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: Columbia University, New York, NY

REPORT DATE: October 2023

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE
October 2023

2. REPORT TYPE
Annual

3. DATES COVERED
15Sep2022-14Sep2023

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

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

Columbia University
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New York, NY 10032

8. PERFORMING ORGANIZATION REPORT NUMBER

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

U.S. Army Medical Research and Development 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 subcloned the catalytic domain of optogenetically activated PI kinases and administer the viral vector into mouse hippocampi to enrich $PI(4,5)P_2$. We have tested for amelioration of behavioral deficits associated with memory.

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 biospecimens. Biofluids and tissues will be analyzed for lipid content using targeted lipidomics. 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 focusing on case histories of traumatic brain injury (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 future prophylactic or therapeutic development of these targets.

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
					USAMRDC
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
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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 a genetic mouse model of AD-associated behavioral 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. 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. 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 behavioral deficits. 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. Biofluids and tissues will be analyzed for lipid content using targeted lipidomics as well as amyloid and tau pathologies. We will request clinical diagnoses 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.
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.
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.
Milestone #1: manuscript describing generation of in vivo tools for PI manipulation and potential ameliorative effects on AD associated behavior deficits.

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.
Milestone #2: Manuscript describing generation of in vivo tools for PI manipulation and potential ameliorative effects on AD associated behavior deficits

What was accomplished under these goals?

Major activities and accomplishments Specific Aim 1:

Specific Aim 1: Major Task 1: Construct development of optogenetic constructs and viral infection in cell lines and mouse embryonic stem cell derived neurons. COMPLETED
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. COMPLETED
Major Task 3: Experimental testing of contribution of phosphoinositide metabolism to behavioral outcome in AD mouse models. Express optogenetic constructs under control of optimized stimulation paradigm followed by behavioral analyses. Data analysis ongoing.
Milestone #1: manuscript describing generation of in vivo tools for PI manipulation and potential ameliorative effects on AD associated behavior deficits is currently in preparation.
Dr McIntire was the Initiating PI and Dr. Hussaini was previously the Partnering PI in the mechanism Convergence Science Research Award (CSRA) of the Peer Reviewed Alzheimer's Research Program (PRARP). Dr. Hussaini is now the approved PI of the award. Dr. McIntire is now Adjunct Assistant Professor, Department of Pathology and Cell Biology, Columbia University Medical School from 2/1/2022 – 12/31/2023 and is also Assistant Professor, Department of Radiology, Weill Cornell Medicine as of 2/1/2022.

The no-cost extension was approved 11/20/2023 for Period of performance: 15 September 2019 – 14 March 2024.

We have used an optogenetic protocol to introduce maintenance of phosphatidylinositol 4,5-bisphosphate in the hippocampi of a mouse model of Alzheimer's disease to ameliorate deficits in phosphoinositide metabolism and behavior. We injected AAV9 virus particles encoding the catalytic region of a light inducible dimerizable phosphoinositide kinase (PIP5K2A) into the hippocampus of a mouse model of Alzheimer's disease and cannulae were implanted above the injection site. Upon light stimulation, the kinase is recruited to the plasma membrane by binding to co-expressed CIBN-CAAX.

We have completed the viral injection, cannulae implantation, behavior and tissue harvesting and planned to determine the extent of AAV9 viral particle infection and induction of the catalytic domain of PIP5K2A (mCherry). The intensity of the mCherry is not sufficient for detection using standard microscopy, therefore, we will consider RNAscope based detection of PIP5K2A to determine the level of expression in future studies.

The brains were harvested from mice with either wild type (WT) or overexpressing amyloid precursor protein with the Swedish mutation (Tg^{+}) and optogenetically stimulated with either blue light or yellow light (control). Therefore, we had the following experimental groups [wild type-yellow light stimulation control (WTY), wild type-blue light stimulation (WTB), transgenic – yellow light stimulation control (TGY) and transgenic – blue light stimulation (TGB). In this study phosphoinositide and poly-phosphorylated phosphatidylinositide species were hypothesized to change due to the recruitment of the phosphoinositide kinase to the plasma membrane after blue light stimulation, but not yellow light stimulation. Mouse brains have been harvested and bisected. Half the brain was flash frozen in liquid nitrogen for analysis using imaging mass spectrometry with desorption electrospray ionization (DESI) imaging mass spectrometry (IMS) which has been completed.

We have optimized the Imaging Mass Spectrometry protocols and methods to determine regional distribution of changes of lipids, specifically of phosphoinositide modulation in mouse brain tissue. We developed methods for specific phosphoinositide detection of phosphoinositide (PI), phosphatidylinositol 4-phosphaste (PI4P) and phosphatidylinositol 4,5-bisphosphate using specific standards using the Waters Synpt-G2si for detection of phosphoinositides in brain tissue. Lipid content will be quantified regionally in brain using Desorption Electrospray Ionization (DESI)-mass spectrometry imaging using the HDI software available from Waters. We have used imaging mass spectrometry to detect regional changes in lipid composition with a focus on phosphoinositide lipids. Because we expect a local change in the lipid composition in the hippocampus, this methodology is particularly appropriate. The optogenetically-treated mouse brains were optimized with various acquisition settings, such as targeted (MS/MS) and untargeted; with (Trap Energy 40) and without (Trap Energy 4) fragmentation of precursor ions; as well as with (HDMS) and without (MS) Ion Mobility Separation. All these acquisitions were obtained by Synapt G2-Si DESI Imaging Mass Spectrometer from Waters. For that reason, several standards were purchased commercially for optimization of acquisition conditions for each phosphoinositide species. The standards and the mass to charge ratio (m/z), ionization mode, acquisition mode, resolution and scan rate are listed in the **Table 1**.

Table 1. Phosphoinositide and poly-phosphorylated phosphoinositide species

PI(P) Standard	m/z	Ionization mode	Acquisition mode	Resolution & Scan Rate
18:0-20:4 PI(4)P	965.5104 (z=1) 482.207 (z=2)	Negative	Sensitivity	60µm x 60µm 180µm/sec
18:0-20:4 PI(4,5)P_2	1045.4663 (z=1) 522.2317 (z=2)	Negative	Sensitivity	60µm x 60µm 180µm/sec
16:0-18:1 PI(4)P	915.4928 (z=1) 457.2017 (z=2)	Negative	Sensitivity	60µm x 60µm 180µm/sec
18:1 PI(4,5)P_2	1021.478 (z=1) 510.2322 (z=2)	Negative	Sensitivity	60µm x 60µm 180µm/sec
18:0 PI	865.5723 (z=1)	Negative	Sensitivity	60µm x 60µm 180µm/sec
18:0-20:4 PI	885.6172 (z=1)	Negative	Sensitivity	60µm x 60µm 180µm/sec
18:1 PI	861.5368 (z=1)	Negative	Sensitivity	60µm x 60µm 180µm/sec

We have optimized the Imaging Mass Spectrometry protocols and methods to determine regional distribution of changes of lipids, specifically of phosphoinositide modulation in mouse brain tissue. We developed methods for specific phosphoinositide detection of phosphoinositide (PI), phosphatidylinositol 4-phosphaste (PI4P) and phosphatidylinositol 4,5-bisphosphate using specific standards using the Waters Synpt-G2si for detection of phosphoinositides in brain tissue (Figure 1). Acquisition of m/z values were obtained by Synapt G2-Si DESI Imaging Mass Spectrometer from Waters. Because we expect a local change in the lipid composition in the hippocampus, this methodology is appropriate.

