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Direct Imaging of Modulatory Neurotransmitters Using Synthetic Nanosensors to Understand and Treat Parkinson's Disease

PRINCIPAL INVESTIGATOR: Dr. Jackson T. Del Bonis-O'Donnell

CONTRACTING ORGANIZATION: The Regents of the University of California

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

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14. ABSTRACT

Nanosensors for the optical detection of dopamine in brain slice has been validated in wildtype C57/C6 mice as well as a Parkinson's model mouse. Using nanosensors, endogenous dopamine release in striatal brain slice is triggered by electrical stimulation and quantified using microscopy. We have established the workflow for this procedure and have prepared a population of Parkinson's and wildtype mice to image in the coming quarter. We have confirmed that the method can quantitatively distinguish dopamine release between subregions of the striatum, a critical step in confirming the method for use in Parkinson's studies. COVID-19 shutdowns stalled research starting in Mar. Research facilities are reopening and the project is expected to resume this quarter.

15. SUBJECT TERMS

NONE LISTED

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TABLE OF CONTENTS

1. INTRODUCTION:

Our work aims to enable the direct visualization of dopamine transmission in living brain tissue of a PD mouse model leveraging our expertise with a new class of infrared dopamine nanosensors. Detailed measurements of neurotransmission at the molecular level with high spatial and temporal resolution will provide unprecedented insight into the mechanism of dopaminergic aberrations in PD and their effects on neuroplasticity. Additionally, these methods will aid in understanding the interplay of the prescribed drug L-DOPA and changes to dopamine neurotransmission and dyskinesia side-effects.

2. KEYWORDS:

Fluorescence imaging – Nanomaterials – Sensing – Dopamine – Parkinson's Disease – Neuromodulators – Dyskinesia

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Major Task 1: Image nanosensors embedded within acute striatal mice brain slice and confirm dopamine sensing

Subtask 1: Optimize nanosensor injection into extracellular space of acute brain slices. Monitor distribution using infrared fluorescence microscopy. *Completed as of Nov 2018.*

Subtask 2: Image nanosensor response to stimulated dopamine release using potassium and optogenetic stimulation. *Completed as of Nov 2018.*

Subtask 3: Monitor neuroinflammation resulting from nanosensor injections. *Ongoing.*

Major Task 2: Quantify differences in dopamine release and reuptake from near-infrared imaging of acute slices from PD model mice

Subtask 1: Preparation of LPS and alpha-synuclein (AS) lesioned mice. Pilot group of mice received LPS and sham injections. Imaging workflow for dopamine established. Issues with TH-immunohistochemical labeling of slices needs to be overcome with new protocol. *Months 10-30. 50% complete.*

Subtask 2: Near-infrared imaging of nanosensor injected slices of PD and control mice to compare dopamine release and uptake dynamics. Simultaneous GCaMP and/or FSCV measurements. *Ongoing. Months 10-30.*

Subtask 3: Analyze image data and quantify release dynamics and fit to dynamic and kinetic models. Compare to GCamp and FSCV. *Ongoing. Months 10-30.*

Major Task 3: Image changes in neurotransmission between PD and WT mice resulting from L- DOPA treatment

Subtask 1: Prepare L-DOPA treated mice of both PD and WT. *Months 15-30.*

Subtask 2: Perform near-infrared microscopy of acute striatal slices for PD and WT mice treated with L- DOPA. Repeat for control mice. *Months 15-30.*

Subtask 3: Analyze image data and quantify release dynamics and fit to dynamic and kinetic models. Determine impact of L-DOPA treatment on dopamine neurotransmission in PD and WT mice. *Months 15-30.*

What was accomplished under these goals?

1) Major Activities:

We finalized the formulation of our near-infrared dopamine nanosensors and established a workflow for their use to image dopamine from brain slices obtained from mice. The full details of the technique that will be utilized for the remainder of the project is outlined in our recent publication (see Appendix I). (Major Task 1)

We performed stereotaxic injections of lipopolysaccharide (LPS) into the substantia nigra pars compacta (SNc) of C57/B6J mice to induce a Parkinsonian phenotype as outlined in our statement of work and approved animal use protocol. Acute brain sections from the dorsal striatum were taken from these mice for imaging. Dopamine nanosensors were introduced into the tissue and electrical stimulation was used to induce the release of dopamine. Imaging the changes to the near-infrared fluorescence of the nanosensors provide us with a quantification of the amount of dopamine released by the tissue. Since the finalization of the slice preparation and stimulation protocols, we have collected and quantified images of dopamine release from striatal tissue from n=3 LPS injected mice and n=2 PBS (sham/control) injected mice. We collect images from both the lesioned and non-lesioned hemispheres of the dorsal striatum. (subtasks of Major Task 2)

