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

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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. In this aim we injected α-synuclein (α-syn) pre-formed fibrils (PFF) in the right olfactory bulbs of mice to model PD then exposed them to nano-particulate matter (nPM) for four weeks after injections. Ten weeks later, we euthanized the mice. The Brundin lab confirmed that PFF injections induced the expected phosphorylated α-syn pathology throughout olfactory areas. nPM exposure increased phosphorylated α-syn pathology only in the cortical amygdala. Importantly, we determined that nPM exposure did not show the expected increase of inflammatory or oxidative markers suggesting that these nPM effects may have been resolved during this extended period of time (10 weeks) when mice were not exposed to nPM.

15. SUBJECT TERMS
Pre-formed fibrils (PFFs), α-synuclein (α-syn), nano-particulate matter (nPM), neuroinflammation, Parkinson's disease (PD)

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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.

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 (Q1)

    Aim 1 injections (n=64) accomplished 1/18/18 (Q2)

2. Expose C57BL/6J mice to nPM.

    Validation experiment accomplished (n=32), 11/09/17 (Q1)

    Aim 1 exposure (n=64) accomplished 2/14/18 (Q2)

Milestones:

Major task: Study preparation

1. Obtain IACUC approval at USC, **completed on 06/07/17**
2. Obtain ACURO regulatory approval, **completed on 09/06/17 (Q1)**
3. Preparation of nPM, **collected (Q1)**

Major task: Determine the effects of exposing mice after triggering of pathology

1. Inject mice with PFFs (performed at USC by VARI postdoc)
   Validation exp done (n=32), 10/12/17 (Q1)
   Aim 1 injections (N=64) completed 01/18/18 (Q2)
2. Expose mice to nPM
   Validation exp done (n=32), 11/09/17 (Q1)
   Aim 1 exposure (N=64) completed 02/14/18 (Q2)

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 (Q3)
4. Biochemical analyses
   Validation exp, not proposed
   Aim 1, completed 07/31/18 (Q4)

Specific Aims 2, 3 & 4: **not yet initiated**

What was accomplished under these goals?

1) Major activities:
1. Obtain IACUC and ACURO regulator approval (Q1).
2. Collect nPM for Aim 1 (Q1)
3. Prepare for first collaborative study involving personnel from USC and VARI (Q1).
4. Inject C57BL/6J mice (Validation exp) with PFFs (Q1).
5. Expose C57BL/6J mice to nPM (Q1).
6. Collect brains and mail to VARI (Q1).
7. Inject C57BL/6J mice (Aim 1) with PFFs (with VARI personnel) (Q2).
8. Expose C57BL/6J mice to nPM (at USC) (Q2)
9. Collect brains and mail to VARI (Q3).
10. Biochemical analysis of Aim 1 tissues (Q4)

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:
Q1: Prior to initiating our first collaborative study we held a teleconference with USC and VARI PIs and researchers to refine our strategy. After much discussion we decided to exclude one of the experimental groups, monomeric a-synuclein. Our rationale was that this group was not needed since monomeric a-synuclein will not aggregate and spread into the brain (our primary outcome). Therefore, this group is redundant with the saline control group. Additionally, since we had never worked together and since the study required Dr. Nolwen Rey (VARI) to travel to USC to sonicate the PFFs and perform the precise surgical injections, we wanted to first do a smaller set of animals. This validation study was needed to ensure that all procedures could be properly executed at USC. Therefore, our initial study was on 32 mice, 16 fibrillar a-syn injected + 16 saline injected.

Dr. Rey arrived at USC on October 9th. She and the USC team immediately spent the entire afternoon setting up the Vivarium procedure room for their planned surgeries. Two full days of surgery occurred on October 10th and 12th, each day lasting nearly 10 hours. On October 11th, the USC team was trained on perfusion and collection techniques to ensure that brains could be processed at VARI. The day after each surgery, injected mice were subjected to their first day of nano-sized particle matter (nPM) exposure (5 hours). Following 60 hours of exposure (5 hours per day, 3 days per week), mice were perfused and brains collected by the USC team. After additional processing, brains were shipped to VARI.

