Eugene Mosharov, PhD

Project Title: Control of L-type channel activity by alpha-synuclein

Contents

Vote Tabulations

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Reviewer's Comments

- An interesting proposal. LTCCs are of considerable interest in PD pathogenesis and the idea of regulation by aSyn is a logical concept. Investigators have the requisite technical skills and the experiments outlined seem solid. Not sure this requires 2 years of funding.

- While the applicants are proposing some interesting projects, there is already mounting data of altering calcium channels in humans with PD. The applicants fail to provide implications for how their proposed work might be useful in humans, especially in the context of past and ongoing clinical studies.

ABF Full Application

Applicant Information

Principal investigator's contact information:

Prefix

First Name
Eugene
Last Name
Mosharov
Suffix

Title
Research Scientist
Institution Name
RFMH/NYSPI
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6467745025
Office Fax

Project Details

Project Title
Control of L-type channel activity by alpha-synuclein

Project Start Date
February 01, 2019
Project End Date
July 30, 2020
Disease focus
Select up to 4 options

Movement Disorders
Specific Disease Focus
Parkinson’s disease

There is an opportunity to upload supporting documentation (i.e. images, graphs, etc.) later in the application.

Project Summary/Abstract

The cardinal motor symptoms of Parkinson’s disease (PD) result from degeneration of dopamine (DA) neurons in the substantia nigra (SN) and their axonal projections to the striatum. A central unanswered question in PD neuropathology is why these neurons are susceptible to neurodegeneration

while other closely related populations, including ventral tegmental area (VTA) dopamine neurons, are less affected. Both α-synuclein (aSyn) and Ca2+ have been consistently shown to play a role in predisposing SN neurons to degeneration, but it is still debated whether these stressors are independent and additive, or they act within the same toxicity pathway. Also, whether the actions of aSyn and Ca2+ and their potential interaction are related to selective vulnerability of SN neurons is unknown. Our preliminary data indicate that aSyn directly controls L-type Ca2+ channels (LTCC) activity, a novel and unexpected relationship between these two well-established players of PD pathogenesis. Under this proposal, we will use electrophysiological recordings to investigate the effect of aSyn deficiency, overexpression and mutation on the activities of the Cav1.2 and Cav1.3 channels, the two isoforms expressed in SN and VTA neurons. The results will provide information about the function of aSyn and LTCC, will serve as a foundation for future studies of cell type- specific interaction between these two well-known risk factors in PD, and will help to design better strategies to counter pathological changes in PD and related neurodegenerative disorders.

Lay Summary

Description of your project for a non-neuroscientist audience

The motor symptoms of Parkinson's disease (PD) result from degeneration of substantia nigra (SN) neurons. Both alpha-synuclein and L-type Ca2+ channels (LTCC) play important roles in PD pathogenesis and therapies to counter dysregulated alpha-synuclein and Ca2+ are currently in clinical trials. Our results point to a novel SN neuron-specific functional interaction between LTCC and alpha-synuclein that may lead to a revision of existing and future PD therapies when they are applied alone or in combination.

Project Narrative

An unanswered central question of PD neuropathology is why SN neurons undergo degeneration while other, even closely related populations such as the VTA DA neurons, are far less affected. PD is mostly sporadic, with an estimated 10 percent prevalence of familial cases. aSyn, encoded by the SNCA gene, plays a central role in both sporadic and familial PD [1]. Nevertheless, aSyn is expressed at similar levels in SN and VTA neurons [2] and aSyn pathology occurs throughout the nervous system in PD patients and does not correlate well with cell death [3], suggesting that the protein may be necessary but not sufficient for PD neurodegeneration. Dysregulation of Ca2+ homeostasis is observed in models of both sporadic and familial PD and recent studies demonstrate that selective death of SN neurons can result from the activity of the LTCC, which produce larger currents in SN than VTA neurons [2,4,5]. It is unknown, however, whether aSyn and Ca2+ present additive stress factors or act within the same toxicity pathway. Our recent data show that aSyn deletion provides protection to SN dopamine neurons that is similar in effect to, and not additive with, a pharmacological blockade of LTCC; both aSyn knock-out and dihydropyridines partially rescue SN neurons from neurotoxins and minimize the differences between SN and VTA neurons [2,4] (Fig. 1). Furthermore, LTCC currents that are larger in SN than VTA neurons are greatly decreased in SN neurons from aSyn deficient animals (Fig. 2), indicating a functional link between the two proteins.

Facilities and Equipment

Dr. Mosharov's lab and office are situated on a newly renovated 6,000 square feet floor shared among 4 PIs in the Division of Molecular Therapeutics in the New York State Psychiatric Institute (NYSPI) Kolb Research Annex. This building also houses the research laboratories of the Departments of Neuroscience, Pharmacology, Psychiatry, and Physiology & Cellular Biophysics making this an ideal environment for scientific exchange and collaborations. Administrative help is provided through the Division of Molecular Therapeutics. All animals will be housed in the Animal Care Facility located in NYSPI Kolb Research Annex Building.
The laboratory has two inverted microscopes (Zeiss Axiovert 100) used for fluorescence imaging (Zeiss Axiocam MRm with assorted filter cubes) and electrophysiology (Two Axopatch 200B amplifiers, Instrutech A/D board, Picospritzer, and assorted micromanipulators). Also available are, an upright microscope for acute brain slice recordings (Olympus BX50WI microscope, Axopatch 200B amplifier, Instrutech A/D board), and a Prairie multiphoton upright microscope. Various other equipment includes low-magnification microscopes for electrode fabrication, Sutter microelectrode puller, two sectioning system for brain slices preparation (a vibrotome and a freezing microtome), PCR machine (PE GeneAmp9700), gel boxes with power supplies (BioRad), spectrophotometer (BioRad), bench-top centrifuges, water purification system (Millipore), water baths, HPLC system (Agilent) with ESA Coulochem electrochemical detection, +4C refrigerator and a -80C freezer. Core facilities at Columbia and NYSPI are outstanding, including high throughput microarray, next generation sequencing, drug screening and proteomics cores, the Genome Center, and confocal microscopy.

Specific Aims

The main Aim of this study is to test a hypothesis that aSyn directly controls LTCC activity.
Aim I: To evaluate the effect of aSyn deficiency on LTCC channel activity, patch-clamp recordings of total and L-type-specific Ca2+ currents will be performed in SN and VTA DA neurons in acute midbrain slices from 1) wild-type (WT) mice, 2) aSyn null mice, and 3) adult WT mice following acute aSyn knock-down. The relative roles of Cav1.2 and Cav1.3, the two LTCC isoforms expressed in midbrain DA neurons, will be studied in slices from knock-in mice expressing a dihydropyridine-insensitive Cav1.2 mutant (Cav1.2DHP-/-), in which LTCC blockers will only inhibit Cav1.3 activity [6].
Aim II: To investigate whether overexpression or PD-like mutation of aSyn affects LTCC activity, we will measure Cav1.2- and Cav1.3-specific currents in SN and VTA neurons in slices from WT mice following virus-mediated overexpression of the human WT or A53T mutant aSyn.

After the completion of this project, we will have built a solid foundation for further studies of biochemical and structural interactions of aSyn with the

Cav1.2 and/or Cav1.3 subunits. These experiments will determine the unique mechanisms that predispose SN neurons to degeneration and will help to design better strategies to counter pathological changes associated with PD and related neurodegenerative disorders.

Research Strategy

Significance, Innovation, Approach, Timeline

Significance: Strategies aimed at countering the impact of dysregulated aSyn and Ca2+ are currently undergoing clinical trials as disease-modifying treatments for PD [7], however, to predict the efficacy of these therapies alone or in combination, better understanding of the interactions between these PD risk factors is required. Our preliminary data indicate that aSyn directly controls LTCC activity, which warrants further study of the relationship between the activity of LTCC in cells susceptible in PD and aSyn expression. These findings will provide insight into normal and pathological functions of aSyn and will help to design better strategies to prevent the demise or restore the function of neurons damaged in PD.

Innovation: Although previous studies have suggested an interaction between aSyn and intracellular Ca2+, our preliminary results point to a novel cell-type specific functional interaction between LTCC and aSyn. These experiments will serve as a foundation for future studies of the cell-specific interaction between the two well-established players of PD pathogenesis.

Approach: Perforated-patch recordings of the plasma membrane Ca2+ currents will be conducted in SN and VTA neurons in acute coronal midbrain slices (see Fig 2). We expect to see a correlation between the levels of aSyn expression and the activity of the LTCC in SN but not VTA neurons. As controls we will use DAT-tdTomato mice derived by crossing DAT-IREScre and Ai9 floxed tdTomato reporter lines. A cross between DAT-tdTomato and aSyn-KO mice (aSynKO-DAT-tdTomato; maintained in the lab) will be used to study the effect of aSyn deletion. To differentiate between the effects of aSyn on the Cav1.2 and Cav1.3 channels, we will cross DAT-tdTomato line with dihydropyridine-insensitive Cav1.2 mutant mice [6]. Dihydropyridine treatment will thus reveal the contribution of either both L-type channels (in DAT-tdTomato) or Cav1.3 channel only (in Cav1.2DHP-/--DAT-tdTomato). Using Cav1.2DHP-/- instead of the Cav1.3 null mice is preferable as it circumvents possible compensatory changes due to channel deletion.
As acute aSyn KD may have different effects than aSyn genetic deletion [8], we will record from DAT-tdTomato and Cav1.2DHP-/--DAT-tdTomato mice following stereotaxic injection of an AAV2-aSyn-siRNA and a scrambled control (provided by Dr. Fredric P. Manfredsson [8]). To investigate the effect of aSyn overexpression or mutation, recordings will be done in slices from mice injected with AAV2-WTaSyn (distributed by the MJFF2) or AAV2-A53TaSyn (provided by the late Susan Lindquist). Adult DAT-tdTomato and Cav1.2DHP-/--DAT-tdTomato mice will be injected with viruses stereotaxically and used 7-14 days later. Expression of aSyn will be verified immunohistochemically.