Software was written to process video files, perform spatial analysis, and fit dopamine release and decay curves for the nanosensor data collected. All data from LPS and sham mice was analyzed, including imaging data from lesioned and non-lesioned hemispheres. (subtask of Major Task 1 & 2)

TH-staining was performed in the SNc of mice that received LPS injections. Stained slices were imaged under a fluorescent microscope to determine the number of dopaminergic neurons present in the SNc in the lesioned hemisphere compared to the non-lesioned hemisphere.

Inflammatory markers from SIM-A9 cells were quantified after exposure to the SWNT-based nanosensor to evaluate toxicity and potential complications arising from tissue inflammation. Additionally, electrophysiology measurements were performed to evaluate the impact on firing rate of neurons ex vivo and in the presence of nanosensor.

Nanosensor fluorescence data was collected from acute coronal slice of WT mice (n=5). The differences between density of release sites (speculated), peak evoked dopamine release, and reuptake constants was quantified and compared across the dorsal lateral striatum and nucleus accumbens core.

Due to the COVID-19 shutdown, all research activities were halted in March. Labs are return to operation at an extremely limited capacity. This project is in the process of resuming and actions are being taken to continue work on Major Task 2.

2) Specific objectives:

Our objective was to finalize the new procedure for quantifying dopamine release from brain slice using our nanosensor technology. The results are included in our recent publication (see Appendix I).

One of the major goals of the project is to confirm the expected decrease in dopamine release in the dorsal striatum of mice receiving an LPS injection in the SNc. Such a decrease in overall dopamine levels and release amounts has been shown in the literature using other techniques, such as fast scan cyclic voltammetry and HPLC/MS, albeit without spatial resolution. From this data, we hope to quantify changes to both the release site density, the amount of dopamine released per release site, and changes to reuptake kinetics.

One of our major tasks is to evaluate the biocompatibility of the nanosensors in neuronal tissue. We performed morphological analysis, RNAseq, and electrophysiology experiments to determine the impact that the nanosensors have on neuronal cells.

The ability of nanosensors to reliably quantify dopamine with an accuracy and systematic experimental variance sufficient to distinguish differences in dopamine release between striatal brain regions, between lesioned and non-lesioned hemispheres, and with a consistency that enables comparisons between animals had yet been rigorously evaluated. In the past year, we performed a series of control experiments to confirm that the sensors are accurate enough and our technique and procedures consistent enough to quantify biologically relevant differences in dopamine release and reuptake between different brain regions.

3) Results:

Major Task 1:
Detailed results validating our technique are provided in our publication (Appendix I).

Major Task 2:
Our results demonstrate no statistically significant difference in evoked dopamine release as measured using near-infrared fluorescent dopamine nanosensors and fluorescence microscopy (Major Task 2, Subtask 1 & 2). Results are outlined in **Figure 1**. This is contrary to what is expected for acute lipopolysaccharide (LPS) injection into substantia pars compacta (SNc). For the last 3 months, we worked to determine whether this discrepancy was due to either i) the LPS lesion not inducing dopaminergic cell death, or ii) the dopamine nanosensor not being capable of distinguishing changes in dopamine release. Another possibility is that peak evoked dopamine release is not impacted by LPS lesioning of the SNc even if overall levels decrease. We are reaching out to colleagues for more information on this topic. After this is resolved, we will repeat Subtask 1 & 2 of Major Task 2 and move on to Major task 3.

TH-staining of fixed brain tissue slices obtained 2 weeks after LPS-lesioning show no decrease in the number of cell bodies in the SNc compared to the non-lesioned hemisphere (**Figure 2a**). This suggests that the injections are not inducing a Parkinsonian phenotype, i.e. a loss of dopaminergic neurons and the associated decrease in striatal dopamine. Moving forward, we are trying higher concentrations of LPS (10 ug/uL) as well as obtaining LPS from a different source (Salmonella minnesota, Sigma Aldrich).