We have used Imaging Mass Spectrometry for analysis of hemi-brain of the ontogenetically manipulated mice. There are four mouse phenotypes in this project: wild type treated with yellow light (WTY), wild type treated with blue light (WTB), APP transgenic treated with yellow light (TgY), and transgenic treated with blue light (TgB).

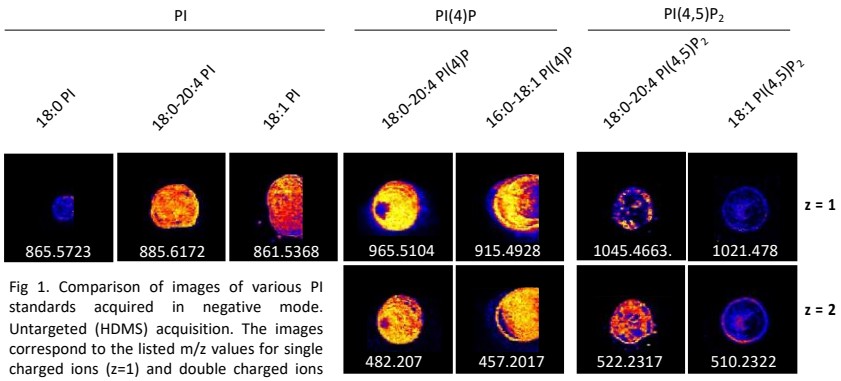

Fig 1. Comparison of images of various PI standards acquired in negative mode. Untargeted (HDMS) acquisition. The images correspond to the listed m/z values for single charged ions (z=1) and double charged ions (z=2).

We previously identified and optimized detection of 11 ions of interest which are phosphoinositide species (**Figure 1**). 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 standards 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 value, respectively [1] 965.5104 [2] 915.4928 [3] 1021.478 [4] 865.5723 [5] 885.6172 [6] 861.5368 [7] 522.2317 (z=2).

Specific objectives and significant results or key outcomes

We hypothesized that the abundance of these eleven ions between the phenotypes in the whole brain and/or hippocampus is altered by the optogenetic manipulation of the PI(4)P5Kinase which translocates to the plasma membrane after blue light stimulation. We developed a pipeline to normalize and quantify the results from the IMS acquisition. Briefly, we load the raw data composed of 18 total samples consisting of 5 WTY, 5 Tg, 4 WTB, and 4 TgB phenotypes into Python. The data is represented by a pixel by ion matrix for each sample. The number of pixels differs for each sample, and the number of total ions is always 4,083 based on the acquisition field. Then, we manually segment the brain using the Python package *mpl-interactions*. Using image *segmenter* we define the brain pixels and to the pixels surrounding the brain are set to zero (**Figure 2**). Highest intensity ions were used to visualize sections and select hippocampal region (**Figure 2).** Hippocampus was selected (mask) using Python package *mpl-interactions* image *segmenter* module (**Figure 2**). The pixel number was an output from image *segmenter*. We then normalize the ions using the total ion current (TIC), acquired during DESI run per pixel. For each pixel, the normalization

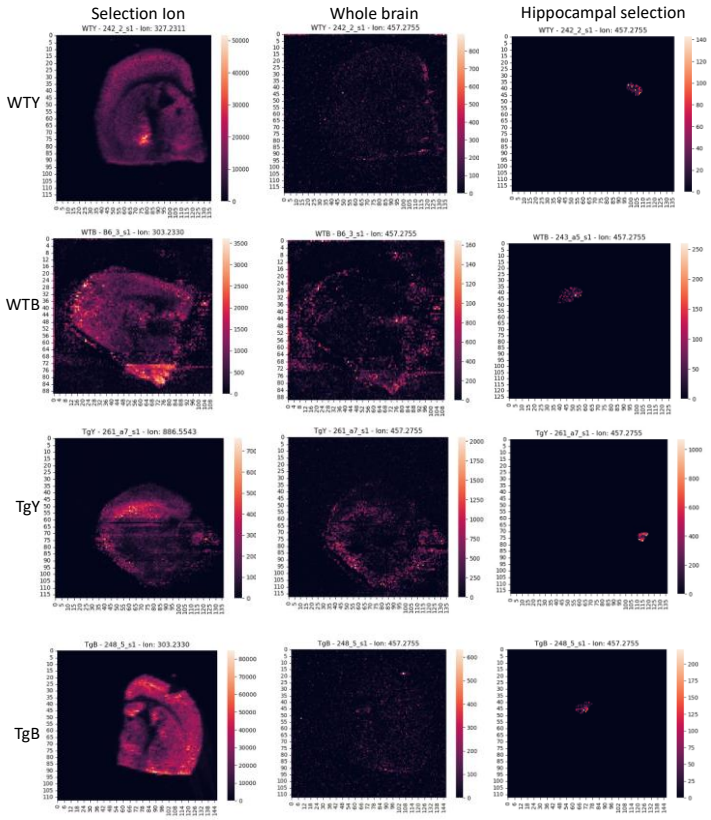

Figure 2. Visualization of DESI acquired images using Python. Whole brain was visualized with ion selected for whole brain intensity (selection ion) and 457.2755 [Note that scales bars represent different maximum for each image. Optimization of visualization by Python is ongoing.

sums all ion intensities and divides each ion by the total intensity which is a widely used method for normalization (ion% within pixel). The ion sums are normalized to brain size by dividing by the total number of pixels in the brain and calculate the sum of sums representing the total intensities of all the normalized ions within the brain across all ions (total brain intensity). The mol % of total brain is determined by dividing each ion sum by the sum of sums.

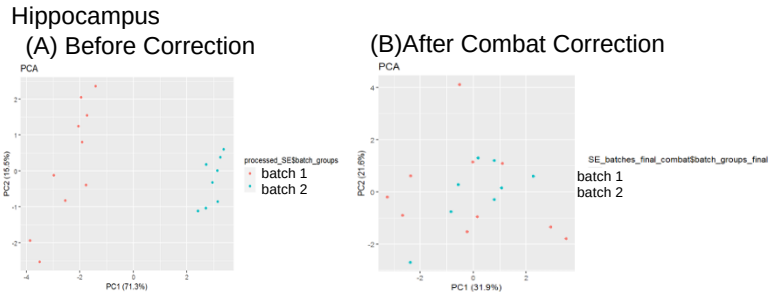

Figure 3. Samples were measured in 2 batches, represented by red and blue dots, respectively. (A) Before batch correction, our samples are completely separable by PCA using the percentages within the total brain of the 11 ions of interest. (B) After combat batch correction, our data is no longer separable by PCA.