Q2: In December 2017, Dr. Nolwen Rey (VARI personnel) reported to VARI and USC personnel preliminary results of the 32-mouse validation experiment: phospho-alpha-synuclein-specific pathology was observed in 6 PFF-injected mice, and no pathology was observed in 6 PBS-injected mice. Subsequently, remaining tissue

from the validation experiment was processed to detect phospho-alpha-synuclein-specific pathology. Further analysis was completed at VARI in Q3.

As stated in the Q1 report, we planned to initiate another collaborative study with USC to inject into mice and obtain tissues for the analyses of all of the proposed endpoints in Aim 1. In January 2018, Dr. Nolwen Rey and Ms. Lindsay Meyerdirk (VARI personnel) traveled to USC to perform PFF injections into 70 C57Bl/6J mice, with USC personnel. The mice were subsequently exposed to nPM by USC personnel for 4 weeks. At ten weeks after nPM exposure, the mice were euthanized and processed for analysis at USC and VARI, according to the Statement of Work.

Q3: As stated in the Q2 report, VARI personnel with assistance from USC personnel performed PFF injections into 70 C57BL/6J mice at USC in January 2018. The mice were subsequently exposed to nPM by USC personnel for 4 weeks. Ten weeks after nPM exposure, the mice were euthanized and processed for analysis at USC and VARI, according to the SOW. Tissues were sent to VARI for receipt on May 1st, 2018. We initiated biochemical analysis: qPCR and Western. RNA was isolated from ipsilateral frontal cortical and olfactory bulb (OB) tissues, cDNA prepared for subsequent qPCR. Protein was isolated from the ipsilateral olfactory bulb.

Q4:
We measured the mRNA levels of select inflammatory markers in ipsilateral frontal cortex and olfactory bulb from mice injected with saline or PFF (α-synuclein pre-formed fibrils) prior to 60 hours of nPM or filtered air (CTL) exposure, followed by 10 weeks of no exposure or additional treatments. In frontal cortex, the mRNA levels of the microglial markers, Iba1 and CD68, as well as the cytokines, TNFa, IL6 and IL10 were unchanged by either injections or exposure (Fig. 1).

