AWARD NUMBER: W81XWH-17-1-0534

TITLE: Airborne pollutants as triggers of Parkinson's disease via the olfactory system

PRINCIPAL INVESTIGATOR: Patrik Brundin, MD, PhD

CONTRACTING ORGANIZATION: Van Andel Research Institute

REPORT DATE: SEPTEMBER 2020

TYPE OF REPORT: Annual

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

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
SEPTEMBER 2020	Annual	1 Sep 2019–31 Aug 2020

4. TITLE AND SUBTITLE

Airborne Pollutants as Triggers of Parkinson's Disease via the Olfactory System

5a. CONTRACT NUMBER
W81XWH-17-1-0534

5b. GRANT NUMBER
PD160021

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

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8. PERFORMING ORGANIZATION REPORT NUMBER

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

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

10. SPONSOR/MONITOR'S ACRONYM(S)

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

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

We are interested in determining whether ambient air pollutants impact the development of Parkinson's disease by increasing α-synuclein (α-syn) pathology via inflammation. After completing Specific Aim 2, wherein we found no differences between experimental groups in spread of α-syn, we discovered our collected nPM did not produce the expected neuroinflammatory changes. We repeated Specific Aim 2 mouse experiments with a new batch of nPM before SARS-CoV-2 quarantine. After restrictions were relaxed, we analyzed spread of α-syn using a novel AI, but found no difference between brains of nPM- and forced air-exposed mice. We are in communication with our collaborators at USC in order to plan and execute novel experiments to explore the effects of LPS-induced olfactory inflammation on spread of α-syn by histological (VARI) and biochemical (USC) analyses under continued quarantine and travel restrictions.

15. SUBJECT TERMS

Pre-formed fibrils (PFFs), α-synuclein (α-syn), phosphorylated serine 129 (pSer129), nano-particulate matter (nPM), neuroinflammation, Parkinson's disease (PD)

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

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

Table of Contents

	Page
1. Introduction…….	4
2. Keywords…….	4
3. Accomplishments……..……...	4
4. Impact……...……	11
5. Changes/Problems...…..……	11
6. Products…….……..…..…….	12
7. Participants & Other Collaborating Organizations…….	12
8. Special Reporting Requirements……	16
9. Appendices……	N/A

1. INTRODUCTION

This project is entitled "*Airborne pollutants as triggers of Parkinson's disease via the olfactory system"* and has two arms: (A) Define the effects of exposure to nano-sized particle matter (nPM) on the development and progression of α-synucleinopathy in olfactory structures by combining two experimental paradigms and the pre-clinical testing of two drugs (ibuprofen and MDSC-0160). (B) Examine the role of ambient air pollutants in olfactory impairment among older adults in order to understand early stages of Parkinson's disease (PD) development. The goal of this multidisciplinary project is to improve our understanding of the early stages of PD development by defining the influence of air pollutants on the development and progression of α-synuclein (α-syn) pathology *in vivo*, and on olfactory dysfunction among older adults. We will pursue experimental (Aims 1-3) and epidemiological (Aims 5-7) studies addressing common research questions. The third year of this project at the Van Andel Research Institute saw the completion of Aim 2.1 analyses, the commencement of Aim 2.2 animal experiments, and subsequently the extraction, processing, and histological analysis of olfactory tissues for presence of Lewy-like α-syn pathology marker, phosphorylated serine 129 (pSer129).

2. KEYWORDS

Pre-formed fibrils (PFFs), α-synuclein (α-syn), phosphorylated serine 129 (pSer129), nano-particulate matter (nPM), neuroinflammation, Parkinson's disease (PD)

3. ACCOMPLISHMENTS:

Major Goals of the Project (from approved SOW):

SPECIFIC AIM 1: Determine the effects of exposing mice to nPM after triggering of pre-formed fibril (PFF) pathology (Months 6-16)

1. Inject C57BL/6NJ mice (n=96) with PFFs.

Validation experiment accomplished (n = 32), 10/12/17 (Y1Q1)

Specific Aim 1 injections (n = 64) accomplished 1/18/18 (Y1Q2)

2. Expose C57BL/6NJ mice to nPM.

Validation experiment accomplished (n = 32), 11/09/17 (Y1Q1)

Specific Aim 1 exposure (n = 64) accomplished 2/14/18 (Y1Q2)

Milestones in this reporting period:

N/A (Completed in Y2Q1)

SPECIFIC AIM 2 (Aim 2.1): Determine the effects of exposing mice to nPM before triggering of PFF pathology (Months 12-30)

1. Pre-expose mice to nPM for 3 weeks before injections

    Specific Aim 2 pre-stereotactic injection exposure (n = 64) initiated 10/8/18, accomplished 10/28/18 (Y2Q1)

2. Inject mice with PFFs (as in Aim 1).

    Specific Aim 2 injections (n = 64) initiated 10/29/18, accomplished 11/1/18 (Y2Q1)

3. Post-expose mice to nPM for 7 weeks after injections

    Specific Aim 2 post-stereotactic injection exposure (n = 64) initiated 11/2/18, accomplished 12/20/18 (Y2Q2)

Milestones in this reporting period:

1. Histological analyses (starts after 1 month for sectioning):
    a. Iba-1 and inflammatory cytokine immunofluorescence double stain optimization **suspended due to USC biochemical analysis of nPM (Y3Q1)**
2. Data analysis, manuscript preparation, and submission **ongoing**