These percentages were imported into *RStudio* for batch correction and z-score transformed. Batch correction was performed using *combat batch* correction, commonly used in -omics analysis and image analysis. Subsequent PCA was performed the overlap of the different batches after the correction is conducted is shown indicating successful batch correction (**Figure 3).** The ion intensity scales are different however, we are currently optimizing the visualization with normalized scales performed to compare the ion abundances of the 11 ions of interest among the treatment groups with FDR multiple testing correction. Since we found significant results for the ANOVA, p-values < 0.05, we conducted t-tests between all combinations of phenotypes (WTY vs TgY, WTB vs TgB, WTY vs TgB, WTB vs TgY, WTY vs WTB, and TgY vs TgB) to determine which groups were driving the significant differences found by the ANOVA and corrected for multiple testing using FDR analysis of the t-test results. None of our finding survived multiple testing correction, but we found increased levels of 16:0- 18:1 PI(4)P (m/z457.2017) in the APPsw mouse while stimulation of the PI(4)P5 kinase with blue light reduced the amount of PI(4)P only in the APPsw mice. This is consistent with the PI(4)P dysregulation the APPsw mouse model and responding to stimulation as the substrate for the PI(4)P5 kinase leading to consumption of this $PI(4,5)P_2$ precursor lipid. We also found that the levels of di18:1 $PI(4,5)P_2$ (m/z1021.478) were increased significantly in the WT mice and the same trend was seen in the increased median of the APPsw mouse hippocampus (Figure 4.)

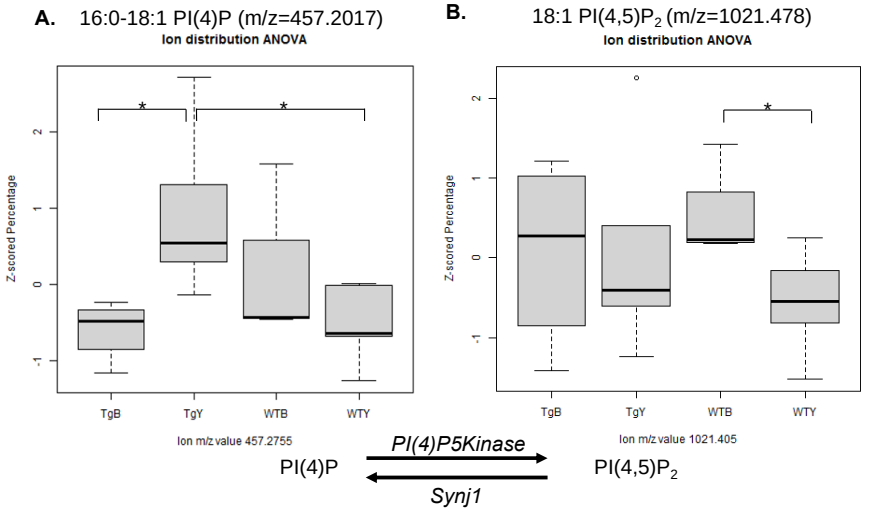

Figure 4. (A) Blue light stimulates the production of $PI(4,5)P_2$ at the expense of PI(4)P in the TgB group. Tg control mice (TgY) have significantly more PI(4)P than the wild type mice (WTY) stimulated with control (yellow) light. A significant loss of PI(4)P is seen after blue light stimulation in the Tg mice which ameliorates the PI(4)P2 to wild type levels. (B) Significant increase in $PI(4,5)P_2$ in hippocampus from wild type mice stimulated with blue light. A trend is seen in the Tg mice stimulated with blue light. Box plot shows median, quartiles and range. T-test without multiple comparison correction.

Interestingly, we also identified ions 885.5492 and 834.5284 and which are consistent with phosphatdiylinositol (PI) 38:4 and phosphatidylserine (PS) 40:6 respectively which were significantly reduced in the APPsw mouse hippocampus (**Figure 5**). These ions were also identified in a non targeted study of APPsw mice compared to WT mice over age. In this corresponding study, we first identified the most intense ions using the sum of sums, or total brain intensity. Then we used K-means cluster analysis, defined the number of clusters to 6 and visualized the clusters with t-SNE plots. We took the union of the top three clusters for each age (4 months, 12 months and 22 months) and identified the top 15 ions of interest (**Table 2**). PI38:4 is typically the precursor for PI(4)P and subsequently $PI(4,5)P_2$. PS transport has been shown to be regulated by PI(4)P levels.

We have used desorption electrospray ionization (DESI), untargted imaging mass spectrometry to detect phosphoinositides and phosphorylated phosphatidylinositides (**Figure 6).** We using this mass spectrometry based method of detection of phosphoinositides and continue to optimize the mass spectrometry methods for detection of PI(4,5)P2 species in brain tissue.

Table 2 Identity

m/z	(delta < 0.01)
281.2486	FA 18:1
283.2643	FA 18:0
303.233	FA 20:4
327.233	FA 22:6
834.5291	PS 40:6
886.5604	PS 44:8
788.5447	PS 36:1
836.5447	PS 40:5
790.5392	PE O-40:7;O/PE40:6
774.5443	PE O-40:7
766.5392	PE O-38:5;O/PE38:4
885.5499	PI 38:4/DG 32:3
835.5342	PI 34:1
747.5182	PG 34:1/LBPA34:1
888.624	SHexCer 42:2;O2

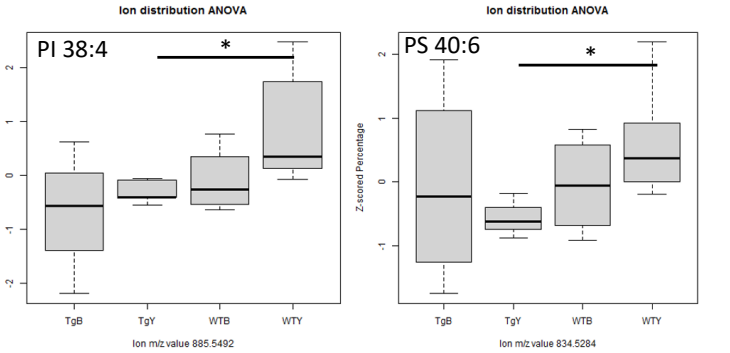

Figure 5. Top 15 ions of interest were identified with K-means clustering, using 6 clusters, of ions from WT and APPsw mouse model of 3 different ages (4 month, 12 month, 22 month). T-SNE plots were used to visualize the data and the union of the top 3 clusters (Table 1). Two of the same ions were identified in our analysis for significantly differential lipids, prior to FDR correction.

We have finished the mass spectrometry acquisition and are currently analyzing these data and preparing for publications of the findings. The second half of the brain will be processed for liquid-chromatography and HPLC-suppressed conductivity detection of anionic lipids after acquisition of a replacement HPLC/IC. In addition to imaging mass spectrometry, we have also We have also received notification of Award for replacement of the HPLC which we can use in future studies to confirm the imaging mass spectrometry results. We have received notification that our grant was approved by the Office of Naval Research to support acquisition of a new HPLC/IC-suppressed conductivity system.