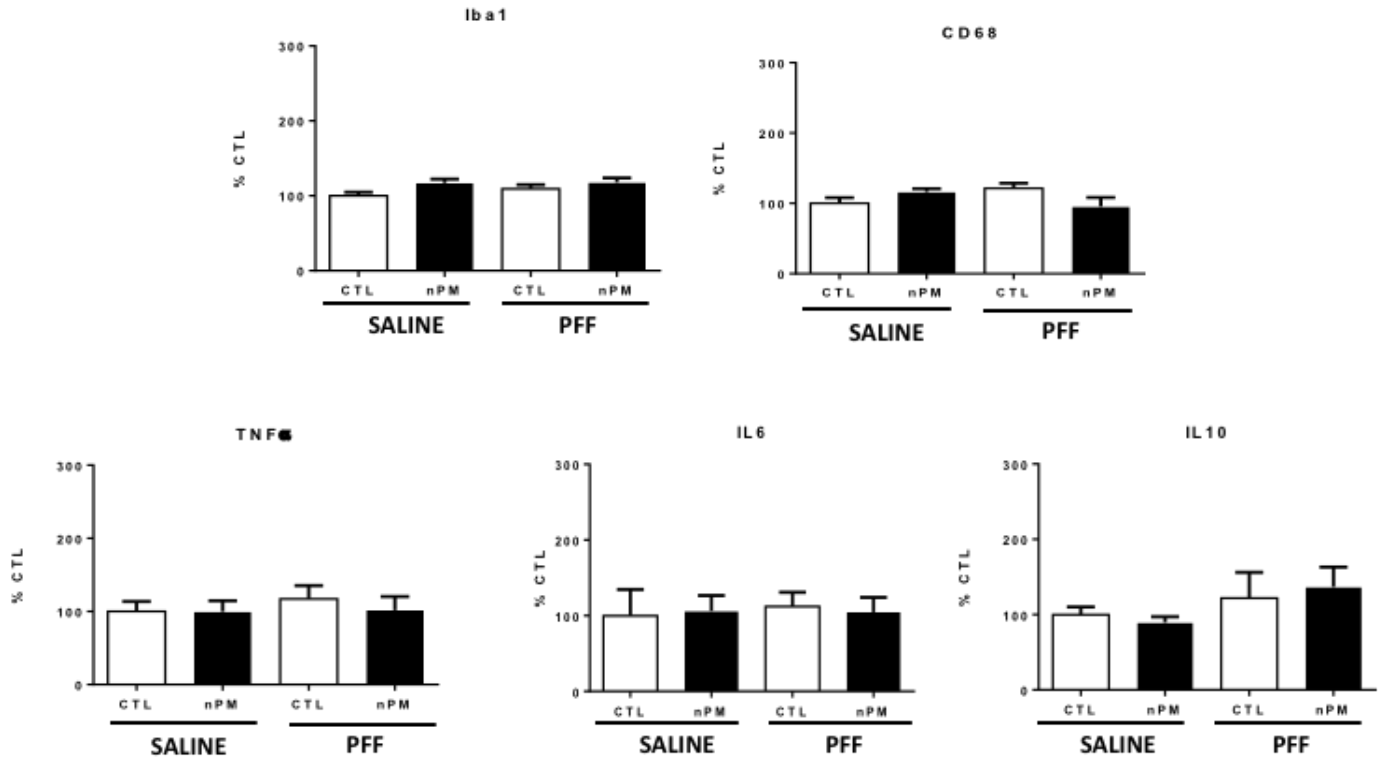


Fig. 1. ***In vivo*** **nanoparticulate matter (nPM) exposure did not significantly induce inflammation in the frontal cortex mice injected with either saline or PFF solutions.** Mice received a saline or PFF (α-synuclein pre-formed fibrils) solution injections prior to 60 hours nPM exposure. The ipsilateral frontal cortex was collected 10 weeks after the final nPM exposure, RNA was Trizol extracted and analyzed. The mRNA levels of the inflammatory/microglia markers showed no significant differences among the injected groups in nPM or filtered air (CTL) exposed mice. (4 groups, n= 8 mice/group).

In the OB, the mRNA levels of Iba1, CD68, TNFa, IL1a and IL10 were also unchanged by treatments (Fig. 2). Additionally, Iba1 protein levels were unchanged in the OB (Fig. 3A). We also measured the oxidative marker, 4-hydroxynonenel (4HNE), by Western blot in protein lysates from OB. 4HNE levels were unchanged by nPM exposure, PFF injection or the combination (Fig. 3B).

