

AWARD NUMBER: W81XWH-18-1-0233

TITLE: Harnessing Neuroplasticity Genes to Combat Synucleinopathy-Mediated Axonopathy

PRINCIPAL INVESTIGATOR: Joseph Patterson PhD

CONTRACTING ORGANIZATION: Michigan State University
Grand Rapids MI, 49503

REPORT DATE: JANUARY 2024

TYPE OF REPORT: Final

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

DISTRIBUTION STATEMENT: Approved for public release; distribution unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**

1. REPORT DATE JANUARY 2024		2. REPORT TYPE FINAL		3. DATES COVERED (From - To) 1 Jun 2018 - 30 SEPT 2023	
4. TITLE AND SUBTITLE Harnessing Neuroplasticity Genes to Combat Synucleinopathy-Mediated Axonopathy				5a. CONTRACT NUMBER W81XWH-18-1-0233	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Joseph Patterson, and Caryl Sortwell				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Michigan State University Grand Rapids Research Center 400 NW Monroe Ave. Grand Rapids MI, 49503				8. PERFORMING ORGANIZATION REPORT NUMBER	
				10. SPONSOR/MONITOR'S ACRONYM(S)	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S Army Medical Research and Development Command Fort Detrick, Maryland 21702-5012				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
				12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution unlimited	
13. SUPPLEMENTARY NOTES					
14. ABSTRACT The earliest stages of synucleinopathy have been difficult to study due to the fact that most animal models of Parkinson's disease (PD) fail to recapitulate the progression of synucleinopathy to neurodegeneration. The alpha-synuclein (α -syn) preformed fibril (PFF) synucleinopathy model exhibits a distinct stage of accumulation of α -syn inclusions in tyrosine hydroxylase immunoreactive (THir) neurons in the substantia nigra pars compacta (SNpc) months prior to the ultimate degeneration of the nigrostriatal system. In the context of the early phases of synucleinopathy in the α -syn PFF model, laser capture microdissection was used to collect phosphorylated α -syn (pSyn) immunoreactive SNpc neurons in PFF-injected rats and SNpc THir neurons in control-injected rats. RNA was isolated and RNASeq used to identify gene expression changes between SNpc neurons with and without pSyn inclusions. Results from male and female rats have identified 326 candidate transcripts. Of these candidates, <i>Gap43</i> , a gene related to neuroplasticity/synaptic plasticity, was selected to be overexpressed via adeno-associated viral vector. The overall goal is to determine if <i>Gap43</i> expression is sufficient to mitigate neurodegeneration in a model of synucleinopathy.					
15. SUBJECT TERMS Synuclein, Synucleinopathy, neurodegeneration, RNASeq					
16. SECURITY CLASSIFICATION OF: U			17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES 16	19a. NAME OF RESPONSIBLE PERSON USAMRDC
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U			19b. TELEPHONE NUMBER (include area code)

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39-18

TABLE OF CONTENTS

	<u>Page</u>
1. Introduction.....	4
2. Keywords.....	4
3. Accomplishments.....	4
4. Impact.....	7
5. Changes/Problems.....	9
6. Products.....	10
7. Participants & Other Collaborating Organizations.....	14
8. Special Reporting Requirements.....	15
9. Appendices.....	16

1. INTRODUCTION:

Parkinson's disease (PD) is a progressive neurodegenerative disease with hallmark features of motor impairment, progressive degeneration of the nigrostriatal dopamine neurons and the accumulation of Lewy Bodies, or cellular inclusions containing alpha-synuclein (a-syn). In PD significant irreversible neurodegeneration occurs before onset of symptoms, as such early diagnosis and understanding of disease progression is critical to development of therapeutics and disease modifying treatments. To address this issue, we use the a-syn preformed fibril (PFF) model in rats, which induces a synchronized synucleinopathy with a well characterized progression which includes Lewy body-like pathology, neurodegeneration, and motor impairments. The overall goals of the project were to leverage rat PFF model to examine transcriptomic changes associated with early synucleinopathy, followed by using viral vector mediated gene expression to "correct" the effects of early synucleinopathy in the attempt to mitigate neurodegeneration.

2. KEYWORDS:

Parkinson's Disease, Synucleinopathy, Transcriptome, RNASeq, Neurodegeneration

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Specific Aim 1: Optimization of isolation of nigrostriatal dopamine neurons using laser capture microdissection.