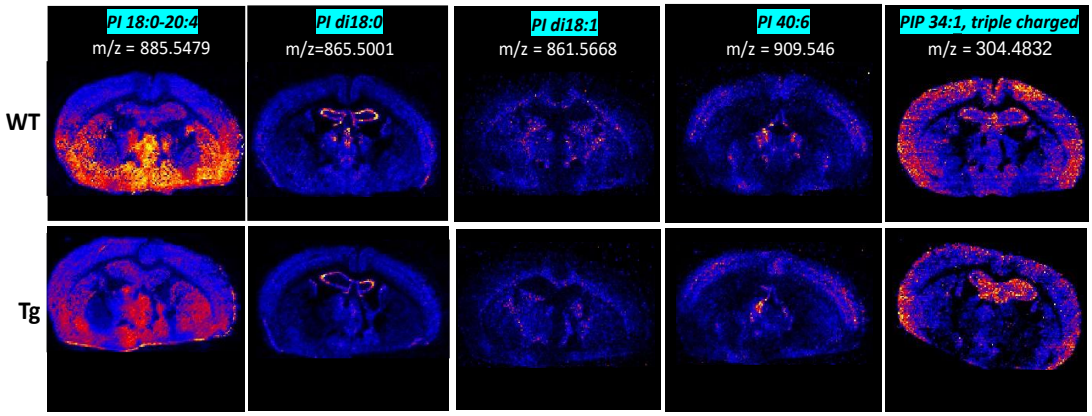

Figure 6. DESI images of WT and Tg mouse brains for the ions m/z = 885.5479; 865.5001; 861.5668; 909.546 and 304.4832 acquired in negative mode. The m/z are consistent the lipid identities in blue determined by the standards or by interrogation of similar m/z in Lipid Maps Datatbase. The 6 month old WT and Tg brains are representative of biological triplicates.

We have acquired the instrumentation necessary for Ion Chromatography dedicated to anionic lipid and phosphoinositide detection using suppressed conductivity, (Thermo Electron North America) Full Integrion system, including HPIC Pump, 6-port injection valve, 25 µL injection loop, 2 L eluent bottle, one electrolytic power outlet (for suppressor), low-pressure eluent degasser, high pressure RFIC-EG degasser, and an additional two electrolytic power outlets (for eluent generation) apparatus equipped with a conductivity detector for detection of negatively charged lipids. The setup includes an autosampler, tablet for external instrument control and Chromelean software. Purchase of this instrument has been approved by Department of Defense (DOD) University Research Instrumentation Program 10/01/2022– 12/31/2023 (DURIP) McIntire, LB (PI), GRANT13369767/GRANT13710486 Phospholipid detection in prognosis for Traumatic Brain Injury and Alzheimer's disease Department of the Army; Office of Naval Research.

We will use a targeted panel of lipid standards to optimize a protocol to detect phosphoinositides based on our previous work (McIntire et al., 2012). Phosphoinositides will be quantified using bulk targeted from homogenate (relative to mole percent of total lipid) as well as spatially within tissue using imaging mass spectrometry. The instrument was installed and we received a two day training for lab members. We will be able to detect the polyphosphorylated species including phosphatidic acid (PA) at a retention time of 15.733 – 15.77 minutes and phosphatidylinositol (3,5)-bisphosphate [PI(3,5)P_2] with a retention time of 55.080 – 55.120 minutes. The A

doublet of phosphatidylinositoal (4,5)-bisphosphate [PI(4,5)P_2] is expected to be detected at 55.690 minutes**.** Our previous method also allows detection of phosphatidylinositol, cardiolipin (retention time approximately 13.39 minutes, phosphatidylserine (13.77 minutes), phosphatidic acid (14.08 minutes), phosphatidylinositol 4-phosphate (20.99 minutes), phospatidylinositiol 3-phosphate (21.48 minutes).

We have used desorption electrospray ionization (DESI), imaging mass spectrometry to detect phosphoinositides and phosphorylated phosphatidylinositides (**Figure 1-2).** We have prioritized using this mass spectrometry based method of detection of phosphoinositides and continue to optimize the mass spectrometry methods for detection of PI(4,5)P_2 species in brain tissue. We will use HPLC detection of polyphosphorylated phosphoinositides, specifically PI(3,4)P_2 and PI(4,5)P_2 which we can use in future studies to confirm the mass spectrometry results.

Our data indicate that optogenetic stimulation of PI(4,5)P2 accumulation at the plasma membrane (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 synthesis at 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 blue light stimulated mice (Tg-Opto) through optogenetic stimulation. Control animals are stimulated with control yellow light (λ=595). No motor effects were evident in the distance travelled, center crossings, speed or time spent in the center of an open field test. We are currently preparing these data for publication.

Discussion of stated goals not met:

Since we have positive results from the behavioral assessment and expect to find significant results from lipid analyses described above, we will not pursue the acute infusion of Aβ. Acute models through infusion of Aβ peptide are likely to be highly variable and unlikely to produce robust results. Therefore, we will have accomplished our goal to test the hypothesis that optogenetically mediated enrichment of phosphoinositide levels in mouse brain will ameliorate AD associated behavioral deficits in genetic mouse models of AD-associated cognitive and synaptic deficits.

Milestone #1: manuscript describing generation of in vivo tools for PI manipulation and potential ameliorative effects on AD associated behavior deficits. 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.

Major activities and accomplishments Specific Aim 2:

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, CSF and plasma in human context of AD and TBI (Months 1-36).

We have previously received approval for the Columbia University IRB for the change of PI to Dr. James Noble and the continuation AAAT8658(M00Y02) Approved 10/27/2022 Expires 10/26/2023 which was approved by the U.S. Army Medical Research and Development Command (USAMRDC), Office of Human and Animal Research Oversight (OHARO), Office of Human Research Oversight (OHRO) on 22 March 2022. The Columbia University Medical Center Institutional Review Board approved the protocol continuation on 24 August 2023;

this approval will expire on 23 August 2024. The USAMRDC OHARO OHRO received the Columbia University Medical Center Institutional Review Board (IRB) approval on 19 October 2023 and correspondence on October 19, 2023 acknowledged OHRO receipt of the continuing review documents for the protocol. No further action related to this continuing review is needed at this time.