SPECIFIC AIM 2 REPEAT (Aim 2.2): Determine the effects of exposing mice to nPM before triggering of PFF pathology (Months 28-38)

1. Pre-expose mice to nPM for 3 weeks before injections

    Specific Aim 2.2 pre-stereotactic injection exposure (n = 88) initiated 1/13/20, accomplished 2/2/20 (Y3Q2)

2. Inject mice with PFFs (as in Aims 1 and 2).

    Specific Aim 2.2 injections (n = 88) initiated 2/3/20, accomplished 2/6/20 (Y3Q2)

3. Post-expose mice to nPM for 7 weeks after injections

    Specific Aim 2.2 post-stereotactic injection exposure (n = 88) initiated 2/7/20, accomplished 3/26/20 (Y3Q3)

Milestones in this reporting period:

1. Complete collection and delivery of brains to VARI **collection initiated 3/23/20 at USC; brains received 5/18/20 at VARI due to SARS-CoV-2 quarantine (Y3Q3)**
2. Biochemical analyses **ongoing at USC**
3. Histological analyses (starts after 1 month for sectioning):
    a. pSer129 Aiforia AI densitometry analysis **completed 9/14/20 (Y3Q4)**

b. Iba-1 and inflammatory cytokine immunofluorescence double stain optimization **suspended due to results from biochemical and histological analyses (Y3Q4)**

Data analysis, manuscript preparation, and submission **ongoing**

Specific Aim 3: **not yet initiated**

What was accomplished under these goals?

1) *Major activities:*

   a. Complete quantitative analyses for Aim 2.1 (by VARI personnel) (Y3Q1).

   b. Pre-stereotactic injection exposure of Aim 2.2 C57BL/6NJ mice to nPM at USC (by USC personnel) (Y3Q2).

   c. Stereotactic injection of Aim 2.2 C57BL/6NJ mice with PFFs at USC (by VARI personnel) (Y3Q2).

   d. Post-stereotactic injection exposure of Aim 2.2 C57BL/6NJ mice to nPM at USC (by USC personnel (Y3Q2, Y3Q3).

   e. Collect Aim 2.2 experiment brains at USC and deliver to VARI (Y3Q3).

   f. Sectioning of Aim 2.2 experiment brains (by VARI personnel) (Y3Q3).

   g. Staining, imaging, and Aiforia AI densitometry analysis of pathologic α-syn (pSer129) in Aim 2.2 brains (by VARI personnel) (Y3Q4).

2) *Specific objectives:* Perform the first collaborative study to examine effects of exposure to nano-sized particle matter (nPM) on the development and progression of α-synucleinopathy in olfactory structures.

3) *Significant results or key outcomes:*

– Y3Q1: Aim 2.1 quantitative analyses were completed, and microglial and cytokine marker immunofluorescent double staining was optimized. Aiforia AI algorithm development continued, utilizing Aim 2.1 brains for AI optimization.

Microglia and cytokine immunofluorescent double staining optimization: We optimized a protocol for double immunostaining of the microglia marker TMEM119 and cytokine TNF-α (Figure 1) after trying several combinations of microglial markers and various cytokines. This double immunostaining will be imaged with fluorescence confocal microscopy to assess changes in localized expression of this microglial marker and cytokine. In combination with analyses of microglial morphology by volumetric quantification, this will allow us to define the inflammatory response to nPM and triggering of α-syn pathology. In order to further validate this protocol for use in our experiments, we generated more positive control tissue from mice intrastriatally injected with lipopolysaccharide (LPS), which induces inflammation and cytokine activation.

