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|---|---|
| AWARD NUMBER: | W81XWH-17-1-0564 |
| LOG NUMBER: | PD160079 |
| PROJECT TITLE: | Mechanisms of Olfactory Deficits in Parkinson's Disease |
| PRINCIPAL INVESTIGATOR NAME: | Sreeganga Chandra |
| PRINCIPAL INVESTIGATOR ORGANIZATION AND ADDRESS: | Yale University; 295 Congress Avenue, New Haven, CT 06536 |
| REPORT DATE: | SEPTEMBER 2020 |
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PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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Mechanisms of Olfactory Deficits in Parkinson's Disease

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**5b. GRANT NUMBER**
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**5c. PROGRAM ELEMENT NUMBER**

**6. AUTHOR(S)**

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Charles Greer

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**5d. PROJECT NUMBER**

**5e. TASK NUMBER**

**5f. WORK UNIT NUMBER**

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

Yale University
15 York Street
New Haven, CT 06520-8327

**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

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**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<br>USAMRMC |
|---|---|---|---|---|---|
| a. REPORT<br>Unclassified | b. ABSTRACT<br>Unclassified | c. THIS PAGE<br>Unclassified | Unclassified | 10 | 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.

Our progress this year has been slow because of COVID-19 related shutdowns, decreased lab activities and difficulties with mouse breeding/maintenance. This has been compounded by the inability to work in other labs and perform methods such as the gCAMP6 imaging needed to complete this study. Therefore, we requested and were granted a no-cost extension of this grant.

In spite of these serious setbacks, we have made progress in finishing Aims 1 and 2. As noted in previous reports, our data suggests that aged α-synuclein transgenic mice (A30P) have decreased synapse density throughout the olfactory system starting with the first afferent synapse in the olfactory bulb glomerulus. We have carried out both light microscopy with synaptic markers as well as detailed morphometric analysis of electron micrographs to establish lower synapse numbers. This suggests that as a result of decreased afferent input from the olfactory sensory neurons that the olfactory system as a whole may be less sensitive with a higher threshold for odor detection and discrimination.

To confirm this, we carried out a series of behavioral tests in which we compared the ability of the A30P mice to wildtype controls in detecting/finding food rewards hidden in the cage bedding. Consistent with the loss of primary afferent synapses, we found the transgenic mice had a mean time of 78.4 seconds +/- 17.8m seconds to find the food reward while the wildtype controls found it in 25.3 seconds +/- 16.7 ($t = 2.84$, $p<.006$). These differences were consistent across multiple trials and over the course of 3 weeks.

Given both synaptic structural and behavioral deficits in A30P mice, we determined if these mice show functional deficits by gCAMP6 measuring calcium transients. The rationale for including gCAMP6 in our experiments as an independent measure of synapse integrity in the olfactory bulbs of the transgenic mice. This work has been delayed to COVID.

Complementing our analyses of primary afferent input to the olfactory bulb glomeruli we have also begun to assess synaptic organization in the first cortical representation of output from the olfactory bulb, piriform cortex, a 3-layer paleo- or allocortex. We think this assessment is critical because when we assessed the expression of alpha-synuclein we found a particularly high expression in piriform cortex neurons, both in anterior (**Figure**) as well as posterior divisions. This is ongoing work but thus far our counts of primary input synapses onto the apical dendrites of piriform cortex pyramidal cells in layer I of piriform are identical for both the transgenic and control (3.03/27$\mu m^2$ and 3.33/ 27$\mu m^2$, respectively. The lack of a difference between the two lines of mice is also apparent when the counts are broken down for asymmetric (excitatory) and symmetric (inhibitory). Similarly, we see no difference at this stage in the number of mitochondria associated with synapses in piriform cortex of the transgenics versus controls.

