Cheng-Chia Wu, **Laura Beth J. McIntire** and Carrie J. Shawber. Novel neuroprotective FLASH radiotherapy and emerging role of meningeal lymphatics in memory loss and cognitive decline post-radiotherapy. Lymphatic Forum 2021 May 31 – June 5, 2021

Website(s) or other Internet site(s)

Nothing to Report

Technologies or techniques

Once phosphoinositide kinase catalytic domain constructs are validated and an optogenetic 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.

DESI-Mass Spectrometry Imaging is a powerful emerging technology which uniquely allows the detection of lipids in intact flash frozen brain histology sections. We are using this technology to identify regions of interest in the mouse brain that are enriched in phosphoinositide lipids. Further, we are using this technology to determine the spatial enrichment of phosphoinositides after optogenetic stimulation.

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: 8 months [9/15/2020 – 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 obtained IRB approval to use human brain tissue from the Columbia University Alzheimer's Disease Research Center Brain Bank and the NYC Brain Bank at Columbia University. She has submitted the ORP_Cadaver use approval forms (submitted 10/15/2021). 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 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. Dr. McIntire has obtained the approved animal protocol to ACURO and required documentation.

Dr. Artur Lazarian
Project Role: Postdoctoral Research Scientist
Research Identifier: al4094@cumc.columbia.edu
Nearest person month worked: 7 months [9/15/2020 – current].
Contribution to Project: 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 performed experiments with PC12 cells for investigation of the distribution of phosphoinositides due to optogeneitc stimulation in a cell model. He has developed and optimized effective strategies for detecting phosphoinositides in mouse brain tissue using DESI-Imaging Mass Spectrometry. His effort has been reduced to 50% 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. Dr. Hussaini also recommended using Vector BioLabs for generation of AAV9 viral encoded optogenetic constructs. Dr. Husaaini advised optogenetic stimulation paradigms.

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.

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, behavior and surgery. He has completed multiple rounds of optogenetic stimulation paradigms.

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 80% to 60% effort. Dr. Lararian's effort has been reduced from 70% to 50% 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:

National Institute of Aging, 3.6 CM calendar months direct cost
NIH 1R01AG072794-01
9/01/2021 - 08/31/2026
Role:PI

a) Title: Acyl chain remodeling and regional lipid dysregulation in Alzheimer's disease

b) Funding Agency: NIH/NIA
c) Goals of the project:
d) Specific Aims/tasks:

SPECIFIC AIM 1: To test the hypothesis that lipid species and classes are regionally depleted in association with age and in mouse models of AD. We will test the hypothesis that polyunsaturated acyl chain composition among multiple phospholipid classes are disrupted in regions responsible for learning and memory such as the hippocampus. Un-targeted lipidomics using Desorption Electrospray Ionization (DESI) Imaging Mass Spectrometry and coordinate targeted lipidomics using liquid chromatrography/mass spectrometry (LC/MS) studies will be first in class to define the exquisitely specific regional distribution of lipids in the brain and to demonstrate ability to manipulate lipid content using exogenously administered lipids. Altered lipid content will be determined using Imaging Mass Spectrometry in brain regions over a range of ages in wild type animals and in a mouse model of AD (Tg2576 and TRAPOE4) for integration of a publically available lipid brain atlas.
Specific Aim 2. We will test the hypothesis that genetic manipulation of brain lipid content using Acsl6 knock-in (KI) can lead to changes in lipid composition and performance in behavioral tasks. Spatial memory, which is dependent on hippocampal function has been shown to decline during aging and in AD mice and will be assessed in rodents using 2-day Water Maze, Barnes Maze, spontaneous alternations Y-maze.
Specific Aim 3. We will test the hypothesis that Acsl6 KI will ameliorate functional defects in AD mouse models such as electrophysiological correlate to learning and memory, long term potentiation (LTP) and AD pathology and neuroinflammation.

e) Start and end date: 04/01/2021 – 08/31/2021
f) Level (%) of effort in the project: 30%
g) Point of contact at the funding agency: Amanda DiBatista
OVERLAP
There is no scientific overlap.

Completed

National Institute of Aging, 3.6 CM calendar months direct cost
NIH 1R56AG072794-01
4/01/2021 - 08/31/2021
Role:PI

a) Title: Acyl chain remodeling and regional lipid dysregulation in Alzheimer's disease

