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Award Number: W81XWH-17-1-0564

TITLE: Mechanisms of Olfactory Deficits in Parkinson's Disease

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REPORT DATE: September 2018

TYPE OF REPORT: Annual

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
Sept 2018	Annual	1 Sep 2017 - 31 Aug 2018

4. TITLE AND SUBTITLE

Mechanisms of Olfactory Deficits in Parkinson's Disease

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-17-1-0564

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

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8. PERFORMING ORGANIZATION REPORT NUMBER

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

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

10. SPONSOR/MONITOR'S ACRONYM(S)

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

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT: Most Parkinson's disease patients lose their sense of smell, sometimes decades before they develop the symptoms typically associated with the disease, such as tremors and difficulty walking. Olfactory deficits, hyposmia or anosmia, is now recognized as a feature of early Parkinson's disease and an indication that the disease is progressing insidiously. In this FY16 PRP Focused Idea Award application, we will examine the underlying causes for olfactory deficits seen in Parkinson's patients. The focus of our application is directly in line with the Focus Area of '*Identification and evaluation of mechanisms in early Parkinson's disease involving olfactory, microbiome, gastrointestinal, and/or autonomic nervous systems'*. In this grant, we will examine how a protein known as alpha-synuclein, that forms abnormal deposits that are a signature of Parkinson's disease, impacts nerve connections or synapses in the olfactory system. We suggest that in Parkinson's disease, due to the presence of these abnormal alpha-synuclein deposits, both the structure and function of olfactory nerve connections is not proper. We will examine this question through our complementary expertise in the olfactory system and alpha-synuclein biology, using high resolution microscopic methods on mouse models that express mutant forms of alpha-synuclein that are linked to the disease. When we have successfully finished this study, we anticipate having a detailed picture of the synaptic abnormalities in the olfactory system in an animal model of early Parkinson's disease. This will allow us to treat the loss of smell in early Parkinson's disease patients in 5-10 years. But more importantly, as the nose and the olfactory system are thought to be a conduit through which alpha-synuclein deposits spreads, eventually reaching the mid-brain, a greater understanding of this pathway will permit us to slow the progression or even prevent Parkinson's disease. This is the ultimate goal of all Parkinson's disease research.

15. SUBJECT TERMS
Hyposmia, Olfactory, Alpha-synuclein, Adult Neurogenesis, Parkinson's disease, Transgenic, Confocal, Electron Microscopy

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		19b. TELEPHONE NUMBER *(include area code)*

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INTRODUCTION:

Early in the course of Parkinson's disease (PD), most patients (> 90%) become hyposmic and are unable to detect or discriminate odors. Because these olfactory deficits typically occur prior to the onset of motor symptoms in PD, olfactory acuity tests are now being used as a biomarker to diagnose prodromal and early PD patients. Hypsomia in patients correlates with alpha-synuclein pathology in the olfactory bulb (OB) and anterior olfactory nucleus (AON). It is postulated that the restricted pattern of α-synuclein pathology in the olfactory system in early PD eventually spreads to the nigrostriatal system as the disease progresses and motor symptoms ensue. However, to date there have been no studies that have shed sufficient light on the mechanisms via which alpha-synuclein perturbs olfactory function. We hypothesize a primary effect on the synaptic circuitry of the olfactory system as an underlying mechanism. However, given the heterogeneity of olfactory circuits and their dependence upon adult neurogenesis, the specific effect(s) of alpha-synuclein and its precocious effect on olfactory function remains speculative. Therefore, the goal of our grant is to understand why the majority of newly diagnosed PD are hyposmic, by evaluating the role of synaptic dysfunction as a result of alpha-synuclein pathology in situ in the olfactory circuitry.

KEYWORDS:

Hyposmia, Olfactory, Alpha-synuclein, Adult Neurogenesis, Parkinson's disease, Transgenic, Confocal, Electron Microscopy

ACCOMPLISHMENTS:

What were the major goals of the project?

Specific Aim 1: To characterize the synaptic perturbations in the glomerular and external plexiform layer in α-synuclein transgenic mice.

