AWARD NUMBER: W81XWH-17-1-0535

TITLE: Airborne pollutants as triggers of Parkinsons disease via the olfactory system

PRINCIPAL INVESTIGATOR: Caleb E Finch, PhD

CONTRACTING ORGANIZATION: University of Southern California

REPORT DATE: September 2020

TYPE OF REPORT: Annual

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
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-17-1-0535

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 (PD) by increasing α-synuclein pathology via inflammation. After completing Specific Aim 2, wherein we found no differences between experimental groups in spread of α-syn, nor the expected nPM-induced neuroinflammatory changes. We repeated Specific Aim 2 mouse experiments with a new batch of nPM that showed in vitro activity. We collected tissues during the first week of SARS-CoV-2 quarantine. After restrictions were relaxed, we began analyzing tissues for neuroinflammation. This on-going analysis has yet to show strong inflammatory effects of the in vivo nPM exposure. Due to continued pandemic quarantine and travel restrictions we are discussing option with our collaborators. We will plan and execute novel experiments to explore the effects of LPS-induced olfactory inflammation on spread of α-syn by histological (VAI) and biochemical (USC) analyses.

15. SUBJECT TERMS

Pre-formed fibrils (PFFs), α-synuclein (α-syn), 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	13	19b. TELEPHONE NUMBER *(include area code)*

Standard Form 298 (Rev. 8-98)
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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 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 pathology *in vivo*, and on olfactory dysfunction among older adults. We will pursue experimental (Aims 1-4) and epidemiological (Aims 5-7) studies addressing common research questions.
The third year of this project at the University of Southern California consisted of the completion of Aim 2.1 analyses, the commencement of Aim 2.2 animal experiments, and subsequently the extraction, processing, and analysis of anterior cortex for markers of inflammation.

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 PFF pathology.

1. Inject C57BL/6J mice (n=96) with PFFs.
   Validation experiment accomplished (n=32), 10/12/17 (Y1Q1)
   Aim 1 injections (n=64) accomplished 1/18/18 (Y1Q2)
2. Expose C57BL/6J mice to nPM.
   Validation experiment accomplished (n=32), 11/09/17 (Y1Q1)
   Aim 1 exposure (n=64) accomplished 2/14/18 (Y1Q2)
3. Complete collection of brains and delivery of brains to VARI
   Validation exp done (n=32), 11/21/17
   Aim 1, completed 05/01/18 (Y1Q3)
4. Biochemical analyses
   Validation exp, not proposed
   Aim 1, completed 07/31/18 (Y1Q4)

Specific Aim 2: Determine the effects of exposing mice before triggering of pathology.

1. Expose mice to nPM (3 weeks before)
   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)
   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
   Aim 2 post-stereotactic injection exposure (n = 64) initiated 11/2/18, accomplished 12/20/18 (Y2Q2)
3. Complete collection of brains and delivery of brains to VARI
   Collection completed 12/21/18; brains received 1/15/19 (Y2Q2).
4. Biochemical analyses
   Completed (Y2Q4)

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. Collect nPM (Sioutas group, at USC) **08/31/19 – 10/31/19**
2. Test in vitro activity and perform 3 week in vivo pilot (Finch & Sioutas groups, at USC) **11/01/19 – 12/31/19**
3. Pre-expose mice to nPM for 3 weeks before injections (Finch & Sioutas groups, at USC)
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). (Brundin & Finch groups, at USC)
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 (Finch & Sioutas groups, at USC)
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 due to SARS-CoV-2 quarantine (Y3Q3)**
2. Biochemical analyses (Finch group, at USC) **ongoing**
3. Histological analyses (starts after 1 month for sectioning): (Brundin group, VARI)
    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**

Milestones:

Specific Aims 1**: Described above**

Specific Aims 2**: Described above**

Specific Aims 3: **not yet initiated**

What was accomplished under these goals?
In this reporting period:

1) Major activities:

