AWARD NUMBER: W81XWH-17-1-0534

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

PRINCIPAL INVESTIGATOR: Patrik Brundin, MD, PhD

CONTRACTING ORGANIZATION: Van Andel Research Institute, Grand Rapids, MI

REPORT DATE: October 2021

TYPE OF REPORT: Annual

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

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

4. TITLE AND SUBTITLE

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

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

5b. GRANT NUMBER
PD160021

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

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8. PERFORMING ORGANIZATION REPORT NUMBER

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

U.S. Army Medical Research and 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 by increasing α-synuclein (α-syn) pathology via inflammation. After completing Specific Aim 2, wherein we found no differences between experimental groups in spread of α-syn, we discovered our collected nPM did not produce the expected neuroinflammatory changes. We are now poised to begin Specific Aim 3 mouse experiments to define the effects of LPS-induced olfactory inflammation on spread of α-syn by histological (VARI) and biochemical (USC) analyses (under continued travel restrictions).

15. SUBJECT TERMS

Pre-formed fibrils (PFFs), α-synuclein (α-syn), phosphorylated serine 129 (pSer129), nanoparticulate 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	21	19b. TELEPHONE NUMBER *(include area code)*

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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 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 improve our understanding of the early stages of PD development by defining the influence of air pollutants on the development and progression of α-synuclein (α-syn) pathology in vivo, and on olfactory dysfunction among older adults. We will pursue experimental (Aims 1-3) and epidemiological (Aims 5-7) studies addressing common research questions. In the fourth year of this project at Van Andel Research Institute we completed Aim 2 histological analysis of piriform cortex tissues for presence of Lewy-like α-syn pathology marker, phosphorylated serine 129 (pSer129), and received DOD approval of both amended Aim 3 and our ACURO application for animal work to begin at Van Andel Research Institute.

1. KEYWORDS:

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

2. ACCOMPLISHMENTS:

What were the major goals of the project?

SPECIFIC AIM 1: Determine the effects of exposing mice to nPM after triggering of pre-formed fibril (PFF)
pathology (Months 6-16)
1. Inject C57BL/6NJ mice (n=96) with PFFs.
    Validation experiment accomplished (n = 32), 10/12/17 (Y1Q1)
    Specific Aim 1 injections (n = 64) accomplished 1/18/18 (Y1Q2)
2. Expose C57BL/6NJ mice to nPM.
    Validation experiment accomplished (n = 32), 11/09/17 (Y1Q1)
    Specific Aim 1 exposure (n = 64) accomplished 2/14/18 (Y1Q2)
Milestones in this reporting period: N/A (Completed in Y2Q1)

SPECIFIC AIM 2 (Aim 2.1): Determine the effects of exposing mice to nPM before triggering of PFF pathology
(Months 12-30)
1. Pre-expose mice to nPM for 3 weeks before injections
Specific Aim 2 pre-stereotactic injection exposure (n = 64) initiated 10/8/18, accomplished 10/28/18 (Y2Q1)
2. Inject mice with PFFs (as in Aim 1).
Specific Aim 2 injections (n = 64) initiated 10/29/18, accomplished 11/1/18 (Y2Q1)
3. Post-expose mice to nPM for 7 weeks after injections
Specific Aim 2 post-stereotactic injection exposure (n = 64) initiated 11/2/18, accomplished 12/20/18 (Y2Q2)
Milestones in this reporting period:
N/A (Completed/suspended in Y3Q1)

SPECIFIC AIM 2 REPEAT (Aim 2.2): Determine the effects of exposing mice to nPM before triggering of PFF
pathology (Months 28-38)
1. Pre-expose mice to nPM for 3 weeks before injections
Specific Aim 2.2 pre-stereotactic injection exposure (n = 88) initiated 1/13/20, accomplished 2/2/20
(Y3Q2)
2. Inject mice with PFFs (as in Aims 1 and 2).
Specific Aim 2.2 injections (n = 88) initiated 2/3/20, accomplished 2/6/20 (Y3Q2)
3. Post-expose mice to nPM for 7 weeks after injections
Specific Aim 2.2 post-stereotactic injection exposure (n = 88) initiated 2/7/20, accomplished 3/26/20
(Y3Q3)
Milestones in this reporting period:

1. Complete collection and delivery of brains to VARI initiated 3/23/20 at USC; brains received 5/18/20 at VARI due to SARS-CoV-2 quarantine (Y3Q3).
2. Biochemical analysis completed at USC (Y3Q4).
3. Histological analyses (starts after 1 month for sectioning):
    a. pSer129 Aiforia AI densitometry analysis completed 11/15/2020 (Y4Q1).
    b. Iba-1 and inflammatory cytokine immunofluorescence double stain optimization suspended due to results from biochemical and histological analyses (Y3Q4).

