**AWARD NUMBER:** W81XWH-17-1-0535

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

**PRINCIPAL INVESTIGATOR:** Caleb E Finch, PhD

**CONTRACTING ORGANIZATION:** University of Southern California

**REPORT DATE:** October 2021

**TYPE OF REPORT:** Annual

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

**DISTRIBUTION STATEMENT:** Approved for Public Release; Distribution Unlimited

# REPORT DOCUMENTATION PAGE

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*OMB No. 0704-0188*



| 1. REPORT DATE | 2. REPORT TYPE | 3. DATES COVERED |
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| October 2021 | Annual | 01Sep2020-31Aug2021 |

**4. TITLE AND SUBTITLE:**

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

| 5a. CONTRACT NUMBER | 5b. GRANT NUMBER | 5c. PROGRAM ELEMENT NUMBER |
|---|---|---|
| W81XWH-17-1-0535 | PD160021P1 | |

**6. AUTHOR(S)**

Caleb E Finch, PhD

E-Mail: cefinch@usc.edu

| 5d. PROJECT NUMBER | 5e. TASK NUMBER | 5f. WORK UNIT NUMBER |
|---|---|---|
| | | |

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

Univ of Southern California
3720 S Flower St THIRD FLOOR
Los Angeles CA 90089-0001

**8. PERFORMING ORGANIZATION REPORT NUMBER**

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

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

**10. SPONSOR/MONITOR'S ACRONYM(S)**

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

**12. DISTRIBUTION / AVAILABILITY STATEMENT**

Approved for Public Release; Distribution Unlimited

**13. SUPPLEMENTARY NOTES**

**14. ABSTRACT**

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 | | | USAMRDC |
| Unclassified | Unclassified | Unclassified | Unclassified | 30 | 19b. TELEPHONE NUMBER *(include area code)* |

TABLE OF CONTENTS

**Page**

1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

This project is entitled "Airborne pollutants as triggers of Parkinson's disease via the olfactory system" and has two aims: (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 preclinical 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 define 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. In the fourth year of this project at University of Southern California we completed Aim 2 analysis of olfactory bulb for effects on the expression of inflammation-related and neuronal genes, and received DOD approval of both amended Aim 3 and our ACURO application for animal work to begin at Van Andel Research Institute.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

Pre-formed fibrils (PFFs), α-synuclein (α-syn), inflammation, Iba1, synergetic effect, GluA1, nanoparticulate matter (nPM), neuroinflammation, Parkinson's disease (PD)

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

**What were the major goals of the project?**

*List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.*

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 2.1: Determine the effects of exposing mice before triggering of pathology
1. Expose mice to nPM (3 weeks before and 7 weeks after injections). Aim 2.1 exposure (n = 64) completed at USC by USC personnel initiated 10/8/18, accomplished 10/28/18.
2. Inject mice with PFFs (as in Aim 1). Aim 2.1 injections (n=64) at USC with VARI personnel assisted by USC personnel, accomplished 11/1/18.
3. Post-expose mice to nPM for 7 weeks after injections. Aim 2.1 post-injection exposure (n = 64) initiated 11/2/18, accomplished 12/20/18.

Milestones
1. Complete collection of brains and delivery of brains to VARI collection completed 12/18&20/18 by USC and VARI personnel; brains sent to VARI on 01/15/19.
2. Biochemical analyses anterior cortex and olfactory bulb Western completed at USC.
3. Histological analyses: pSer129 histology completed 6/12/19, Iba-1 histology completed 12/4/19 at VARI.
4. Data analysis and manuscript preparation.

Specific Aims 2.2 Repeat: using a nPM collection that has been verified to have inflammatory activity.
1. Collect nPM on filters for Aim 2.2 exposure. Performed 09/01/19 to 10/31/19.
2. Verify in vitro (NF-kB activation assay) and in vivo (3-wk pilot exposure) activity. Completed.
3. Expose mice to nPM (3 weeks before and 7 weeks after injections). Aim 2.2 exposure (n = 88) completed at USC by USC personnel: 01/13/20 – 03/25/20.
4. Inject mice with PFFs (as in Aim 1). Aim 2.2 injections (n=88) at USC with VARI personnel assisted by USC personnel: 02/03 – 02/06.
Milestones
1. Complete collection of brains and delivery of brains to VARI, initiated 3/23/20 at USC, accomplished 5/4/20 due to COVID-19 Shelter-in-Place quarantine (USC only open to essential, COVID19 research).
2. Biochemical analyses initiated at USC on 6/8/20 (limited access to lab due to USC Research Restart requirements – 10% capacity maximum for now). Completed. OB results reported (Y4Q1 and Y4Q2).
3. Histological analyses (starts after 1 month for sectioning) initiated at VARI on 6/11/20; pSer129 staining completed 6/12/20.
4. Data analysis, manuscript preparation, and submission.
Two Publications (See attachment files):
Zhang et al. Urban Air Pollution Nanoparticles from Los Angeles: Recently Decreased Neurotoxicity. J Alzheimers Dis. 82(1):307-316, 2021. (PMID 33967042)
Jennifer Ailshire and Caleb E Finch. Recently decreased association of air pollution with cognitive impairment in a population-based aging cohort and in a mouse model. Letter to Alzheimer and Dementia, accepted August 10, 2021. ADJ-D-21-00733 - [EMID:faa92f7ed956f72e]

**What was accomplished under these goals?**

*For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.*

Collaboration lab at VARI has received the approval for our Request for Modification of Aim 3 on April 13, 2021, received ACURO approval on July 15, 2021. We applied for a no-cost extension, which was approved on August 3, 2021. Finally, we report final data on Specific Aim 2 samples from Y4Q1 and Y4Q2, which will help us in Specific Aim 3 analysis.

**Aim 2.1 Determine the effects of exposing mice before triggering of pathology**

During this reporting period, we finished our analysis of Specific Aim 2 brains of nPM- and forced-air exposed mice, with a focus on the altered expression of both inflammation-related and neuronal genes in the olfactory bulb (OB) and cortex. The inflammation-related genes: Iba1 (microglia activation), CD68, IL1b, MyD88, IL10 and others; neuronal genes: GluA1, EPAC2, PLPPR4, and FLVRC2. The selection of genes of interest genes is because they are critical mediators for inflammation process or their expression is altered by nPM (GluA1, EPAC2, PLPPR4, and FLVRC2) based on our prior studies using different batches of nPM.

Y4Q1: Olfactory bulb tissue from the ipsi- and contr-lateral sides [relative PFFs (pre-formed fibrillary α-synuclein) injection] were processed for PCR. Results demonstrate no effect of nPM or PFF for the mRNAs of Iba1 (Fig. 1A,B), CD68 (Fig. 1.C,D), IL-1b (Fig. 2A,B) or IL-10 (Fig. 2C,D). Furthermore, nPM alone or PFF alone had no effect on the mRNAs of MyD88 (Fig. 3A,B) or GluA1 (Fig. 3C,D). However, nPM and PFF in combination decreased both mRNAs.