To confirm that the dopamine nanosensors are capable of quantifying differences in dopamine release, we sought to map out the differences in evoked dopamine release in a single slice. A dorsal to ventral decrease in peak evoked dopamine release has been previously reported in literature. We confirmed that our nanosensor platform can optically quantify this gradient across the striatum **(Figure 3)** from n=3 mice. These results give us confidence that the platform is robust enough to provide insight into biologically relevant variation in dopamine release dynamics, specifically those induced by Parkinson's disease models as part of our Major Task 1 &2.

Furthermore, we began leveraging the unique imaging data obtained using our technique to investigate the spatial dependencies of dopamine release at a micron scale. This is unique in that no other technique to date has been able to leverage and quantify this type of information. **Figure 4** shows results analyzing population statistics of 20µM square ROIs shows which shows clear distinctions between DLS and NAc as well as subpopulations distinguished by their peak release and decay times. The ability to quantify these distinctions at such a fine spatial resolution will allow for new ways to investigate and probe the molecular underpinnings that drive circuits regulated by dopamine. Such analysis is particularly pertinent to understanding the details of dysregulation in Parkinson's disease.

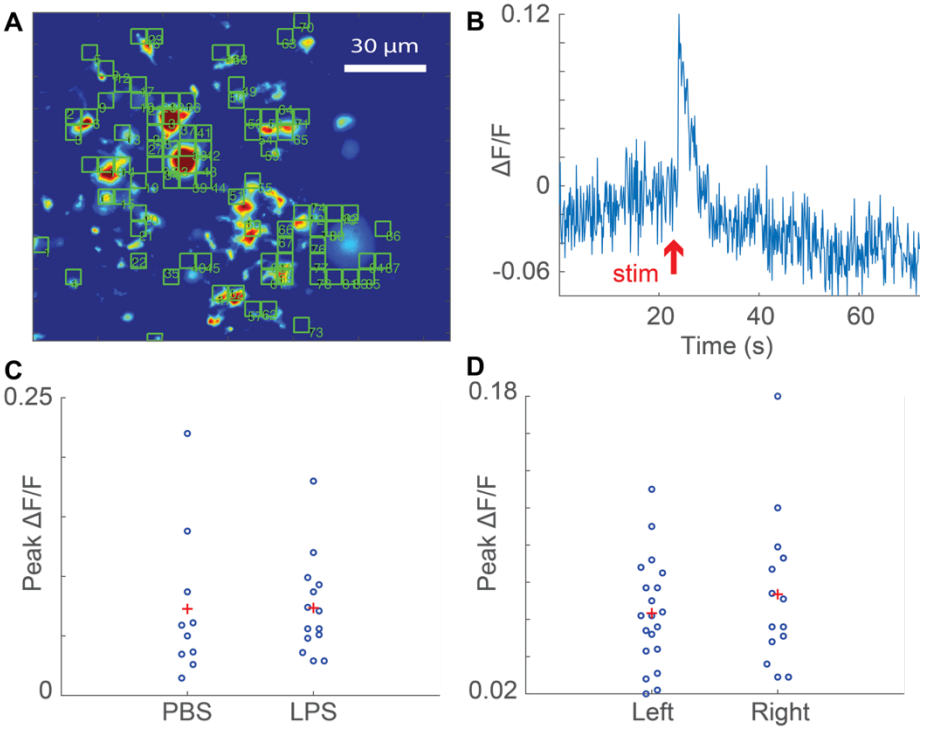

Figure 1 (**A**) Representative mean projection image generated from a video recording of acute brain slice of dorsal striatum from mouse. Fluorescence signal is generated by our near-infrared nanosensors infused in the tissue. Images are obtained using an InGaAs short wavelength near-infrared camera. Our algorithm identifies regions of interest (green squares) in each video where a statistically significant increase in fluorescence intensity occurs following electrical stimulation. (**B**) Representative trace of change in fluorescence intensity (dF/F) of a region of interest over time. A red arrow indicates the time of electrical stimulation. The increase in dF/F following this time point indicates that dopaminergic neurons are releasing dopamine into the extracellular space and binding to our nanosensors. (**C**) Preliminary data collecting peak dF/F signal after stimulation for n=2 PBS injected mice and n=3 LPS injected mice.

Images were collected from the right (injected) hemisphere. (**D**) Preliminary data collecting peak dF/F signal after stimulation and comparing the left (non-lesioned) hemisphere to the right (lesioned) hemisphere for n=3 LPS injected mice.