SPECIFIC AIM 3: not yet initiated

What was accomplished under these goals?

We report that we received the approval for our Request for Modification of Aim 3 on April 13, 2021. We subsequently applied for ACURO approval on April 28, 2021 and received approval on July 15, 2021. Next, 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, which will help us in Specific Aim 3 analysis.

Aim 2.2 analysis of α-syn pSer129 presence in piriform cortex of nPM- and forced air-exposed brains using Aiforia AI algorithm

During this reporting period, we finished our analysis of Specific Aim 2 brains of nPM- and forced-air exposed mice, with a focus on α-synuclein (α-syn) pSer129 presence. Because there appears to be a possible ceiling effect in pSer129 detection in regions such as the anterior olfactory nucleus (see Y3 annual report), we decided to quantify the pSer129 presence in brain regions more distant from the site of injection (ROB). Thus, we analyzed pSer129 presence in the piriform cortex (PCX), a region that is also one synapse away from the OBs that still exhibits pathology without oversaturation.

Analysis using the Aiforia AI shows no statistical difference in pSer129 presence in the PCX (Figure 1). These results further support our conclusion that the second batch of nPM was inactive and therefore unable to alter synucleinopathy, and reinforces our decision to conduct Aim 3 experiments using the robustly inflammatory endotoxin lipopolysaccharide (LPS)—which is found in airborne pollutants—in order to test our hypothesis (see Request for Modification of Aim 3, approved July 15, 2021).

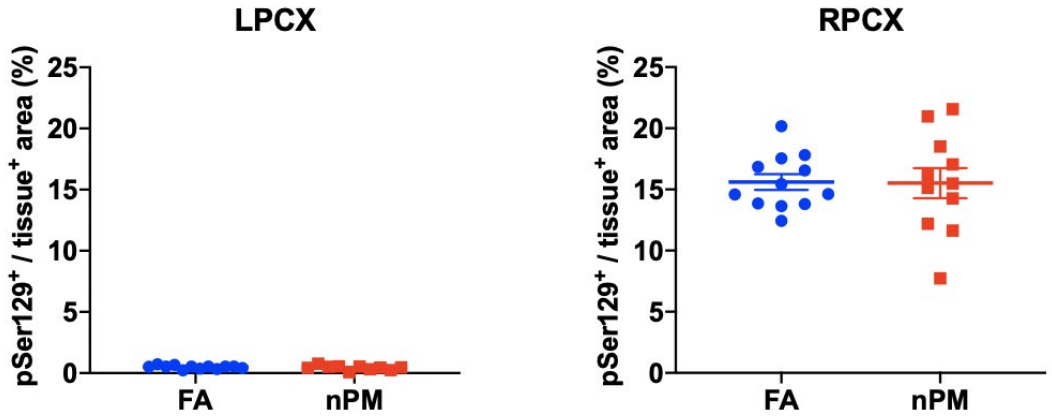


Figure 1: Pathological α-syn (pSer129) presence as percent of total tissue area in left (L) and right (R) piriform cortex (PCX) of forced air (FA, blue)- and nano-particulate matter (nPM, red)-exposed mice after ten total weeks (three weeks pre-, seven weeks post-PFF injection) of exposure. No statistical significance detected. Mean ± s.e.m.

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?

We will initiate the mouse injections as outlined in Specific Aim 3.

4. IMPACT:

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:

The anticipated arrival of Dr. Mohit Kwatra to begin the third aim of the project was delayed due t

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

Nothing to report.

Changes that had a significant impact on expenditures

As mentioned above, Dr. Mohit Kwatra was delayed in arriving, thus we have underspent in salaries during this period. In addition, waiting periods for approvals (for the Request for Modification and ACURO) led to lower associated animal and project costs during this project period.