With variance from our data, effect size=0.3, α=0.05 and power=0.95, each group should have ~15 cell recordings (G*Power software). Although previous reports did not find sex differences in electrophysiological properties of GABA or DA [9] neurons in slices, we will consider this factor in the analyses and have powered the study accordingly.

Timeline: Electrophysiological recordings from WT, aSyn KO and aSyn KD mice will be completed within 6 month. In parallel, breeding of Cav1.2DHP-/--DAT-tdTomato mice will proceed, followed by recordings from these as well as virus-injected mice. All experiments will be completed within 1-1.5 years.

List up to 5 milestones you will reach within the first 6 months of your study.

1. Patch-clamp recordings of LTCC activity in SN and VTA neurons with aSyn knock-out and knock-down.
2. Breading and genotyping of Cav1.2DHP-/--DAT-tdTomato transgenic mice.
3. Recordings from Cav1.2DHP-/--DAT-tdTomato mice to establish which LTCC isoform is affected by changes in aSyn level.
4. Recordings from DAT-tdTomato and Cav1.2DHP-/--DAT-tdTomato mice following virus-mediated overexpression of WT and A53T mutant aSyn.

Age of Population Group(s) that will potentially benefit from this research
(check boxes that apply)

All Ages

Scientific Literature References

Please upload **one** PDF that includes up to **ten** scientific literature references.

References.pdf

Budget, Attachments and Acknowledgements

Budget

We recognize that changes may have occurred since the time you submitted your Letter of Intent. Please share the most recent accurate numbers below:

Total Project Budget

120000

Total existing funding or in-kind support

60000

Amount to be raised through crowdfunding campaign

60000

Please upload a detailed budget and budget justification

PHS398 BUDGET_ABF.xls

Additional Attachments

Please consolidate all supporting documentation to be included with your proposal, i.e. images, graphs, etc. into one PDF and upload here.

Figs.pdf

Evidence of institutional support. Recommended to have a letter from your department chair that includes information about the value of the department's support (in-kind or otherwise).

Institutional Support Letter.pdf

Documentation of IRB/IUCAC approval or exemption, if applicable.

Acute slice Animal Protocol.pdf

Acknowledgements

I understand that the ABF will not list approved projects for general public crowdfunding campaigns until documentation of IRB/IUCAC approval or exemption is provided.

Yes

I understand that approval of the project to be shared on the crowdfunding campaign site is dependent on providing and working with the ABF staff to create the requisite materials that present the project in an engaging, easy-to-understand website presentation. I am amenable to working with the ABF staff to create such materials.

Yes

I understand that approval once a project has been completed, I will be required to submit a summary of my findings to be posted online (one page), and will submit this in a reasonably timely fashion. I also agree to submit a financial report, and to co-sign a thank you letter with the ABF that will be sent to donors.

Yes

I understand and agree that the ABF may share the information that I provide (including but not limited to the project description and relevant biographical/background details) in conversations with other potential funders outside the website to bolster fundraising efforts.

Yes

American Brain Foundation Release Agreement

American Brain Foundation
Release Agreement – Research

1. **Grant**. For good and valuable consideration, the receipt and sufficiency of which is hereby acknowledged, I grant to the American Brain Foundation ("ABF") and to the ABF's affiliates (including the American Academy of Neurology), and their respective contractors, agents, assigns, licensees, and successors (collectively, the "ABF Group"), a worldwide, royalty-free, perpetual, irrevocable right to take and use my image, likeness, voice, verbal statements, written testimonials and name and all images, videos, sound recordings, and written and verbal materials that I provide to the ABF (collectively, the "Materials "), in all forms and media, including composite or modified representations, for the purpose of promoting and supporting the missions of the ABF. For

the avoidance of doubt, the Materials include all research project

proposal information, project reports and other research-related information submitted to the ABF. I understand and agree that the ABF may publish the Materials on any and all media, including printed matter, promotional materials, e-mail, websites and social media platforms.

2. **Acknowledgement of Use**. I understand that the ABF Group may use the Materials on any and all media, including printed matter, promotional materials, e-mail, websites and social media platforms. I understand that the ABF's use of the Materials may intentionally or unintentionally give rise to the impression that either I or a family member suffers from brain/neurologic disease, and I nevertheless consent to this use. The ABF is not obligated to utilize any of the rights granted in this agreement. I waive the right to inspect or approve any uses of the Materials in connection with this grant.
3. **Warranty**. I warrant that I have the full power to enter into this agreement and to grant the aforementioned rights.
4. **Release**. I release the ABF Group from all liability for any claims that may arise regarding the use of Materials, including any claims of defamation, invasion of privacy, or infringement of moral rights, rights of publicity, or copyright. The ABF is permitted, although not obligated, to include my name as a credit in connection with any use of the Materials.**I have read and understood this agreement, I understand that it contains a release of liability, and I am over the age of 18**. This agreement expresses the complete understanding of the parties and shall be binding on me and my heirs, legal representatives and assigns. I understand that I am entering into a legally binding agreement and that clicking "I Accept" below shall have the same legal effect as my signature on this Release Agreement.

I Accept

Application Fee

There is a $100 non-refundable crowdfunding application fee. Applications will not be considered until the fee payment has been received. Click on the link below to submit your payment online or send a check made out to American Brain Foundation to 201 Chicago Ave, Minneapolis, MN 55415.

Submit my application fee now!

Research Foundation for Mental Hygiene, Inc.
New York Psychiatric Institute Division
1051 Riverside Drive • New York, New York 10032
(646) 774-6500 • (646)-774-6540

November 28, 2018

American Brain Foundation

To the Committee Members,

The enclosed application entitled "Control of L-type channel activity by alpha-synuclein" aims to investigate a functional interaction between alpha-synuclein and the activities of the $Ca_V1.2$ and $Ca_V1.3$ channels, the two L-type calcium channels isoforms expressed in midbrain dopaminergic neurons. These findings will provide insight into both pathological and normal functions of alpha-synuclein, will serve as a foundation for future studies of the cell-specific interaction between several well-established players of Parkinson Disease pathogenesis, and will help to design better strategies to counter pathological changes associated with Parkinson Disease and related neurodegenerative disorders.

Given the strong research plan and the importance of this project, we are pleased to give Dr. Mosharov and his team our full institutional support for this project. The Division of Molecular Therapeutics at New York State Psychiatric Institute will provide laboratory space and any other support Dr. Mosharov will need for this project. We are committed to supporting this type of innovative research and will fully support Dr. Mosharov in this endeavor.

Sincerely,

Jonathan A. Javitch, M.D., Ph.D.
Chief
Department of Molecular
Therapeutics, NYSPI

Jeffery A. Lieberman, M.D.
Professor & Chair, Dept. of Psychiatry
Director, NYSPI

Janelle Greenhill, M.P.H.
Director of Administration
RFMH @NYSPI

New York State Psychiatric Institute Animal Care and Use Committee
Protocol Summary Form for Approval of Animal Research

For IACUC Use Only
Protocol Number (s):
Meeting Date:
Approval Date:

Protocol Title(s): Optical and electrophysiological recordings in brain slice preparation. 1450.

Principal Investigator: David Sulzer **Email Address**: ds43@columbia.edu
Department: Psychiatry **Mailbox**: **Telephone #: 646-774-5024** **Fax #:**
Contact Person: **Ellen Kanter** **Telephone #:** 646-774-5064 **Fax #: 774-8438**
Contact person's email address: **ek289@cumc.columbia.edu**

This application for approval of animal research is: ☐New (New Research) ☐New (Ongoing Research)
☒ Continuation

Is this a Pilot Study? _____Yes _x____No

List all Funding Sources providing ALL grant titles and reference numbers (add rows as required)**:**

Agency	Grant #	CUMC or RFMH Account # **(Required)**	Grant Title
JPB	471	PG006614	Cellular and physiological mechanisms of Parkinson's Disease
Simons Foundation		PG008927	Regulation of Striatal Neuronal Development by mTOR/Macroautophagy
Private Foundation		PG006650	
NIH RO1	NIDA	GG010336	Presynaptic mechanisms in dopamine neurotransmission
NIH	NIMH	GG009410	Development of Fluorescent False Neurotransmtters

Simons Foundation grant ("Regulation of Striatal Neuronal Development by mTOR/Macroautophagy") utilizes the brain slices techniques and experiments described in this animal protocol. The experiments proposed in this grant are related to the study of Striatal neurons, Medium Spiny Neurons (MSNs), and the role of mTOR pathways and Macroautophagy in the regulation of Striatal neuronal development. In particular, this animal protocol describes 5 projects in the lab, and the first two are directly related to the Simons Grant:

1. "Analysis of synaptic changes in the striatum in mouse Parkinson's disease models and in response to drugs of abuse.
2. A study on how the synapses of one of the major efferent pathway of the striatum are functioning in the Parkinsonian brain.