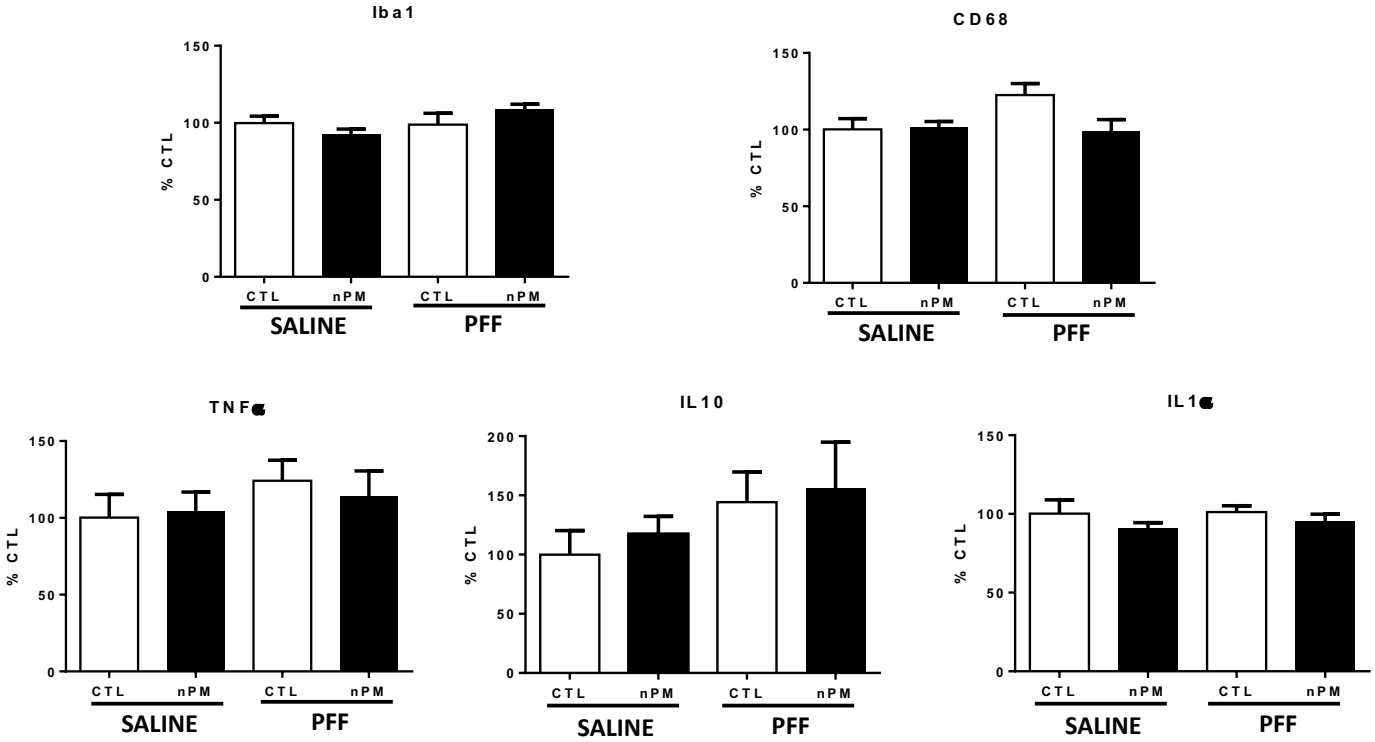


Fig. 2. ***In vivo*** **nanoparticulate matter (nPM) exposure did not significantly induce inflammation in OB mice injected with either saline or PFF solutions.** Mice received a saline or PFF (α-synuclein pre-formed fibrils) solution injections prior to 60 hours nPM exposure. The ipsilateral OB was collected 10 weeks after the final nPM exposure, RNA was Trizol extracted and analyzed. The mRNA levels of the inflammatory/microglia markers showed no significant differences among the injected groups in nPM or filtered air (CTL) exposed mice. (4 groups, n= 8 mice/group).

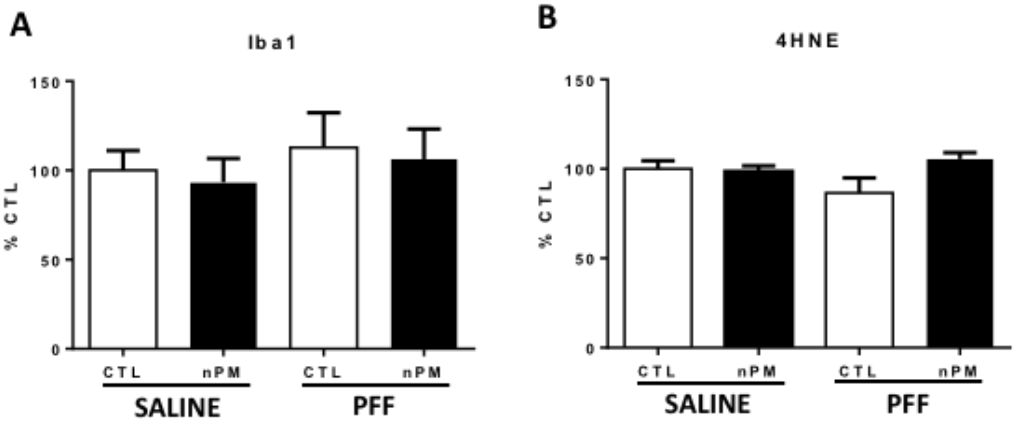


Fig. 3. ***In vivo*** **nPM exposure did not significantly induce microglial activation or oxidative stress in OB mice injected with either saline or PFF solutions.** Mice received a saline or PFF solution injections prior to 60 hours nPM exposure. The ipsilateral OB was collected 10 weeks after the final nPM exposure, proteins was Trizol extracted and analyzed. (A) Protein levels of the microglial marker, Iba1, was unchanged by either injections or exposure. (B) The oxidative stress marker, 4-Hydroxynoneral (4-HNE), also did not change in both groups injected with saline solution or PFF when exposed to nPM. (4 groups, n= 8 mice/group).