1. Collect nPM for Aim 2 (Y3Q1).
2. Test in vitro activity and perform 3 week in vivo pilot (Y3Q1)
2. Pre-stereotactic injection exposure of Aim 2.2 C57BL/6NJ mice to nPM at USC (by USC personnel) (Y3Q2).
3. Stereotactic injection of Aim 2.2 C57BL/6NJ mice with PFFs at USC (by VARI personnel (Y3Q2).
4. Post-stereotactic injection exposure of Aim 2.2 C57BL/6NJ mice to nPM at USC (by USC personnel) (Y3Q2).
5. Collect Aim 2.2 experiment brains at USC and deliver to VARI (Y3Q3).
6. Biochemical analysis of Aim 2 tissues (Y3Q4).

2) Specific objectives: Perform Aim 2.2 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: In order to collect sufficient nPM for Aim 2.2, nPM was collected on filters over 60 days. After elution into sterile water by sonication nPM was aliquoted and stored at -20˚C. The NF-kB activation assay was performed on the new batch of nPM (2019-05; to be used for Aim 2.2), the previous batch of nPM (2018-03, previously used for Aim 2), and lipopolysaccharide, LPS (as positive control) (Fig. 1). The new batch of nPM significantly increased NF-kB activation above control. Based on this positive result, we initiated a 3-wk exposure on 7-wk C57BL/6NJ male and female mice (N=6).

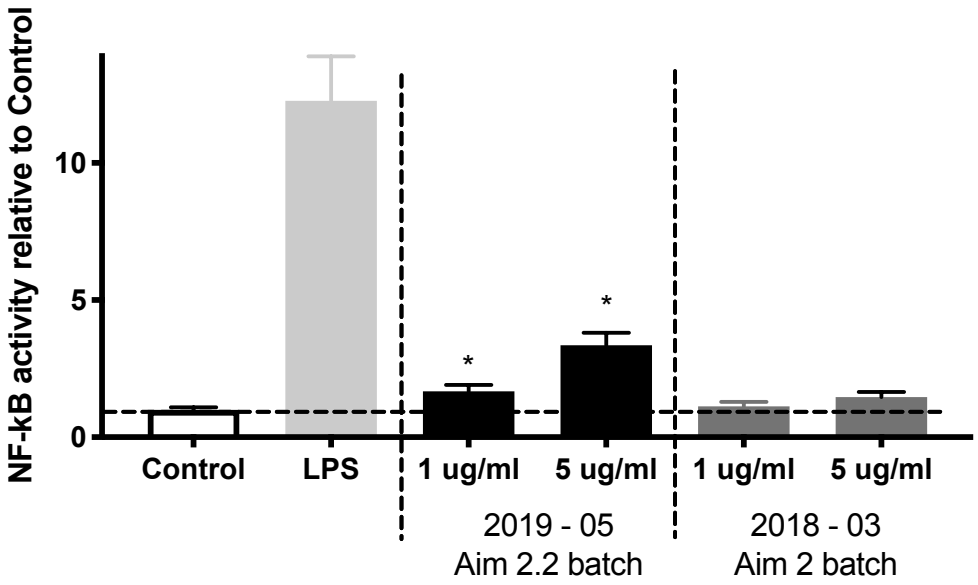
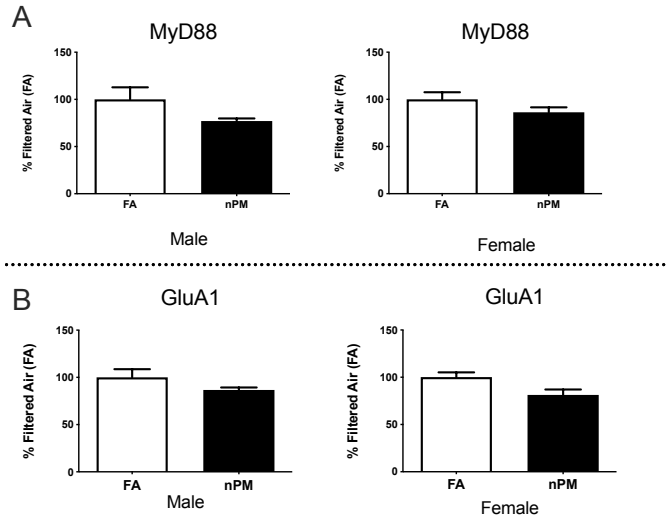