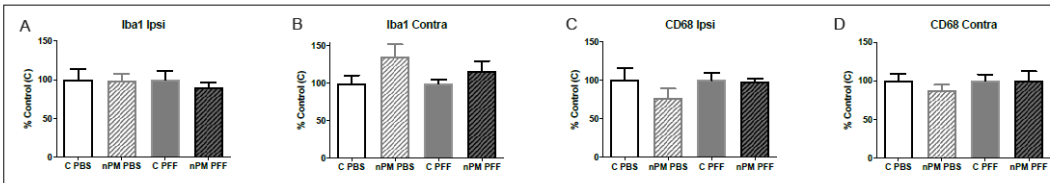



Fig. 1. Microglial Iba1 and CD68 mRNA response to nPM +/- PFF. Iba1 mRNA levels were unchanged by nPM alone or in combination with PFF in the ipsilateral (A) and contralateral (B) OB. CD68 mRNA levels were unchanged by nPM alone or in combination with PFF in the ipsilateral (C) and contralateral (D) OB. Mean±SEM; N=10

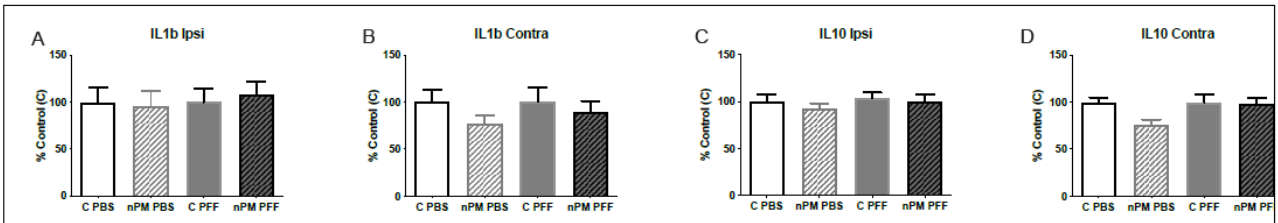



Fig.2. The proinflammatory cytokine, IL1b, and the anti-inflammatory cytokine, IL10, mRNA response to nPM +/- PFF. IL1b mRNA levels were unchanged by nPM alone or in combination with PFF in the ipsilateral (A) and contralateral (B) OB. IL10 mRNA levels were unchanged by nPM alone or in combination with PFF in the ipsilateral (C) and contralateral (D) OB. Mean±SEM; N=10

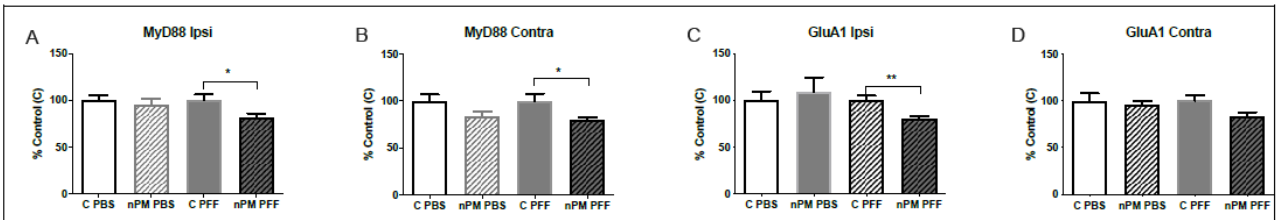
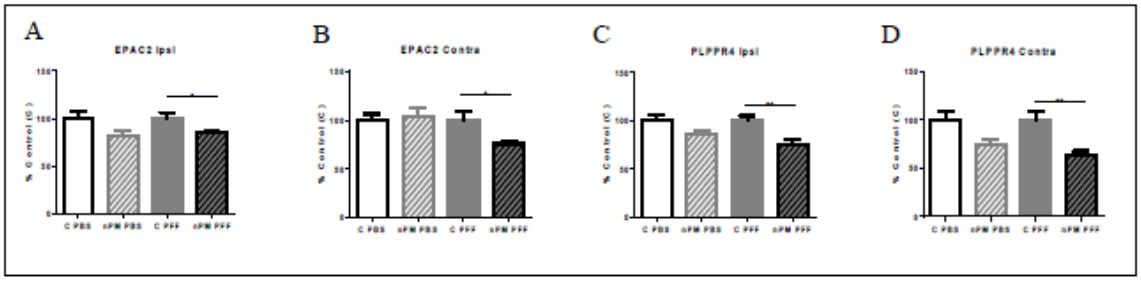
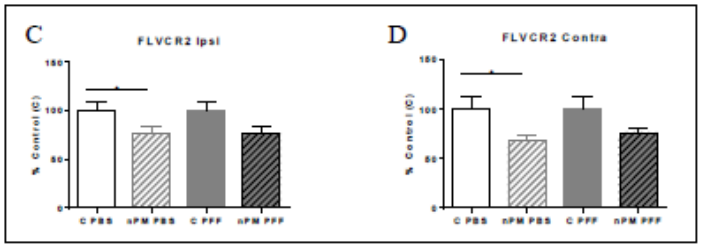



Fig. 3. The inflammatory signaling pathway transducer, MyD88, and the glutamatergic synaptic receptor, GluA1, mRNA response to nPM +/- PFF. MyD88 mRNA levels were unchanged by nPM alone or PFF alone in the ipsilateral (A) and contralateral (B) OB. MyD88 mRNA levels were decreased in the nPM-PFF group in both the ipsi- and contra-lateral OB. GluA1 mRNA levels were unchanged by nPM alone or PFF alone in the ipsilateral (C) and contralateral (D) OB. In the ipsilateral OB, GluA1 mRNA was decreased in the nPM-PFF group. N=10, *p<0.05; **p<0.01

Y4Q2: We analyzed the expression of several neuronal genes (EPAC2, PLPPR4, and FLVRC2) in the anterior cortex tissue from the ipsi- and contra-lateral sides [relative PFFs (pre-formed fibrillary α-synuclein) injection], the expression of these genes were altered in cortex of mice exposed to a different batch of Npm in a prior study (RNASeq analysis) (Haghani et al 2020 PMID320048735). PCR results demonstrated that nPM and PFF together had a synergetic effect on EPAC2 (Fig. A-B) and PLPPR4 (Fig. C-D) genes. The effect of nPM by itself was visible only on FLVRC2 gene (Fig.C-D), but not in the other neuronal genes.



Y4Q3: optimization of Jess western blot system to get ready for analyzing Aim3 samples while waiting for Aim3 animal exposure, which will be conducted at VARI.
Y4Q4: Nothing to report.

**What opportunities for training and professional development has the project provided?**
*If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.*

Nothing to report.

**How were the results disseminated to communities of interest?**
*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.*

The results from this project have contributed to 2 publications, which are available online to scientific communities (see attached files)
Zhang et al. Urban Air Pollution Nanoparticles from Los Angeles: Recently Decreased Neurotoxicity. J Alzheimers Dis. 82(1):307-316, 2021. (PMID 33967042)

Jennifer Ailshire and Caleb E Finch. Recently decreased association of air pollution with cognitive impairment in a population-based aging cohort and in a mouse model. Letter to Alzheimer and Dementia, accepted August 10, 2021. ADJ-D-21-00733 - [EMID:faa92f7ed956f72e]

**What do you plan to do during the next reporting period to accomplish the goals?**
*If this is the final report, state "Nothing to Report."*

*Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.*

| |
|---|
| We will initiate the mouse injections and analyze the samples as outlined in Specific Aim 3. |

***4.*** **IMPACT:** D*escribe 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?**
*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).*

| |
|---|
| The results from this project have contributed to 2 publications, which are available online to scientific communities (See attached files).<br>Zhang et al. Urban Air Pollution Nanoparticles from Los Angeles: Recently Decreased Neurotoxicity. J Alzheimers Dis. 82(1):307-316, 2021. (PMID 33967042)<br><br>Jennifer Ailshire and Caleb E Finch. Recently decreased association of air pollution with cognitive impairment in a population-based aging cohort and in a mouse model. Letter to Alzheimer and Dementia, accepted August 10, 2021. ADJ-D-21-00733 - [EMID:faa92f7ed956f72e] |

**What was the impact on other disciplines?**
*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.*

| |
|---|
| Nothing to report. |

**What was the impact on technology transfer?**
*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:*

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report.