Importantly, analyses of inflammatory and oxidative markers in the ipsilateral olfactory bulb did not reveal the expected nPM inflammatory or oxidative effects, as documented in our previous work (Cheng et al 2016 Environ Health Perspect 124:1537-1546 PMID27187980). In our experimental setup we chose to examine the brains after a 10-week period (after α-syn PFF injection and nPM exposure) to allow for the spread of the α-syn PFF-induced pathology, as documented in the Brundin lab (Rey et al 2016 J Exp Med 213:1759-1778 PMID27503075). We hypothesize that the expected nPM effects may have resolved during this extended period of time (10 weeks) when mice were not exposed to nPM.

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?

Initiate Aim 2.

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

- **Changes in approach and reasons for change**
  - Nothing to Report
- **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

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: 0.6 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: 2.4 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: Nikoo Safi
Project Role: Technician, 09/01/17 – 03/16/18
Researcher Identifier:
Nearest person month worked: 6.0 month per year
Contribution to Project: Nikoo assisted with the surgical injections, the nano-sized particle matter (nPM) exposure and the tissue collection. She prepared the brains for transport to VARI.

Name: Madhura Sachindra Lotlikar
Project Role: Technician, 07/09/18 - present
Researcher Identifier:
Nearest person month worked: 6.0 month per year
Contribution to Project: Madhura assisted Carla with the qPCR and Westerns.

Sioutas Lab:
Name: Mohammad Sowlat
Project Role: Graduate Research Assistant
Commons ID: MOHAMMADSOWLAT
Cumulative Person Months: 0.69 calendar
Current Period Person Months: 0.21 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: 0.66 calendar
Current Period Person Months: 0.21 calendar
Contribution to Project: Collection and characterization of particle samples.

Name: Christopher Lovett
Project Role: Graduate Research Assistant
Commons ID: CLOVETT
Cumulative Person Months: 0.55 calendar
Current Period Person Months: 0.35 calendar
Contribution to Project: Collection and characterization of particle samples.

Name: Constantinos Sioutas
Project Role: Co-Investigator
Commons ID: SIOUTAS
Cumulative Person Months: 0.15 calendar
Current Period Person Months: 0.15 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?

Yes; see below:

CE Finch:

RF1 AG051521-01 (Finch) 09/30-2015-09-29/2020 1.20 calendar
NIH/NIA
$405,674 Annual Direct Cost
Amyloid and inflammation: modulation by apoE, gender, air pollution, and drugs

P50 AG05142-31 (Chui) 04//2015- 03/2020 0.20 calendar
NIH/NIA $1,338,249 Alzheimer Disease Research Center (ADRC).
The ADRC project focuses on the molecular and cellular analysis of changes during Alzheimer's disease and on clinical methods for early detection and the identification of subtypes. No overlap. Role: Co-investigator

R21 AG05020 (Finch PI) 04/01/2016 – 03/31/2019 NCE 1.0 calendar
NIH/NIA
$247,500
Air pollution nano-particulate matter, APP processing, and glutamate receptors.

PD160021P1 (CE Finch) 0/01/2017 - 09/30/2021 0.6 calendar
DoD, CDMRP
$225,628
Ariborne Pollutants as Triggers of Parkinson's Disease via the Olfactory System
The two major goals of this proposal are to: (i) define how exposure to air pollutants (specificall nano-sized particulate matter) influence the development and the progression of α-syn pathology

using in vivo systems; (ii) define the relationships between exposure to air pollutants, hyposmia and risk for Parkinson's disease (PD).