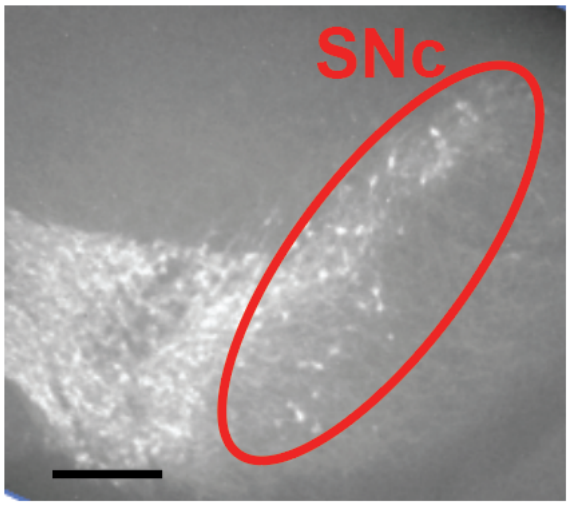

Figure 2 Immunohistochemical labeling of a brain slice obtained from an LPS-lesioned mouse using a tyrosine-hydroxylase antibody. No loss of dopaminergic neurons was observed.

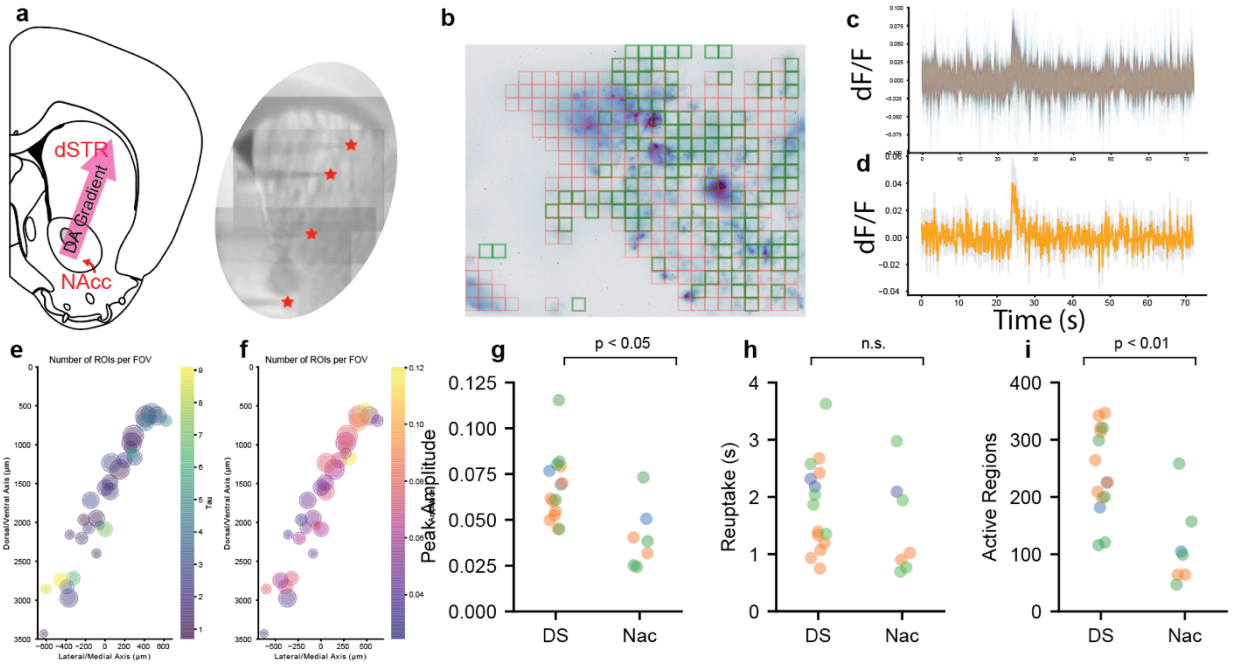

Figure 3. (a) Schematic depicting where electrically evoked dopamine was measured. We expect peak DA repease to be greater in DLS than in NAc. (b) Schematic depicting algorithmic processing of video data where active and inactive zones of dopamine release are identified and marked for further analysis. (c) dF/F signal from nanosensors plotted from each active ROI in a single field of view (top) and average trace with bounds depicting standard deviation. Stimulation occurs at the 24 s mark. (e,f) Plots depicting spatial difference in number of active ROIs (circle diameter) and color depicting decay constant of transient (e) and peak amplitude (f). Plots of peak amplitude (g), decay constant (h), and number of active regions (i) averaged over FOV for different regions and n=3 mice.