*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:*

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report.

5. **CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Nothing to report.

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

*Describe problems or delays encountered during the reporting period and actions or plans to resolve them.*

Nothing to report.

**Changes that had a significant impact on expenditures**

*Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.*

Nothing to report

**Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

*Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.*

**Significant changes in use or care of human subjects**

Not applicable.

**Significant changes in use or care of vertebrate animals**

Current IACUC protocol approved on 8/4/2021 to change the entire animal use protocol to Category E as some mice in Segment 6 (unrelated to DOD-funded work) were considered Category 3. This animal use protocol also contains all DOD- and ACURO-approved plans that were approved 7/1/2021 (during this reporting period).

**Significant changes in use of biohazards and/or select agents**

| |
|---|
| 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**
  *Report only the major publication(s) resulting from the work under this award.*

  **Journal publications.** *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

| |
|---|
| The results from this project have contributed to 2 publications (See attached files).<br>Zhang et al. Urban Air Pollution Nanoparticles from Los Angeles: Recently Decreased Neurotoxicity. J Alzheimers Dis. 82(1):307-316, 2021. (PMID 33967042)<br><br>Jennifer Ailshire and Caleb E Finch. Recently decreased association of air pollution with cognitive impairment in a population-based aging cohort and in a mouse model. Letter to Alzheimer and Dementia, accepted August 10, 2021. ADJ-D-21-00733 - |

  **Books or other non-periodical, one-time publications.** *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

| |
|---|
| Nothing to report. |

**Other publications, conference papers and presentations**. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to report.

- *A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.*

Nothing to report.

- **Technologies or techniques**
*Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.*

Nothing to report.

- **Inventions, patent applications, and/or licenses**
*Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.*

Nothing to report.

- **Other Products**

  *Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:*

  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

| |
|---|
| Nothing to report. |

## 7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

**What individuals have worked on the project?**

*Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".*

*Example:*

| | |
|---|---|
| *Name:* | *Mary Smith* |
| *Project Role:* | *Graduate Student* |
| *Researcher Identifier (e.g. ORCID ID):* | *1234567* |
| *Nearest person month worked:* | *5* |
| *Contribution to Project:* | *Ms. Smith has performed work in the area of combined error-control and constrained coding.* |

*Funding Support:* *The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

1. Name: Caleb E Finch
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.

2. 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.

3. Name: Carla D'Agostino, DCLS
Project Role: Researcher
Researcher Identifier (Commons ID): cdagostino
Nearest person month worked: 6.0 month per year
Contribution to Project: Carla is in charge of the biochemical analysis. She extracted protein from cortical tissues for Western analyses. She performed the Western analyses on the samples. She extracted protein from olfactory bulb tissues.

4. 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.

5. 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:
1. Name: Constantinos Sioutas
Project Role: Co-Investigator
Commons ID: SIOUTAS
Cumulative Person Months (Y3): 0.17 calendar
Current Period Person Months: 0.17 calendar
Contribution to Project: Project analysis, reporting, GRA supervision.

Sioutas lab (continue)

2. Name: Sina Taghvaee
Project Role: Graduate Research Assistant
Commons ID: TAGHVAEE
Cumulative Person Months (Y3): 0.57 calendar
Current Period Person Months: 0.15 calendar
Contribution to Project: Collection and characterization of particle samples.

3. Name: Milad Pirhadi
Project Role: Graduate Research Assistant
Commons ID: PIRHADI
Cumulative Person Months: 0.23 calendar
Current Period Person Months: 0.15 calendar
Contribution to Project: Collection and characterization of particle samples.

4. Name: Ehsan Soleimanian
Project Role: Graduate Research Assistant
Commons ID: EHSANSOL
Cumulative Person Months: 0.43 calendar
Current Period Person Months: 0.26 calendar
Contribution to Project: Collection and characterization of particle samples.

**Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**
*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.*

Dr. Todd Morgan retired from the institute on 3/15/2021 and Carla D'Agostino quitted the institute on 04/30/2021.

**What other organizations were involved as partners?**
*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial*

*or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.*

*Provide the following information for each partnership:*
*Organization Name:*
*Location of Organization: (if foreign location list country)*
*Partner's contribution to the project (identify one or more)*

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

| |
|---|
| None identified outside of our funded DoD collaborations. |

## 8. SPECIAL REPORTING REQUIREMENTS

See attached Quad Chart (Next page).

## 9. APPENDICES:

Copies of publications

Project Title: Airborne Pollutants as Triggers of Parkinson's Disease via the Olfactory System
Log Number: PD 160021P1; Annual report Year 4
Award Number: W81XWH-17-1-0535

**PI:** C.E. Finch, PhD **Org:** University of Southern California **Award Amount:** $1,456,165.00

## Study Aims

- Aim1: Determine the effects of nPM exposure on α-synucleinopathy *after* microinjection of fibrillar α-syn in the OB.
- Aim2.1: Determine the effects of nPM exposure on α-synucleinopathy prior to microinjection of fibrillar α-syn in the OB.
- Aim2.2: Repeat Aim 2.1 using a nPM collection that has been verified to have inflammatory activity.
- Aim 3: Define the effects of systemic administration of ibuprofen on the development of α-syn pathology.
- Aim3.1 Determine the effects of LPS-induced olfactory or systemic inflammation on spread of pathology
- Aim3.2 Define the effects of systemic administration of ibuprofen on the development of α-syn pathology triggered by LPS and microinjection of α-syn .

**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.

## 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) | | | | |
| Estimated Budget ($1,456,165) | $354,548 | $363,030 | $365,426 | $373,462 |

**Updated:** 06/11/21

Aim 2:
Major findings:
nPM and PFF together have synergistic effects on decreasing the expression of MyD88, an adaptor protein in the inflammatory signaling, and GluA1, the receptor for neurotransmitter glutamate, in OB; and neuronal genes dEPAC2 and PLPPR4 in cortex.

Accomplishments: two publications.

Zhang et al. Urban Air Pollution Nanoparticles from Los Angeles: Recently Decreased Neurotoxicity. J Alzheimers Dis. 82(1):307-316, 2021. (PMID 33967042)

Jennifer Ailshire and Caleb E Finch. Recently decreased association of air pollution with cognitive impairment in a population-based aging cohort and in a mouse model. Letter to Alzheimer and Dementia, accepted August 10, 2021. ADJ-D-21-00733 - [EMID:faa92f7ed956f72e]

### 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 exposure; 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 2); 5) data analysis/manuscript prep and submission
CY21: 1) Initiate aim 3; 2) Inject mice with PFFs (Aim 3) at VARI; 3) LPS expose at VARI; 4) obtain brains from VARI; 5) Biochemical analysis (Aim 3); 6) data analysis/manuscript prep and submission

### Comments/Challenges/Issues/Concerns

- Nothing to report.