Fig. 1. The newly collected nPM batch (2019 - 05) increased NF-kB activity. The previously used nPM batch (2018 - 03) did not activate NF-kB. LPS (10 ng/ml) was used as positive control. Assay was performed in triplicate. Mean +/- SEM, *p<0.05

Y3Q2: A 3-wk pilot exposure on 7-wk C57BL/6NJ male and female mice (N=6) was performed. Cerebral cortices were collected; RNA and protein extracts were obtained. Replicating our recently reported data (Haghand et al 2020 PMID32004873), MyD88 and GluA1 mRNA levels were decreased in the cerebral cortices of both males and females exposed to nPM (Fig. 2). MyD88 is an adapter protein involved in the Toll-like receptor and IL-1 receptor signaling pathway leading to NF-kappa-B activation, cytokine secretion and the inflammatory response. GluA1 is a glutamate receptor is involved in synaptic plasticity.



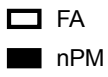
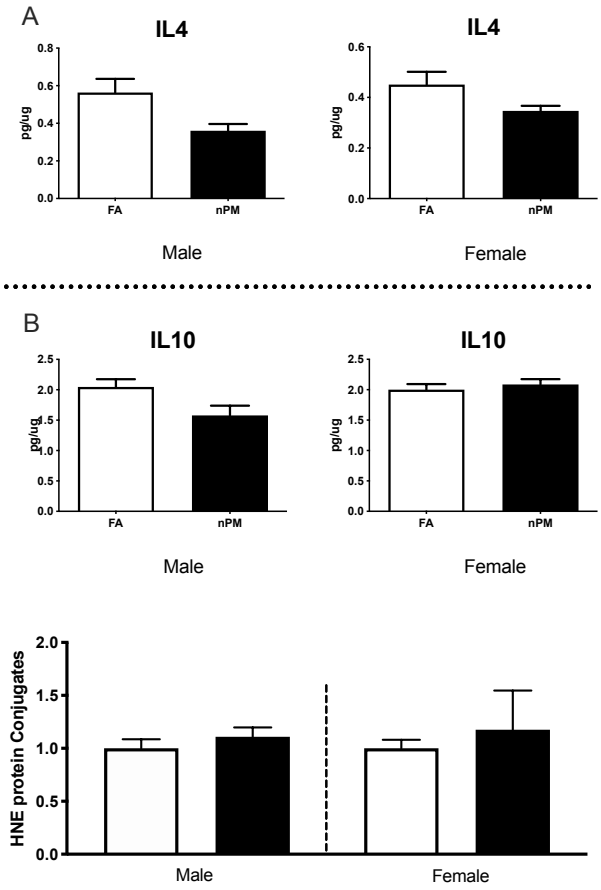


Fig. 2. The newly collected nPM batch (2019 - 05) decreased the mRNA levels of MyD88 (A) and GluA1 (B). qPCR was performed on mRNA isolated from cerebral cortices of males and female mice. N=6.

The inflammatory cytokines, IL-4 and IL-10, were also decreased (Fig. 3). 4-HNE, the hydroxyalkenal that is produced by lipid peroxidation, was slightly increased in the cerebral cortices of male and female mice exposed to nPM (Fig. 4). These data suggest that this batch of nPM induces inflammatory and oxidative responses in the cortex. Given these results, we proceeded with Aim 2.2.



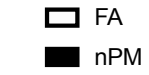


Fig. 3. The newly collected nPM batch (2019 - 05) decreased IL4 (A) and IL10 (B). Protein extracts were analyzed by Elisa. N=6.

Fig. 4. 4-HNE increased in the cerebral cortex of male and female mice exposed to nPM. Protein extracts were analyzed by Western blot. N=6.

We initiated Aim 2.2 nPM exposure.