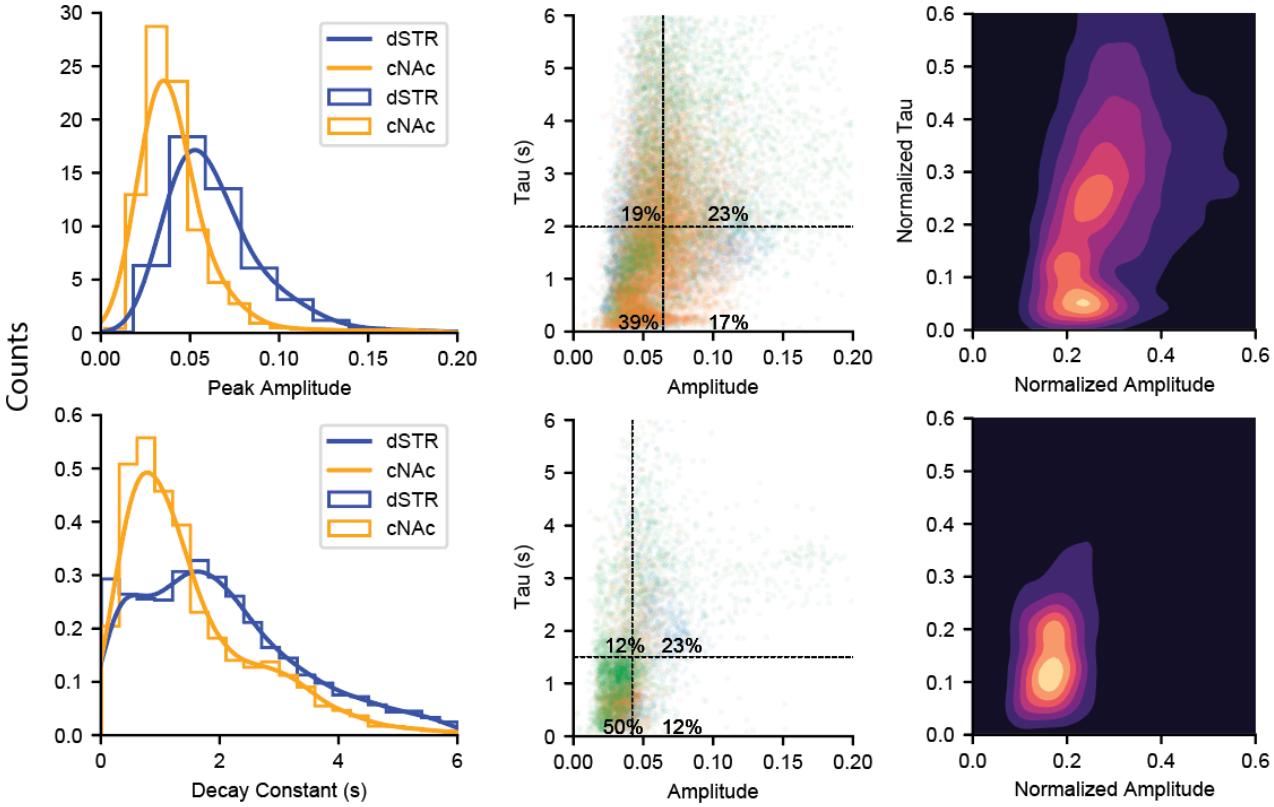

Figure 4. (left) Data pooled from nanosensor fluorescence data on an ROI by ROI basis from n=3 mice. Data is fit using a kernel density estimator to show distinct population differences between 20 µm square regions of the DLS (top) and NAc (bottom). (center) Differences in regional subpopulations of dopamine dynamics based on their peak dopamine release volume and decay constant (analogous to reuptake). Clear distinctions are shown between dorsal striatum (top) and nucleus accumbens (bottom). (right) Same as center plots, but contours fit using a kernel density enstimator. These results highlight that spatial hetereogeneity in dopamine release and modulation between different sub-nuclei of the striatum exist at the 10s of micron scale.

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."