### Budget Expenditure to Date

Projected Expenditure: $1,456,165
Actual Expenditure: $1,447,795

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# Urban Air Pollution Nanoparticles from Los Angeles: Recently Decreased Neurotoxicity

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**Abstract**.
**Background:** Air pollution is widely associated with accelerated cognitive decline at later ages and risk of Alzheimer's disease (AD). Correspondingly, rodent models demonstrate the neurotoxicity of ambient air pollution and its components. Our studies with nano-sized particulate matter (nPM) from urban Los Angeles collected since 2009 have shown pro-amyloidogenic and pro-inflammatory responses. However, recent batches of nPM have diminished induction of the glutamate receptor GluA1 subunit, Iba1, TNFα, $A\beta_{42}$ peptide, and white matter damage. The same methods, materials, and mouse genotypes were used throughout.
**Objective:** Expand the nPM batch comparisons and evaluate archived brain samples to identify the earliest change in nPM potency.
**Methods:** Batches of nPM were analyzed by *in vitro* cell assays for NF-κB and Nrf2 induction for comparison with *in vivo* responses of mouse brain regions from mice exposed to these batches, analyzed by PCR and western blot.
**Results:** Five older nPM batches (2009–2017) and four recent nPM batches (2018, 2019) for NF-κB and Nrf2 induction showed declines in nPM potency after 2017 that paralleled declines of *in vivo* activity from independent exposures in different years.
**Conclusion:** Transcription-based *in vitro* assays of nPM corresponded to the loss of *in vivo* potency for inflammatory and oxidative responses. These recent decreases of nPM neurotoxicity give a rationale for evaluating possible benefits to the risk of dementia and stroke in Los Angeles populations.

Keywords: Air pollution, Alzheimer's disease, microglia, mouse brain, ultrafine particulate matter

## INTRODUCTION

Exposure to particulate matter (PM) in urban air pollution is associated with accelerated cognitive decline and increased risk of Alzheimer's disease (AD) in many populations [1–4]. Urban airborne

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PM is a mixture from diverse sources that can vary widely in chemical composition and size distribution. Rodent models for the neurotoxicity of urban air document neurite atrophy, glial activation, and oxidative damage in association with increased production of amyloid peptides, inflammatory responses, and Nrf2-mediated antioxidant response [2, 5–7]. Experimental studies have used PM from diverse sources: concentrated total ambient urban PM [8, 9], ambient air from a traffic tunnel [10], size-selected PM collected on filters [11–13], and dust-storm PM [14]. Air pollution components include exposure to diesel exhaust particles [5, 15, 16], zinc nanoPM [17], iron-coated silica nanoPM [18], carbon-nanotubes [19], and ozone [7, 20].

Protocols used by our laboratory since 2010 [21] expose rodents to nPM, a subfraction of ultrafine PM0.2 [1, 11, 21–35]. Urban ultrafine PM (PM0.2) are continuously collected on filters from the same urban freeway corridor in Los Angeles. Filters are sonicated in water to yield an eluate we designated as nPM in distinction from total ultrafine PM [21]. The nPM subfraction of PM0.2 has equivalent oxidative activity to total PM0.2 assayed *in vitro* [36], despite its lower content of transition metals and the absence of polycyclic aromatic hydrocarbons [11]. Exposure of mice to nPM for 3–12 weeks can activate microglia in multiple brain regions [25, 28, 35]; induce pro-inflammatory cytokines in brain (TNFα and IL-1β) [21, 25, 28]; decrease neuronal glutamate receptor subunit GluA1 in hippocampus and cortex [1, 21, 28]; and cause atrophy of hippocampal CA1 neurites [1, 28]. The nPM and other air pollution components are also pro-amyloidogenic in AD-transgenic mice [1, 7, 10] and in wild-type rodents: the $A\beta_{42}$ peptide was increased by diesel exhaust in F344 rats [37] and by nPM in C57BL/6 mice [36]. The combination of nPM with chronic cerebral hypoperfusion from bilateral carotid artery stenosis (BCAS) caused increased white matter damage and activated microglia [27].

We recently characterized seven nPM batches collected from 2016 to 2018 for inflammatory cytokine responses and lipid peroxidation in cultured monocytes [36]. Several recent batches of nPM showed lower activity for NF-κB induction and lipid peroxidation that corresponded to decreased *in vivo* microglial activation and amyloidogenic response [36]. Here, we extend these findings to a wider time range of nPM batches, 2009–2020. The nPM batches are compared for *in vivo* inflammatory responses, glutamate signaling, amyloid production, and white matter damage in mouse models. A new *in vitro* assay was developed for simultaneously assessing the activation of Nrf2 and NF-κB in monocytes, which are increased in lung by chronic exposure to air pollution [38].

## METHODS AND MATERIALS

### *Reagents*

RPMI 1640 cell culture media and TRIzol reagent were from Thermal Fisher (Rockford, IL). QUANTI-Blue was from InvivoGen (San Diego, CA); reverse transcription reagent and qPCR master mixture were from BioPioneer Inc (San Diego, CA); other chemicals, were from Sigma-Aldrich (St. Louis, MO). Antibodies of Iba1, MAG, and dMBP were from Abcam (Cambridge, MA). $A\beta_{42}$ was assayed by ELISA Meso Scale Discovery (Rockville, MD).

### *Collection and preparation of nPM*

All nPM batches were collected from Los Angeles city within 150 m downwind of the I-110 Freeway [39]. These aerosols represent a mix of fresh ambient PM mostly from vehicular traffic. PM0.2 was collected on Zeflour PTFE filters (Pall Life Sciences, Ann Arbor, MI) with a High-Volume Ultrafine Particle Sampler [39] at 400 L/min flow. Filter-deposited nPM was eluted by sonication into deionized sterile water and stored in –20°C. Table 1 shows the collection dates of nPM batches.

### *Animals and nPM exposure*

Animal procedures were approved by the University of Southern California (USC) Institutional Animal Care and Use Committee (IACUC). Young adult C57BL/6J male and female mice were purchased from Jackson Laboratories. Mice were exposed to nPM on the same protocol used since 2010 [11]. For each exposure, mice were transferred from home cages into sealed exposure chambers with ample ventilation. The nPM group was exposed to re-aerosolized nPM at 300 $\mu g/m^3$, while controls received filtered air in parallel chambers. Exposure schedules were 5 h/d, 3 d/wk; the duration varied by experiment, 3 to 15 wk (Table 1). Mass concentration of the re-aerosolized nPM exposure stream was gravimetrically assessed on filters in parallel to the exposure stream before and after exposures. The nebulizer maintained a similar size distribution of re-aerosolized PM0.2. In one study, mice were

Table 1
nPM Batch, Exposure paradigm, and Reference to publication or current data

| Batch | Collection date | Genotype and Sex (C57BL/6 and transgenics) | Exposure duration | Reference |
|---|---|---|---|---|
| 2009 | Nov-Jan, 2008-2009 | M | 10 wk | [21], Fig. 2B |
| 2010a | Mar, 2010 | M | 10 wk | [22, 40] |
| 2010b | Mar-Apr, 2010 | F | Prenatal 10 wk | [23, 24, 28, 29, 31] |
| 2011 | Mar-Jun, 2011 | M (LDL$^{-/-}$) | 10 wk | [41] |
| 2012 | Aug-Sep, 2012 | M, M (ApoE-TR), F (EFAD) | 1, 4, 9 d, 15 wk, 15 wk | [1, 25, 33] |
| 2014 | Oct-Feb, 2014-2015 | M | 20 wk, prenatal to adult 3 mo | [32] |
| 2015a | Oct-Dec, 2015 | M | 3 wk | [27, 35] |
| 2015b | Nov-Feb, 2015-2016 | M (rat) | 28 wk, prenatal to adult 5 mo | [30] |
| 2016a | Feb-Apr, 2016 | M | 10 | Table 2, Fig. 2B |
| 2016b | Oct-Dec, 2016 | M (J20 hAPPSwe) | 10 | [42] |
| 1 | Apr-June, 2016 | *In vitro* only | | [36], Fig. 1A, B |
| 2 | June-Aug, 2016 | M | prenatal, or 8 wk | [34–36, 43], Fig. 2A, C, G, H |
| 3 | May-Sep, 2016 | *In vitro* only | | Fig. 1A, B |
| 4 | Nov-Jan, 2016-2017 | *Caenorhabditis elegans* | | [44] |
| 5 | May-Jun, 2017 | M | 3 wk | [11, 33] |
| 6 | Jan-Mar, 2018 | M, F | 8 wk | [33, 36] |
| 7 | May-Jul, 2018 | F | 8 wk | Fig. 2G |
| 8 | Feb-Mar, 2019 | F | 10 wk | Fig. 2. D, E, F |
| 9 | Sep-Nov, 2019 | M, F | 3 wk | Table 2, Fig. 2G, 2H |
| | | F | 10 wk | |
| 10 | Jan-Mar, 2020 | *In vitro* only | | Fig. 1A, B |