RF1AG054442-01 (PI Kaplan) 04/15/2017 – 03/31/2022 0.6 calendar
NIH/NIA $3,891,345
Brain atrophy, cognitive impairment and Alzheimer's in a low CVD-risk population
Little is known about the incidence, prevalence, and predictors of Alzheimer's disease (AD) in populations living traditional pre-industrial lifestyles similar to those experienced over human pre-history. This information is critical to determine whether AD is a byproduct of modern environments. Compared to age-matched industrialized populations, Tsimane exhibit: a) delayed atherosclerosis progression over their lifetime; b) low prevalence of diabetes and hypertension; and c) a near absence of atrial fibrillation, stroke and myocardial infarctions. At the same time Tsimane experience high rates of infection and inflammation throughout life. The two major goals of this proposal are to: 1) measure rates of cerebral atrophy and cognitive decline in association with atherosclerotic and inflammatory burden, APOE genotype and schooling, and 2) generate estimates of the prevalence and incidence of all-cause dementia and AD. Our central hypothesis is that compared to Westerners, the low rate of atherosclerosis among Tsimane will be paralleled by a slower rate of cerebral atrophy, and reduced age-related cognitive impairment. We will test the alternative hypothesis that infection and inflammation are associated with accelerated rates of cerebral atrophy and cognitive impairment. Co-Investigator (subcontractor)

R01 AG058068 (Pike, Gatz, LaDu) 09/01/17 – 08/31/22 0.6 Cal
NIH/NIA $470,484 Months

Sex differences in the relationship between APOE and AD: Role of sexual differentiation
This project uses human and rodent models to investigate the role of early neural development in sex differences in AD risk and how this modulated by *APOE4* genotype

P01 AG055367-01A1 (CE Finch, JC Chen) 04/01/2018 - 03/31/2023 Admin/Proj 0.6 Cal each
NIH/NIA
$12,626,207, total requested
Urban Air Pollution and Alzheimer's Disease: Risk, Heterogeneity, and Mechanisms.
A multi-disciplinary and multi-institutional team from the University of Southern California and their collaborators will study how traffic-related air pollution (TRAP) from metropolitan areas where most older Americans reside contributes to accelerated brain aging and risk of dementia. In the next 5 years, the team will study human populations and experimental models to examine the brain pathways that are specific for Alzheimer disease for gender and age differences in vulnerability to TRAP.

TE Morgan:		
R01 AG051521 (CE Finch) NIH/NIA *Amyloid and inflammation: modulation by apoE, gender, air pollution, and drug*s This project tests novel inflammation-gender-environment (air pollution) interactions in the EFAD mouse model for Alzheimer disease (AD).	09/30/2015 – 08/31/2020 $405,674 annual directs	0.6 Cal Months
OVERLAP: None		
R21 AG050201 (CE Finch) NIH/NIA *Air pollution nano-particulate matter, APP processing, and glutamate receptors* This proposal examines the impact of nPM exposure (C57BL/6 mice) on hippocampal neuronal physiology associated with cognitive processes, and associations of pro-amyloidogenic amyloid precursor protein (APP) processing with GluA1 changes, including experimental manipulation of APP processing with novel drugs.	04/01/2016 – 03/31/2019 (NCE) $137,500 annual directs	0.0 Cal Months
OVERLAP: None		
PO1 AG026572 (RD Brinton) NIH/NIA *Perimenopause in Brain Aging and Alzheimer's Disease.* Project 2 (CJ Pike, PI) The Perimenopause in Brain Aging and Alzheimer's Disease Program Project will determine how the brain changes during the perimenopausal transition and how these changes can lead to development of early risk actors for developing Alzheimer's disease.	06/01/2016 - 05/31/2021 $125,000 annual directs	0.0 Cal Months
OVERLAP: None		
R01 ES023780: H Volk (PI), TE Morgan (PI, Subaward), NIH/NIEHS *Prospective Evaluation of Air Pollution, Cognition, and Autism from Birth Onward.* This project focuses on the risk of air pollution exposure on developing autism using both human and mouse samples.	08/01/2015-04/30/2019 (NCE) $81,000, annual directs	0.6 Cal Months
OVERLAP: None		