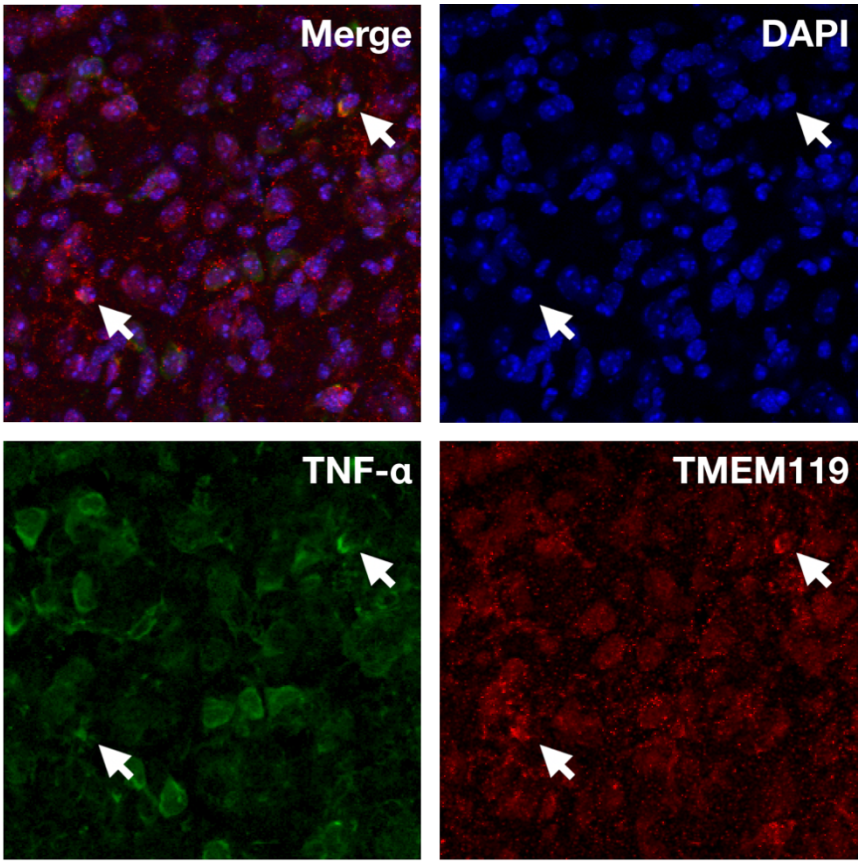


Figure 1: Double immunofluorescence images captured with confocal microscopy show TNF-α (green) and TMEM119 (red) in LPS-injected striatum. DAPI indicates cell nucleus. White arrows indicate two microglial cells positive for TNF-α.

Aiforia AI optimization: The Aiforia cloud-based AI algorithm for unbiased quantification of α-syn pSer129 presence through olfactory regions reached its third iteration. We scanned all our previously stained slides for this project. Scanned slides were uploaded to Aiforia for further fine-tuning of its recognition of Lewy-like pathology for use in our upcoming experiments (Figure 2). The algorithm, being far more accurate (particularly with its recognition of Lewy neurites), will replace ImageJ densitometry analyses.

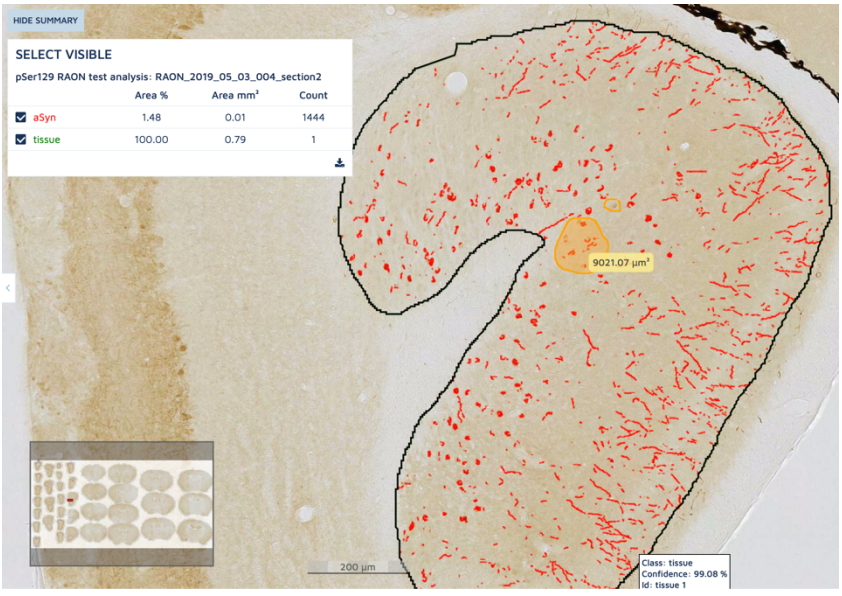


Figure 2: Representative image of a scanned right anterior olfactory nucleus section processed and analyzed using our Aiforia pSer129 AI algorithm, version 3. Area marked in red represents AI-recognized Lewy pathology. Part of our optimization of this algorithm includes identifying missed signal (light staining in orange-highlighted areas). The algorithm will provide quantification of total Lewy pathology across the entirety of that region's area within a section.

– **Y3Q2:** Repeat of Specific Aim 2 (Aim 2.2) was initiated at USC with exposure to forced air or nPM of experimental mice. VARI personnel traveled to USC to perform stereotactic PFF injections on experimental mice. Optimization of Aiforia AI continued over successive iterations of the α-syn pathology analysis algorithm. Microglia and cytokine immunofluorescent double staining protocol was validated using newly-generated positive control tissue.

Aim 2.2 nPM exposure and PFF injections: From February 2–6, 2020, after initial 3-week exposure of mice to nPM or forced air at USC, Dr. Christopher Tulisiak and Ms. Allison Lindquist (VARI personnel) traveled to USC to perform microinjections of PFFs or saline into the right olfactory bulb of 88 C57Bl/6NJ mice with USC personnel. The number of mice in Aim 2.2 was increased from 64 to 88 after a power analysis showed we would be able to detect effect sizes nearly 20% smaller (from 1.92 to 1.75 for pSer129) with the addition of 6 mice per group. This would have been advantageous in the event that the new batch of nPM produced smaller changes to neuroinflammation and pSer129 presence than anticipated. The increased number comes at no additional cost because the budget for the project allowed for 64 additional animals for Aim 4 (n = 128), which was removed in the approved Request for Modification of Aims 3 and 4 (submitted 11/8/19), and which Aim 2.2 (n = 88) has replaced.

Continued validation of artificial intelligence algorithm (produced by Aiforia) for efficient analysis of presence of pathological α-syn marker pSer129: Four new iterations (v4-v7) of our artificial intelligence algorithm for analysis of the presence of pSer129 were generated during this reporting period. These new iterations reduced the false positives and negatives in both tissue and pSer129 detection (Figure 3) when using scanned images with 3-µm z-depth (a lower resolution compared to initial 1 µm z-depth images used in previous iterations). Use of 3-µm z-depth images has allowed us to reduce the time required to image our slides by 40% but, because of their lower resolution, additional fine-tuning of the AI algorithms was required.