- Optimization of laser capture microdissection - **Completed**
- RNA isolation and cDNA synthesis to confirm isolation - **Completed**

Specific Aim 2: Identification of genetic signatures associated with early synucleinopathy.

- Generation of an early synucleinopathy cohort of rats - **Completed**
- Laser capture microdissection and downstream RNA sequencing - **Completed**
- RNA sequencing analysis and identification of potential neuroplasticity related target for Specific Aim 3. - **Completed**

Specific Aim 3: Mitigation of neurodegeneration via modification of neuroplasticity gene expression.

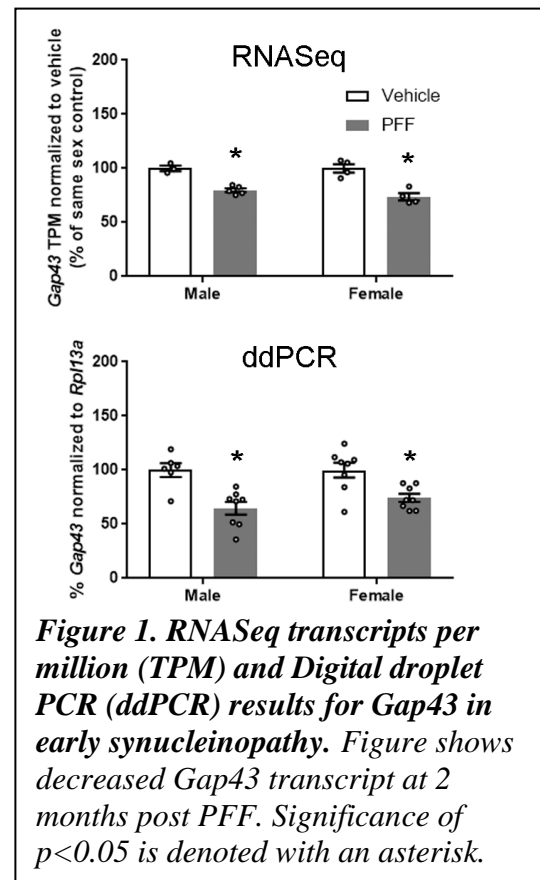
- Production of rAAV vector to modify gene expression - **Completed**
- Optimization of rAAV (titer experiment to ensure expression with low toxicity) - **Completed**
- Assessment of rAAV use in the PFF model to determine if it is neuroprotective – **Data analysis and manuscript preparation in progress**

What was accomplished under these goals?

The RNASeq experiment and analysis have been outlined in previous reports. During this final reporting period a manuscript on that portion of the project (covering Specific Aims 1 and 2) was submitted and accepted (PMID: **38172128**). In addition to the publication, we have been working to leverage the RNASeq results to select and modify a neuroplasticity related gene to determine if it could be neuroprotective in our synucleinopathy model. The gene selected was *Gap43*.

In early synucleinopathy in both male and female rats, we observed the downregulation of *Gap43* (growth and plasticity 43), also known as neuromodulin. *Gap43* is a neuroplasticity gene which plays a key role in neurite formation and regeneration, axon pathfinding/regeneration and neuronal growth cone formation during development, synaptic endocytosis, and synaptic plasticity and long-term potentiation. GAP43 functions downstream of BDNF and is an essential effector of BDNF-driven neuroprotection. In PD, *GAP43* transcript is decreased in the remaining dopamine neurons in the SNpc, GAP43 protein is decreased in the neuropil in the SN, and there is a reduction in GAP43 protein in the CSF. Similarly, in the context of early synucleinopathy in our study, we see a decrease in *Gap43* transcript (Figure 1). Early decrease in *Gap43* suggests damaged nigrostriatal neurons will lack the capacity to recover from damage associated with the synuclein burden. This leads us to hypothesize that modulation, specifically overexpression, of *Gap43* could be important to the survival of the dopamine neurons.