And, in the Simons grant, specifically in SubAim 1.3 (SubAim 1.3: "Elucidate changes in Kir currents during MSN development.") In "**Approach:** We will measure surface expression and subcellular localization of Kir channels in the striatum of P18 and P28 mice to determine the portion of Kir protein on cell surface, lysosomes, autophagosomes, ER and Golgi.
Surface expression **will be measured in acute brain slices**…"…" *Please note the Simons grant experiments are also covered by our RASCAL AAAV9459 protocol for the majority of experiments conducted in the Black Building.

1. **NIH GG010336** grant ("Presynaptic Mechanisms in Dopamine Neurotransmission") covers the projects 3,4 and 5 - described in this protocol.The relative grant aims are these (PAGE 39)-Aim 2. To characterize how presynaptic DA terminals are selected by neural activity. Hypothesis: Once formed by rules explored in Aim 1, DA terminals can exhibit a wide range of synaptic vesicle fusion probabilities, with a large fraction of presynaptically "silent" synapses. The conversion

between silent and active synapses and the release probability from individual synapses are regulated by D2 DA autoreceptors and other local neurotransmitter input via presynaptic heteroreceptors.
Plan: Synaptic vesicle exocytosis from DA terminals in corticostriatal brain slices will be imaged with a novel FFN and DAT substrate, FFN102, together with the endocytic synaptic vesicle probe FM1-43. We will determine the dependence of DA terminal synapse selection on the pattern of DA neuron activity, extracellular Ca2+, and on auto- and heteroreceptor activation by endogenous neurotransmitters

2. NIH grant, GG009410 ("Development of FFNs") covers the projects described in this protocol - "We use electrophysiology, fast-scan cyclic voltammetry, and 2-photon microscopy imaging of newly developed fluorescent compounds to study synaptic activity in the basal ganglia in living mouse brain slices. These studies elucidate rules of synaptic connectivity in brain circuits that are affected in the above mentioned disorders and will provide insight for directed therapeutic pharmacological intervention. Projects 3 and 4: 3 - Development of new imaging tools to visualize catecholamine, serotonin, and glutamate release. 4 - A study on the modulation of dopamine release within the basal ganglia, we will use pharmacology and 2- photon imaging of a fluorescent probe in mouse brain slices.
The relative aims in the grant are these (Page 2) - Aim 1. Characterization of Dopamine FFN Probes (DA-FFNs) in vivo in the Striatum. We recently designed probe FFN102 as a dual fluorescent substrate for both the dopamine transporter (DAT) and vesicular monoamine transporter 2 (VMAT2) to serve as an optical mimic of DA. FFN102 shows high selectivity for DA presynaptic terminals in acute brain slices, and due to its pH-sensing properties, enables the multi-photon microscopy imaging of the release of synaptic vesicle content at DA boutons evoked by single action potentials. In preparation for the in vivo experiments, we will fully characterize FFN102 in acute slice in the dorsal striatum, including determination of the fluorescence signal-to-background ratio (S/B), signal stability, selectivity of labeling, activity dependent signal (ΔF/F), and kinetics of release at individual DA presynaptic varicosities. The release parameters will be compared to calcium concentration transients in the same boutons measured by GCaMP calcium indicators specifically expressed in DA neurons. We will optimize the surgical approaches and the imaging parameters in anesthetized mice in the striatum. The release properties of ensembles of individual DA presynaptic terminals will be examined in vivo as evoked by tonic and phasic firing where the stimulation mode will be induced by electrical stimulation of the medial forebrain bundle (MFB) and optogenetic stimulation of DA neuronal cell bodies in the substantia nigra and ventral tegmental area (SN/VTA)..Aim 2. Development of Norepinephrine FFN Probes (NE-FFNs). We recently developed compound FFN270 as a dual fluorescent substrate of the norepinephrine transporter (NET) and VMAT2. Preliminary data in anesthetized mice show that FFN270 labels NE axons and presynaptic terminals in the somatosensory (barrel) cortex and is released in vivo by systemic administration of amphetamine. NE release in the cortex modulates sensory and behavioral decision processes and is implicated in many CNS disorders. We will examine the selectivity of this probe for NE versus DA synapses in several cortical areas in vivo; optimize the administration, labeling, and imaging parameters; and determine the release properties of NE terminals in the cortex as in Aim 1 (in collaboration with Dr. Gary Ashton Jones).
Aim 3. Development of Serotonin FFN Probes (5HT-FFNs). We have developed compound lead FFN245 as a selective, fluorescent substrate of the serotonin transporter (SERT) and VMAT2. We have also synthesized a focused library of compounds around this structural motif to enable optimization of photophysical and transport properties using cell culture assays developed in the PI's laboratory (SERT- and VMAT2-transfected HEK cells). The best compound(s) will be selected and examined in acute brain slice and in vivo in the CA1 region of the hippocampus (in collaboration with Drs. Atilla Losonczy and Scott Thompson). This locus contains a high density of 5HT axonal fibers that potentiate excitatory neurotransmission. The new probes will be characterized in acute slices and in vivo using the imaging parameters similar to those described in Aims 1 and 2. The new in vivo imaging methods will unlock the possibilities of examining fundamental aspects of synaptic control of monoamine neurotransmission in intact circuitry of living rodent brain.

1. NIDA Grant "Presynaptic Mechanisms in Dopamine Neurotransmission" covers Projects # 3,4 and 5
Relevant Aim:
To characterize how presynaptic DA terminals are selected by neural activity.
Hypothesis: Once formed by rules explored in Aim 1, DA terminals can exhibit a wide range of synaptic vesicle fusion probabilities, with a large fraction of presynaptically "silent" synapses. The conversion between silent and active synapses and the release probability from individual synapses are regulated by D2 DA autoreceptors and other local neurotransmitter input via presynaptic heteroreceptors.
Plan: Synaptic vesicle exocytosis from DA terminals in corticostriatal brain slices will be imaged with a novel FFN and DAT substrate, FFN102, together with the endocytic synaptic vesicle probe FM1-43. We will determine the dependence of DA terminal synapse selection on the pattern of DA neuron activity, extracellular Ca2+, and on auto- and heteroreceptor activation by endogenous neurotransmitters.

2. JPB Foundation Grant " Cellular and Physiological mechanisms of Parkinson's Disease Pathogenesis" covers projects #1 and 2
Relevant Aim:
In Aim 3, we develop new means to optically measure neurotransmission of the two most damaged neurotransmitter systems in PD, dopamine and norepinephrine. Loss of the first causes PD motor disorders, and loss of the second is involved in PD cognitive symptoms. By inventing new compounds known as fluorescent false neurotransmitters, we have discovered new phenomena in the activity of these synapses, and demonstrate the first optical measure of neurotransmitter release in the brain of living animals. These techniques will have wide applicability for studying normal and disease behavior including PD.

Grant Submission Status:____ New Application _____ Date of Submission
____Competitive Renewal _____ Noncompetitive Renewal

Grant Submission Made to the Office of: ☒ RFMH
☒Columbia (For Columbia submissions, you must submit a separate protocol for each species used.)

*****Attach a copy of the Research Plan and Vertebrate Animal Sections of the grants supporting this protocol and clearly mark the sections relevant to this protocol*****

Does Your Experiment Require the Use of (Please complete the relevant form(s) providing approval signatures from the relevant offices):

☒ Use of Hazardous Chemicals ☐ Use of Radioactivity or Radiation
☐ Animal Transportation (Outside of NYSPI) ☐ Use of an MRI Facility

"EXCEPTIONS" as outlined in the guide from the National Research Council (check if appropriate):
☐ Multiple Survival Surgery ☐ Food Restrictions ☐ Water Restrictions ☐Physical Restrictions
☐Physical Restraint of a Non-human Primate > 12 hr

Species (List Below) **Total Number Projected to be Used for 3 yrs = 1355**
Mus musculus_____ Number projected for use in Year 1 = 451
Number projected for use in Year 2= 451
Number projected for use in Year 3= 451

You must discuss space and caging requirements with the Veterinarian before this protocol can be considered for approval

INSTRUCTIONS

- Please make sure you are using the most current Application for Approval of Animal Research.
- Please be sure that you list all of the grant/award titles, grant numbers, CUMC and/or RFMH account numbers associated with this protocol on the face page (**required)**.
- If your experiment will require the use of MRI equipment, Hazardous Chemicals, or Radioisotopes, you MUST fill separate forms for approval to use these items. **If you will require the use of Radiation, i.e., X-Rays, you must submit your protocol for review by the Radiation Safety Office at Columbia University.** All forms for approval MUST BE reviewed, approved, signed and attached to your

application before submitting it to the PI IACUC Office. These forms are to be submitted **ANNUALLY** (RSO) and **TRIENNIALLY** (EHS and MRI).

- Please type your responses underneath the appropriate question, answering all questions as required. Please proofread your text. **DO NOT ALTER THE FORMAT OF THIS PROTOCOL SUMMARY FORM,** for the sake of institutional protocol, format consistency and efficient protocol review.

- Please be as concise as possible. If possible, do not exceed 25 pages.

- You MUST sign your application, scan it and submit it electronically. **PROTOCOL SUMMARY FORMS ARE ONLY BEING ACCEPTED ELECTONICALLY.**

- In justifying your choice of procedures, the USDA requires that you indicate the sources you consulted in making your decisions. For literature searches, state what databases you consulted, at least two. Indicate the key words used, especially those related to finding alternatives, e.g. "alternative," "computer model," etc. Give the range of dates of the literature searched. And finally, state the date you most recently performed the search.

- Note that your application will be returned if these instructions are not followed.