The PI, Dr. Jackson Del Bonis-O'Donnell, has been trained in basic mouse husbandry and breeding, anesthesia, perfusion, preparation of brain slices for imaging and electrophysiology, as well as performing sterile survival stereotaxic injection surgeries of agents into the brain of mice. These are skills essential to the current project and allow for data to be collected at a faster pace because both he and a collaborator can prepare mice for the project. These are also essential skills for future projects as he transitions into an independent research career involving topics in quantitative neurobiology.
Additionally, the project provided research experience for a undergraduate/lab tech who has now successfully transitioned into a graduate research program elsewhere.

How were the results disseminated to communities of interest?

The PI, Dr. Jackson Del Bonis-O'Donnell, has attended several conferences in which the methodologies utilized in this project, i.e. using nanosensors to image dopamine release from neurons, were shared. These included talks given at the Annual Meeting of the American Institute of Chemical Engineers in November 2018. This meeting has thousands of attendees and provides an excellent opportunity to share our research with many undergraduate students, graduate students and early-career industry and government employees. Dr. Del Bonis-O'Donnell also gave a talk at a DARPA meeting related to brain machine interfaces in April 2019. This provided a unique opportunity to interface with many different labs and government teams interested in ways that new techniques can provide quantitative feedback to use for interfacing with the brain. Additionally, Dr. Del Bonis-O'Donnell presented aspects of this work at the American Chemical Society Fall meeting 2019.
Dr. Del Bonis-O'Donnell has published analysis code in MATLAB in an app available to the public at www.github.com/jtdbod/

What do you plan to do during the next reporting period to accomplish the goals?

Nothing to report. PI unable to find a faculty position (3 years attempted) and has left academia.

4. **IMPACT:**
What was the impact on the development of the principal discipline(s) of the project?

Many neurodegenerative diseases and psychiatric disorders are related to changes in how neurons release chemicals known as neuromodulators. Most drug therapies attempt to manipulate the levels of neuromodulators in your brain to correct for any imbalances. However, there exist few, if any, ways in which to accurately measure how these drugs impact how much neuromodulator a neuron releases as well as how these levels change in real time. Using our engineered nanosensors, we can visualize the release of the neuromodulator dopamine and easily see how the amount of dopamine release changes as a result of a disease, disorder, or therapy, potentially at the single neuron level. For Parkinson's disease, this means we can explore the details underlying changes to dopamine release and how it impacts brain function. Additionally, we can use this technique to better understand why the most common Parkinson's therapy, levodopa, produces is negative side-effects, and potentially inform new ways to reduce these effects and improve therapies.

What was the impact on other disciplines?

The ability to image and quantify catecholamines, such as dopamine, in real-time with high spatial resolution will be a powerful tool for the study of the neurobiology of disease as well as inform fundamental understanding of brain function at a chemical level. The more quantitatively we understand these systems, the better the pharmaceutical industry can develop and test new therapies aimed towards difficult to treat patients ranging from neurodegenerative diseases to psychiatric disorders.

What was the impact on technology transfer?

The methods we use for this project are included in a recent patent filing by Landry, Markita Del Carpio P. (Berkeley, CA, US), Wilbrecht, Linda A. (Berkeley, CA, US), Del Bonis-O'Donnell, Jackson Travis (Berkeley, CA, US), Beyene, Abraham G. (Berkeley, CA, US). Application number: 16/373542, Filing date: 04/02/2019.

What was the impact on society beyond science and technology?

It is the hope of the principal investigator that the methods and results of this project will help to better inform the development of drug therapies for patients suffering from neurodegenerative disease and psychiatric disorders. An improved understanding of the molecular and chemical mechanisms of brain function could ultimately improve the quality of life for millions of Americans, promote new growth in the pharmaceutical sectors, and influence policy choices related to these illnesses for public health.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

Nothing to report.

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

Due to COVID-19, our research facilities are only operational in a very limited capacity. As such, availability of equipment for performing the necessary surgeries and measurements will be limited. Currently, Dr. Jackson Del Bonis-O'Donnell has limited laboratory time and is the sole contributor to the project. As such, we anticipate output to be ~25% of what can typically be expected given the current restrictions.

Changes that had a significant impact on expenditures

Animal experiments were delayed because of a staff change on the part of the collaborator performing the stereotaxic injections outlined in the approved AUP. During that time, Dr. Del Bonis-O'Donnell went through training and certification and is now approved and routinely performing stereotaxic injections for the project. This delay postponed much of the animal and surgical costs. Additionally, expenditures for electrodes has been delayed because we have been using custom demo electrodes while we determine the optimal fabrication. Once finalized, we will be purchasing enough for the remainder of the experiments in the project.