In the previous report, we detailed the titer issues that we were working to overcome. In brief, viral vector titers up to 1E12 dis not sufficiently increase Gap43 protein in the substantia nigra or striatum. We suspect that the primary issue is that Gap43 is normally expressed broadly enough in the brain that we would not be able to detect an increase in a small population of neurons. Addressing this issue, we injected the highest titer we had available (1E13). At this titer we see ~2 fold increase in the substantia nigra, but no change in the terminals in the striatum. The increase in the striatum cannot be appreciated likely because the other innervations in the striatum (also containing Gap43) vastly outnumber those from the nigra. Based on these results, we proceeded on to the neuroprotection



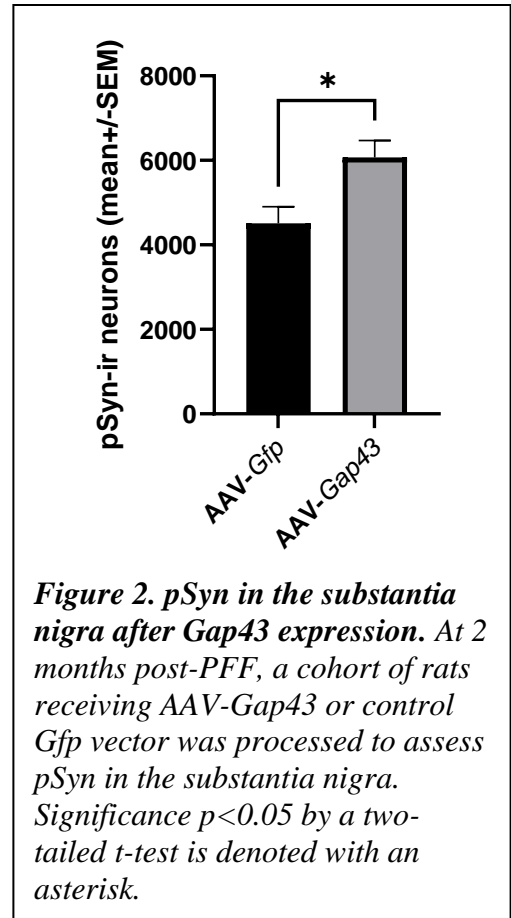
studies. In these studies, AAV-*Gap43* or a control vector (AAV-*Gfp*) were injected into the substantia nigra 4 weeks before PFFs were injected into the striatum. Brains were collected from these rats at either 2- or 6-months post PFF when we expect to see the peak in phosphorylated a-syn (pSyn) pathology or nigrostriatal degeneration respectively.

At 2 months post-PFF, rats receiving the AAV-*Gap43* vector had more pSyn containing neurons in the substantia nigra (Figure 2). There was no qualitative difference in tyrosine hydroxylase immunoreactive neurons in the substantia nigra, and stereology by an individual blinded to the groups is ongoing. We predict the reason for increased pSyn in the *Gap43* group is due to increased neurite formation and axonal branching, which would increase access points in the striatum for PFF uptake. We are currently working on quantifying TH and DAT in the striatum to determine if *Gap43* overexpression did increase the number of projections to the striatum.

In addition to the 2-month cohort, we are also in the process of analyzing the results from the 6-month cohort. These samples have been processed and stained but are still in the queue for stereology. Once this has finished, we will submit the manuscript including these results to a journal (to be determined).

In addition to the results directly from the project, we also wanted to add that the RNASeq results have spurred on other project in the lab, including a focus on neuroinflammation in early synucleinopathy (PMID: 38228253), a project examining the effects of age on glycolytic/energy related genes (funded internally at MSU), a project examining earlier timepoints in synucleinopathy (funded internally at MSU), and currently the submission of five other grants (one CDMRP, one MJFF, two R21s, and one R01).

What opportunities for training and professional development has the project provided?



The project was not originally intended to provide training opportunities, however, with the success of the laser capture microdissection and RNASeq, a follow-up project is in progress within the lab. The follow-up project is focused on inflammation and the microglia in early synucleinopathy. The graduate student is being mentored by the PI of this project who is overseeing their training in laser capture microdissection, RNA isolation, and RNASeq analysis. This student (expected to graduate in August 2024) has presented posters at Grand Challenges in Parkinson's Disease (Grand Rapids, MI), the local Michigan Society for Neuroscience meeting, and a Gordon Research Conference in Switzerland.

How were the results disseminated to communities of interest?