- If you have any questions, call PI IACUC Office at iacuc@nyspi.columbia.edu.

Instructions for the Literature Search for Alternatives

Adapted from Animal Welfare Information Center (AWIC), 3/26/2004 Website: www.nal.usda.gov/awic

In order to submit a complete IACUC application, you must conduct literature searches to determine if alternatives to the use of animals exist, and to determine whether your protocol unnecessarily duplicates previous research. When searching for alternatives, the Animal Welfare Information Center (AWIC) refers to the 3R's of W.M.S. Russell and R.L. Burch described in their book "The Principles of Humane Experimental Techniques" (1959). The 3R's are reduction in the number of animals used, refinement of techniques and procedures to reduce pain and distress, and replacement of animals with non-animal techniques or use of less-sentient species.

1. **List any potential alternatives** (3R's of Reduction, Refinement and Replacement) of which you are aware (e.g., alternate models, modified techniques, housing modifications, computer simulations, etc.).

2. **List key *keywords/concepts*** using terminology from your responses to questions above. Keywords will help the searcher determine if there is unintentional duplication, how many animals are necessary, etc. It is important for the researcher to review some of the articles found.
Keywords should include those for alternatives such as "vitro", "culture" or "simulation". The word "alternative" may also be included. The selected animal model, other species and the word "model" will help retrieve animal and non-animal models as potential alternatives.
Keeping your concepts separate will assist you in creating a good search strategy (e.g. Keywords of concept 1 – heart or cardiac or cardiovascular disease (include synonymous terms; and Keywords of concept 2 – atherosclerosis or arterial plaques (include synonymous terms).

3. Database selection
Choose databases that are appropriate for the area of study: PubMed, Index Medicus, EMBASE, BIOSIS, CAB, AGRICOLA, TOXNET, NTIS, REDRIP, PSYCHLIT. Examples of other sources are: conference attendance, committee membership, professional expertise, training, etc.

Include alternative data bases and web resources: the AWIC Alternatives and Searches Database, Bibliography on Alternatives to Animal Testing, InvitroDerm, AVAR's Alternatives in Education Database, Altweb's Anesthesia/Analgesia and Human Endpoints Databases, AnimAlt-ZEBET, etc.

4. Years of coverage.
The researcher should record the years included in the search, based on database coverage or the years selected by the searcher within a search strategy (e.g., 1988 – 2004).

5. Results of Search.
Note alternatives found in the search and cite relevant articles, whether or not they present clear alternatives to the use of live animals for the studies proposed, and if not useful, why not.

QUESTIONAIRE

1. **Project Summary**

   A. **In nontechnical language, summarize the objectives and rationale for the proposed studies**. This statement may be used by administrators to communicate the goals of your study to the media and the general population. This section should be clear to individuals who do not share your expertise. **Please limit your response to 300 words.**

Understanding the regulation of dopamine cell activity, as well as dopamine's effects on synaptic circuitry, and adaptive changes in the brain will help to develop new ways of intervention for Parkinson's disease, schizophrenia, and substance abuse.
We use electrophysiology, fast-scan cyclic voltammetry, and 2-photon microscopy imaging of newly developed fluorescent compounds to study synaptic activity in the basal ganglia in living mouse brain slices. These studies elucidate rules of synaptic connectivity in brain circuits that are affected in the above mentioned disorders and will provide insight for directed therapeutic pharmacological intervention.
This work consists of 5 different projects:

3. Analysis of synaptic changes in the striatum in mouse Parkinson's disease models and in response to drugs of abuse.
4. A study on how the synapses of one of the major efferent pathway of the striatum are functioning in the Parkinsonian brain.
5. Development of new imaging tools to visualize catecholamine, serotonin, and glutamate release.
6. A study on the modulation of dopamine release within the basal ganglia, we will use pharmacology and 2- photon imaging of a fluorescent probe in mouse brain slices.
7. A study on striatal cholinergic interneurons and their response to amphetamine.

   B. What are your hypotheses?

1. Some of the adaptive changes in striatal cells in response to altered dopamine input caused by disease underlie the development of dyskinesia in response to L-Dopa treatment.
2. Regulation of GABA release from striatal projections is altered in Parkinson's Disease.
3. We develop new imaging tools to visualize presynaptic activity at catecholamine, serotonin, and glutamate synapses in brain tissue.
4. Dopamine release is studied for the first time in the globus pallidus using imaging and its regulation is compared to release in the striatum.
5. Striatal cholinergic interneurons respond to amphetamine with altered firing patterns and this is at least partly caused by amphetamine acting as an agonist at nicotinic receptors.

2. If this is a **revised protocol**, briefly summarize the changes in the application, e.g., addition of drug class, addition of procedure, etc. Include a detailed description in your letter accompanying the revised protocol. Be sure to highlight the changed and new areas in grey or yellow on this protocol.

3. Why are living animals required for this work? What literature sources (for example, the Animal Welfare Information center, the Agricultural Library, Pubmed, or experts in the field) did you consult to determine if there are **alternatives** to experiments involving living animals? **For literature searches, state**

what databases you consulted, at least two. Indicate the key words used, especially those related to finding alternatives, e.g. "alternative," "computer model," etc. Give the range of dates of the literature searched. And finally, state the date you most recently performed the search. ***See page 3 for additional instructions for conducting literature searches.***

(Note: The searches in Section 3 are for alternatives to using living animals while the searches in Section 8 are for alternatives to using painful procedures.)

Please use the following search example as your guide:

As these studies investigate synaptic interactions in certain brain areas that are comparable to circuits in the human brain, it is necessary to use living brain slices derived from rodents. Based on our knowledge of the literature, and searches of Medline (1975 – present), BIOSIS (1975 – present), and Embase (1975 – present) there is no other alternative.

	Pubmed	BIOSIS	Embase
brain slice + mouse + alternative	29	67	19

The hits were either on brain slice organotypic cultures or on imaging the whole brain or were irrelevant. While whole brain imaging can provide information of activity changes in different brain areas this method does not allow for understanding the underlying synaptic adaptations. Similarly, the data obtained by this work can be used for computer modeling, but modeling itself cannot substitute for examining the adaptations in receptor and channel activity that actually occur.

The most recent search was accomplished 08 November, 2016

4. **Species**

A. What species will you use? Why did you choose this species for these studies? What is the source of the species you will be working with?

Mus musculus: We will use mouse lines (sources indicated in table below) that carry genetic alterations that are critical for identification of specific cell types and for manipulating the activity of cells and intracellular pathways. The mice will both be bred in house and obtained from Jackson Laboratories.

Mice transferred from CUMC (referred to in table below as "100% from BB") come only from BB room 1905 or BB room 317 and transfer is subject to regular veterinary review. The Sulzer lab will insure that NYSPI DCM has on file the most recent health status reports for the relevant Columbia housing locations. Other criteria and procedures relating to use of mice housed in CUMC BB are described in Section 5.

B. Will you be using different strains of the same species, or genetically-modified versions of the same species? If yes, please complete the following table listing for each variant its name, estimated rate of breeding success and any known behavioral or physical issues associated with that variant.

Experimental Strain (Genotype)	Percentage of Strain to be Used Experimentally	Known Phenotypic Issues
DAT IRES-Cre Jax#006660	25% for experiments	None
D1-Cre MMRRC#034258-UCD	25% for experiments	None
C57/Bl6J (Charles River)	100% for experiments (100% from BB)	None
Drd1a-tdTomato JAX # 016204	25% for experiments (100%	None

	from BB)	
Ai9tdTomato JAX #007909	25% for experiments	None
GCamp6 JAX # 024105	25% for experiments	None
Adora 2aCre MMRRC#031168-UCD	25% for experiments	None
ChATCre Jax#006410	25% for experiments	None
ChR2 flox/flox-YFP Jax#024109	25% for experiments	None
D2 flox/flox Jax#020631	25% for experiments	None
alpha 7 flox/flox Jax #026965	25% for experiments	None
beta2 nAchR KO	100% from BB	None
ChAT eGFP	100% from BB	None
GCaMP3 X ChAT Cre	100% from BB	None
ChR2 X ChAT Cre	100% from BB	None
ChR2 X DAT Cre	100% from BB	None
ATg7 f/f X D1 tomato	100% from BB	None
ATg7 f/f X a2a Cre X D1 tomato	100% from BB	None
Pitx3 KO	100% from BB	None
Pitx3 KO X D2 tomato	100% from BB	None
D1 Cre X ATg7f/f X D1 tomato	100% from BB	None

5. If your experiment requires animals being removed from their housing rooms to approved laboratories, you must provide in detail the type of transport cage/ chair, route you'll be using to transport the animals (e.g., freight elevator, stairs) to the laboratory, and how often the transport cages or chairs will be sanitized**.** If you **transport nonhuman primates,** you MUST follow the Standard Operating Procedure for Bites and Scratches, as well as for sanitization of the transport vehicle. You MUST also have a copy of the S.O.P and sufficient disinfectant solution with you.

   C57/BL6/J mice and transgenic mice will be housed in Kolb 1032 pathogen-free barrier animal facility for breeding. Mice at postnatal week 1 or older will be transported according to IACUC policy 403 in disposable containers provided by DCM from the housing in 1032 using the freight elevator to the physiology room 431 in Kolb 4 where they will be euthanized within one hour to prepare brain slice preparations.

   Mice to be transferred from CUMC Black Building

   We will use a maximum of ~20 experimental mice for each of the 12 mouse lines listed above (highlighted), thus will use a total of 240 mice until the end of January 2018, that will be transported from the Black Building to Kolb 4 for acute experiments. These mice will be used for completing experiments for project 1 and 5 and are already included in the estimated number of animals needed (statistical justification).