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

Significant changes in use or care of human subjects

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:

- **Publications, conference papers, and presentations**

Journal publications.

Nothing to report.

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

Nothing to report.

Other publications, conference papers and presentations.

Nothing to report.

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

Nothing to report.

- **Technologies or techniques**

Nothing to report.

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

Nothing to report.

- **Other Products**

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

1. Name: Patrik Brundin
   Project role: Principal Investigator/Project Director
   Researcher identifier: ORCID ID: https://orcid.org/0000-0003-2924-5186
   Nearest person month worked: 1.2 cal months (or 10% effort)

   Contribution to Project: Dr. Brundin has performed work to organize and oversee the project, including participating in teleconferences and email correspondence.

2. Name: Christopher Tulisiak
   Project role: Post-doctoral fellow
   Researcher identifier: ORCID ID: https://orcid.org/0000-0002-0793-1823
   Nearest person month worked: 4 cal months (or 33% effort) *left VAI 12/31/2020*
   Contribution to Project: Dr. Tulisiak drove Aim 2.2 analysis, the request for modification of Aim 3, and the corresponding documentation for the latter.

3. Name: Jennifer Steiner
   Project role: Senior laboratory manager
   Researcher identifier: ORCID ID: https://orcid.org/0000-0003-0953-1310
   Nearest person month worked: 2.4 cal months (or 20% effort)
   Contribution to Project: Dr. Steiner has performed work to help the Finch laboratory transport materials to and from USC and to coordinate work at VARI necessary to amend and complete the Statement of Work.

4. Name: Emily Kuhn
   Project role: Research technician
   Researcher identifier: ORCID ID: https://orcid.org/0000-0001-9715-7941
   Nearest person month worked: 2.3 cal Months (or 30% effort of 7.8 Months – *Emily was on medical leave 3/4/21 – 7/11/21*)
   Contribution to Project: Ms. Kuhn contributed to the processing of Aim 2.2 brains and to PFF production.

5. Name: Allison Lindquist
   Project role: Research technician
   Researcher identifier: ORCID ID: https://orcid.org/0000-0001-7377-3504
   Nearest person month worked: 3.9 cal months (or 32% effort)
   Contribution to Project: Ms. Lindquist performed control PBS microinjection surgeries for Aim 2.2 with Dr. Tulisiak. She also contributed to processing of Aim 2.2 brains and to PFF production.

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

Yes; see below:

Recently Completed Support

R21NS105436 (Brundin, P. & Taylor, M) 01/01/2018 - 12/31/2020 (No Cost Extension)
0.12 Cal Mths or 1% Effort
NIH/NINDS Total Cost
Promoting Survival of Dopamine Neurons in Models of Parkinson's Disease Using a Novel Transcriptional Regulator
The major goal of this project is to define how up regulating a novel transcriptional cascade (PM-Nato3) influences the survival of dopamine neurons in models of Parkinson's disease.
Specific Aim 1: Define the effects of PM-Nato3 expression in MPP+ and α-synuclein toxicity models of PD using cultured human DA neurons.
Specific Aim 2: Define the effects of PM-Nato3 expression in En1 haploinsufficient and α-synuclein animal models of PD.
Role: PD/PI
Agency contact: Vicky R Haines; email: vhaines@mail.nih.gov.
Overlap: None

N/A (Brundin, P.) 01/11/2016 - 07/10/2021 0.00 Cal Mths or 0% Effort
Hoffmann-La Roche Total Cost
SPRA: The role of inflammation in alpha-synuclein propagation w/visiting scientist Nazia Maroof (*No Cost Extension*)
The major goal of this project is to determine whether aSyn pathology can propagate from the ENS to the Central Nervous System (CNS) and what the role of the immune system may be in this process.
Specific aims: 1) To determine whether inflammation following acute (1 week)/chronic (4 weeks) DSS colitis has a long term impact on aSyn accumulation in the ENS and CNS and what role it plays in aSyn propagation from ENS to CNS. 2) To ascertain whether aSyn isolated from faecal samples from PD patients and transgenic aSyn overexpressing mice which have been subjected to DSS colitis, causes seeding and propagation of aSyn aggregate pathology through the nervous system following injection into the olfactory bulb. 3) To investigate whether colitis in early life of Thy1-(A30P)aSyn tg mice leads to aSyn pathology and gliosis in brain over longer period of time. 4) To explore epigenetic changes in nuclei of enteric nerves after DSS colitis in Thy1-(A30P)aSyn tg mice.
Agency contact: Markus Britschgi; email: markus.britschgi@roche.com
Role: PD/PI
Overlap: None