The only results currently published directly from this project is the RNASeq data, which is published in an open access journal (Nature Partner Journal Parkinson's Disease; PMID: 38172128). The raw data files for analysis by other researchers are all available on the Gene Expression Omnibus (GEO) through NCBI (Accession number GSE246112). These results have been presented as the project developed at multiple meetings (Society for Neuroscience, World Parkinson's Congress, Movement Disorders, Grand Challenges in Parkinson's Disease). Of these meetings, Movement Disorders was a good platform to present information to other researchers and clinicians, and World Parkinson's Congress was a good platform to present information to PD patients and caregivers, clinicians, and other researchers.

In addition to the paper that directly came from this grant, findings from RNASeq informed decisions on neuroinflammatory genes published on in a collaborator's lab (PMID: 38228253).

Though this is the final report, we are still working on publishing the GAP43 portion of this project. Similarly, we have other offshoots from the RNASeq performed in this project that we are working on which focus on transcriptomic changes in the microglia earlier in synucleinopathy, and the effects of aging on the transcriptome of energy/glycolysis associated genes, among other projects in progress.

4. IMPACT:

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

It is too early to determine how impactful our findings will be in translating to humans with Parkinson's Disease. Our major findings are an RNA sequencing data set which focuses on the gene expression changes which occur early in the progression of a synucleinopathy (neurodegeneration with a-synuclein pathology present). We have already started to use the lists of differentially expressed genes in the development of other projects examining the neuroinflammatory response, energy requirements, and effects of aging on synucleinopathy.

What was the impact on other disciplines?

Nothing to report

What was the impact on technology transfer?

Nothing to report

What was the impact on society beyond science and technology?

Nothing to report

5. CHANGES/PROBLEMS:

The majority of challenges/problems (rat vendor genotype/breeding issue, lengthy wait time for sequencing, other COVID related delays) were already presented in previous reports. The only recent challenge was with viral titrating, which we needed to repeat to get sufficient gene expression. This took a few months longer to achieve than predicted. Availability of the surgical team also pushed back the stereotactic surgeries for the final experiment, this, and waiting on the 6-month timepoint has delayed sample processing.

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

Though this is the final report, we are continuing to process tissue and will be working on submitting a manuscript on the GAP43 portion of the project in the next few months.

Changes that had a significant impact on expenditures

No significant changes that impacted expenditures.

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

Human subjects/tissue was not used.

Early in the project we had tissue from a cohort of rats that needed to be removed from the study (vendor had a breeding issue and sent wild-type rats with a mixed genotype). Costs were refunded and new rats delivered, the only significant loss was time. This was reported in a previous technical report.

Significant changes in use of biohazards and/or select agents

No significant changes

6. PRODUCTS:

- **Publications, conference papers, and presentations**
Journal publications.

Patterson JR, Kochmanski J, Stoll AC, Kubik M, Kemp CJ, Duffy MF, Thompson K, Howe JW, Cole-Strauss A, Kuhn NC, Miller KM, Nelson S, Onyekpe CU, Beck JS, Counts SE, Bernstein AI, Steece-Collier K, Luk KC, Sortwell CE. Transcriptomic profiling of early synucleinopathy in rats induced with preformed fibrils. *NPJ Parkinsons Dis.* 2024 Jan 3;10(1):7. doi: 10.1038/s41531-023-00620-y. PMID: 38172128; PMCID: PMC10764951.

Other publication still in preparation

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

Nothing to report

Other publications, conference papers and presentations.

Poster Presentations

Howe JW. Kemp CJ. Stoll AC. Luk KC. Sortwell CE. **Patterson JR.** (2023). Identification of the early microglial transcriptomic response to alpha-synuclein inclusion formation in the substantia nigra following preformed fibril injection. Trainee Poster presentation. *Grand Challenges in Parkinson's Disease*. Grand Rapids, Michigan.

Howe JW. Kemp CJ. Stoll AC. Luk KC. Sortwell CE. **Patterson JR.** (2023). Identification of the early microglial transcriptomic response to alpha-synuclein inclusion formation in the substantia nigra following preformed fibril injection. Trainee Poster presentation. *53rd Annual Michigan Society for Neuroscience*. East Lansing, Michigan.