Specific objectives and significant results or key outcomes:
We have received approval for the Columbia University IRB for the change of PI to Dr. James Noble and the continuation AAAT8658(M00Y02) Approved 10/27/2022 Expires 10/26/2023. On November 30, 2022 we received the Continuing Review Acceptance for the Protocol, "Lipid Dysregulation in Alzheimer's Disease and Traumatic Brain Injury," Submitted by Dr. Laura Beth McIntire, PhD, Columbia University, in Support of the Proposal, "Optogenetic Regulation of Phosphoinositide Metabolism in Susceptibility, Resistance, and Resiliency to Alzheimer's Disease-Associated Deficits and Pathology," Submitted by Dr. Laura Beth McIntire, PhD, Columbia University Medical Center, New York, New York, Proposal Log Number AZ180124, Award Number W81XWH-19-1-0817, OHRO Log Number E01016.2a from Ms. Jill Graygo, MPH, MSE; Human Subjects Protection Scientist; Office of Human Research Oversight; Office of Human and Animal Research Oversight; U.S. Army Medical Research and Development Command; Email: jill.m.graygo.civ@health.mil The OHRO point of contact for this study is Mrs. Angela Urbina, BS, Human Subjects Protection Scientist, at 301-619-2370/angela.c.urbina.ctr@health.mil

The Columbia University Medical Center Institutional Review Board approved the protocol continuation on 24 August 2023; this approval will expire on 23 August 2024. The USAMRDC OHARO OHRO received the Columbia University Medical Center Institutional Review Board (IRB) approval on 19 October 2023 and correspondence on October 19, 2023 acknowledged OHRO receipt of the continuing review documents for the protocol. No further action related to this continuing review is needed at this time.

We submitted a request for no-cost extension August 2023 and the no-cost extension was approved 11/20/2023 for Period of performance: 15 September 2019 – 14 March 2024.

Discussion of stated goals not met:
We are preparing to submit samples for lipidomic analysis in the Biomarkers Core at Columbia University. We will accomplish data analysis, figure preparation and manuscript preparation.

Milestone #2: Manuscript describing generation of in vivo tools for phosphoinositide manipulation and potential ameliorative effects on AD associated behavior deficits. We expect to submit our findings from human tissues after completing analysis of phospholipid content. These results may be submitted as a single publication with results from Specific Aim 1.

Based on the current scope of the project we are not pursuing a second IRB approval to pursue lipidomic studies with di-identified, previously collect human biospecimens as previously described 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 will not pursue any additional IRB approvals at this time.

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.

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.
Subtask 3: Lipid analysis of brain from multiple stimulation paradigms using HPLC suppressed conductivity detection of anionic lipids. Western analysis will be done of brain tissue to determine expression level of protein of interest including mCherry-CRY2-PI4P-5Kinase; mCherry-CRY2-PI4KinaseIIα.
Milestone #1: *manuscript describing generation of in vivo tools for phosphoinositide manipulation and potential ameliorative effects on AD associated behavior deficits*

We are completing analysis of quantified levels of phosphoinositides using imaging mass spectrometry on the mouse brain from animals that underwent behavioral testing. The brains were bisected to reserve half the brain for Imaging Mass Spectrometry, analysis has been completed and figures are being prepared for publication. Using targeted panel of phosphoinositides we have developed a pipeline for quantification of the relative fold change in lipid content through batch correction and statistical testing. Though our current analysis did not survive multiple testing correction, we expect sufficient sample numbers (n) would allow us to obtain power to detect significant differences in phosphoinositide lipid content. The trends we observed were consistent with the hypothesis that PI4P5K was sufficiently recruited to the PM to allow the accumulation of the enzymatic product $PI(4,5)P_2$.

We are preparing a manuscript for publication describing generation of in vivo tools for PI manipulation and potential ameliorative effects on AD associated behavior deficits. 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.

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.
Major Task 1: LC/MS-MS targeted lipidomics of brain, CSF and plasma in human context of AD and TBI

Acquire biospecimens, brain tissue, CSF and blood from ADRC anonymized AD and age matched control groups. Human brain tissue, from ADRC and New York Brain Bank (Columbia University). Samples will be acquired from bio-bank storage facility at Columbia University Medical Center on dry ice and stored at -80C until lipid extraction, Subtask 2.

Subtask 2. Lipid extraction of samples storage at -80C until analysis in subtask 3 (HPLC) and subtask 4 (LC/MS-MS).

Subtask 3: HPLC for phosphoinositide and anionic phospholipid

Subtask 4: Targeted Lipidomics using LC/MS-MS for plasma, CSF and brain tissue. Statistical methods for brain tissue between paired groups (AD and control) will be compared using Student's t-test to test for significance at $p<0.05$ with 2-tailed distribution and equal variance. For statistical analysis for plasma and CSF ANOVA will be used for comparison of multiple groups, (AD, control and AD+TBI). Planned comparisons will be used for post-hoc analysis. Significance will be set for $p<0.05$. ANOVA will be appropriate for comparison of lipidomic analyses from multiple groups including specimens from those with diagnosed AD, TBI and control groups.

Milestone #2: *Manuscript describing generation of in vivo tools for PI manipulation and potential ameliorative effects on AD associated behavior deficits*

The above represent our original aims. We are currently in the process of identifying and acquiring biospecimens from the Columbia University Medical Center Alzheimer's Disease Research Center (ADRC). Due to unavailability of matched EDTA plasma and brain tissue, we have now requested EDTA plasma from normal controls and those with self-reported TBI and will request brain tissue in a future request. We have prioritized plasma and brain tissue at this time for analysis over CSF and will determine if CSF analysis is necessary after results are obtained from plasma and brain tissue. If plasma and brain tissue show no difference between control and TBI, we will not analyze CSF with lipidomic methods.

We will prioritize targeted lipidomics analysis of phosphoinositide and phospholipid levels using mass spectrometry over detection of anionic lipids using HPLC due to the availability of lipidomic mass spectrometry at Columbia University.

We will submit the samples for lipid extraction and targeted lipidomic analysis by the Biomarker's Core run by Dr. Nandakumar at Columbia University Medical Center. All biospecimens and data are de-identified. Samples to be stored at -80C until lipid extraction and targeted lipidomics in collaboration with the Irving Institute Biomarker's core facility at Columbia University.

We will prepare a manuscript describing the findings from the lipidomic analysis which may be combined with our findings from Aim 1.

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. 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. 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. Our technology will be of value to multiple fields in which phosphoinositides play a critical role in cellular function. In addition to a neuronal function, phosphoinositides also play a critical role in cancer biology.

What was the impact on technology transfer?

Nothing to Report

What was the impact on society beyond science and technology?

We have identified a potential new treatment target for Alzheimer's disease. Ultimate development of this strategy for amelioration of lipid levels in the brain has potential to impact the patients, caregivers and the medical support networks through discovery of novel pathways in lipid metabolism which can be harnessed for potential prophylactic or ameliorative therapies.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

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.*

Due to unavailability of matched EDTA plasma and brain tissue, we have now requested EDTA plasma from normal controls and those with self-reported TBI and will request brain tissue in a future request. We have prioritized plasma and brain tissue at this time for analysis over CSF and will determine if CSF analysis is necessary after results are obtained from plasma and brain tissue. If plasma and brain tissue show no difference between control and TBI, we will not analyze CSF with lipidomic methods.

We will prioritize targeted lipidomics analysis of phosphoinositide and phospholipid levels using mass spectrometry over detection of anionic lipids using HPLC (subtask 3.) due to the availability of lipidomic mass spectrometry at Columbia University.

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

Nothing to Report

Changes that had a significant impact on expenditures

Nothing to Report

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.