   Due to limited availability of specific strains necessary for work proposed in this protocol from approved sources or our breeding colony at NYSPI, we will transport mice on an experiment-by-experiment basis from the Black Building to Kolb 4 for terminal slice experiments performed on Kolb 4. It will also be subject to regular review by the NYSPI veterinarian; the veterinarian can stop transfer of mice from Black at any time. The procedures to be followed for the transfer and use of these mice are described below.

   a) Locations :
      1. Housing locations of these mice in the CU Black Building (BB) are listed under question 4.A.
      2. Mice will be transported directly to Kolb 431, or Kolb 427 for habituation or euthanasia (preparation of brain slices).

b) Transport route and procedure

Mice will be transported in DCM-approved disposable containers. The containers will be transported in a ventilated secondary container or on a cart covered with opaque, breathable material. Mice will be transported via the BB freight elevator to the BB service entrance or service tunnels connecting BB to the Milstein Bldg. Upon exiting BB or Milstein, they will be transported 1-2 blocks to the Kolb Building at 40 Haven Ave, and via the Kolb service elevator to Kolb 4. Mouse containers will enter through Kolb 416 where the secondary container will be misted with 1% Virkon, then moved to Kolb 431 or 427 to be unpacked into clean disposable habituation chambers. Mice will be habituated in these containers. Following habituation, mice will be euthanized for acute slice recording experiments described under Question 6.

c) Disposal and sanitation of materials and carcasses.

All materials used for transport, handling, habituation, dissection and other procedures on the mice will be disposed of in a red regulated medical waste container in 431 or 427. No live mice or mouse carcasses will enter the recording rooms or common-use areas of Kolb 4 labs. All instruments used in dissection of mice transferred from BB will either be disposed of in sharps containers or sterilized using a hot bead sterilizer or a cold sterilization station permanently located in Kolb 432.

d)Personnel

Personnel will wear complete personal protective equipment including booties, gown, mask, bonnet and gloves while working with mice from BB or related materials. The experimental will remove PPE and dispose of it in Kolb 431 or 427, and wash hands and arms up to the elbow before entering common areas of Kolb 4. To the extent possible, personnel working with mice from BB will be segregated from personnel that perform work in NYSPI mouse holding locations. Personnel responsible for care of breeding colonies at NYSPI will not be exposed to BB mice or materials and avoid rooms where these materials are used. Experimenters using mice from BB will not enter any NYSPI holding area, including Kolb 10, Kolb 9 and DCM satellite locations on Kolb L1, Kolb 1, Pardes 5 and 4, within 72 hrs after exposure to mice or materials from BB. Prior to entry to NYSPI facilities, these experiments must have showered and may not wear clothing, footware, jewelry worn during procedures on BB mice unless first sanitized.

6. Describe, in detail, the planned use of animals.

Begin with a brief **(300 words or less)** overall description of the experimental procedures. It is particularly helpful for you to describe a longitudinal view of the experiment for each group of animals including the number and types of procedures that will be tested in each group: a study flow chart may also be used to provide an efficient overview. Please limit procedural details in the overview, but provide them in the appropriate sections below.

Overview

C57/BL6/J mice and transgenic mice will be housed in Kolb 1032 pathogen-free barrier animal facility for breeding. Mice aged 1 week to adult (3-4 months) will be transported in disposable containers provided by DCM from the housing in 1032 to the physiology room 431 in Kolb 4 where they will be euthanized within

one hour to prepare brain slice preparations. As a temporary accommodation, mice of a few mouse lines not listed for Kolb DCM, and some crosses of lines (all listed in the table as "100% from BB") that are still kept in the CUMC Black Building ICM facility, will be transported to Kolb 4 ONLY for experiments and euthanized after a short habituation period.

A. How will individual animals be identified? You must justify any possibly painful procedures to be used (e.g., ear notching, tattooing) and indicate the sources you have checked to determine that there are no alternative procedures.

Some mice will be identified by placement of an ear tag. It is a less painful method than toe clipping and more reliable than ear notching. At the same time a tail biopsy is taken (0.2-0.5 cm) using a sharp scissor that has been sterilized between individual tails clippings high temperature (250 Celsius bead sterilizer). The area of the tail to be clipped with be disinfected with 70% ethanol or isopropanol. The local anesthetic lidocaine is applied before and Kwikstop with benzocaine after biopsy. The tail tissue sample will be used to determine the genotype of each individual mouse. Typically we genotype the mice between 10 and 17 days, but in some cases we have to quickly genotype new born pups before we prepare cell cultures. For new born pups, a piece of tail is clipped, Kwikstop is applied, and mice are identified with a sharpie mark on their back. They stay with the mother until genotyping is completed (3-4 hours) and are then euthanized for cell culturing.

Some mice will have to be marked by toe clipping (postnatal day 10-17). Toe clipping is the most reliable way to mark mice as ear tags can fall out or be torn out by other mice. Therefore, we will use toe clipping for mice that will grow up to a certain age and thus spend a considerable time together with other mice in a cage. Toe clipping serves to identify the animal at the same time as sampling tissue for genotyping. The to be clipped toe is treated with topical viscous lidocaine (1%), then using a pair of sharp scissors, the portion of the toe distal to the first (most distal) metatarsal joint is removed, and Kwikstop with benzocaine is applied. Scissors are wiped with 70% ethanol and will be sterilized using a bead sterilizer between mice (see justification in 8).

B. Where will the animals be housed and will there be any special housing requirements? If so, describe these requirements. Discuss the environmental enrichment program that you will provide for your animals wherever applicable.

Mice will be housed and bred in the NYSPI DCM barrier holding facility Kolb 1032. All cages will be provided with nesting material and additional DCM-approved enrichment (e.g. hutches, toys). Animals will be housed in social groups whenever possible (exceptions are if a litter has 6 males after weaning or breeding males that are not actively used)

C. Please provide the following information for your experimental procedures, the name of the experimental procedure, indicate **if its surgical or nonsurgical and the exact room location for the procedure(s) to be performed.**

Breeding, identification and tail-clipping will be performed in the DCM holding rooms where the mice are housed (see above).
Mice aged 1 week- adult (3-4 months) will be transported from Kolb 1032 to Kolb 432 where transcardial perfusion fixation takes place.

D. If your experiments require restriction of animals' intake of food or water, you must:

There will be no food or water restriction.

a. Specify the level and duration (short-term or long-term) of restriction (e.g., food restriction resulting in 80% of body weight) and describe how you will achieve and maintain this level.

b. Justify both the use of the restriction and the particular level of restriction.

c. If animals' access to water is to be restricted, describe how you plan to monitor the animals for dehydration?

E. **Describe all nonsurgical procedures to be used whether they are painful or not (including breeding method). If you are conducting more then one procedure please categorize the pain for EACH procedure**. SEE QUESTION #8 for the applicable pain categories.

Holding & Breeding of animals (Pain category B):
Mice will be kept according to the standard operating procedures of the DCM animal facility. Two female mice per breeding cage will be housed with one male. We will remove either the male or one female prior to the birth of pups. If we remove the male from a trio breeding cage and more than 10 pups are born, they must be separated by the time the oldest litter is 12 days old to prevent overcrowding (Policy 400). Standby breeder males will be singly housed.
Animals will be weaned at three weeks of age and single sex housed in conditions of 5 animals per cage. If a litter has 6 males after weaning, one will be singly housed. Weaning will be delayed until 28 days when pups are too small to be weaned at 3 weeks of age.
Tail and toe biopsy before PND 17 (Pain category D): Tail or toe biopsies will be performed before PND 17 in accordance with NYSPI IACUC Policy 702. The area of the tail or toe (the first, most distal metatarsal joint) to be clipped will be disinfected with 70% ethanol or isopropanol. Tail (0.2-0.5 cm) or toe biopsy will be taken using recently sharpened scissor that have been autoclaved and wiped clean with ethanol with a sterile 4x4 pad between individual tails clippings. The local anesthetic lidocaine will be applied before and Kwikstop with benzocaine after biopsy.
Ear tagging (Pain category C): The animals will be identified by placement of an ear tag at the time of tail clipping or weaning using small animal ear tags (purchased from National Band and Tag co.). In accordance with NYSPI IACUC Policy 702, tags will be disinfected with 70% ethanol or isopropanol prior to the procedure. With the mouse gently restrained, the tag will be placed low on the pinnae and into the cartilage of the ear, but not into the ear canal.

Drug effects: Please, note that all of the drugs listed in the table in section 9 will be only applied in vitro to brain slices.