C57BL/6 mice and designated transgenic strains, and Sprague-Dawley rats were exposed to nPM at 300 μg/m$^3$, 5 h/d, 3 d/wk for the stated number of weeks.

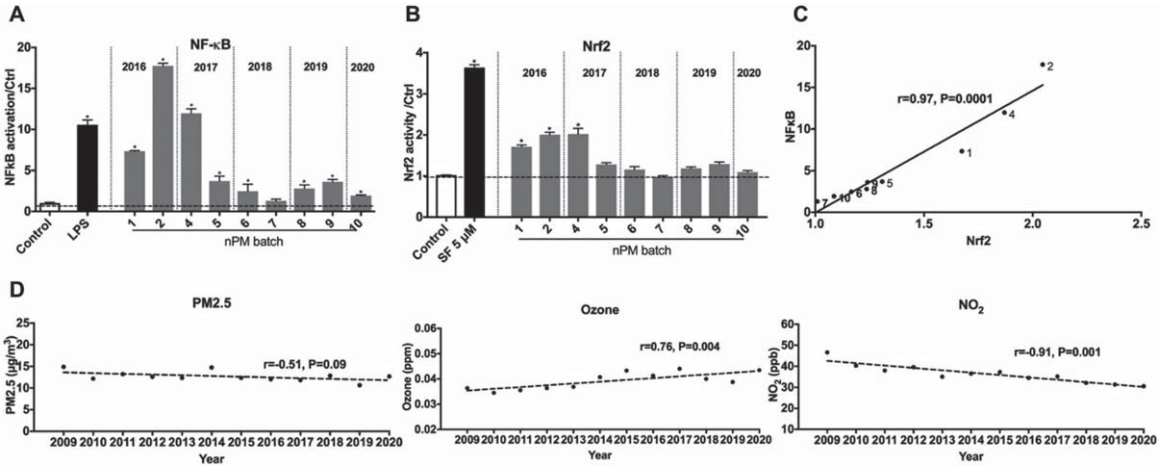


Fig. 1. *In vitro* biological activities of nPM batches. A) NF-κB induction; LPS, lipopolysaccharide (5 ng/ml). B) Nrf2 induction; SF, sulforaphane (5 μM). $^*p<0.05$ versus control, four wells per assay plate. C) Co-plot of NFκB and Nrf2; $R^2$, 0.94, $p=0.0001$; bolded numbers are batch ID since 2018 (Batch 6–10). The correlation of NF-κB and Nrf2 was analyzed by two-tailed Pearson correlation analysis. D) Historical change of air pollutants. Levels of PM2.5, ozone and $NO_2$ in central LA (5 miles from nPM collection site) from 2009 to 2020 showed linear trends: PM2.5: $R^2=0.26$, $p=0.09$; $O_3$: $R^2=0.58$, $p=0.004$; $NO_2$: $R^2=0.83$, $p=0.001$.

given bilateral carotid artery stenosis (BCAS) at 4 weeks before the final nPM exposure [45]. After euthanization by cardiac perfusion under anesthesia, brains were dissected, frozen on dry ice, and stored at –80°C.

*mRNA*

Cerebral cortex (alternate hemispheres) was extracted for RNA with TRIzol reagent, followed by qPCR with specific primers [11].