Specific Aim 2: To examine the migration of newly generated neuroblasts into the OB and their integration into olfactory circuitry on α-synuclein overexpression.

Specific Aim 3: To monitor synaptic vesicle trafficking in OSNs and granule cells in α-synuclein transgenic mice.

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.

Milestone(s) Achieved: Local IACUC Approval: We have obtained IACUC approval for the all the proposed experiments. 1-2 months (10/2017-11/2017)
Milestone Achieved: HRPO/ACURO Approval: We have obtained ACURO approval for the proposed experiments. 3 months (12/2017)
Subtask 1: Perform electron microscopy on OB of α-synuclein transgenic mice and controls. We have perfused A30P and A53T α-synuclein transgenic mice and controls and prepared samples for immunofluorescence and electron microscopy. We have performed several immunofluorescence experiments staining for alpha-synuclein (Figure 1).

We have also prepared samples for electron microscopy and are beginning to perform synaptic morphometric analysis of these samples. Features that are being measured include, number of vesicles, lengths of pre- and postsynaptic specializations, and mitochondria (Figure 2).

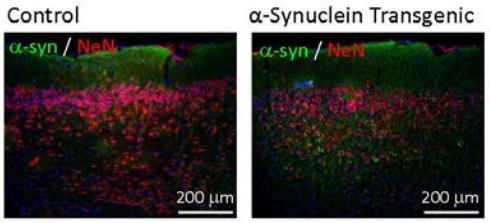


Figure 1: Piriform cortex (pPC) sections show immunostaining of alpha-synuclein (green) and NeuN (red) in pPC with nuclei counterstained with DAPI (blue). NeuN is used to show mature neurons in pPC in both wildtype and human A30P alpha-synuclein transgenic animals. An overexpression of alpha-synuclein was detected in pyramidal neurons of layer III in pPC. Scale bars of 200 µm.

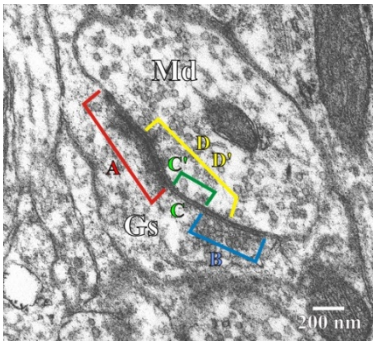


Figure 2: Synaptology quantification. Typical D:D synapse between a MC dendrite and GC spine. A, red line, length of postsynaptic specialization in GC; B, blue line, length of postsynaptic specialization in MC; C and C', green line, distance between pre- and postsynaptic specializations in MC and in GC; D and D', distance between the midpoints of the Gray Type 1 and Gray Type 2 specializations in the GC and in the MC. Not shown, hemispheric Sholl analysis to quantify vesicle number as a function of distance from the presynaptic specialization. Note: lines indicating measurements are placed for ease of viewing and not necessarily in the target processes. MC, mitral cell; GC, granule cell, Md, mitral dendrite; Gs, granule cell spine.

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 continue to do morphometric analysis on the wildtype and synuclein transgenic samples. We will also perform immunoelectron microscopy with PhosphoSer129 α-synuclein antibody to determine which specific synapses in the olfactory circuit are impacted by α-synuclein pathology.

IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Our results will help us understand why most newly diagnosed PD patients are hyposmic, i.e. have lost their sense of smell. This proposal is significant and impactful for several reasons. As the olfactory deficits occur prior to the onset of motor symptoms, and the olfactory system is thought to be a conduit through which α-synuclein pathology spreads, eventually reaching the mid-brain, a greater understanding of this pathway will permit us to slow the progression or even prevent Parkinson's disease. As all available treatments for PD are symptomatic and do not halt or retard

the underlying causative neurodegeneration, the translational prospects of our proposal is particularly important.

What was the impact on other disciplines?

We are learning more synapse structure and function in the olfactory circuit and this is important for the fields of synaptic biology and olfaction."

What was the impact on technology transfer?

Nothing to Report

What was the impact on society beyond science and technology?