F. In **table format** specify the number of animals to be used for each procedure and provide statistical or other justification for that number. The total number of animals to be used listed on the face page must match the total number from all procedures. ***If you are breeding animals to obtain a specific genotype please include in your total number of animals, the number to be used experimentally and the number to be bred but discarded.***

Procedure	Mouse line/Rat line	Number required
Breeding	**DAT IRES-Cre**	3 breeding pairs plus 5 for crosses with DAT-Cre
Breeding	**C57/Bl6J**	5 breeding pairs
Breeding	**Drd1a-tdTomato**	2 breeding pairs, plus 5 for cross with ATG7f/f
Breeding	**Ai9tdTomato**	2 breeding pairs plus a total of 10 for crosses with D1-Cre, and Adora 2aCre
Breeding	**GCamp6**	2 breeding pairs and 6 for crosses
Breeding	**ATG7 Flox/Flox**	2 breeding pairs, plus 10 for cross with Adora 2aCre and D1-Cre
Breeding	**Adora 2aCre**	2 breeding pairs plus 5 for cross with ATG7f/f
Breeding	**CHATCre**	2 breeding pairs 3 for crosses with D2flox flox and 3 for GCamp6
Breeding	**Alpha 7 flox/flox**	2 breeding pairs 3 for crosses with DATCre
Breeding	**D2 flox/flox**	2 breeding pairs 3 for crosses with CHATCre
Breeding	**ChR2 flox/flox-YFP**	2 breeding pairs 6 for crosses
Total number of mice for breeding (for experimental mice plus number of mice with undesired genotype see below)		**117**

Experimental mice:

1. For a study on the synaptic mechanisms involved in L-Dopa-induced dyskinesia in PD, we will study the modulation of excitability of medium spiny neurons by cholinergic transmission in the striatal brain slices. For the electrophysiological recording, we will need 8-10 slices to obtain statistically significant data ($p<0.05$ using student t-test, GraphPad Prism 5). Since experiments have a failure rate of about 20% due to technical difficulty of recording living cells in brain slices, the actual number of slices needed per condition is 13 slices. Usually 3-4 slices per mouse are usable; therefore we will need 4 mice per condition.

a) We measure 5 different parameters (I/V relationship, input resistance, and input/output response in current clamp mode; synaptic efficacy in response to cortex stimulation, and voltage dependent K+/Ca+ current in voltage clamp mode), thus we will need 5x4=20 mice.

b) To study how the excitability of medium spiny neuron and glutamatergic synaptic transmission are affected by activation of cholinergic interneurons in current and in voltage clamp mode (ChR2), we will need 4x4=16 mice for current clamp recording and 4x4=16 for voltage clamp recording, that is a total of 32 mice.
c) To study the induction of synaptic plasticity in medium spiny neuron in dependence of cholinergic interneuron activity we estimate to need 4x4=16 mice.
d) To determine the effect of 10 different receptor antagonists and agonists of endogenous neurotransmitters, we will need 10x2x4=80 mice.
In total, we will need 20+32+16+80=148 mice. All are transgenics (either CHATcre/Chr2f/f crosses or CHATCre/Ai9 tomato crosses.Thus the total is 148 mice for experiments plus 464 mice that won't have the desired genotype: **612 mice.**

2. For a study on how the synapses of one of the major efferent pathway of the striatum are functioning in the Parkinsonian brain, we will examine the mechanisms involved in the loss of feedback inhibition by GABAb receptors. In a series of pharmacological and genetic experiments, we will test a combination of the GABAb receptor agonist baclofen and inhibitors of potassium channels (Ba2+, TEA, 4AP), GTPS, Gallein (G-proten inhibitor), CCG 50014 (RGS4 inhibitor) and pertussis toxin. To obtain a statistical significance 0.05, and an effect size of 0.8 and a power of 0.95, a priori power analysis revealed that we need 42 slices per treatment group (Calculated with G*Power). In a typical experiment we prepare 3 slices per mouse brain. Thus, in total we expect to use 7*42/3 = 98 mice for these experiments. In addition we will make use of transgenic mice in which the fluorescent calcium binding protein GCaMP is targeted to selective neuronal populations by Cre recombinase. These experiments will compare calcium dynamics in the presynaptic terminals of the striatal efferents in normal and Parkinsonian mice. For these experiments we need both D1Cre and iresDATcre mice to obtain the conditional expression of GCaMP in striatal efferents and dopamine terminals, respectively. With the same statistical parameters as above, we need 2*42/3 = 28 mice for these experiments. In total we therefore predict to use 126 mice in this part of the project. 28 mice are transgenics plus 84 mice that won't have the desired genotype: The total is 98+84+28=**210 mice**.

3. We will use the new FFN102 probe to study release probability of individual dopaminergic synapses of the dorsal striatum. We have been able to show fluorescent signal changes associated with a single action potential, but are still working on determining the spatial relationship between the synapses that make up a population. According to preliminary data, we expect 10 – 15 mice will be required to reach statistical significance when measuring single action potential events due to the very small fluorescent changes and high background signal (Working between 0-5% F/F). As a result, when comparing the fluorescent changes in response to D2 receptor agonist or antagonist, we expect 30-45 mice for these experiments. In addition, we will test a new compound that is a promising candidate to be specific for serotonergic neurons. As with already tested compounds, we estimate that we will require ~ 25 mice to fully characterize for specific serotonergic loading (7 control and 7 SERT-inhibited, ANOVA with post hoc Tukey test, GraphPad Prism5) and uptake into vesicles (7 control and 7 VMAT2-inhibited, similar analysis). If the compound passes these tests, we will then determine release kinetic parameters, which we estimate would require another ~25 mice (Both electrical and chemical stimulation in the presence and absence of inhibitors).
The total for this project is 45+25+25=**95 mice** (all wildtype).

4. For a study on the modulation of dopamine release within the basal ganglia, we will use pharmacology and 2-photon imaging of a fluorescent probe in mouse brain slices. For the imaging, we measure the amount of fluorescent probe released from a field of view within a medial sagittal slice of mouse brain. This imaging can be conducted under different pharmacological manipulations. For each manipulation, we will need 5-6 mice to obtain statistically significant data ($p<0.05$ using student t-test, GraphPad Prism).

a) To test the role of six pharmacological agents on the release of fluorescent neurotransmitter probes, we will apply agonists and antagonists for D1 receptor, D2 receptor, and kappa-opioid receptor, cocaine and amphetamine. To conduct experiments under these conditions for these requires 6x6 = 36 mice.
b) To study how extracellular calcium levels modulate the release of fluorescent neurotransmitter probes, we will use three levels of calcium and apply our electrical stimulation to produce release. This will require 6x3 = 18 mice.
c) To study the differences between how globus pallidus and striatum dopamine is modulated, we will repeat experiments using the six pharmacological agents and the extracellular calcium manipulations while imaging in the striatum. This will require 36+18=54 mice.
Total mice required for this study is then **108 mice** (all wildtype).

5. Striatal Cholinergic interneuron physiology.
Depending on the kind of experiment (electrophysiology or electrochemistry), we use one of the following two criteria to decide the minimal and maximal number of mice to be tested per condition. These criteria state the amount of data required for (1) determining data distribution of our samples and (2) obtaining good power from the statistical analysis. From one mouse brain, we obtain 3 slices from the striatum, which are cut by half to obtain 6 slices in total (3 slices x 2 hemispheres). Depending on the type of experiment, we used from 3 to 6 slices per mouse. Based on the study of N.M. Razali, et al. (Journal of Statistical Modeling and Analytics, v.2, 2011), it has been claimed that for meeting the first criterion at least 30 data points are required to obtain a good power in the Shapiro-Wilk test for normal distribution, which is considered the best analysis for determining normal distribution and the one that requires less data. This implies we need from 5 (30/6) to 10 (30/3) mice to meet this criterion per tested condition. To meet the second criterion, we rely on our previous data (over 200 experiment performed in control conditions) to determine the minimum of data required to obtain a power greater than 80% (www.dssresearch.com, Statistical Power Calculator).
a) Cholinergic interneurons basal firing rate
Electrophysiological recordings on acute brain slices from ChAT-eGFP mice will be employed for easy visualization of cholinergic interneurons. In addition, we expect to use CHAT and DAT specific D2 and alpha 7 KO mice. We expect to test ligands (agonist and agonist+antagonist) for histamine receptor 1, norepinephrine receptor 1, serotonin receptors 6 and 7 in the ChAT-eGFP mice. In addition, staurosporine (broad-spectrum kinase
inhibitor), calyculin A (protein phosphatases 1 and 2A inhibitor), and ZD7288 (HCN channel inhibitor). Therefore, (4 receptors x 2 ligands) + (3 other drugs) + control= 11 different conditions. In the D1/D5 KO mice, we expect to use only D2R-like agonist and antagonist for additional 5 conditions ([2 mice x 2 ligands] + control), therefore we will screen 16 different conditions for this study. Because we don't have a good approximation of the population mean and variance to calculate the power of the statistical analyses for these experiments, we used the first criterion to determine the number of mice. But, we would like to highlight that drugs that shows no tendency in the first 10 slices (2 mice) are treated as no effect. In the case that all drugs induced an effect, we need from 80 (16x5) to 160 (16x10) mice to accomplish the experiments. We expect to accomplish one third of these experiments during the following year, therefore we will be using from 27 to 53 mice.
b) Amphetamine modulation of cholinergic interneuron firing rate
Electrophysiological recordings on acute brain slices from conditional D2R and alpha 7 KO mice will be used in this study. We will test amphetamine (1 and 10 M) and amphetamine along with D2R-like and D1R-like antagonist for a total 12 conditions ([3 mice x 2 amphetamine concentration] + [3 mice x 2 amphetamine+antagonist]). By the time we perform these experiments, we will have enough data from the previous project to calculate a good estimate of the statistical power, but as a first estimation we use the first criterion, therefore we will need from 60 to 120 mice in a three year period to accomplish this study. We expect to use one third of the total mice following year, therefore we will be using from 20 to 40 mice.

The maximum total for this study is 53+40=93 mice. For the 40 transgenic mice there will be 120 mice with undesired phenotype thus the total is 120+93= **213 mice**.

The total number of mice for these studies is 612+210+95+108+213+117(breeders): 1355.