Other Publications with Dr. McIntire as co-author

Neel H Mehta, Liangdong Zhou, Yi Li, **Laura Beth McIntire**, Anna Nordvig, Tracy Butler, Mony de Leon, Gloria C Chiang for the Alzheimer's Disease Neuroimaging Initiative. (2023) Altered peripheral immune cell ratios in Alzheimer's are associated with cortical amyloid deposition and longitudinal cognitive decline. Science Reports 2023 May 31;13(1):8847

Mikaila Ann Bantugan, Haotian Xian, Victoria Solomon, Maria Del Sol Gómez Loarte, Mitchell Lee, Cristiana Meuret, Alfred Fonteh, Meredith Braskie, **Laura Beth J. McIntire,** Luica Jurin, Sarah Oberlin, James Evans, Roderick Davis, Laila Abdullah, Hussein N. Yassine. (2023) Associations of ApoE4 status and DHA supplementation on plasma and CSF lipid profiles and entorhinal cortex thickness. Journal of Lipid Research 2023 Jun;64(6):100354

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. (2023) mTORC2-NDRG1-Cdc42 axis couples fasting to mitochondrial fission. Nature Cell Biol. 2023 Jul;25(7):989-1003

Mara De Martino, Camille Daviaud, Hanna E. Minns, Artur Lazarian, Anja Wacker, Ana Paula Costa, Nabeel Attarwala, Qiuying Chen, Seung-Won Choi, Raùl Rabadàn, **Laura Beth J. McIntire**, Robyn D. Gartrell, James M. Kelly, Evagelia C. Laiakis, Claire Vanpouille-Box, (2023) Radiation therapy promotes unsaturated fatty acids to maintain survival of glioblastoma, Cancer Letters, 2023 Aug 28;570:216329, ISSN 0304-3835

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

Nothing to Report

Other publications, conference papers, and presentations.

Oluwafunke Kolawole, Merav Antman-Passig, PhD, Laura Beth McIntire, PhD, Daniel Heller, PhD. Carbon Nanotubes Amyloid Beta Sensor Responds to Aβ Associated to Lipids Annual Biomedical Research Conference for Minoritized Scientists (ABRCMS), Anaheim, CA Nov 9-12, 2022

Costa A.P., Lazarian A., Batra R., Krumsiek J., Butler T., Glodzik L., Xi K., Wang X., Initiative A.A., Leon M.J., McIntire L.B. Functional role of the endogenous nanoparticles in Alzheimer's disease. Selected Oral Presentation. Alzheimer's Disease and Parkinson's Disease International Conference (ADPD), Gotenborg, Sweden March 28 – April 1, 2023, (#1075) 3/31/2023

Lazarian A., Costa A.P., Kim Y.A, McIntire L.B., Tremendous Spatial Lipid Dyshomeostasis revealed by DESI Imaging Mass Spectrometry between normal aging and Alzheimer's disease mouse brains. Alzheimer's Disease and Parkinson's Disease International Conference (ADPD), Goteborg, Sweden March 28 – April 1, 2023, (#1868) Selected Oral Presentation 3/30/2023

McIntire, LBJ., Lazarian A., Costa A.P., Mares J., Nuriel T., Karan C., Pampou S., Nandakumar R., Xu O., Changule J., Xu Y., Kolawole O., Antman-Passig M., Heller D., Menon V, Consequences of Acyl chain dysregulation in Alzheimer's disease. Alzheimer's Disease and Parkinson's Disease International Conference (ADPD), Goteborg, Sweden March 28 – April 1, 2023, #1858 Selected Oral Presentation 3/30/2023

Leon M.J., Wang X., Xi K., Mehta N., Tanzi E., Spector E. Zhou L., Kelly J. Butler T., Pahlajani S., Nordvig A.S., Glodzik L., McIntire L.B., Dyke J., Okamura N., Carare R., Rusinek H., Chiang G.C., Li Y. CSF drainage pathways are reduced in normal aging using PET with [11C]-butanol and associated with brain amyloid. Alzheimer's Disease and Parkinson's Disease International Conference (ADPD), Goteborg, Sweden March 28 – April 1, 2023, (#1143) Selected Oral Presentation 4/01/2023

Erica Acquarone, Ana Paula Costa, Jonathan Van de Loo, Damian Williams, Hong Zhang, Agnieszka Staniszewski, Luciano D'Adamio, Daniela Puzzo, Laura Beth McIntire, Andrew Teich, Ottavio Arancio. Presynaptic amyloid precursor protein is necessary for the impairment of synaptic function and memory caused by extracellular amyloid-beta and tau oligomers. Alzheimer's Association International Conference (AAIC) 2023, Amsterdam Netherlands, 07/16-07/20/2023 (75844) 7/19/2023

Krista Wartchow, Ana Paula Costa, Artur Lazarian, Nicholas Bartelo, Vilas Menon, Jason Mares, Tal Nuriel PhD, Piali Mukherjee, Laura Beth McIntire. Dysregulated Lipid Metabolism and ACSL6 Expression in Alzheimer's Disease: Insights from Spatial Lipidomic and Transcriptomic Analysis in a Mouse Model. Kern Lipid Conference Lipids in Aging, Lifespan and Aging-Associated Diseases 8/14-8/16, Vail, CO. 08/15/23

William Jones Dartora, Ana Paula Costa, Gloria Chiang, Richa Batra, Jan Krumsiek, **Laura Beth McIntire**, with the Alzheimer's Disease Neuroimaging Initiative (ADNI). Investigating the association between lipid profiles and Alzheimer's disease outcomes: an analysis of the ADNI study. Kern Lipid Conference Lipids in Aging, Lifespan and Aging-Associated Diseases 8/14-8/16, Vail, CO. 08/15/23

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) as a

description of the methods we have employed. Since we have generated new constructs which have not yet been described, we expect publication in a high impact journal due to the novelty of our approach.

Desorption electrospray ionization (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?

Dr. Syed Abid Hussaini

Project Role: Principle investigator (previously Partnering Investigator)
Nearest person month worked: voluntary
Contribution to Project: Dr. Hussaini was the partnering PI in the mechanism Convergence Science Research Award (CSRA) of the Peer Reviewed Alzheimer's Research Program (PRARP). We requested Dr. Abid Hussaini, who has been the Partnering PI to transition to the PI of the grant. The change in PI has been approved along with a no-cost extension on 11/18/2022. We submitted a request for no-cost extension August 2023 and the no-cost extension was approved 11/20/2023 for Period of performance: 15 September 2019 – 14 March 2024.

Laura Beth McIntire

Project Role: Previously Principle Investigator, now Significant Collaborating Investigator
Nearest person month worked: voluntary
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. Dr. McIntire is now Adjunct Assistant Professor, Department of Pathology and Cell Biology, Columbia University Medical School (2/01/22 – 12/31/2023) and has also been appointed Assistant Professor, Department of Radiology, Director of the Lipidomics and Biomarker Discovery Lab in the Brain Health Imaging Institute at Weill Cornell Medicine as of 2/1/2022. The change in PI has been approved along with a no-cost extension 11/18/2022. Dr. McIntire remains dedicated to the completion of the research. Dr. McIntire is guiding the work with imaging mass spectrometry quantification of phosphoinositide species.