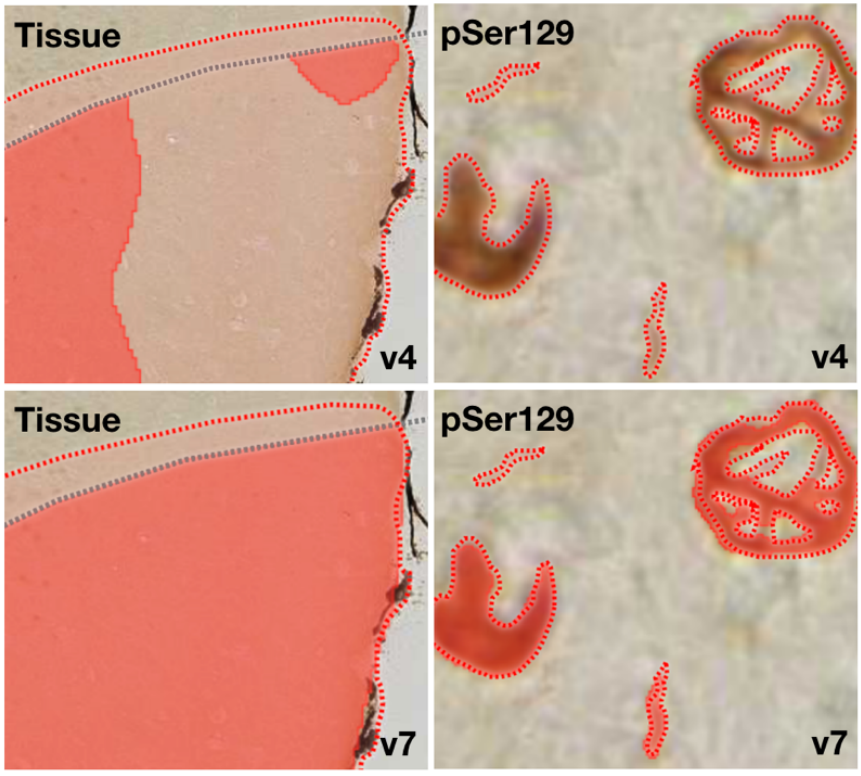


Figure 3: Top: tissue and α-syn analysis algorithm version 4. Note the outlined tissue and pSer129 pathology that is not highlighted. This indicates that this version of the algorithm did not recognize the pathology (false negative). Bottom: tissue and α-syn analysis algorithm version 7. Note the same outlined tissue and pSer129 pathology is now highlighted in red, indicating true positive detection of pSer129 signal. Gray outline in tissue images indicates region of interest for validation purposes.

Microglia and cytokine immunofluorescent double staining validation: Additional validation of optimized microglia marker TMEM119 and cytokine TNF-α antibodies for use in immunofluorescent

double staining and imaging continued during this reporting period. Positive control practice mice were injected intrastriatally with LPS, which generated a robust neuroinflammatory response, and then stained with our optimized antibodies. Efficacy of the double staining protocol generated in Y3Q1 was validated.

– **Y3Q3:** SARS-CoV-2 quarantine began for VARI on 3/15/20. nPM and forced air exposures were completed at USC on 3/23/20. Although VARI research technicians were permitted entrance to the building to receive experimental brains on 5/18/20, Dr. Tulisiak was first permitted back to VARI post-quarantine on 5/26/20. Aim 2.2 histological analyses were initiated at this time as outlined below. Development of Aiforia AI for analysis of α-syn pSer129 presence was completed during this period, and the AI was published for use by the Brundin laboratory.

Aim 2.2 sectioning and pSer129 staining: nPM and forced air exposures were completed by USC personnel (3/23/20). At this time, personnel at both USC and VARI were forbidden from entering buildings except for essential, time-sensitive tasks due to the COVID-19 pandemic. Therefore, USC was allowed to complete perfusions of experimental mice. VARI personnel were unable to initiate histological analyses until end of quarantine. USC was able to ship experimental brains, and brains were received by essential VARI personnel (Ms. Meyerdirk) on 5/18/20. Sectioning was initiated upon end of Michigan quarantine on 5/26/20 and completed 6/10/20. Staining of mouse brain tissue for α-syn pSer129 was initiated on 6/11/20 and completed 6/12/20.

Release of artificial intelligence algorithm (produced by Aiforia) for efficient analysis of presence of α-syn pSer129: Our artificial intelligence (AI) algorithm produced by Aiforia Technologies was released on 5/22/20 for use in laboratory analyses after additional fine tuning during this quarter. These adjustments have resulted in an algorithm that detects tissue area in a given region of interest (ROI) with 0.51% error, and pSer129 α-syn area in a given region of interest (ROI) with 0.03% error (Figure 4). Both error rates are exceptionally low (optimal error rates for these AIs are < 1%), and at this point we are able to conduct highly accurate quantification of α-syn pSer129 presence. This has been a great improvement in comparison to the limited semi-quantitative, densitometric, and stereological analyses used previously.