b) Funding Agency: NIH/NIA
c) Goals of the project:
d) Specific Aims/tasks:
SPECIFIC AIM 1: To test the hypothesis that lipid species and classes are regionally depleted in association with age and in mouse models of AD. We will test the hypothesis that polyunsaturated acyl chain composition among multiple phospholipid classes are disrupted in regions responsible for learning and memory such as the hippocampus. Un-targeted lipidomics using Desorption Electrospray Ionization (DESI) Imaging Mass Spectrometry and coordinate targeted lipidomics using liquid chromatrography/mass spectrometry (LC/MS) studies will be first in class to define the exquisitely specific regional distribution of lipids in the brain and to demonstrate ability to manipulate lipid content using exogenously administered lipids. Altered lipid content will be determined using Imaging Mass Spectrometry in brain regions over a range of ages in wild type animals and in a mouse model of AD (Tg2576 and TRAPOE4) for integration of a publically available lipid brain atlas.
Specific Aim 2. We will test the hypothesis that genetic manipulation of brain lipid content using Acsl6 knock-in (KI) can lead to changes in lipid composition and performance in behavioral tasks. Spatial memory, which is dependent on hippocampal function has been shown to decline during aging and in AD mice and will be assessed in rodents using 2-day Water Maze, Barnes Maze, spontaneous alternations Y-maze.
Specific Aim 3. We will test the hypothesis that Acsl6 KI will ameliorate functional defects in AD mouse models such as electrophysiological correlate to learning and memory, long term potentiation (LTP) and AD pathology and neuroinflammation.
e) Start and end date: 04/01/2021 – 08/31/2021
f) Level (%) of effort in the project: 30%
g) Point of contact at the funding agency: Amanda DiBatista
OVERLAP
There is no scientific overlap.

Completed
1R56AG062271-01A1 0 calendar months/no effort direct cost
06/15/2019 – 05/31/2021 (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.

Completed
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

Completed
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:
National Institute of Aging, 6.0 CM calendar months direct cost
NIH 1R01AG072794-01
9/01/2021 - 08/31/2026
Role:PI
a) Title: Acyl chain remodeling and regional lipid dysregulation in Alzheimer's disease
b) Funding Agency: NIH/NIA
c) Goals of the project:
d) Specific Aims/tasks:
SPECIFIC AIM 1: To test the hypothesis that lipid species and classes are regionally depleted in association with age and in mouse models of AD. We will test the hypothesis that polyunsaturated acyl chain composition among multiple phospholipid classes are disrupted in regions responsible for learning and memory such as the hippocampus. Un-targeted lipidomics using Desorption Electrospray Ionization (DESI) Imaging Mass Spectrometry and coordinate targeted lipidomics using liquid chromatrography/mass spectrometry (LC/MS) studies will be first in class to define the exquisitely specific regional distribution of lipids in the brain and to demonstrate ability to manipulate lipid content using exogenously administered lipids. Altered lipid content will be determined using Imaging Mass Spectrometry in brain regions over a range of ages in wild type animals and in a mouse model of AD (Tg2576 and TRAPOE4) for integration of a publically available lipid brain atlas.
Specific Aim 2. We will test the hypothesis that genetic manipulation of brain lipid content using Acsl6 knock-in (KI) can lead to changes in lipid composition and performance in behavioral tasks. Spatial memory, which is dependent on hippocampal function has been shown to decline during aging and in AD mice and will be assessed in rodents using 2-day Water Maze, Barnes Maze, spontaneous alternations Y-maze.
Specific Aim 3. We will test the hypothesis that Acsl6 KI will ameliorate functional defects in AD mouse models such as electrophysiological correlate to learning and memory, long term potentiation (LTP) and AD pathology and neuroinflammation.
e) Start and end date: 04/01/2021 – 08/31/2021
f) Level (%) of effort in the project: 30%
g) Point of contact at the funding agency: Amanda DiBatista
OVERLAP
There is no scientific overlap.

Completed
National Institute of Aging, 3.6 CM calendar months direct cost
NIH 1R56AG072794-01
4/01/2021 - 08/31/2021
Role:PI
a) Title: Acyl chain remodeling and regional lipid dysregulation in Alzheimer's disease
b) Funding Agency: NIH/NIA
c) Goals of the project:
d) Specific Aims/tasks:
SPECIFIC AIM 1: To test the hypothesis that lipid species and classes are regionally depleted in association with age and in mouse models of AD. We will test the hypothesis that polyunsaturated acyl chain composition among multiple phospholipid classes are disrupted in regions responsible for learning and memory such as the hippocampus. Un-targeted lipidomics using Desorption Electrospray Ionization (DESI) Imaging Mass Spectrometry and coordinate targeted lipidomics using liquid chromatrography/mass spectrometry (LC/MS) studies will be first in class to define the exquisitely specific regional distribution of lipids in the brain and to

demonstrate ability to manipulate lipid content using exogenously administered lipids. Altered lipid content will be determined using Imaging Mass Spectrometry in brain regions over a range of ages in wild type animals and in a mouse model of AD (Tg2576 and TRAPOE4) for integration of a publically available lipid brain atlas.
Specific Aim 2. We will test the hypothesis that genetic manipulation of brain lipid content using Acsl6 knock-in (KI) can lead to changes in lipid composition and performance in behavioral tasks. Spatial memory, which is dependent on hippocampal function has been shown to decline during aging and in AD mice and will be assessed in rodents using 2-day Water Maze, Barnes Maze, spontaneous alternations Y-maze.
Specific Aim 3. We will test the hypothesis that Acsl6 KI will ameliorate functional defects in AD mouse models such as electrophysiological correlate to learning and memory, long term potentiation (LTP) and AD pathology and neuroinflammation.
e) Start and end date: 04/01/2021 – 08/31/2021
f) Level (%) of effort in the project: 30%
g) Point of contact at the funding agency: Amanda DiBatista
OVERLAP
There is no scientific overlap.

Completed
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

Completed
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**

APPENDICES: N/A