Nothing to Report

CHANGES/PROBLEMS:

Nothing to Report

Changes in approach and reasons for change: Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them: Nothing to Report

Changes that had a significant impact on expenditures: Nothing to Report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents: Nothing to Report

Significant changes in use or care of human subjects: Not applicable

Significant changes in use or care of vertebrate animals: Nothing to Report

Significant changes in use of biohazards and/or select agents: Nothing to Report

PRODUCTS:

Publications, conference papers, and presentations: Nothing to Report

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

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:	Sreeganga Chandra
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	https://orcid.org/0000-0001-9035-1733
Nearest person month worked:	.36
Contribution to Project:	Dr. Chandra has obtained IACUC and other regulatory approval. She also has organized the mice breeding for the experiments.
Funding Support:	$15,390

Name:	Charles Greer
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	.24

Contribution to Project:	Dr. Greer has obtained local IACUC and ACURO approvals. He has also supervised the ongoing experiments.
Funding Support:	4765.53

Name:	Eduardo Martin-Lopez
Project Role:	Post Doc
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	6
Contribution to Project:	Dr. Martin-Lopez has organized the alpha-synuclein transgenic mouse colony.
Funding Support:	$9016.36

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.

What other organizations were involved as partners?

Nothing to Report

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.

SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: Not Applicable

QUAD CHARTS: Attached.

APPENDICES: *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc. Reminder: Pages shall be consecutively numbered throughout the report.* ***DO NOT RENUMBER PAGES IN THE APPENDICES.***

Mechanisms of Olfactory Deficits in Parkinson's Disease

Log # PD160079
W81XWH-17-1-0564

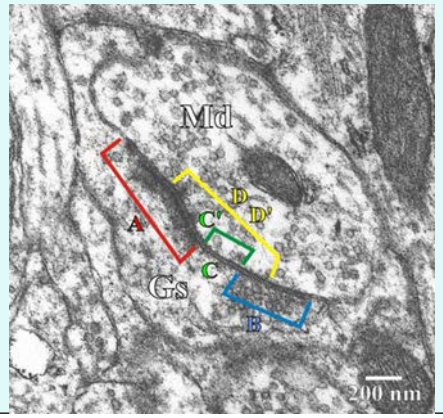
PI: Sreeganga S. Chandra; Charles Greer **Org: Yale University** **Award Amount: $**586,250

Study/Product Aim(s)

- ***Aim 1:*** To characterize the synaptic perturbations in the glomerular and external plexiform layer in alpha-synuclein transgenic mice.
- ***Aim 2:*** To examine the migration of newly generated neuroblasts into the OB and their integration into olfactory circuitry on alpha-synuclein overexpression.
- . ***Aim 3:*** To monitor synaptic vesicle trafficking in OSNs and granule cells in alpha-synuclein transgenic mice.

Approach

Using established mouse models of alpha-synuclein expression we will use high resolution confocal and electron microscopy to determine the effects of alpha-synuclein expression on synaptic structure in the olfactory circuit.



Synaptology quantification: We are quantifying Md, mitral dendrite; Gs, granule cell spine synapses in wildtype and alpha-synuclein transgenics.

Timeline and Cost

Activities CY	17	18	19	20
EM on a-synuclein transgenics				
Morphometric analysis				
BrdU labelling				
SypHy imaging				
Estimated Budget ($K)	**$000**	**$132**	**$235**	**$219**

Updated: (11/8/2018)

Goals/Milestones (Example)

CY17 Goal– EM on a-synuclein transgenics and controls

CY18 Goals – EM and phosphoSer129 immuno-EM on a-synuclein transgenics and controls.

CY19 Goal – To label and monitor newly generated neuroblasts in the olfactory bulb

CY20 Goal – To monitor odorant-evoked synaptic vesicle trafficking in OSNs and granule cells of α-synuclein transgenic mice and controls

Comments/Challenges/Issues/Concerns

We are on track to achieve this goals

Budget Expenditure to Date

Projected Expenditure:$212,375

Actual Expenditure: $132,270.18