LAS_aSyn_V7_3umOnly | aSyn_3umonly_GT_25Ki_LASV3 Completed in 47 hours 13 minutes 54 seconds
Iterations: 23778

	Type	Complexity	Area Error	Error
Tissue	Region	Extra Complex	0.51 % (11357324/2238713742)	0.91 % 11357324/1251260892.5)
tissue			0.51 % (11357324/2238713742)	0.91 % 11357324/1251260892.5)
nSyn_seg	Region	Extra Complex	0.03 % (159193/508914777.5)	59.66 % (159193/266851)
aSyn			0.03 % (159193/508914777.5)	59.66 % (159193/266851)

Figure 4: Area error for AI algorithm developed for analysis of pSer129. Tissue area recognition error is 0.51%, while pSer129 α-syn area recognition error is 0.03% (red box).

– **Y3Q4:** During this reporting period, sections were mounted to slides and scanned into a digital image format. These images were uploaded to the Aiforia cloud for analysis of presence of α-syn pSer129 in olfactory structures. Statistical analysis of results from this analysis indicated no effect of nPM on presence of α-syn pSer129 in olfactory bulb or anterior olfactory nucleus structures (where majority of pathology is observed).

Aim 2.2 analysis of α-syn pSer129 presence in olfactory structures of nPM- and forced air-exposed brains using Aiforia AI algorithm: After staining was completed in the previous reporting period, sections were mounted on slides (completed 6/26/20). Slides were then coverslipped and cleaned

(completed 7/3/20) before scanning using the AxioScanZ.1 scanner (completed 7/10/20). Thereafter, scanned images were uploaded to the Aiforia cloud platform for analysis. After running the AI analysis using our pSer129 algorithm, statistics were completed 9/14/20 (Dr. Tulisiak was on paternity leave July 15 – August 25, 2020). To quantify α-syn pSer129 presence, we used the quotient of the total pSer129-immunopositive area divided by the total tissue area detected by the AI algorithm for each brain region analyzed in each subject. As with our previous aims, Aim 2.2 PBS-injected brains did not exhibit any α-syn pSer129 pathology in olfactory structures including ipsilateral (right) olfactory bulb (OB), which was the site of PFF injection, nor contralateral (left) OB, ipsilateral anterior olfactory nucleus (AON), or contralateral AON. Therefore, two-tailed Student's t-test was used to compare the effects of nPM on presence of α-syn pSer129 in the left and right OBs and left and right AONs. Analyses showed no effect of nPM on altering pSer129 presence through these olfactory structures (Figure 5).

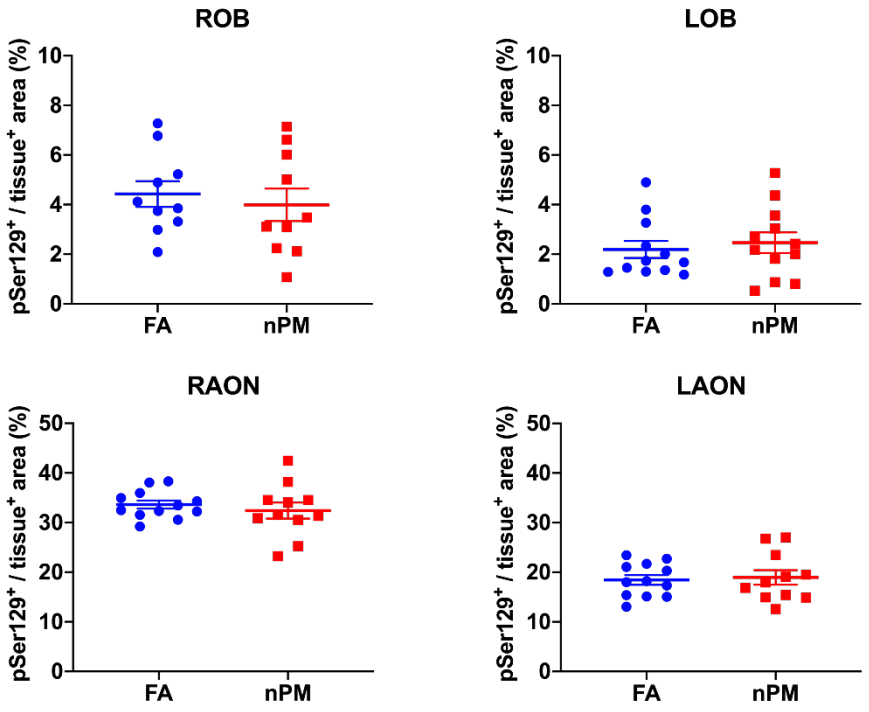


Figure 5: Pathological α-syn (pSer129) presence as percent of total tissue area in olfactory structures of forced air (FA, blue)- and nano-particulate matter (nPM, red)-exposed mice after ten total weeks (three weeks pre-, seven weeks post-PFF injection) of exposure. Olfactory structures include right and left olfactory bulbs (OBs) and anterior olfactory nuclei (AONs). ROB was the site of PFF injection. No statistical significance detected for any of the olfactory structures analyzed. Mean ± s.e.m.

In conjunction with results from the biochemical analyses performed by USC (see their Y3 annual report, award number W81XWH-17-1-0535), we were unable to confirm our hypothesis—that ambient air pollutants induce inflammation that impacts the development of α-syn pathology in our model of prodromal PD—with the present batch of nPM. It is important to mention that the capability of the new batches of nPM to elicit a neuroinflammatory response has diminished when compared to batches used in years prior to the start of this project.