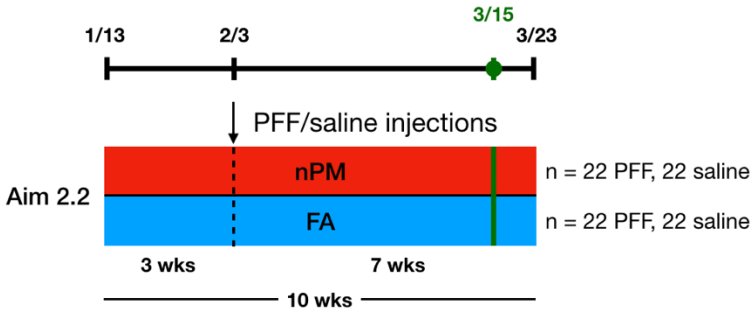
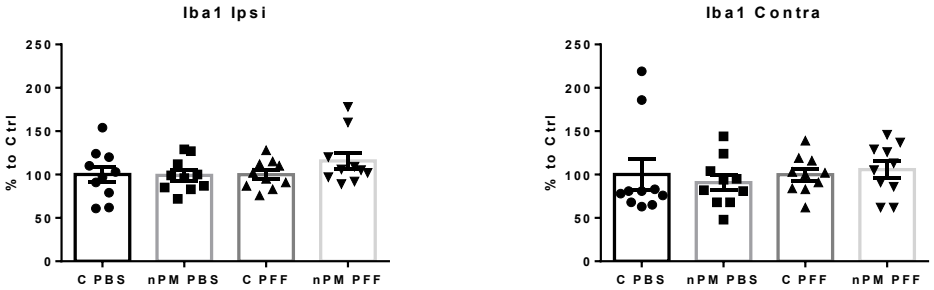


Figure 5: Timeline and experimental schematic. Aim 2.2 nPM and forced air (FA) exposures began January 13, 2020. PFF and saline microinjections into the right olfactory bulb of the 88 nPM- or FA-exposed mice occurred from February 3–6, 2020. Post-injection exposures will end and brains will be collected from March 23–25.

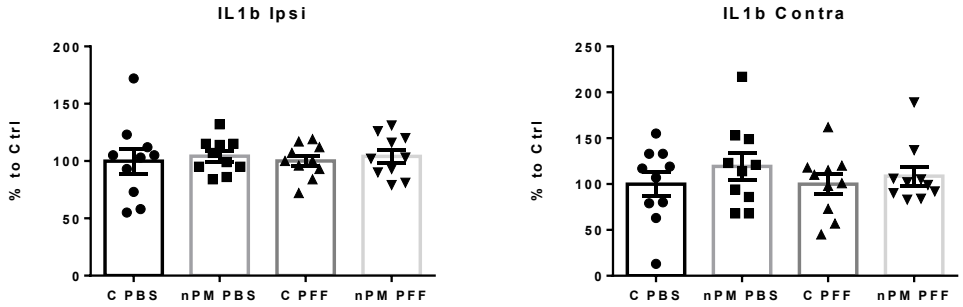
Y3Q3: During this quarter, Aim 2.2 nPM and forced air exposures were completed (Fig. 5). At this time, personnel at both USC and VARI were forbidden from entering buildings except for essential, time-sensitive tasks due to the COVID-19 pandemic. Therefore, USC personnel were only allowed to complete perfusions of experimental mice and collect tissues. Brains were shipped to VARI on 5/4/20. Since USC was still closed to research no activities could be performed. On a limited basis, USC personnel initiated biochemical analyses on 6/8/20. However, USC Research Restart limits capacity to 10% so activities are extremely slowed.

Y3Q4: Anterior cortexes from all 4 groups (C PBS; nPM PBS; C PFF; nPM PFF)(C=Filtered Air; nPM=nanosized particulate matter; PBS=saline control; PFF= Pre-formed α-synuclein fibrils), both ipsi and contralateral, (80 samples) were processed to extract RNA. qPCR analysis were performed to measure Iba1, IL1ß, IL10, MyD88 levels (Fig. 6). nPM exposure did not change mRNA levels for these genes. Only MyD88 mRNA was decreased in the nPM-PFF group.