COVID-19 has impacted our ability to purchase from the grant. However, the re-budgeting has allowed for the PI to continue the project through an NCE and the remaining funds are now allocated for the remaining animal and care costs. All other remaining supplies necessary for the project are already purchased and available.

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
Nothing to report.

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.

6. PRODUCTS:

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

Journal publications.

- Beyene, A.G., Delevich, K., Del Bonis-O'Donnell, J.T., Piekarski, D.J., Lin, W.C., Thomas, A.W., Yang, S.J., Kosillo, P., Yang, D., Prounis, G.S. and Wilbrecht, L., 2019. Imaging striatal dopamine release using a nongenetically encoded near infrared fluorescent catecholamine nanosensor. Science advances, 5(7), p.eaaw3108. Acknowledgment of federal support: no, PI was not yet funded by grant for the data collected in this manuscript.
- Yang, S.J., Del Bonis-O'Donnell, J.T., Beyene, A.G. *et al.* Near-infrared catecholamine nanosensors for high spatiotemporal dopamine imaging. *Nat Protoc* **16,** 3026–3048 (2021). https://doi.org/10.1038/s41596-021-00530-4. Acknowledgement of federal support is included.
- Darwin Yang, Sarah J. Yang, Jackson Travis Del Bonis-O'Donnell, Rebecca L. Pinals, and Markita P. Landry. *ACS Nano* **2020** *14* (10), 13794-13805. DOI: 10.1021/acsnano.0c06154. Acknowledgement of federal support is included.

Books or other non-periodical, one-time publications.
Nothing to report.

Other publications, conference papers and presentations.
Presentations:
American Institute of Chemical Engineers Annual Meeting. November 2018.
DARPA Brain Machine Interfaces Workgroup Meeting. April 2019.
American Chemical Society Fall Meeting. August 2019.

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

https://github.com/jtdbod/Nanosensor-Brain-Imaging - website hosting video analysis code.
https://github.com/jtdbod/Nanosensor-Imaging-App - website hosting new code

landrylab.com – group website highlighting results, publications and research focus.

- **Technologies or techniques**
The techniques used are outlined in the above publication and subsequent patent.

- **Inventions, patent applications, and/or licenses**
  Title: Imaging Neurotransmitters In Vivo Using Functionalized Carbon Nanotubes
  United States Patent Application 20190224342
  Kind Code: A1
  Application number: 16/373542
  Filing Date: 04/02/2019

  The contents of the above patent is related to the techniques used and optimized for the currently funded project.

- **Other Products**
  Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Jackson Del Bonis-O'Donnell
Project Role: PI/Postdoc
Project Researcher Identifier: orcid.org/0000-0002-9135-2102
Nearest person month worked: 12
Contribution to Project: Prepared and imaged ex vivo brain slices obtained from LPS and sham injected mice. Further improved upon image processing and data analysis software. Analyzed imaging data obtained from imaging experiments. Optimized electrical stimulation protocol to increase robustness and repeatability of dopamine release and quantification. Constructing second imaging system to increase experimental throughput. Received training in stereotaxic surgery and performed LPS and PBS injections in SNc.

Name: Rhea Misra
Project Role: Collaborator/Research Associate
Research Identifier: N/A
Nearest Person Month Worked: N/A
Contribution to Project: Rhea is a research assistant in the Saijo laboratory (co-PI and mentor to Jackson Del Bonis-O'Donnell) that aided the PI (J. Del Bonis-O'Donnell) in performing stereotaxic injections of LPS and immunohistochemistry staining.
Funding Source: NIH (K. Saijo)

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

Dr. Jackson DelBonis-O'Donnell is now primarily advised by Prof. Dr. Linda Wilbrecht of the Helen Wills Neuroscience Institute at UC Berkeley. He continues this project through collaboration with Prof. Saijo and Prof. Landry.

What other organizations were involved as partners?

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:
QUAD CHARTS: See Appendix III

9. APPENDICES:

- **I.** Published manuscripts related to techniques partially developed for this project.
- **II.** Patent Application
- **III.** Quad chart

Appendix I N/A

Appendix II N/A

Appendix III N/A