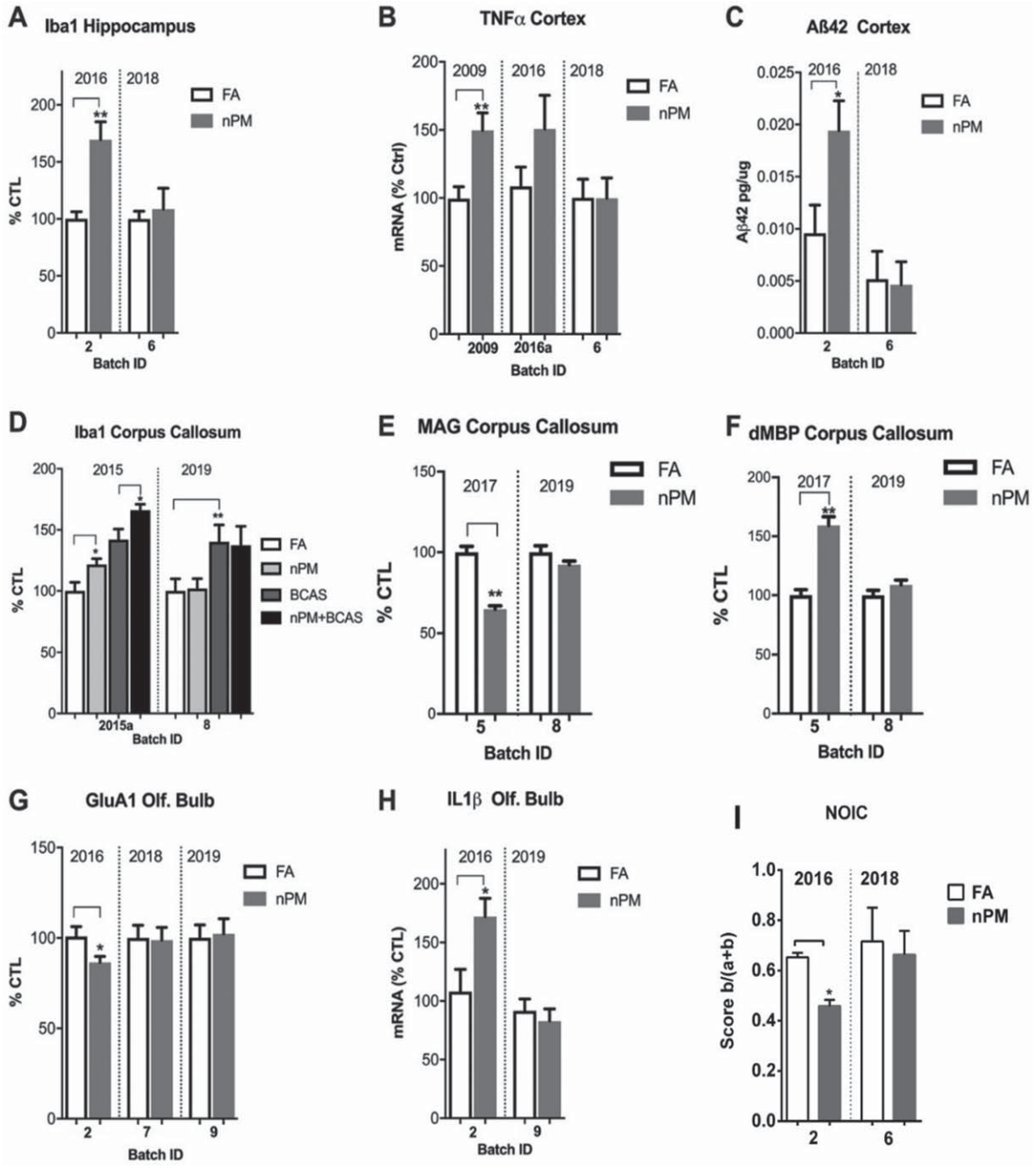


Fig. 2. *In vivo* brain responses to nPM by batch for 120 to 150 h (see Table 1 for exposure details and primary citations). A) Microglial Iba1 immunostaining in hippocampus; Batch 2 data are shown from our initial report [36]. B) TNFα mRNA in cerebral cortex; Batch 2009 [21]. C) $A\beta_{42}$ peptide in cerebral cortex; Batch 2 data [36]. D) Microglial Iba1 immunostaining in corpus callosum; Batch 2015a [35]. E) MAG (myelin-associated glycoprotein) immunostaining in corpus callosum; Batch 5 data [Q. Liu and W.J. Mack, unpublished]. F) dMBP (debris of myelin basic protein) immunostaining in corpus callosum [Q. Liu and W.J. Mack, unpublished]. G) GluA1 in olfactory bulb; Batch 2 and Batch 9: mRNA by qPCR, Batch 7: protein by western blot. H) IL-1β mRNA in olfactory bulb. Mean ± SD, N = 7–10, $^{*}p < 0.05$, $^{**}p < 0.01$. I) Novel Object in Context (NOIC) score for memory assessment. Batch comparisons were from exposures for 8 or 10 weeks conducted in different years. The standardized effect sizes differed across batch collection years ($p = 0.001$); effect sizes in 2018 and 2019 were smaller than prior years ($p = 0.003$).

*Immunohistochemistry*

Iba1 [45], myelin-associated glycoprotein (MAG), and debris of myelin basic protein (dMBP) [26] were immunostained by standard procedures in cited references.

*NF-κB and Nrf2 activity*

To simultaneously assay both activities, we engineered THP-1 Blue NF-κB reporter monocytes by transfection with a Nrf2 reporter. This THP-1 line expresses the reporter NF-κB-SEAP (secreted embryonic alkaline phosphatase) regulated by 5-tandem IκB cis-elements [36]. The NF-κB reporter measures activity but cannot differentiate which NF-κB family member causes activation. THP-1 NF-κB/Nrf2 reporters were developed by transducing THP-1 Blue NF-κB monocytes with ARE Luciferase Reporter Lentivirus (BPS Bioscience, San Diego, CA). The ARE Luciferase reporter is regulated by 3-tandem antioxidant-response elements. Cells were grown at density of $4 \times 10^5$–$1 \times 10^6$ cells/ml under 5% $CO_2$/37°C in RPMI 1640 medium containing 10% fetal bovine serum (FBS) and antibiotics (1% penicillin/streptomycin, 100 μg/ml normocin, 10 μg/ml blasticidin). Cells were treated with 5 μg/ml nPM for 8 h; NF-κB activity was assayed by QUANTI-Blue and Nrf2/luciferase activity by luminometry; both were calculated relative to vehicle ($H_2O$). This new cell line is available on request.

*Behavioral testing*

Novel Object in Context (NOIC) test was used to assess hippocampal dependent object and context recognition [46].

*Statistical analysis*

Data were analyzed by GraphPad Prism v.7 and Stata v.16.1 (College Station, TX). Batch group comparisons were analyzed by either Student t-test (for 2 groups) or one-way ANOVA (>2 groups). Pairwise comparisons of later batches to the first batch were corrected for multiple hypothesis testing using the False Discovery Rate of Benjamini, Krieger and Yekutieli [47]. For trend analysis, a time variable (in months since first sample batch) was calculated according to the nPM collection date, with Batch 1 represented as time 0. The date for each batch used the midpoint of the date range (Table 1); the month time variable ranged from 0–47 months. Linear regressions evaluated NF-κB and Nrf2 activity as dependent variables, and months as the independent variable. For effect size analysis of each data collection period in Fig. 2A-I, standardized treatment effect sizes (nPM versus control) were calculated as the mean difference between groups, divided by the pooled standard deviation. The standardized effect sizes were compared over collection year, using a mixed effects linear model specifying a fixed effect for collection year, and a random effect for study (Fig. 2A-I). Historical data 2009–2020 of PM2.5, ozone and $NO_2$ was obtained from Chemical Speciation Network (CSN) provided by the US Environmental Protection Agency (US EPA) [48] for a site in Los Angeles city (1630 North Main St., Los Angeles, CA 90012), 5 miles from the nPM collection site (3400 South Hope St., Los Angeles, CA 90089). $p < 0.05$ was considered significant for all statistical tests.

## RESULTS

*In vitro activity*

Inflammation and oxidative stress are two putative mechanisms of how nPM causes neurotoxicity. *In vitro* activity of nPM was assessed by its capacity to induce two key transcription factors: NF-κB, the master transcriptional regulator of inflammatory response, and Nrf2, which controls the antioxidant response to oxidative stress [49]. The reporter genes were transfected into a monocyte cell line, as a model for respiratory tract monocyte/macrophages that directly receive inhaled PM [36]. Exposure of NF-κB/Nrf2 reporter cells to nPM increased the activities of both NF-κB and Nrf2, with dose-dependence over a 40-fold range (0.25 –10 μg/ml, data not shown).

The nPM batches differed in potency for inducing NF-κB (Fig. 1A) and Nrf2 (Fig. 1B). Both transcriptional responses were strongly correlated ($r = 0.97$, Fig. 1C). The trend analysis showed a significant decrease in the induction of NF-κB and Nrf2 by collection dates ($p < 0.01$ for both NF-κB and Nrf2). For both NF-κB and Nrf2, Batches 2 and 3 were significantly higher than Batch 1 ($p < 0.05$), and Batches 6–10 (2018-2019) were significantly lower than Batch 1. These data suggest that the potency of nPM to induce NF-κB and Nrf2 varies by batch and has declined since 2017.

Trends of major air pollutants near the nPM collection site were linear during 2009–2020. PM2.5