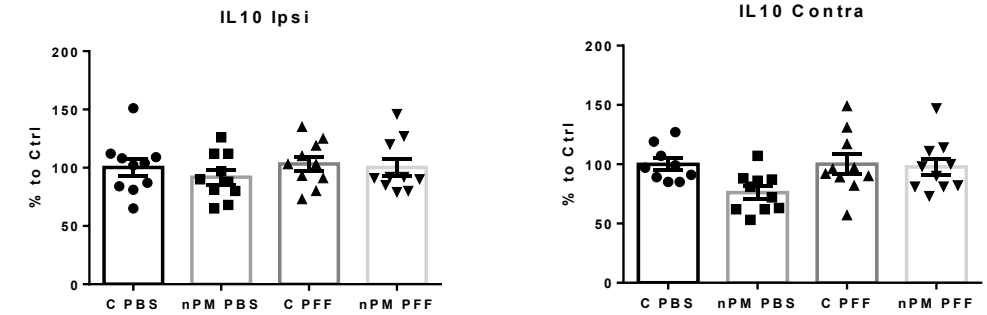
A. Iba1 (Microglia)



B. IL1ß



C. IL-10



D. MyD88

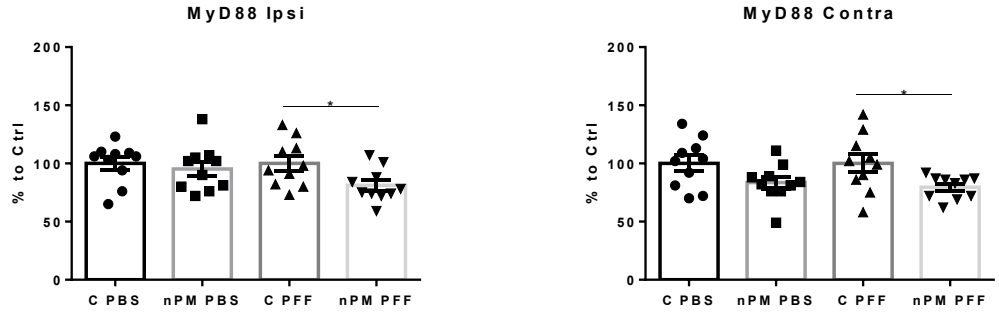
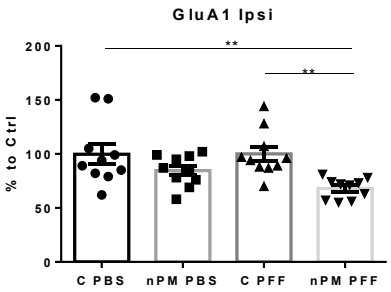


Fig. 6. The mRNA levels for Iba1, IL1ß, IL10, MyD88, in the anterior cortex were unchanged by nPM exposure. Only MyD88 was reduced in the nPM-PFF group. N=10, *p<0.05

We also measured GluA1 mRNA (Fig. 7) since we have shown that GluA1 is decreased by nPM exposure in numerous published studies (e.g., Morgan et al 2011 PMID21724521; Woodward et al 2017 PMID28212893; Cacciottolo et al 2017 PMID21724521; Haghani et al 2020 PMID32004873).