7. If the experiments **will involve surgery**, indicate the type (**nonsurvival, major or minor survival, multiple survival**) and **describe the presurgical, surgical and post surgical procedures in detail**. This description must include a list of all anesthetic agents and drugs to be given before, during, and after surgery and a post-operative monitoring plan. Also indicate who will be responsible for monitoring the depth of anesthesia during surgery and for providing postoperative care.
Note that multiple survival surgeries on a single animal MUST be justified.

No surgery will be performed other than euthanasia by transcardial perfusion.

8. **USDA categories.** The following are four USDA categories of pain induced in animals by biomedical experiments. For each experimental procedure detailed in 6 and 7, please describe which of these categories applies. Note that all procedures rated category D, or E must include additional information detailed after the descriptions of the categories.

B. Breeding or colony management procedures: number of animals being bred, conditioned, or held for use in teaching, testing, experiments, research, or surgery but not yet used for such purposes.
- Animal breeding, pregnancy, parturition and lactation
- Restraint and preventative medical procedures such as vaccination
- Husbandry procedures such as non-stressful transporting animal from one housing location to another

C. No painful procedures: number of animals upon which, teaching, research, experiments, or tests were conducted involving no pain, distress, or of use pain-relieving drugs.
- Restraint and husbandry procedures, such as applying identification tags, ear notching, tattoos, etc.
- Transporting of animals from one housing location to another over several hours
- Insertion of per-cutaneous catheters, cannula or ID systems
- Positive reinforcement behavioral modification
- Blood sampling
- Euthanasia alone using AVMA approved methods

D. Pain or distress appropriately relieved with pain relieving drugs: number of animals upon which experiments, teaching, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic, or tranquilizing drugs were used.
- Approved euthanasia methods following terminal procedures with anesthesia
- Surgeries with local and or general anesthesia conducted by trained personnel in accordance with standard veterinary practice
- Painful or stressful post-operative circumstances with analgesics
- Transport of animals with tranquilizers

E. Pain or distress or potential pain or distress without pain relieving drugs: number of animals upon which teaching, experiments, research, surgery or tests were conducted involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic, analgesic, or tranquilizing drugs would have adversely affected the procedures, results or interpretation of the teaching, research, experiments, surgery, or tests.

- Negative conditioning via electric shocks that would cause pain in humans
- Restraint for long periods of time (days to weeks)
- Chairing of non-human primates not conditioned to the procedure for the time period used
- Death as an endpoint study
- Inductions of self-mutilation

Category D and Category E experiments present an explicit responsibility on the investigator to explore alternative designs to ensure that animal distress is minimized or eliminated.

- **If you answered D or E** you must provide literature searches documenting that you have investigated alternative procedures that are less painful. S**tate which databases you consulted (at least two) and indicate the key words used, especially those related to finding alternatives, e.g. "alternative," "computer model," etc. Give the range of dates of the literature searched. And finally, state the date you most recently performed the search for each painful procedure:**

a) Describe the animals' response to the procedure.

b) Indicate how the animals will be monitored and cared for during the procedure.

c) If pain is to be alleviated by the use of drugs, list these drugs, their actions, the dosages to be used, and their routes of administration.

d) If the pain or stress cannot be alleviated by drugs, explain why and justify.

e) What reference sources did you consult to determine that there are no alternatives to this painful or stressful procedure. ***For literature searches, state what databases you consulted, at least two. Indicate the key words used, especially those related to finding alternatives, e.g. "alternative," "computer model," etc. Give the range of dates of the literature searched. And finally, state the date you most recently performed the search.***

Tail and toe clipping (Pain Category D):
Tail clipping:
a) For genotyping purposes we will use tail clipping on mouse pups age 17 days or younger. Tail clipping (0.2-0.5 cm) provides sufficient material for genotyping and causes little distress for the mice.
b) and c) Pups respond to tail clipping with a brief distress response which subsides as soon as Kwikstop with benzocaine is applied. Pups will be monitored for bleeding and Kwiskstop will be reapplied if necessary.
d) N/A
e) Tail clipping is the most commonly used procedure used for genotyping of mice. A literature search conducted on 8/29/16 (from 1975 until present) using "tail clipping AND alternative AND mouse" resulted in 5 hits in PubMed: one reference discussed the permanence of tattooing on different mouse body parts the other four discussed hemophilic mice. The same search in BIOSIS gave 9 hits: the

additional hits were on blood clotting and one on alternatives to opioids in inducing hypnosis and immobility.

Toe clipping:

a) Toe clipping (done on pups up to PND 17) is the most reliable way to mark mice as ear tags can fall out or be torn out by other mice. Therefore we will use toe clipping for mice that will grow up to a certain age and thus spend a considerable time together with other mice in a cage. Toe clipping provides at the same time material for genotyping.

b) and c) Pups respond to toe clipping with a brief distress response which subsides quickly. Lidocaine is applied to the toe before and after clipping to numb the pain. Usually there is no prolonged bleeding but pups are monitored and in case bleeding should occur Kwikstop will be applied.

d) N/A

e) We have carefully reviewed the available literature on alternatives to toe clipping, prompted by the NIH policy that such clipping must be scientifically justified after consideration of alternatives. Of the various methods suggested by the NIH only ear punching obtains tissue for genotyping with the same intervention as identification. This method will not work for our studies because (1) it must be performed in older (> 2 weeks old) mice, making it difficult or impossible to complete genotyping before weaning, and (2) ear marks heal after several weeks and can be unreliable after healing. The only other permanent method of identifying animals that is acceptable for neonates is tattooing. Since tattooing does not result in the acquisition of tissue for genotyping, it would need to be combined with tail clipping or ear punch for tissue acquisition. We tried in the past simultaneous neonatal tattooing plus tail clipping as an alternative form of identifying animals. In well over two-thirds of the animals tattooed in the neonatal period, the tattoo faded over time, a well-known problem with tattooing. We had to re-tattoo the animals, subjecting them to further discomfort. Therefore we propose to use toe clipping. The advantage of using toe clips to identify mice is that this provides simultaneously tissue for genotyping.

We have examined the standard references for this type of work, consulted several senior investigators in the field, and thus far we have not found a less painful alternative for genotyping. To continue searching for alternatives which allow labeling and genotyping of animals, we conducted literature searches (from 1975-present) in Pubmed (3 references) and BIOSIS (same 3 references) using "genotyping AND animal identification AND toe clipping" on 11/08/16. The 3 references suggested that toe clipping is the preferable identification method, inducing the least stress for the animals. Paluch et al. 2014 state that toe clipping at PND 7 or 17 has no effect on the well-being and development of mice.

1. Identification methods in newborn C57BL/6 mice: a developmental and behavioural evaluation. Castelhano-Carlos MJ, et al. Lab Anim. 2010 Apr;44(2):88-103.

2. Analysis of physiological and behavioural parameters in mice after toe clipping as newborns. Schaefer DC et al. Lab Anim. 2010 Jan;44(1):7-13.

3. Developmental and behavioral effects of toe clipping on neonatal and preweanling mice with and without vapocoolant anesthesia. Paluch LR, Lieggi CC, Dumont M, Monette S, Riedel ER, Lipman NS. J Am Assoc Lab Anim Sci. 2014 Mar;53(2):132-403.

Euthanasia by transcardial perfusion (pain category D):

a)-d) For preparing living brain slices, mice will be anesthetized with ketamine/xylazine (100mg/kg and 7mg/kg respectively) or euthanized with euthasol (100mg/kg) followed by transcardial superfusion with ice-cold saline followed by decapitation.

No procedures will be performed until mice are deeply anesthetized, as determined by toe-pinch. The animal will be euthanized by perfusion with exsanguination while completely anesthetized.

e) A literature search on 12/01/16 (from 1975-present) using the terms "transcardial and perfusion and mouse" resulted in 31 references in Pubmed and 34 references in BIOSIS. None of these references presented an alternative. (Adding the term "alternative" to the search resulted in 0 hits in both data bases)

9. In **table format**, list **all drugs** used in all procedures, surgical and nonsurgical, including the purpose, dosage, and route of administration of each drug. **After the Table please describe the expected side effects of the drugs.**

Drug Name:	Dosage:	Route:	Purpose:
ketamine	100mg/kg	intraperitoneal	Anesthesia/followed by euthanasia
xylazine	6mg/kg	intraperitoneal	Anesthesia/ followed by euthanasia
Euthasol	100mg/kg	intraperitoneal	euthanasia
amphetamine	1-10 microM	only in vitro for brain slice superfusion	experimental
cocaine	10 microM	only for in vitro brain slice superfusion	experimental
GABA receptor related controlled drugs: benzodiazepines diazepam and midazolam and nonbenzodiazepine sedative zolpidem	3nM to 1 microM	only for in vitro brain slice superfusion	experimental
various transmitter receptor agonists and antagonist, that are not controlled substances	1-10 microM range	only for in vitro brain slice superfusion	experimental

10. If animals are to be **euthanized** at the completion of experiments**, please provide the location and room number(s) where this procedure will be performed, indicate the method to be used and justify your choice**. If the method of euthanasia requires that animals not be anesthetized, explain why. Specify the sources you have consulted to assure that there are no alternatives to this procedure. If animals are not to be euthanized, what is your plan for the animals at the end of the study?