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

- Training was provided to postdoctoral fellow Dr. Christopher Tulisiak in development and optimization of artificial intelligence algorithms for histological analyses using the Aiforia Technologies platform. Dr. Tulisiak was also trained in the use of a confocal microscope and the AxioScan slide scanner for bright field and fluorescent imaging of slide-mounted biological samples.

- Professional development was provided to all VARI researchers through regular seminars and journal clubs pertaining to neurodegenerative diseases, including Parkinson's disease.

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?

- Submit Request for Modification of Specific Aim 3 in light of continued travel restrictions related to SARS-CoV-2 and Aim 2.2 results using nPM that we have shown is inactive *in vivo* (please refer to section 5 B).
- Commence work on a revised Specific Aim 3 of project.

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?***
  - Nothing to Report
- ***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

A. Changes in approach and reasons for change

- As described in the Request for Modification of Specific Aims 3, 4 submitted 11/8/2019, we removed Aim 4 and added a repeat of Aim 2 (Aim 2.2) with a newly generated batch of nPM. Our collaborators at USC confirmed that the new batch was active *in vitro* after biochemical analysis of the nPM batch used in Aims 1 and 2.1 demonstrated that this original nPM batch was inactive. Furthermore, after a power analysis outlined in the Y3Q2 progress report, we increased the number of experimental mice from 64 to 88 at no increase in cost (since the 124-mouse Specific Aim 4 was removed in the Request for Modification of Specific Aims 3, 4) in order improve our ability to detect subtle differences in α-syn pSer129 presence by 20%.

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

- The SARS-CoV-2 pandemic halted laboratory work from March 16 – May 26, 2020. During that time, literature searches were performed, online courses were taken, and, most importantly, validation of the α-syn pSer129 AI algorithm produced by Aiforia Technologies was completed.

This algorithm is now ready for use. Since laboratory work has been permitted again, and with much of the non-laboratory work having been tended to during quarantine, processing and analysis of Aim 2.2 brains for α-syn pSer129 has been completed. Because of these efforts, we have remained on schedule according to the SOW.

- As described above in the Y3Q4 section, we observed no effect of nPM on presence of α-syn pSer129 in olfactory structures. Furthermore, our collaborators at USC did not observe changes to expression of cytokine and inflammatory marker genes in the brains of nPM-exposed mice. As discussed previously, not all batches of nPM cause severe inflammatory responses, as a report published by our collaborators at USC after the initiation of this project indicates (PMID: 31542466; see also their Y2 annual report, award number W81XWH-17-1-0535). Because our collective results indicate that the batch of nPM used in Aim 2.2 was also inactive, we plan to submit a Request for Modification of Aim 3 and to execute the newly proposed experiments as soon as possible. In short, due to continued quarantine and travel restrictions, we plan to perform intranasal LPS injections at VARI to induce olfactory inflammation, in conjunction with PFF (or PBS) injections as in Aims 1 - 2.2, before collecting brains and shipping half to our collaborators at USC for biochemical analyses. Our aim, therefore, will be to test the hypothesis that eliciting inflammation in the olfactory bulb exacerbates presence and spread of synucleinopathy through olfactory structures. While the batches of nPM generated were unable to induce the desired inflammation *in vivo*, LPS is known to induce neuroinflammation, and so will provide an artificial model of effects of active nPM. Please note that before initiating any instructions we will submit the necessary IACUC protocols required by VARI and the USAMRMC ACURO as soon as possible; only after these are approved will animal experiments begin at VARI for this project.

C. Changes that had a significant impact on expenditures

- Nothing to Report

6. PRODUCTS

List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**
  Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

1. Name: Patrik Brundin
   Project role: Principal Investigator/Project Director
   Researcher identifier: ORCID ID: https://orcid.org/0000-0003-2924-5186
   Nearest person month worked: 1.2 cal months (or 10% effort)
   Contribution to Project: Dr. Brundin has performed work to organize and oversee the project, including participating in teleconferences and email correspondence.

2. Name: Christopher Tulisiak
   Project role: Post-doctoral fellow
   Researcher identifier: ORCID ID: https://orcid.org/0000-0002-0793-1823
   Nearest person month worked: 12 cal months (or 100% effort)
   Contribution to Project: Dr. Tulisiak performed PFF microinjections for Aim 2.2 at USC. He has driven the optimization of both the Aiforia AI algorithm and immunofluorescent double staining, and performed sectioning and staining for Aim 2.2 brains. He has performed all histological analyses for Aim 2.2.

3. Name: Jennifer Steiner
   Project role: Senior laboratory manager
   Researcher identifier: ORCID ID: https://orcid.org/0000-0003-0953-1310
   Nearest person month worked: 2.3 cal months (or 19% effort)
   Contribution to Project: Dr. Steiner has performed work to help the Finch laboratory transport materials to and from USC and to coordinate work at VARI necessary to amend and complete the Statement of Work.

4. Name: Emily Kuhn
   Project role: Research technician
   Researcher identifier: ORCID ID: https://orcid.org/0000-0001-9715-7941
   Nearest person month worked: 2.9 cal months (or 24% effort)
   Contribution to Project: Ms. Kuhn contributed to optimization of immunofluorescent double staining and processing (sectioning, mounting, coverslipping) of Aim 2.2 brains.