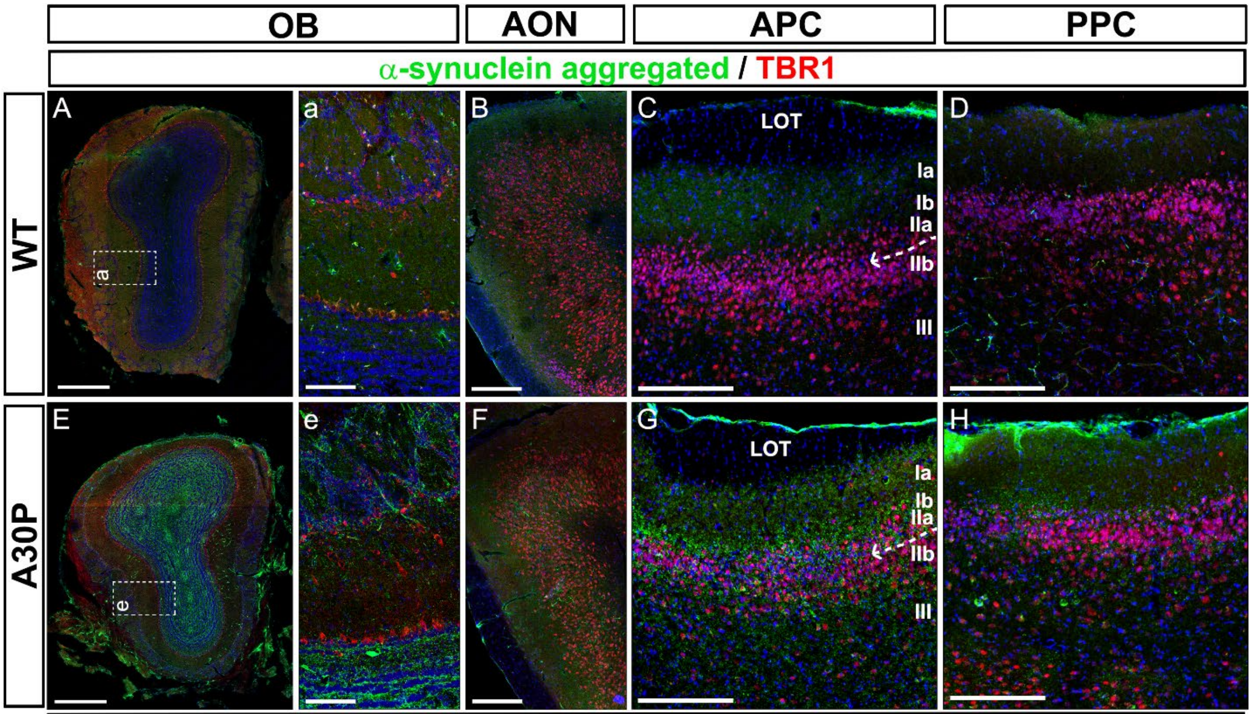



**Figure Legend. Expression of a-synuclein aggregated in projection neurons of the olfactory pathway**. The presence of either a-syn aggregated (green) is indicative of pathological a-syn. Projection neurons are labeled with TBR1 (red). Nuclei counterstained with DAPI (blue). a-syn aggregated is absent along the olfactory pathway in WT animals (A-D). In A30P mutants (E-H) a-syn aggregated is overexpressed in fibers of the granule cell layer of the OB (E, e) and in layer Ib of APC, where apical dendrites of piriform cortex neurons and LOT axons synapse (G). This expression is reduced in PPC, but some cell bodies appear labeled in layer III of PPC (H). AON lacks a-syn aggregated in both groups (B, F). It is notable that alpha-synuclein transgenic lines there is a subjective decrease in the TBR1 pyramidal neurons of piriform cortex suggesting some of these cells may be lost which would be consistent with our behavioral data. Abbreviations: A30P: an a-synuclein gene mutation where the amino acid alanine is replaced with the amino acid proline at position 30; AON: anterior olfactory nucleus; APC: anterior piriform cortex; LOT: lateral olfactory tract; OB: olfactory bulb; PPC: posterior piriform cortex; WT: wild type. Scale bars: A, E: 500 µm; C, D, G, H, K: 200 µm; a, e: 100 µm

We have also carried out BrDU experiments to monitor neurogenesis and migration of new neuroblasts into the olfactory bulb. To generate objective data that we can test statistically and pursue the hypothesis that a decrease in this process may lead to a perturbation of olfactory bulb circuits in the transgenic mice we must wait for them to age until such time as when they begin to exhibit motor systems due to the over-expression of alpha synuclein. Successful breeding of these important mice is poor and motor symptoms are seen at the earliest around 8-12 months postnatal. Thus, this is a very long-term study. The electron microscopy reported above afforded more flexibility because we could use tissue from mice that died prior to sacrifice. The neurogenesis studies, and those with the gCAMP6 reported below, require live animals.

**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 complete the immunoelectron microscopy with PhosphoSer129 α-synuclein antibody to determine which specific synapses in the olfactory circuit are impacted by α-synuclein pathology. We will continue our live gCAMP6 imaging in wildtype and α-synuclein transgenic mice to understand how α-synuclein pathology impacts neuronal activity. We also plan to complete this study and publish our findings.

**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. Given that hyposmia is a common symptom of COVID-19, these studies may lead to greater insight into why this is the case.

**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:**

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

**Actual or anticipated problems or delays and actions or plans to resolve them**: We are delayed in completing the goals of this grant, especially Aim 3 as we lost cohorts of mice due to the lockdown and our inability to work at full time. We were granted a No-Cost-Extension for this grant and plan to avail of this additional year to complete the proposed studies.

**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:**

**Journal publications.**

**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. She has also supervised the ongoing 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 performed the confocal and electron microscopic experiments. |
| 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 projec**t *(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:**

# Mechanisms of Olfactory Deficits in Parkinson's Disease

Log # PD160079
W81XWH-17-1-0564

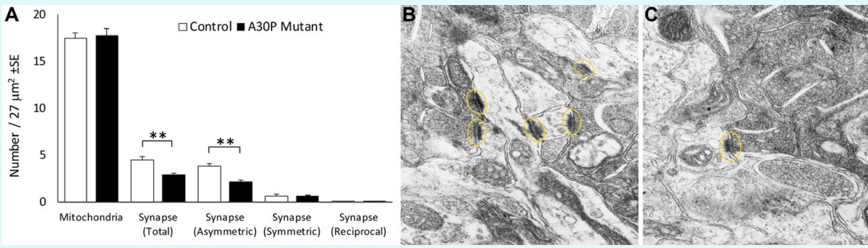
**PI: Sreeganga S. Chandra; Charles Greer** **Org: Yale University** **Award Amount: $594,650**

## 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.




Quantification of Synapse Number in the Olfactory Bulb of Wildtype and Alpha-Synuclein Transgenics.

## Timeline and Cost

| Activities | CY 17 | 18 | 19 | 20 |
|---|---|---|---|---|
| EM on a-synuclein transgenics | | | | |
| Morphometric analysis | | | | |
| BrdU labelling | | | | |
| SypHy/gCAMP6 imaging | | | | |
| **Estimated Budget ($K)** | **$000** | **$132** | **$235** | **$219** |

**Updated:** (9/30/2019)

**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:$293,078

Actual Expenditure: $293,078