NA (Brundin, P) 3/1/2019 – 7/31/2021 0.00 Cal Mths or 0% Effort
Farmer Family Foundati Total Cost
Infections as triggers of Parkinson's disease (iPark)
The major goal of this project is to identify bacterial and viral pathogens that can act as triggers of Parkinson's disease (PD) and to characterize their mechanism of action.
Specific aims: Not applicable
Agency contact: Available upon request
Role: SubProject PI
Overlap: None

W81XWH-18-1-0512 (Brundin, P) 08/15/2018 – 08/14/2021 0.36 Cal Mths or 3% Effort
DoD/Army Total Cost
Epigenetic control of TET2 as a mechanism affecting PD neuropathology
Major goal for this project is to determine whether epigenetic dysregulation of TET2 contributes to the development and progression of Parkinson's disease neuropathology.
Specific Aim 1: determine the extent of DNA methylation abnormalities affecting the TET2 gene in neurons of PD patients and quantify the resulting effects on TET2 transcript levels
Specific Aim 2: determine whether Tet2 inactivation causes DNA methylation and transcriptional abnormalities at PD-relevant genes in the parkin pathway
Agency Contact: Karen L. Petrore, Grant Specialist, USAMRAA, US Army Medical Research Acquisition Activity Building 817A, 820 Chandler Street, Ft. Detrick, MD 21702, Phone: , Karen.L.Petrore.civ@mail.mil
Role: PD/PI
Overlap: None

1R21NS112614-01 (Brundin, P) 09/15/2019 – 08/31/2021 1.44 Cal Mths or 12% Effort
NIH/NINDS Total Cost
Molecular signatures of Parkinson's disease in the gut and brain
The major goal of this project is to investigate whether there are changes in the autophagy-lysosomal pathway in the Parkinson's disease appendix and brain.
Specific Aim 1: Identify DNA methylation abnormalities in the PD appendix that affect the function of the ALP
Specific Aim 2: Identify aging changes in DNA methylation that are disrupted in the PD appendix
Role: PD/PI
Agency Contact: NIH Grants Management Specialist, Karen Molina, Ph. , Email: karen.molina@nih.gov
Overlap: None

Current Research Support (Changes indicated in bold)

R01DC016519 (Brundin, P.) 07/01/2017 - 06/30/2022 **1.56 Cal Mths or 13% Effort**
NIH/NIDCD Total Cost
Linking Synucleinopathy and Dysfunction of Olfactory Pathways
The main goal of this project is to establish how the progressive spreading of aggregated a-synuclein from the olfactory bulb to other olfactory structures causes loss of olfaction.
Specific Aim 1: Determine the cellular mechanisms associated with synucleinopathy that underlie olfactory deficits.
Specific Aim 2: Establish the effects of immunotherapy on olfactory deficits associated with the progression of synucleinopathy.
Specific Aim 3: Define the role of microglia in the development of synucleinopathy and olfactory deficits.
Role: PD/PI
Agency contact: Maria Garcia; email: mg421s@nih.gov
Overlap: None

(THIS AWARD)
W81XWH-17-1-0534 (Brundin, P) 09/01/2017 - 08/31/**2022 (NCE)**
1.20 Cal Mths or 10% Effort
DoD Total Cost
Airborne pollutants as triggers of Parkinson's disease via the olfactory system
The major goal of this project is to determine the influence of air pollutants in the development of PD.
Specific Aim 1: Determine the effects of nPM exposure after microinjection of fibrillar α-syn in the OB.
Specific Aim 2.1: Determine the effects of nPM exposure before microinjection of fibrillar α-syn in the OB.
Specific Aim 2.2: Repeat Specific Aim 2.1 with new batch of active nPM confirmed in vitro to be active.
Specific Aim 3: Define the effects of systemic administration of ibuprofen on the development of
α-syn pathology.
Specific Aim 4: Examine the effect of long-term exposures to ambient PM2.5 and NO2 on hyposmia.
Specific Aim 5: Examine whether early PD pathogenesis is exacerbated by ambient air pollutants.
Specific Aim 6: Examine whether lifetime use of NSAIDs, ibuprofen in particular, modify potential adverse effects of air pollutants on hyposmia.
Agency contact: David Adosci; email: David.n.Adosci.civ@mail.mil, Ph. Role: PD/PI
Overlap: N/A