Dr. James Noble

Project Role: Co-Investigator, IRB Principle Investigator
Nearest person month worked: voluntary
Contribution to Project: Dr. Noble met with Dr. McIntire to advise on IRB submission and ADRC at Columbia University. Dr. Noble advised Dr. McIntire on needed IRB approvals. The U.S. Army Medical Research and Development Command (USAMRDC), Office of Human and Animal Research Oversight (OHARO), Office of Human Research Oversight (OHRO) approved the subject protocol on 22 March 2022. The amendment which allows for change in the protocol site investigator from Laura Beth McIntire, PhD to James Noble, MD with continuation AAAT8658(M00Y02) was approved 10/27/2022 and expires 10/26/2023 by the Columbia University on 27 October 2022. On November 30, 2022 we received the Continuing Review Acceptance for the Protocol, "Lipid Dysregulation in Alzheimer's Disease and Traumatic Brain Injury," Submitted by Dr. Laura Beth McIntire, PhD, Columbia University, in Support of the Proposal, "Optogenetic Regulation of Phosphoinositide

Metabolism in Susceptibility, Resistance, and Resiliency to Alzheimer's Disease-Associated Deficits and Pathology," Submitted by Dr. Laura Beth McIntire, PhD, Columbia University Medical Center, New York, New York, Proposal Log Number AZ180124, Award Number W81XWH-19-1-0817, OHRO Log Number E01016.2a from Ms. Jill Graygo, MPH, MSE; Human Subjects Protection Scientist; Office of Human Research Oversight; Office of Human and Animal Research Oversight; U.S. Army Medical Research and Development Command; Email: jill.m.graygo.civ@health.mil The OHRO point of contact for this study is Mrs. Angela Urbina, BS, Human Subjects Protection Scientist, at 301-619-2370/angela.c.urbina.ctr@health.mil. The USAMRDC OHARO OHRO received the Columbia University Medical Center Institutional Review Board (IRB) approval on 19 October 2023. The Columbia University Medical Center IRB approved continuation of the subject protocol on 24 August 2023; this approval will expire on 23 August 2024. The USAMRDC OHARO OHRO received the Columbia University Medical Center Institutional Review Board (IRB) approval on 19 October 2023 and correspondence on October 19, 2023 acknowledged OHRO receipt of the continuing review documents for the protocol.

Dr. Artur Lazarian, Weill Cornell Medicine
Project Role: Postdoctoral Research Scientist, Brain Health Imaging Institute, Department of Radiology, Weill Cornell Medicine
Nearest person month worked: volunteer
Contribution to Project: Dr. Lazarian has developed and optimized effective strategies for detecting phosphoinositides in mouse brain tissue using DESI-Imaging Mass Spectrometry.

Nicholas Bartelo
Project Role: Graduate student, Physiology, Biophysics and Systems Biology (PBSB) Graduate Program Rotation, Weill Cornell Medicine
Nearest person month worked: volunteer
Contribution to Project: Quantification and analyses of imaging mass spectrometry data.

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

Dr. Syed Abid Hussaini, PhD. – Other Support
Active research support:
Title: Electrophysiological Evaluation of Brain Regions Vulnerable to Alzheimer's Disease
Supporting Agency: NIH/NIA R01AG064066-03S1
PI: Hussaini, Syed Abid
Name and Address of the Funding Agency Officer: Newman, Elizabeth, newmanela@mail.nih.gov
Performance Period: 09/01/2022 – 03/31/2024
Level of Funding (Including Indirect Costs):
Project Goal and Aims: The major goal of this supplement is to make the existing in vivo electrophysiology data open source by building converters and making it available freely for computational analysis. Further, it will incorporate tools for sorting and analyzing data via open-source platforms.
Overlap: None

Title: A computational approach to predict early symptoms of AD
Supporting Agency: ALZA AARG-20-685513
PI: Hussaini, Syed Abid
Name and Address of the Funding Agency Officer: grantsapp@alz.org
Performance Period: 07/01/2020 – 12/31/2023 (NCE)
Level of Funding (Including Indirect Costs):
Project Goal and Aims: The goal of this project is to probe the Lateral Entorhinal Cortex (LEC)- one of the most vulnerable regions affected by AD and apply a computational approach to identify dysfunction earlier than

currently possible. The project will combine in vivo electrophysiology with LEC-specific object- and odor-memory tasks to monitor neuronal function in a mouse model of AD. Using machine learning algorithms, the project aims to detect early signs of neuronal vulnerability prior to onset of cognitive symptoms.
Overlap: None

Title: Electrophysiological Evaluation of Brain Regions Vulnerable to Alzheimer's Disease
Supporting Agency: NIH/NIA R01AG064066
PI: Hussaini, Syed Abid
Name and Address of the Funding Agency Officer: Newman, Elizabeth, newmanela@mail.nih.gov
Performance Period: 04/15/2020 – 03/31/2025
Level of Funding (Including Indirect Costs):
Project Goal and Aims: The major goal of the project is to identify how vulnerable brain regions are affected by tau and abeta pathology. We will use in vivo electrophysiology and optogenetic methods combined with machine learning approach to identify and reverse neuronal dysfunction.
Overlap: None

Completed research support:
Title: Decoding Early Signs of Alzheimer's Disease in The Lateral Entorhinal Cortex Using Machine Learning
Supporting Agency: NIH/NIA R21AG066168
PI: Hussaini, Syed Abid
Name and Address of the Funding Agency Officer: Dibattista, Amanda amanda.dibattista@nih.gov,
Performance Period: 09/15/2019 – 05/31/2023 (2nd NCE)
Level of Funding (Including Indirect Costs):
Project Goal: The major goal of this project is to identify early signs of neuronal dysfunction in the lateral entorhinal cortex, which is selectively vulnerable in Alzheimer's disease.
List of the Specific Aims:
Aim 1: Evaluate LEC function in the younger mice in order to detect neuronal changes prior to behavioral deficits.
Aim 2: Record LEC activity with silicon probes and test responses towards objects, odors and passage of time.
Aim 3: Use a computational approach such as machine learning to determine if ensemble properties of LEC neurons are affected by tau and Aβ.
Overlap: None

Title: Mechanistic Monitoring of Ultrasound Neuromodulation
Supporting Agency: NIH/NIBIB R01EB027576
PI: Konofagou, Elisa
Name and Address of the Funding Agency Officer: King, Randy Lee, randy.king@nih.gov,
Performance Period: 04/04/2019 – 12/31/2023 (NCE)
Level of Funding (Including Indirect Costs):
Project Goal: In this study, we will develop novel tools for improvement of focused ultrasound by monitoring its physiological and physical effects on the brain in real time.
List of the Specific Aims:
Aim 1: To harness from the technical expertise available by the group of investigators so as to develop monitoring of the underlying physical and physiological mechanisms in vivo and in real time and simultaneously sync technologies that will allow translation to humans.
Aim 2: To optimize targeting and efficacy of FUS neuromodulation by mapping the physical mechanism so as to better explore noninvasive modulation of motor and motivation responses in humans for the first time for the ultimate treatment of conditions ranging from movement to psychiatric disorders.
Overlap: None