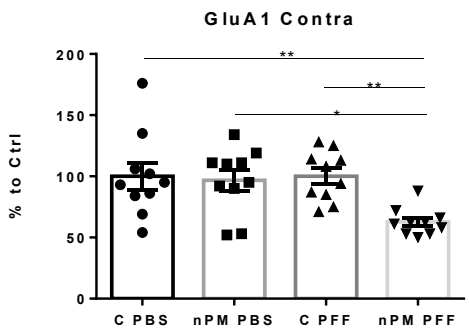
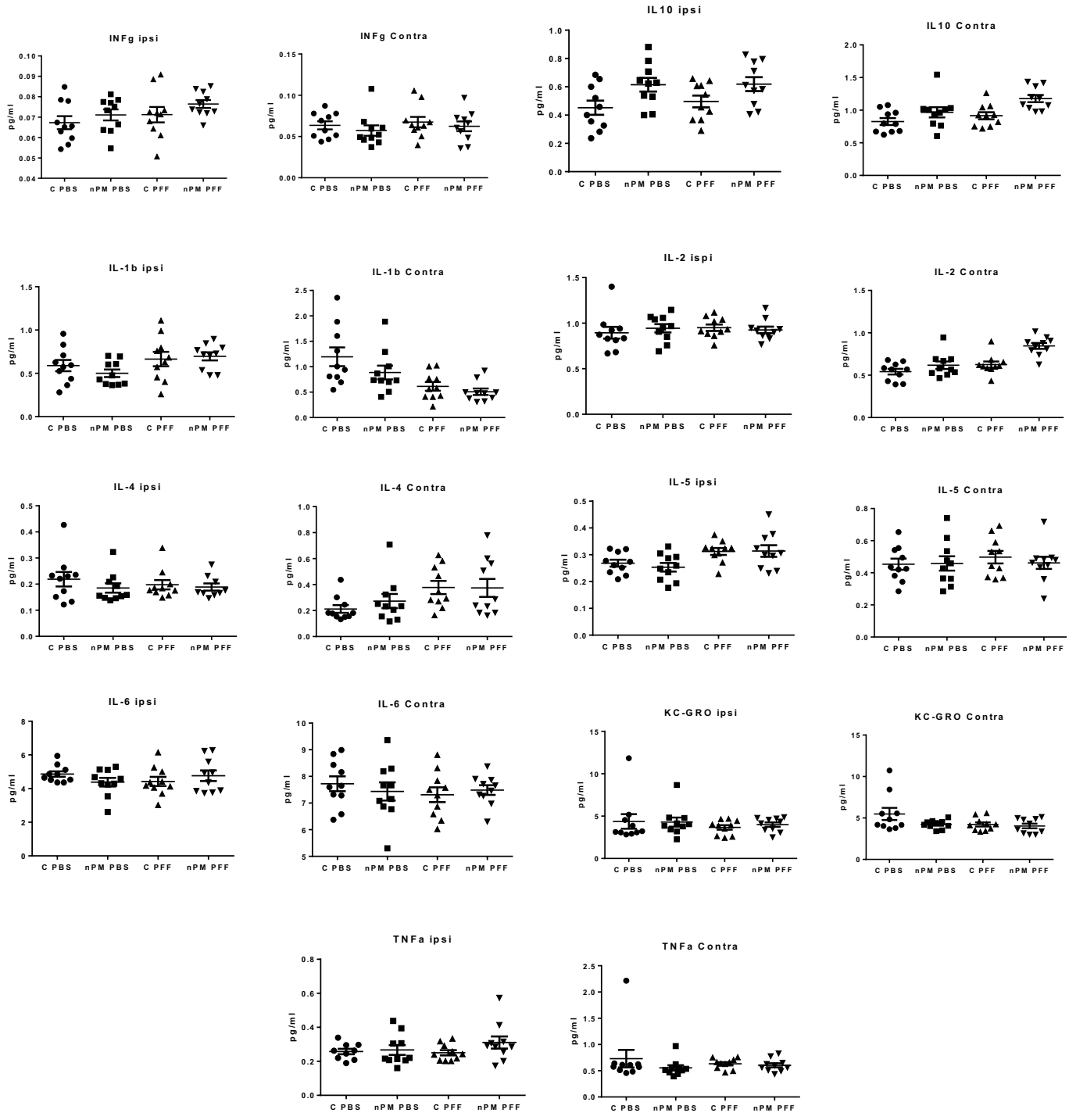


Fig. 7. GluA1 mRNA levels decreased in the anterior cortex in nPM+PFF mice. Both ipsi- and contra-lateral sides were affected. N=10, *p<0.05; **p<0.01

Protein was also extracted from the anterior cortex for analysis of proinflammatory proteins using MSD ELISA Proinflammatory Panel kit. No significant changes were measured (Fig. 8).