R21NS106078-01A1 (Brundin & Kordower) 01/15/2019 - 12/31/**2021 (NCE)**
0.72 Cal Mths or 6% Effort
NIH/NINDS Total Cost
Combining synucleinopathy and mitochondrial deficits in a novel mouse model of Parkinson's disease
The major goal of this project is to develop a new mouse model of PD combining mitochondrial deficits and alpha synuclein pathology.
Specific aims: 1) Determine effects of combining mitochondrial dysfunction and α-syn aggregation on
neurodegeneration. 2) Determine effects of combining mitochondrial dysfunction and α-syn aggregation on motor behaviors relevant to PD.
Agency contact: Crystal L Anderson; email: andersoncl@mail.nih.gov
Role: PD/PI
Overlap: None

N/A (Brundin, P & Brundin, L) 02/01/2019 – 01/31/2022 0.00 Cal Mths or 0% Effort
Peter C & Emajean Cook Foundation Total Cost
Defining the role of a genetic risk factor in the development of Parkinson's disease.
The major goal of this project is to define the effects of up– and down–regulating ACMSD in vivo.
Specific aims: Not applicable
Agency contact: Carrie Boer, 2900 Charlevoix Drive SE Ste 130 Grand Rapids MI 49546
Role: PD/PI
Overlap: None

R01NS113894 (Coetzee, G) 04/01/2020 – 03/31/2025 0.24 Cal Mths or 2% Effort
NIH/NINDS Total Cost
Epigenetic contributions to symptom asymmetry in Parkinson's disease
The major goal of this project is to determine the molecular signatures enabling hemisphere differences in neuronal dysfunction and symptom asymmetry in PD.
Specific Aim 1: Identify hemispheric differences in epigenetic gene regulation in neurons involved in PD.
Specific Aim 2: Determine hemispheric asymmetry in the epigenome and transcriptome of neurons across aging in health and PD.
Specific Aim 3: Determine whether the arrival and accumulation of PD-like neuropathology in mice induces gene regulatory changes relevant to hemisphere asymmetry.
Role: Co-Investigator
Agency Contact: NIH Grants Management Specialist, Karen Molina, Ph. , Email: karen.molina@nih.gov
Overlap: None
***New since last report**

R01NS114409 (Brundin, P) 07/01/2020 – 06/30/2025 1.92 Cal Mths or 16% Effort
NIH/NINDS Total Cost
The contribution of the vermiform appendix to Parkinson's disease
The major goal of this project is to determine how the appendix, and the α-syn aggregates within, can impact the development of PD.
Specific Aim 1: Identify abnormalities in gene regulation in the PD appendix.
Specific Aim 2: Identify aberrant α-syn proteoforms in the PD appendix.
Specific Aim 3: Determine whether α-syn pathology in the appendix is capable of inducing PD-like neuropathology in the brain.
Role: PD/PI
Agency Contact: NIH Grants Management Specialist, Karen Molina, Ph. , Email: karen.molina@nih.gov
Overlap: None
***New since last report**

MJFF-010238 (Gregor, P) 12/01/2020 – 06/30/2022 0.6 Cal Mths or 5% Effort
MJFF Total Cost
A Novel Preclinical Development Candidate for the Treatment of Parkinson's Disease
The major goal of this project is to determine if GISMO compounds can inhibit development of alpha-synuclein pathology.
Specific Aim 1: Confirm efficacy of Lead Compound GTC-3062 and evaluate the efficacy of its structural analog GTC-3309, in a mouse model of α-synucleinopathy.
Specific Aim 2: Evaluate 'drug-like' properties of the selected Lead Compound in standard pharmaceutically acceptable assays.
Specific Aim 3: Performing an in vivo efficacy studies for the selected lead compound at three doses
Role: Subaward PD/PI
Agency Contact: Liliana Menalled, x254, lmenalled@michaeljfox.org Overlap: None
***New since last report**