Howe JW. Kemp CJ. Stoll AC. Luk KC. Sortwell CE. **Patterson JR.** (2023). Identification of the early microglial transcriptomic response to alpha-synuclein inclusion formation in the substantia nigra following preformed fibril injection. Trainee Poster presentation. *Gordon Research Conference; Parkinson's Disease*. Les Diablerets, Switzerland.

Patterson JR. Kochmanski J. Stoll AC. Kubik M. Kemp CJ. Howe JW. Duffy MF. Miller KM. Cole-Strauss A. Beck JS. Counts SE. Steece-Collier K. Luk KC. Sortwell CE. (2022). Early synucleinopathy is associated with differential regulation of neurotransmission and immune related genes in the rat preformed fibril model. Poster presentation. *Grand Challenges in Parkinson's Disease*. Grand Rapids, Michigan.

Patterson JR. Kochmanski J. Stoll AC. Kubik M. Kemp CJ. Howe JW. Duffy MF. Miller KM. Cole-Strauss A. Beck JS. Counts SE. Steece-Collier K. Luk KC. Sortwell CE. (2022). Early synucleinopathy is associated with differential regulation of neurotransmission and immune related genes. Poster presentation. *MDS*. Madrid, Spain.

Patterson JR. Kochmanski J. Stoll AC. Kubik M. Kemp CJ. Howe JW. Duffy MF. Miller KM. Cole-Strauss A. Beck JS. Counts SE. Steece-Collier K. Luk KC. Sortwell CE. (2021). Early Synucleinopathy is Associated with Downregulation of Dopamine Signaling and Upregulation of Neuroinflammation: A Transcriptomic Study in the Rat Pre-formed Fibril Model. Poster presentation. *51st Annual Michigan Society for Neuroscience*. Virtual.

Patterson JR. Kemp CJ. Duffy MF. Howe JW. Russell CP. Stoll AC. Miller KM. Beck JS. Counts SE. Steece-Collier K. Luk KC. Sortwell CE. (2020). Genetic profiling of early synucleinopathy in rat nigrostriatal dopamine neurons. Poster presentation. *MDS Virtual Congress*.

Patterson JR. Kemp CJ. Howe JW. Duffy MF. Stoll AC. Miller KM. Beck JS. Counts SE. Luk KC. Steece-Collier K. Sortwell CE. (2019). Genetic signature of early synucleinopathy in nigrostriatal dopamine neurons. Poster presentation. *49th Annual Society for Neuroscience meeting*. Chicago, Illinois.

Patterson JR. Kemp CJ. Duffy MF. Stoll AC. Miller KM. Beck JS. Counts SE. Luk KC. Sortwell CE. (2019). Temporal genetic profiling of early synucleinopathy in nigrostriatal dopamine neurons. Poster presentation. *5th World Parkinson Congress*. Kyoto, Japan.

Research Talks

Patterson JR. (2019). Features and Applications of Fibril Seeded Synucleinopathy. Research talk. *5th Annual Udall Center for Parkinson's Disease Research Symposium*. Ann Arbor, Michigan.

- **Website(s) or other Internet site(s)**

<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE246112>

- **Technologies or techniques**

Nothing to report

- **Inventions, patent applications, and/or licenses**

NA

- **Other Products**

RNASeq dataset for early synucleinopathy deposited in the Gene Expression Omnibus (GEO) through NCBI (Accession number GSE246112)

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Joseph Patterson
Project Role: PI
Researcher Identifier (ORCID ID): 0000-0003-0926-7396
Nearest person month worked: 4
Contribution to Project: Project oversight, surgeon, tissue processing, data analysis.

Name: Caryl Sortwell
Project Role: Advisor
Nearest person month worked: 1
Contribution to Project: Surgeon for alpha-synuclein preformed fibril and AAV injection surgeries.

Name: Anna Stoll
Project Role: Graduate Student
Nearest person month worked: 1
Contribution to Project: Surgeon for alpha-synuclein preformed fibril and AAV injection surgeries.

Name: Christopher Kemp
Project Role: Lab Technician
Nearest person month worked: 1
Contribution to Project: Surgical support, rat perfusions.

Name: Allyson Cole-Strauss
Project Role: Lab Technician
Nearest person month worked: 2
Contribution to Project: Tissue processing.

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

Nothing to report

What other organizations were involved as partners?

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *N/A*

QUAD CHARTS: *N/A*

9. APPENDICES: *N/A*