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?

- Finish biochemical analyses (PCR) of the OB.
- Submit Request for Modification of Specific Aim 3 in light of 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. We 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.

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

- The SARS-CoV-2 pandemic continues to limit our full research abilities.
- As described above in the Y3Q4 section we have not observe changes to expression of cytokine and inflammatory marker genes in the anterior cortex of nPM-exposures. While we may detect some changes in the OB (work on-going), based on the lack of effect of nPM on presence of α-syn marker pSer129 in olfactory structures (VARI results), we conclude that this batch of nPM did not induce an inflammatory effect. Furthermore, the pandemic does not allow our Michigan collaborators to travel to USC to inject mice. Therefore, we plan to submit a Request for Modification of Aim 3 as soon as possible and to execute a newly proposed experiment. Because of continued quarantine and travel

restrictions, VARI will perform intranasal LPS injections 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 our hypothesis that olfactory inflammation 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.

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?

Finch Lab:
Name: Caleb Finch, PhD
Project Role: Program Director
Researcher Identifier (Commons ID): cefinch
Nearest person month worked: 0.4 month per year
Contribution to Project: Dr. Finch is the Program Director who is overseeing this project.

Name: Todd Morgan, PhD
Project Role: Co-Investigator
Researcher Identifier (Commons ID): temorgan
Nearest person month worked: 1.8 month per year
Contribution to Project: Dr. Morgan is orchestrating the experimental plan, overseeing all aspects of the project, and ensuring regulatory compliance.

Name: Carla D'Agostino, DCLS
Project Role: Post-doctoral fellow
Researcher Identifier (Commons ID): cdagostino
Nearest person month worked: 6.0 month per year
Contribution to Project: Carla was in charge of the nPM exposures. She assisted the VARI team with injections. Carla was also in charge of tissue collection and sending tissue to VARI. She extracted RNA and protein from cortical tissues for qPCR and Western analyses. She performed the qPCR and Western analyses on the samples.

Name: Hongqiao Zhang
Project Role: Researcher
Researcher Identifier (Commons ID): hongqz
Nearest person month worked: 2.4 month per year
Contribution to Project: Hongqiao developed the in vitro NF-kB assay. He is in charge of verifying the in vitro activity of nPM batches. Hongqiao also assists Carla with the Westerns.

Name: Shannon McKay
Project Role: Administrator
Nearest person month worked: 0.6 month per year
Contribution to Project: Shannon is the grants manager.

Sioutas Lab:
Name: Mohammad Sowlat
Project Role: Graduate Research Assistant
Commons ID: MOHAMMADSOWLAT
Cumulative Person Months: 0.48 calendar
Current Period Person Months: 0.00 calendar
Contribution to Project: Collection and characterization of particle samples.

Name: Amirhosein Mousavi Nasabi Shams
Project Role: Graduate Research Assistant
Commons ID: MOUSAVIAMIR
Cumulative Person Months: 0.52 calendar
Current Period Person Months: 0.00 calendar
Contribution to Project: Collection and characterization of particle samples.

Name: Sina Taghvaee
Project Role: Graduate Research Assistant
Commons ID: TAGHVAEE
Cumulative Person Months: 0.77 calendar
Current Period Person Months: 0.20 calendar
Contribution to Project: Collection and characterization of particle samples.

Name: Milad Pirhadi
Project Role: Graduate Research Assistant
Commons ID: PIRHADI
Cumulative Person Months: 0.54 calendar
Current Period Person Months: 0.31 calendar
Contribution to Project: Collection and characterization of particle samples.

Name: Ehsan Soleimanian
Project Role: Graduate Research Assistant
Commons ID: EHSANSOL
Cumulative Person Months: 0.74 calendar
Current Period Person Months: 0.31 calendar
Contribution to Project: Collection and characterization of particle samples.