MJFF-001209 (Brundin, P) 03/01/2021 – 08/31/2022 0.24 Cal Mths or 2% Effort
MJFF Total Cost
Targeting TET2: Epigenetic Suppression of Neurodegeneration in Parkinson's Disease
The major goal of this project is to define changes in TET2 and its molecular network occurring with increasing PD pathology, and to determine if Tet2 prevents neurodegeneration. Specific Aim 1: To define how TET2 epigenetic alterations contribute to transcriptional changes observed in PD neurons.
Specific Aim 2: To determine the neuroprotective effects of Tet2 inactivation on the development of α-synuclein (α-syn) neuropathology and associated motor deficits.
Role: PD/PI
Agency Contact: TBD, grants@michaeljfox.org
Overlap: None
***New since last report**

R21NS122376 (Brundin, P) 04/01/2021 – 09/30/2022 0.6 Cal Mths or 5% Effort
NIH Total Cost
Role of T cells alpha-synuclein pathology
The major goal of this project is to define the contribution of T cells on the development and propagation of alpha-synuclein pathology.
Specific Aim 1: Determine which T cells alter the propagation of α-syn pathology in a preformed fibrils
(PFFs) model.
Specific Aim 2: Define the specific contribution of Treg to α-syn pathology propagation.
Role: PD/PI
Agency Contact: Karen Molina, , karen.molina@nih.gov
Overlap: None
***New since last report**

NA (Brundin, P) 07/15/2021 – 07/14/2023 0.6 Cal Mths or 5% Effort
Farmer Family Foundation Total Cost
Infections as triggers of Parkinson's disease (iPark)
The major goal of this project is to identify bacterial and viral pathogens that can act as triggers of Parkinson's disease (PD) and to characterize their mechanism of action.
Specific aims: Not applicable
Role: SubProject PI
Agency contact: Available upon request
Overlap: None
***New since last report**

What other organizations were involved as partners?

None identified outside of our funded DoD collaborations.

8. SPECIAL REPORTING REQUIREMENTS

See attached Quad Chart.

9. APPENDICES:

N/A

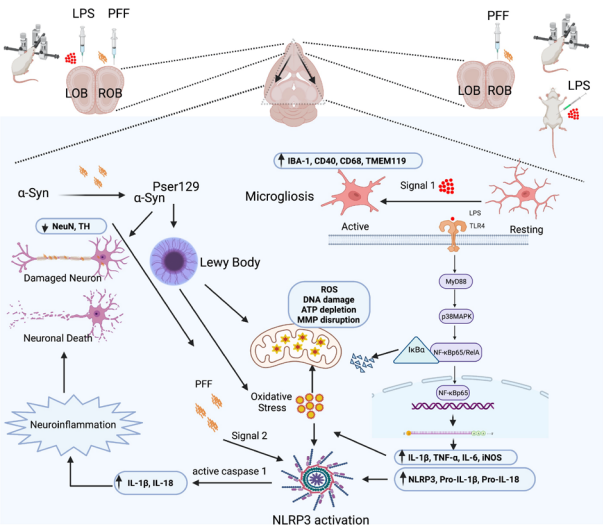
Project Title: Airborne Pollutants as Triggers of Parkinson's Disease via the Olfactory System
Log Number: PD 160021 Annual Report Year 4
Award Number: W81XWH-17-1-0534

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

Study Aims

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

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



Proposed mechanisms of Aim 3: Stereotactic injection of α-Syn PFF (preformed fibrils) into right olfactory bulb (ROB) and LPS, either injected in left olfactory bulb (LOB) or via systemic administration may lead to microglia activation by LPS (signal 1) and PFF (signal 2), initiating inflammatory cascades and NLRP3 (inflammasome) activation with mitochondrial dysfunction.

Timeline and Cost

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

Updated: 9/28/2021

Goals/Milestones

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

Comments/Challenges/Issues/Concerns

- We are awaiting a post-doctoral fellow Dr. Mohit Kwatra, who will arrive shortly but was not able to start before the close of this reporting period.

Budget Expenditure to Date

Projected Expenditure: $746,039
Actual Expenditure: $510,524