Table 2
Cerebral Cortex mRNA Response to nPM by Batch Number and Date

| nPM Batch ID | Collection date | Gender | Exposure duration (wk) | GluA1 | IL-1β | NFκB1 | RelA/p65 | MyD88 | TLR4 |
|---|---|---|---|---|---|---|---|---|---|
| 2016a | Feb-Apr, 2016 | M | 10 | 0.69 ± 0.14* | 1.67 ± 0.34* | 0.71 ± 0.05* | 1.23 ± 0.15 | 1.11 ± 0.22 | 1.02 ± 0.20 |
| 5 | May-Jun, 2017 | M | 3 | 0.63 ± 0.16* | 0.95 ± 0.20 | 0.74 ± 0.26* | 0.93 ± 0.10 | 0.75 ± 0.07* | 0.80 ± 0.12 |
| 6 | Jan-Mar, 2018 | M | 8 | 0.92 ± 0.25 | 1.26 ± 0.73 | 1.01 ± 0.42 | 1.32 ± 0.43 | 0.97 ± 0.21 | 1.33 ± 0.56 |
| 6 | Jan-Mar, 2018 | F | 10 | 0.81 ± 0.40 | 1.15 ± 0.72 | 0.80 ± 0.26 | 0.97 ± 0.18 | 0.90 ± 0.16 | 0.95 ± 0.20 |
| 7 | Apr-Aug, 2018 | F | 10 | 1.00 ± 0.08 | 1.12 ± 0.07 | 0.97 ± 0.10 | 0.95 ± 0.12 | 1.06 ± 0.15 | 0.95 ± 0.12 |
| 8 | Feb-Mar, 2019 | F | 10 | 1.21 ± 0.29 | 0.96 ± 0.37 | 0.81 ± 0.09* | 1.04 ± 0.09 | 1.23 ± 0.06 | 1.03 ± 0.18 |
| 9 | Sep-Nov, 2019 | M | 3 | 0.89 ± 0.08 | 0.75 ± 0.33 | 0.78 ± 0.03* | 0.91 ± 0.09 | 0.82 ± 0.13* | 0.89 ± 0.30 |
| 9 | Sep-Nov, 2019 | F | 3 | 0.92 ± 0.12 | 0.85 ± 0.28 | 0.82 ± 0.11* | 0.94 ± 0.14 | 0.95 ± 0.10 | 1.13 ± 0.23 |
| 9 | Sep-Nov, 2019 | F | 10 | 0.85 ± 0.27 | 0.94 ± 0.27 | 0.85 ± 0.12 | 0.97 ± 0.16 | 0.99 ± 0.18 | 1.04 ± 0.26 |

Archived frozen cerebral cortex of experiments with batches from 2016–2019 was analyzed for mRNAs in the same PCR assay; numbers are fold- change (mean ± SD) of nPM exposure versus controls (filtered air), $^{*}p < 0.05$ versus control, N = 5–8.

declined weakly ($p = 0.09$), while the decline of $NO_2$ was significant (3.6%/y, $p = 0.001$). Opposing these declines was a significant increase of ozone (1.8%/y, $p = 0.004$).

*In vivo responses*

Five older nPM batches (2009–2017) and four recent nPM batches (2018, 2019) (Table 1) were compared from independent 8- or 10-week exposure experiments in different years (Fig. 2A-I). Each panel shows the statistically significant response of earlier nPM batches for comparison with null response to recent nPM. Microglial Iba1 (Fig. 2A) did not respond to Batch 6 (2018). TNFα mRNA (Fig. 2B) induction was robust for two nPM batches collected in 2009 and 2016 [21], contrasting with null response to Batch 6 (2018). Similarly, $A\beta_{42}$ (Fig. 2C) responded to nPM with > 70% increase for Batch 2 (2016) but did not respond to Batch 6 (2018) in an identical protocol 2 years later. In corpus callosum, white matter microglial Iba1 was increased by Batch 2015a (Fig. 2D). Partial ischemia from bilateral carotid artery stenosis (BCAS) plus nPM gave additive effects for Batch 2015a, but not for Batch 8 (2019). The increase of Iba1 from BCAS alone gives an internal control for microglial responsivity in this group of mice. Myelin damage in corpus callosum showed parallel batch differences. MAG was decreased by 35% by Batch 5 (2017) but was not changed by Batch 8 (2019); dMBP was increased by 60% by Batch 5 (2017) but did not respond to Batch 8. In olfactory bulb, the glutamate receptor subunit GluA1 was decreased and IL-1β increased by Batch 2 (2016), but did not respond to Batch 7 (2018) and Batch 9 (2019). Moreover, Batch 2 (2016) caused memory loss assessed by Novel Object in Context (NOIC) test, whereas Batch 6 (2018) did not impair memory. By a mixed effects model, the mean standardized effect size was smaller for nPM batches of 2018 and 2019 compared to 2009–2017 ($p = 0.003$). Overall, these observations indicate that recent nPM batches had less neurotoxicity.

*mRNA responses in vivo*

To extend the examples of Fig. 2, we examined mRNA responses of archived cerebral cortex from mice exposed to different nPM Batches (Table 2). NFκB1, the p105/p50 member of NF-κB family, gave the most consistent response to nPM, and was decreased by most batches (Table 2). IL-1β was increased by Batch 2016a but not by others. MyD88, an adaptor protein in the TLR4-NFκB pathway, was decreased by Batches 5 and 9 in males exposed for 3 weeks, whereas MyD88 did not respond to other batches. GluA1 mRNA was decreased in cortex by Batches 2016a and 5, confirming findings in hippocampus [1, 21, 28], whereas GluA1 mRNA did not respond to recent batches. The mRNAs of RelA/p65 (NF-κB family), and TLR4 responded minimally to all nPM batches. Taken together, Table 2 findings parallel batch responses of Fig. 1 and Fig. 2, suggesting that nPM batches collected since 2018 have decreased potency. This comparison is limited by two factors: exposures varied from 3 to 10 weeks (Table 1) and some batches were not available because of depletion in prior studies.

## DISCUSSION

Air pollution PM varies widely in bioactivity/toxicity by sites of collection and season, as shown

by the DTT assay and other biochemical and *in vitro* cell models [36, 50–55]. The present data extend these findings by comparing the *in vitro* Nrf2 and NF-$\kappa$B transcriptional responses with *in vivo* neurotoxic effects caused by batches of PM0.2 collected over a decade at the same site in urban Los Angeles. These findings show extensive variations in the biological potency of the nPM subfraction of ambient PM0.2, assessed by the same methods, materials, and mouse genotype from the same sources. The new cell-based assay for induction of Nrf2 and NF-$\kappa$B may be useful to evaluate the potency of PM collected from ambient air in animal models of air pollution.

The *in vitro* potency of nPM to induce NF-$\kappa$B and Nrf2 signaling with a monocyte reporter declined in five batches collected after 2016 (Fig. 1). NF-$\kappa$B and Nrf2 were chosen for *in vitro* assays because these transcription factors are key mediators of inflammatory, detoxification, and anti-oxidative responses. The transcriptional gene reporter for NF-$\kappa$B does not identify the relative contribution of its five subunits (RelA/p65, NF$\kappa$B1/p50, NF$\kappa$B2/p52, RelB and RelC) that act by forming hetero- or homo-dimers [54–56], which could include a decrease of the NF$\kappa$B1 mRNA as observed *in vivo*.

The *in vivo* responses of cerebral cortex were expanded to four recent batches collected after 2017 (Batches 6,7,8,9) (Fig. 2, Table 2). Batches 6–9 did not increase TNF$\alpha$, microglial Iba1, A$\beta_{42}$, and dMPB (myelin degeneration), or decrease GluA1 (glutamate receptor subunit), unlike earlier batches. The decreasing neurotoxicity of recent batches since 2016 was consistently observed in four brain regions: cerebral cortex, corpus callosum, hippocampus, and olfactory bulb. These observations further document the declining *in vitro* potency of recent batches and confirm our initial findings that nPM batches vary in the potential to cause inflammatory response and neurotoxic effects [36].