5. Name: Lindsay Meyerdirk
   Project role: Research technician
   Researcher identifier: ORCID ID: https://orcid.org/0000-0003-4640-9517
   Nearest person month worked: 3.8 cal months (or 32% effort)
   Contribution to Project: Ms. Meyerdirk contributed to optimization of immunofluorescent double staining and processing (sectioning, mounting, coverslipping) of Aim 2.2 brains.

6. Name: Liza Bergkvist
   Project role: Post-doctoral fellow
   Researcher identifier: ORCID ID: https://orcid.org/0000-0001-7433-2647
   Nearest person month worked: 2 cal months (or 17% effort)
   Contribution to Project: Dr. Bergkvist participated in Aim 2.1 analysis and optimization of immunofluorescent double staining.

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

Yes; see below:

Recently Completed Support

22044 (Brundin, P) 10/1/2018 - **9/30/2019** 0.00 Cal Mths or 0% Effort
East Tennessee Fdn $25,000 Total Cost
Defining the influence of a genetic risk factor in the development of Parkinson's Disease
The major goal of this project is to determine the negative effects of external insults in these mice and to initiate experiments aiming to counteract the negative effects of lacking ACMSD using a novel drug.

Specific Aim 1: Define the effects of the lack of ACMSD upon exposure to insults known to induce nigral neurodegeneration.
Specific Aim 2: Determine the tolerability and brain penetrance of a novel drug to counteract the negative effects of deleting the ACMSD gene.
Role: PD/PI
Agency contact: Jan Elston
Overlap: None

3R01DC014443-04S2 (Wesson) 7/1/2018 - **6/30/2020** (No Cost Extension) 0.00 Cal Mths or 0% Effort
NIH/NIDCD via U of Florida $142,500 Total Cost
Inter-Regional Coding of Odor Valence by Neural Ensembles
The major goal is to determine neuropathological changes associated with olfactory dysfunction in a mouse model of Alzheimers.

Specific Aim: to define/quantify the levels of brain neuropathology in a mouse model of Alzheimer's disease.
Role: Subaward PD/PI
Agency Contact: Hoai Doan
Overlap: None

R01NS096241 (Krainc) 9/30/2016 - **8/31/2020** 0.24 Cal Mths or 2% Effort
NIH via Northwestern University $591,994 Total Cost
The role of ATP13A2/PARK9 in secretion of exosomes and alpha-synuclein
The major goal of this project is to study the influence of increasing ATP13A2 expression on the development of alpha-synuclein pathology.

Specific aim: To perform in vivo experiments in mice (using a model generated in the Brundin Lab) to define how manipulating the levels of expression of ATP13A2 influences the development of alpha-synuclein pathology and associated deficits in olfactory function.
Role: Subaward PD/PI
Agency contact: Olamide Adeniyi
Overlap: None

Changes to Current Research Support – *Indicated in Bold*

(THIS AWARD)
W81XWH-17-1-0534 (Brundin, P) 9/1/2017 - 8/31/2021 1.20 Cal Mths or 10% Effort
DoD $1,417,473 Total Cost
Airborne pollutants as triggers of Parkinson's disease via the olfactory system
The major goal of this project is to determine the influence of air pollutants in the development of PD.

Specific Aim 1: Determine the effects of nPM exposure *after* microinjection of fibrillar α-syn in the OB.
Specific Aim 2.1: Determine the effects of nPM exposure *before* microinjection of fibrillar α-syn in the OB.
Specific Aim 2.2: Repeat Specific Aim 2.1 with new batch of active nPM confirmed *in vitro* to be active.
Specific Aim 3: Define the effects of systemic administration of ibuprofen on the development of

α-syn pathology.
Specific Aim 4: Examine the effect of long-term exposures to ambient PM2.5 and NO2 on hyposmia.
Specific Aim 5: Examine whether early PD pathogenesis is exacerbated by ambient air pollutants.
Specific Aim 6: Examine whether lifetime use of NSAIDs, ibuprofen in particular, modify potential adverse effects of air pollutants on hyposmia.
Role: PD/PI
Agency contact: Christopher Meinberg
Overlap: N/A

N/A (Brundin, P.) 1/11/2016 - **1/10/2021** 0.00 Cal Mths or 0% Effort
(No Cost Extension)
Hoffmann-La Roche $107,010 Total Cost
SPRA: The role of inflammation in alpha-synuclein propagation w/visiting scientist Nazia Maroof
The major goal of this project is to determine whether aSyn pathology can propagate from the ENS to the Central Nervous System (CNS) and what the role of the immune system may be in this process.

Specific Aim 1: To determine whether inflammation following acute (1 week)/chronic (4 weeks) DSS colitis has a long term impact on aSyn accumulation in the ENS and CNS and what role it plays in aSyn propagation from ENS to CNS.
Specific Aim 2: To ascertain whether aSyn isolated from faecal samples from PD patients and transgenic aSyn overexpressing mice which have been subjected to DSS colitis, causes seeding and propagation of aSyn aggregate pathology through the nervous system following injection into the olfactory bulb.
Specific Aim 3: To investigate whether colitis in early life of Thy1-(A30P)aSyn tg mice leads to aSyn pathology and gliosis in brain over longer period of time.
Specific Aim 4: To explore epigenetic changes in nuclei of enteric nerves after DSS colitis in Thy1-(A30P)aSyn tg mice.
Role: PD/PI
Agency contact: Markus Britschgi
Overlap: None

1R21NS112614-01 (Labrie, V) 09/15/2019 – 08/31/2021 0.12 Cal Mths or 1% Effort
NIH/NINDS $522,500 Total Cost
Molecular signatures of Parkinson's disease in the gut and brain
The major goal of this project is to investigate whether there are changes in the autophagy-lysosomal pathway in the Parkinson’s disease appendix and brain.