MCINTIRE OTHER SUPPORT

Active:

National Institute of Aging
NIH 1 R01 AG078800-01
09/15/2022 – 06/30/2027
Role: Dr. McIntire, Contact PI
Multi-PI, Dr. Vilas Menon, Columbia University Medical Center Dr. Tal Nuriel, Columbia University Medical Center
Title: Spatial dysregulation of the lipidome in Alzheimer's disease human and mouse brain
Funding Agency: NIH/NIA
Goals of the project: In response to RFA-AG-22-019, High Resolution Mapping of Biomolecules in Brain Cells in Aging and Alzheimer's Disease, we will determine the spatial distribution of lipids in human and mouse brain.
Specific Aims/tasks:
Specific Aim 1. To test the hypothesis that lipid(s) and enzymes are implicated in acyl chain remodeling play a role in AD pathology
Specific Aim 2. We will test the hypothesis that young, non-pathology bearing ApoE4 carriers will show characteristic changes in regional lipid composition in human autopsy tissues
Specific Aim 3. We will integrate the lipidomic imaging data into a publicly available database.
Start and end date: 09/15/2022 – 06/30/2027
Level of Funding (Including Indirect Costs):
Level (%) of effort in the project: 40%
Point of contact at the funding agency: Dr. Austin Yang, PhD <Austin.yang@nih.gov>
Overlap: None

National Institute of Aging
NIH 1R01AG072794-01
9/01/2021 - 08/31/2026
Role: Dr. McIntjre, PI
Title: Acyl chain remodeling and regional lipid dysregulation in Alzheimer's disease
Funding Agency: NIH/NIA
Goals of the project: To 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, pathology and neuroinflammation.
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.
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.
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.
Start and end date: 09/01/2021 – 05/31/2026
Level of Funding (Including Indirect Costs):
Level (%) of effort in the project: 30%;
Point of contact at the funding agency: Dr. Amanda DiBattista, PhD <amanda.dibattista@nih.gov>
Overlap: None

Department of Defense (DOD) University Research Instrumentation Program (DURIP)
GRANT13369767/GRANT13710486
02/01/2022– 01/31/2023

Role: Principle Investigator (PI)
Title: Phospholipid detection in prognosis for Traumatic Brain Injury and Alzheimer's disease
Funding Agency: Department of the Army; Office of Naval Research
Goals of the project: To use suppressed conductivity High Performance Liquid Chromatography/Ion Chromatography to detect changes in phospholipids in association with Traumatic Brain Injury and Alzheimer's disease.
Specific Aims/tasks: Acquisition of Instrument HPLC/IC-Suppressed Ion Conductivity Detection –ThermoFisher Integrion Ion Chromatography with Eluent Generation, Integrion IC with Eluent Generation, Integrion system, including HPIC Pump, eluent generator, autosampler. The instrument will be integrated into existing facilities of the McIntire Lab. Justification for Instrument Need: The DoD Defense University Research Instrumentation Program (DURIP) will support funding for instrumentation to support lipid research in a broad range of indications including, but not limited to Traumatic Brain Injury (TBI), aging, Alzheimer's Disease, dopaminergic transmission in Parkinson's disease, and novel lipid sensing of mechanistic target of rapamycin-complex 1(mTOR).
Start and end date: 02/01/2022– 12/31/2023
Level (%) of effort in the project: 0% Instrumentation only
Point of contact at the funding agency: Dr. Timothy B. Bentley, PhD <timothy.b.bentley6.civ@us.navy.mil>; McKee, Lauren G CIV USN (USA) <lauren.g.mckee.civ@us.navy.mil
Overlap: None, instrumentation only

Role: Co-Investigator: McIntire, PD/PI: Tracy Butler (contact), Yi Li, Sudhin Shah
Title: Brain fluid clearance and misfolded protein dynamics following traumatic brain injury
Funding Agency: NIH/NIA 1 R01 AG077576-01A1
Goals of the project: Using Positron Emission Tomography (PET) and Magnetic Resonance Imaging (MRI) performed as soon as possible after TBI, this project will monitor the deposition and rate of clearance of amyloid and tau proteins, with the goal of better understanding how brain clearance may relate to TBI recovery and later risk of neurodegeneration
Start and end date: 07/01/2023-06/30/2028
Level (%) of effort in the project: 5%
Point of contact at the funding agency: rovescaa@mail.nih.gov Alessandra Rovescalli
Overlap: None

Role: Co-Investigator: McIntire, PD/PI: Wang (contact); Chiang (mPI)
Title: Quantitative MRI-based cerebral oxygen metabolism in Alzheimer's disease
Funding Agency: NIH/NIA 1 R01 AG080011-01
Goals of the project: The study team's long-term objective is to develop noninvasive challenge-free MRI-based quantitative mapping of cerebral oxygen metabolism as a biomarker of neuronal viability in Alzheimer's disease.
Start and end date: 07/01/2023-06/30/2028
Level (%) of effort in the project: 5%
Point of contact at the funding agency: dbabcock@mail.nih.gov Debra Babcock
Overlap: None

Pending:
Role: PD/PI: Tracy Butler (contact), Anthony Ahmed, Amy Kuceyeski, Laura Beth McIntire
Title: Understanding Psychosis Heterogeneity In Late Life (UPHILL)
Funding Agency: NIH/NIMH 1 R01MH135154-01 (pending)
Goals of the project: Psychotic symptoms that start much later in life may be due to schizophrenia with unusually late onset or to the earliest stage of a neurodegenerative disease like Dementia with Lewy Bodies (DLB) or Alzheimer's Disease (AD). This project will use highly accurate diagnostic biomarkers for DLB and

AD, detailed clinical and cognitive assessment, and MRI to distinguish these possibilities in order to guide appropriate treatment of patients with late onset psychotic symptoms
Start and end date: 12/01/2023– 11/30/2028
Level (%) of effort in the project: 15%
Overlap: None

What other organizations were involved as partners?

Weill Cornell Medicine**:** Dr. McIntire remains committed to the completion of the Aims of the project as Adjunct Professor of Pathology and Cell Biology at Columbia University Medical Center and as Assistant Professor of Pharmacology in the Department of Radiology, Director of the Lipidomics and Biomarker Discovery Lab, Brain Health Imaging Institute, Weill Cornell Medicine. Dr McIntire originally was the Initiating PI and Dr. Hussaini was the Partnering PI in the mechanism Convergence Science Research Award (CSRA) of the Peer Reviewed Alzheimer's Research Program (PRARP). The change of PI to Dr. Abid Hussaini, has been approved along with a no-cost extension on 11/20/2023 at Columbia University to March 14, 2024.

SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: **N/A**

APPENDICES: N/A