In contrast, some other cerebral cortex mRNA responses were maintained in recent batches, e.g., NF$\kappa$B1 mRNA was still decreased by Batches 8 and 9 (Table 2). Batch 5 (2017) and Batch 9 (2019) showed respectively higher and lower potency *in vitro*, nonetheless both decreased MyD88 mRNA in cortex. Moreover, a separate transcriptomic analysis of male C57BL/6 mice showed Batch 6 induced extensive responses of Nrf2 and NF-$\kappa$B mediated pathways [33]. A hierarchy of atherogenic response to air pollutants was described by Araujo and Nel [56], in which antioxidant defenses responded to lower activity of PM, followed by inflammation and cytotoxicity at higher PM activity. The nPM batches that did not induce brain inflammatory cytokines (Fig. 2, Table 2), but still altered Nrf2 and NF-$\kappa$B [33] may be consistent with this framework. We are planning to compare Batch 6 with Batch 2 for transcriptomic responses of cerebral cortex.

Unexpectedly, the early 2012 Batch did not alter the A$\beta_{42}$ in cerebral cortex of ApoE-TR cerebral cortex, but still decreased mRNA for amyloid production (APP, Psen1) and A$\beta_{42}$ peptide clearance (C3, Vav3) [33]. Note that these data for transgenic mice cannot be compared with the wildtype B6 which was examined in a different study with another nPM batch (Fig. 2C). Current data do not resolve the cause of inconsistent effects of nPM on the brain and whether they are attributable to exposure duration, sex, or difference in PM0.2 composition. Nor do epidemiological associations of air pollution components with dementia risks [1–4] show how ubiquitous inflammatory processes may cause brain aging to go from bad to worse.

The cognitive impact of air pollution must consider multiple interacting factors and neurotoxic pathways [57] including and beyond the glial and neuronal responses being studied in this growing field. The decreased impact of nPM on glial and neuronal responses gives a rationale for examining Los Angeles elderly populations for possible benefits to cognition. The declining activity of nPM per $\mu$g by our measures since 2016 does not parallel the modest declines of PM2.5 and $NO_2$ and is opposite to the stronger trend for increased ozone (Fig. 1D). However, the mass of the nPM fraction is less than 25% of the total PM2.5 at the nPM collection site [58]. We must also consider the concurrent increase of ozone 2009–2020 (Fig. 1D) [20, 59], which was specifically associated with impaired executive function of Los Angeles elderly [60].

The nPM subfraction of urban air pollution is a highly complex mixture, in which altered batch differences of trace components could alter *in vivo* potency. Batches 1–7 varied widely in composition without obvious historical trends for copper, iron, or other transition metals, or toxic organic species [36]. Hierarchical clustering by nPM batch showed weak associations of endotoxin with NF-$\kappa$B activation *in vitro* but did not identify correlations of any chemical element with *in vitro* activities [36]. A more comprehensive analysis of nPM components, including the chemical status of transition metals on particle surface, the microbial components and other factors, may explain the cause of the unequal toxicity of nPM.

We must also consider the chemical state of transition elements. For example, Los Angeles PM2.5 contain multiple species of iron in combination with carbonaceous material ranging from crystalline iron to several types of ferrihydrates [61]. These different iron species arise from engine combustion and from abrasion of brake linings. Additional redox active meals arise from industrial and natural sources [62, 63]. We suggest a broader discussion of urban air pollution components in experimental models that will consider particle surface chemistry as well as composition. A systematic approach could identify broadly shared anthropogenic and natural components that might serve as a standard in comparing experimental findings from the expanding research on air pollution neurotoxicity.

In conclusion, we show that nPM batches from Los Angeles have recently decreased potency for neurotoxic responses relevant to neurodegeneration and AD risk in the same mouse model. The declines in ambient PM2.5 and $NO_2$ of Los Angeles air pollution 2009–2020 (Fig. 1D) in combination with decreased neurotoxicity give a rational for examining possible parallel changes in human brain health. A decline in nPM potency could impact the hospital admission rates or discharge outcomes for patients presenting with stroke or cerebral hypoperfusion in the Los Angeles basin.

*Limitations and strengths*

These post hoc comparisons of nPM by batch (Table 2) were limited by availability of archived nPM and cerebral cortex tissues. The *in vivo* comparisons in Fig. 2 were from independent experiments at different times. Cognitive deficits from earlier nPM batches were not evaluated for the recent batches. Caveats recognized, the data shows progressively declining potency of nPM during the past decade of sampling in urban Los Angeles.

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## SUPPLEMENTARY MATERIAL

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LETTER

# Recently decreased association of air pollution with cognitive impairment in a population-based aging cohort and in a mouse model

Particulate matter air pollution is an environmental risk factor for poor cognitive function in the elderly,[1–3] together with accelerated cognitive loss,[4,5,6] increased risk of dementia,[5,7,8] loss of brain grey and white matter,[9,10] and small vessel disease.[11] Rodent models document the neurotoxicity of air pollution components for cognitive impairments,[12,13] oxidative damage and increased brain amyloid,[5,14,15] and impaired adult neurogenesis.[12,16] Our labs have independently reported indications of recent decreases in the neurotoxicity of air pollution components in human populations[17] and rodent models.[18]

In the US-wide Health and Retirement Study (HRS), Ailshire showed a strong association of cognitive deficits with air pollution for cohorts with lower education in 2004: a 12% higher risk of incident cognitive impairment per 5 $\mu g/m^3$ increase in PM2.5 among adults ages 65 and older with ≤8 years of education.[17] However, by 2014 there was no such association for the corresponding HRS cohort.

Among possibilities for the lack of association in 2014, a likely factor is the reduction in PM2.5 from 2004 to 2014. The mean annual ambient PM2.5 in neighborhoods of HRS respondents was 9.2 $\mu g/m^3$ (SD = 1.7) in 2014, 25% below the 2004 level of 12.4 $\mu g/m^3$ (SD = 2.8).[17] Importantly, in 2014 very few HRS respondents lived in places with an annual average PM2.5 level above the EPA standard of 12.0 $\mu g/m^3$, suggesting a decline in exposure to high pollution among older adults.

Rodent models indicate diminished neurotoxicity of air pollution from urban sites. A nanoscale subfraction of PM2.5 (nPM) from Los Angeles showed sharp declines after 2017 in neurotoxicity for nine parameters, including spatial learning and oxidative damage.[18] These experiments exposed the same mouse genotype (B6) to nPM collected at the same urban site and for the same levels of nPM and duration.

The composition of air pollution was also changing during these observations. The levels of PM2.5 declined US-wide by 50% (from 13 $\mu g/m^3$ in 2000 to 8 $\mu g/m^3$ in 2019), while the Los Angeles urban PM2.5 level declined slightly, from 13 $\mu g/m^3$ in 2009 to 12 $\mu g/m^3$ in 2019.[18] Although US-wide ozone levels continued to decrease,[19] Los Angeles County ozone reversed the prior trends by increasing after 2015.[20] Chemical analysis of Los Angeles nPM did not identify changes attributable to the lower neurotoxicity per $\mu g$.[18,21]

The findings of the HRS cohort are consistent with the lower dementia risk by advancing birth year in other population-based studies.[22–24] The protective role of increased education for dementia risk from air pollution[17] could be a factor in these prior findings, because higher education was increasingly available in the 20th century to both women and men.[25] Nonetheless, air pollution must be considered among environmental factors in the AD-Exposome[26,27] over the lifespan because of the present evidence for recent changes in level and neurotoxicity.

We emphasize that our findings cannot evaluate potential benefits of air pollution improvements to the risk of cognitive decline and dementia. Although PM2.5 levels did decline nationally from 2009 to 2016, the year-over-year increases that have been observed since 2017[20,27] show that improvements in air quality can be reversed. Our findings underscore the importance of efforts to improve air quality in the ambient and indoor environments and the continued importance of parallel demographic and experimental evaluation of air pollution neurotoxicity.

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