Specific Aim 1: Identify DNA methylation abnormalities in the PD appendix that affect the function of the ALP
Specific Aim 2: Identify aging changes in DNA methylation that are disrupted in the PD appendix
Role: Co-Investigator
Agency Contact: NIH Grants Management Specialist, Karen Molina
Overlap: None
***New since last report**

What other organizations were involved as partners?

None identified outside of our funded DoD collaborations.

8. SPECIAL REPORTING REQUIREMENTS

See attached Quad Chart.

9. APPENDICES

N/A

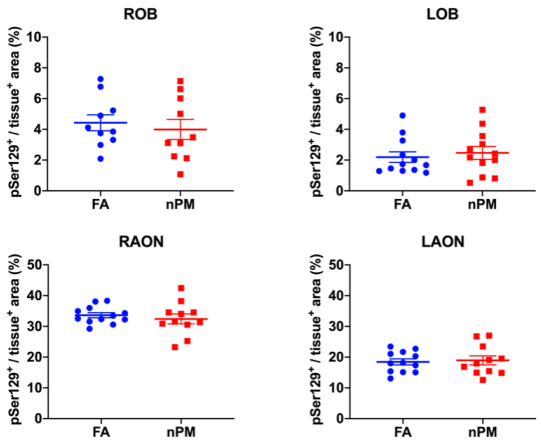
Project Title: Airborne Pollutants as Triggers of Parkinson's Disease via the Olfactory System
Log Number: PD 160021 Annual Report Year 3
Award Number: W81XWH-17-1-0534

PI: Brundin, Patrik **Org:** Van Andel Research Institute **Award Amount:** $ 746,039/Direct

Study Aims

1. Determine the effects of nPM exposure *after* microinjection of fibrillar α-syn in the OB.
2.1. Determine the effects of nPM exposure *before* microinjection of fibrillar α-syn in the OB.
2.2 Repeat Specific Aim 2.1 with new batch of active nPM confirmed *in vitro* to be active.
3. Define the effects of systemic administration of ibuprofen on the development of α-syn pathology.
4. Examine the effect of long-term exposures to ambient $PM_{2.5}$ and NO_2 on hyposmia.
5. Examine whether early PD pathogenesis is exacerbated by ambient air pollutants.
6. Examine whether lifetime use of NSAIDs, ibuprofen in particular, modify potential adverse effects of air pollutants on hyposmia.

Approach: This project ranges from *in vivo* studies, to elucidate the influence of exposure to airborne pollutants on the development of α-syn pathology, to epidemiological studies, to unravel the contribution of relevant factors in PD-like long-time exposure to airborne pollutants, genetic risk score or use of NSAIDs (as well as the interactions among these factors).



Accomplishments: Repeat of Specific Aim 2 was initiated at USC 1/13/20 and analysis with a novel artificial intelligence algorithm for quantification of pSer129 presence in olfactory tissues was completed 9/14/20 (see Y3 annual report). **Statistical analyses show no effect of nPM on altering pSer129 presence throughout olfactory tissues including the left (L) and right (R) olfactory bulbs (OB) or anterior olfactory nuclei (AON). Mean ± s.e.m.** In conjunction with biochemical analyses performed by USC, we are unable to confirm our hypothesis—that ambient air pollutants induce inflammation that impacts the development of α-syn pathology in our model of prodromal Parkinson's disease—with the present batch of nPM.

Timeline and Cost

Activities CY	17/18	19	20	21
Study Prep/Specific Aim 1	$177,530			
Specific Aim 2.1 (see goals/milestones)		$183,808		
Specific Aim 2.2 (see goals/milestones)			$192,162	
Specific Aim 3 (see goals/milestones)				$192,539
Estimated Budget ($746,039)	**$177,530**	**$183,808**	**$192,162**	**$192,539**

Updated: 9/30/20

Goals/Milestones

CY17: 1) ☑ Obtain IACUC approval at USC; 2) ☑ Generation of PFFs
CY18: 1) ☑ Generation of PFFs; 2) ☑ ACURO regulatory approval; PFFs, nPM ready to be used; 3) ☑ Inject mice with PFFs at USC and nPM exposure (aim 1); 4) ☑ Histological analyses (aim 1): pSer129 and Iba-1 quantification
CY19: 1) ☑ nPM exposure and inject mice with PFFs (aim 2); 2) ☑ Histological analysis (aim 2); 3) Data analysis/manuscript prep and submission
CY20: 1) ☑ Inject mice with PFFs (aim 2.2); 2) ☑ Histological analysis (aim 2.2); 3) Data analysis/manuscript prep and submission; 4) Initiate aim 3
CY21: 1) Histological analysis (aim 3); 2) Data analysis/manuscript prep and submission

Comments/Challenges/Issues/Concerns

- SARS-CoV-2 will affect our ability to travel, preventing surgical injection of PFFs in mice to be exposed. We are in the process of submitting a Request for Modification to deal with this challenge (outlined in Y3 annual report).

Budget Expenditure to Date

Projected Expenditure: $556,555
Actual Expenditure: $408,410