Name: Constantinos Sioutas
Project Role: Co-Investigator
Commons ID: SIOUTAS
Cumulative Person Months: 1.00 calendar
Current Period Person Months: 0.83 calendar
Contribution to Project: Project analysis, reporting, GRA supervision

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period? No

What other organizations were involved as partners?
None identified outside of our funded DoD collaborations.

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

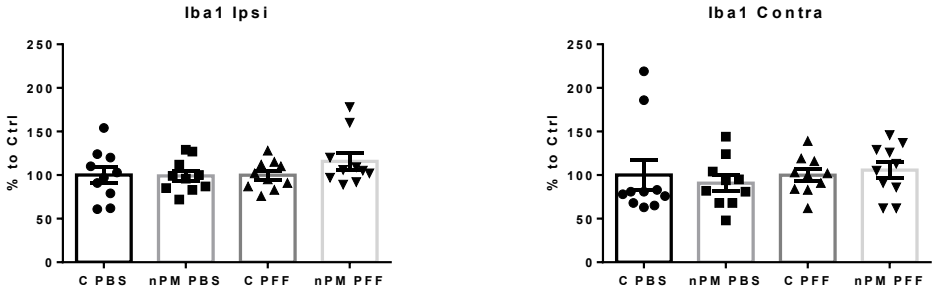
PD160021P1
W81XWH-17-1-0535
PI: C E Finch, PhD **Org:** University of Southern California **Award Amount:** $1,456,165.00

Study/Product Aim(s)

•Aim 1: Determine the effects of nPM exposure after microinjection of fibrillar α-syn in the OB.
• Aim 2.1: Determine the effects of nPM exposure prior to microinjection of fibrillar α-syn in the OB.
• Aim 2.2: Determine the effects of nPM exposure prior to microinjection of fibrillar α-syn in the OB.
• Aim 3: Define the effects of systemic administration of ibuprofen on the development of α-syn pathology.

Approach: This project includes in vivo studies to elucidate the influence of exposure to airborne pollutants (nPM) on the development of α-synpathology and possible interventions with NSAIDs..

Aim 2.2 exposure: No neuroinflammatory effect detected



Accomplishments:
Aim 2.2 exposure was completed. Biochemical analysis of the anterior cortex did not reveal any neuroinflammatory effect of nPM exposure. Microglial Iba1 mRNA levels shown here.

Timeline and Cost

Activities CY	17/18	18/19	19/20	20/21
Study Prep / Specific Aim 1				
Specific Aim 2.1 (see goals/milestones)				
Specific Aim 2.2 (see goals/milestones)				
Specific Aim 3 (see goals/milestones)				
Budget ($1,456,165)	**$354,548**	**$363,030**	**$365,426**	**$373,162**

Updated: 09/30/2020

Goals/Milestones
CY17: ☑ 1) Obtain IACUC approval; ☑ 2) ACURO regulatory approval; ☑ 3) Initiate validation study.
CY18: ☑ 1) Inject mice with PFFs (Aim 1); ☑ 2) nPM expose; 3) ☑ collect & deliver brains to VARI; 4) ☑ Biochem analyses (Aim 1)
CY19: 1) ☑ nPM expose (Aim 2); 2) ☑ Inject mice with PFFs; 3) ☑ collect & deliver brains to VARI; 4) ☑ Biochemical analyses (Aim 2); 5) data analysis/manuscript prep and submission
CY20: ☑ 1) Inject mice with PFFs (Aim 2.2); ☑ 2) nPM expose; ☑ 3) collect & deliver brains to VARI; 4) Biochemical analyses (Aim 3); 5) data analysis/manuscript prep and submission; 6) Inject mice with PFFs (Aim 3); 7) nPM expose; 8) collect & deliver brains to VARI;
CY21: 1) Biochemical analysis (Aim 3); 2) data analysis/manuscript prep and submission
Comments/Challenges/Issues/Concerns None to report
Budget Expenditure to Date
Projected Expenditure: $1,083,004
Actual Expenditure: $1,016,870