PD160021P1 (CE Finch) DoD, CDMRP *Ariborne Pollutants as Triggers of Parkinson's Disease via the Olfactory System* The two major goals of this proposal are to: (i) define how exposure to air pollutants influence the development and the progression of α-syn pathology using *in vivo* systems; (ii) define the relationships between exposure to air pollutants, hyposmia and risk for Parkinson's disease (PD).	09/01/2017 – 08/31/2021 $225,628 annual directs	1.8 Cal Months
OVERLAP: None		
PO1 AG055369 (VD Longo) NIH/NIA *Dietary Restriction, GH/IGF-1 & Mechanisms of Differential Cell. Protection*: Core B (TE Morgan, PI) The Animal and Biostatistics Core works directly with Project Leaders to design, plan, monitor and interpret all animal experiments, as well as, provide specific mouse models and standardized experimental protocols in support of the overall program's common goals to understand how age and genetic background modulate the effects of caloric restriction, prolonged fasting and protein restriction cycles and fasting-mimicking diets on longevity and healthspan.	12/1/ 2017 - 11/30/2022 $380,000, Core B, annual directs	2.0 Cal Months
OVERLAP: None		
P01 AG055367-01A1 (CE Finch, JC Chen) NIH/NIA *Urban Air Pollution and Alzheimer's Disease: Risk, Heterogeneity, and Mechanisms*. A multi-disciplinary and multi-institutional team from the University of Southern California and their collaborators will study how traffic-related air pollution (TRAP) from metropolitan areas where most older Americans reside contributes to accelerated brain aging and risk of dementia. In the next 5 years, the team will study human populations and experimental models to examine the brain pathways that are specific for Alzheimer disease for gender and age differences in vulnerability to TRAP.	04/01/2018 - 03/31/2023 $12,616,207.00, total requested	2.4 Cal Months
OVERLAP: None		

What other organizations were involved as partners?

None identified outside of our funded DoD collaborations.

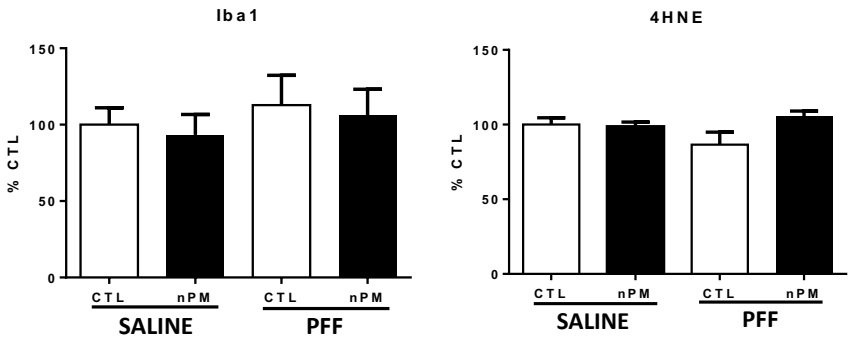
Airborne Pollutants as Triggers of Parkinson's Disease via the Olfactory System

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: 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.
• Aim 4: Define the effects of systemic administration of MSDC-0160 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 and MSDC-0161.



Accomplishment: The protein levels of the microglial marker, Iba1, and the oxidative marker, 4-hydroxynonenal (4HNE), were unchanged in the olfactory bulb (OB) injected with SALINE or PFF (α-synuclein pre-formed fibrils) exposed to either filtered air (CTL) or nPM. Mice received a saline or PFF solution injection prior to 60 hours of nPM or filtered air (CTL) exposure. The injected OB was collected 10 weeks after nPM exposure and protein lysates were analyzed by Western blot. N= 8 / group

Timeline and Cost

Activities CY	17/18	18/19	19/20	20/21
Study Prep / Specific Aim 1				
Specific Aim 2 (see goals/milestones)				
Specific Aim 3 (see goals/milestones)				
Specific Aim 4 (see goals/milestones)				
Budget ($1,456,165)	**$354,548**	**$363,030**	**$365,426**	**$373,162**

Updated: 09/26/2018

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 3); 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 4); 7) nPM expose; 8) collect & deliver brains to VARI;
CY21: 1) Biochemical analysis (Aim 4); 2) data analysis/manuscript prep and submission

Comments/Challenges/Issues/Concerns None to report

Budget Expenditure to Date

Projected Expenditure: $354,548
Actual Expenditure: $321,859