Mice will be euthanized to prepare brain slices in Kolb 432.
For brain slice preparation, we either anesthetize the mice with ketamine and xylazine i.p., or inject Euthasol (100 mg/kg) i.p. and then decapitate, or perfuse transcardially with ice-cold saline and then decapitate.
Transcardial perfusion: Mice are anesthetized with ketamine/xylazine or euthanized with Euthasol (100 mg/kg). Depth of anesthesia is tested by hind limb toe pinch. Once depth of anesthesia has been achieved, the mouse is laid stomach up. The fur is clipped and the skin cleaned with a 70% alcohol swab. The chest is then opened, the right atrium of the heart is punctured, and a 25 gauge needle connected to a line from a pump is

placed into the apex of the heart to pump ice cold physiological saline through the animal at 4ml/min for 5 min. The mouse is then decapitated and the brain removed to prepare brain slices.

In some cases, we use cervical dislocation without anesthesia and then decapitate with sharp scissors. This is for experiments in which tissue dopamine levels should not be altered, as ketamine is known to act as a dopamine releaser (Hancock PJ et al Br J Anesthesia 1999, 82: 603). This is a simple and humane method to euthanize mice. The mouse is held by the tail and placed onto a surface that it can hold onto. The mouse is gently grabbed from the back with thumb and pointing finger moving towards the neck. Once a good grip is achieved the vertebrae are dislocated by quick pressure with the fingers. The head is removed by decapitation and brain slices are prepared with a vibratome. All personnel new to the technique will be trained on anesthetized animals first. All personnel on this protocol have gone through a skill assessment.

All mice transferred from CU BB will be euthanized by cervical dislocation. The materials and carcasses will be disposed of as regulated medical waste and all instruments and surfaces used will be sterilized or sanitized.

11. List all **hazardous agents** (e.g., toxins, carcinogens) that will be used in any procedures, as well as the methods to be used for containment of these agents. If you are taking blood or tissue from nonhuman primates you must include this into the protocol.

    If your project involves the use of any hazardous agents complete the attached form you must obtain approval from the Environmental Health & Safety Office, (EH&S). This form is to be completed triennially or each time you add new hazardous agents and must accompany the protocol at the time of submission.

    The appropriate registration form must be approved by EH&S before submitting your protocol summary application to the IACUC office. If you have any questions or need assistance with the forms, you can the Biological Safety Officer at EH&S at 305-(5)6780. EH&S is located at 601 W. 168th St., Suite 66.
    We have attached the appendix I for controlled substances and Appendix D for use of Lasers.

12. If you plan to use any **radiation or radioisotope**, you must have prior approval from the Radiation Safety Officer.

    Complete the attached form if you're using Radioisotopes in your protocol. If you're using radiation, your must take your protocol to the Office of Radiation Safety for review and approval. The RSO form must be completed, approved and accompany the protocol submission EACH YEAR that you use radioisotopes.

    Please provide the radiation badge numbers for all personnel involved. You can contact Radiation Safety Office at 212-305-0303, send an email for questions regarding the use of radiation or radioisotopes or visit the Radiation Safety Office website for more information.

NA

13. If you plan to use any of the **MRI facilities**, you must have prior approval from the Head of the MRI facility.

Separate forms are to be completed for both the PI and Columbia Institutions. These forms are to be completed, approved and accompany the protocol submission EACH YEAR that you require use of MRI equipment.

And a brief summary in non-technical terms describing your objectives for the experiment needs to be attached to the form as well. The addresses for the MRI facilities and the department heads locations are attached to this application.

NA

14. Provide the following information for **all of your research personnel** who will be involved in your experiment. **Please indicate newly added personnel.**

Name	Email address	AHQ date	OHP date	LATA date **Citi**	Work phone	Emergency phone	Years of experience with animal species	This person is trained on animal procedures described in the protocol
Avery McGuirt	afm2150@cumc.columbia.edu	10.08.2018	07.21.2017	07/11/2017	646-774-5064	704-615-3229	4+	routine care, genotyping, euthanasia
Vanessa Morales	vm2178@columbia.edu	10.08.2018	06/05/2012	07/13/2016	646-774-5064	646-363-3692	8+	routine care, genotyping
Ori Lieberman	ojl2106@columbia.edu	10.08.2018	03/06/2015	07/13/2016	646-774-5064	410-493-4084	4+	routine care, genotyping, euthanasia
Sejoon Choi	sjc2167@columbia.edu	10.08.2018	07/08/2010	07/13/2016	646-774-5064	917-771-3111	7+	routine care, genotyping, euthanasia
Jihang Wang	jw3337@columbia.edu	10.08.2018	03/31/2016	07/13/2016	646-774-5064	646-774-5064	2+	routine care, genotyping, euthanasia
Mahalakshmi Somayaji	ms5335@columbia.edu	10.08.2018	07/13/2016	06/27/2016	646-774-5064	256-653-0248	6+	routine care, genotyping, euthanasia
David Sulzer	ds43@columbia.edu	10.08.2018	01/06/1997	07/13/2016	646-774-5024	917-805-5735	15+	Supervises experiments
Ellen Kanter	ek289@cumc.columbia.edu	10.08.2018	01/31/2001	07/13/2016	646-774-5064	646-641-5324	14+	Contact Person, advises on protocol only
Marian Blanca Ramirez	mbr2159@cumc.columbia.edu	08.13.2018	08.17.2018	08.09.2018	646-774-5064	929-387-7102	0	routine care, genotyping, euthanasia
Maria Concetta Miniaci	mcm2007@cumc.columbia.edu	09.18.2018	09.21.2018	10.02.2018	646-774-5023	929-319-2029	15+	routine care, genotyping, euthanasia
Stefano Cataldi	sc4516@cumc.columbia.edu	10.10.2018	10.10.2018	10.10.2018	646-774-5064	509-861-8649	5+	routine care, genotyping, euthanasia

***OHP date is the date individual was cleared by Work, Health and Safety to begin working with animals.**

After your protocol has been approved, please report any personnel changes to the PI IACUC Office via email by sending a copy of the PI IACUC protocol modification form with the added personnel listed on Page 2 to iacuc@nyspi.columbia.edu. Please provide all the information requested in Item 14 for any new individual.

I have read and will comply with the policies and procedures of the Institutional Animal Care and Use Committee (IACUC) and the Federal Guidelines for Animal Welfare. I am aware of the requirement that I update this application annually or whenever there is a significant change in my procedures. I am also aware that I must notify the IACUC when this research project is terminated. I further certify that these studies do not unnecessarily duplicate previous experiments. I understand that I must have IACUC approval before starting the experiments I have described.

Signature: _____ Date ____10.05.2018 _____
Principal Investigator

Dear Applicant:

In the increasingly complex world of scientific publication, concerns about commercial influence and other possible conflicts make it important for authors to disclose all potential sources of bias. Our system of reviewing conflicts of interest aligns with the policies of the American Academy of Neurology and allows donors to judge whether conflicts exist. Please complete this form, referring to the definitions in the beginning regarding commercial entities, compensation, expert witness, and "immediate family member." At first glance, this task may seem onerous, but will likely take less than 10 minutes.

What to expect: You will be asked whether you have disclosures relating to each question (check yes or no) and will be provided a field in which to list the disclosures. Filling out the forms on the next few screens will be easiest if you have a list of the following items regarding your activity (either commercial or non-profit) and that of any immediate family members during the period of your project. Disclosures are required for any dollar amount, except for gifts valued under $1000. Names of commercial and non-profit entities are required along with specific roles, grant numbers for grants, and specific years. No dollar amounts need to be included. Please indicate complete names of sponsors or companies.

DEFINITIONS

Personal compensation:
Serving on a scientific advisory board
Gifts worth more than $1000
Travel funded by a commercial entity
Serving as a journal editor, associate editor, or on an editorial advisory board
Patents held or pending
Royalties from publishing
Honoraria for speaking engagements
Corporate appointments or consultancies
Speakers' bureaus
Clinical, neurophysiology, or imaging studies in your practice and % effort devoted if the result of this paper will benefit your practice, affiliated unit, or a sponsor

Research support:
Commercial research support
Government research support (including funding organization, grant number, and role)
Academic research support not attributed in the manuscript
Support from a non-profit foundation or society
Stock options for serving on a Board of Directors
License fee payments
Royalty payments from technology or inventions

Stocks, stock options, and royalties
Stock options in a company in which you are (were) an investigator
Stock options in medical industry

Legal proceedings
Expert testimony for a legal proceeding on behalf of industry
Affidavit for a legal proceeding on behalf of industry
Witness or consultant for a legal proceeding on behalf of industry

Optional non-financial
Non-financial disclosures you wish to share

Definitions of Terms in Disclosure Agreement
Commercial entity: A for-profit business that manufactures, distributes, markets, sells, or advertises pharmaceutical or scientific products or medical devices.
Compensation: Anything of monetary value including a salary, honorarium, stipend, gift, or payment of travel-related expenses.
Expert witness: A person who has provided expert medical testimony during a trial or administrative hearing, in a deposition or an affidavit, or in any other type of legal proceeding.
"Immediate family member": Any person who would benefit financially from the publication of the manuscript because of their relationship to the author. This includes a member of an applicant's immediate family or anyone else who has a significant relationship with the applicant.

Please provide all financial relationships (and those of your "immediate family members") from the past two years regardless of whether these relationships are related to the project described in your application.

FINANCIAL DISCLOSURE
Personal Compensation from Commercial and Non-Profit Entities that benefits you directly or indirectly. Within the past two years (and during the course of the study under consideration if the study exceeded two years), I or one of my "immediate family members" received personal compensation for the following:

All compensation received during the past two years regardless of the relationship to your project must be disclosed; for the period exceeding two years, only compensation relevant to the topic of the study needs to be disclosed.

1. Serving on a scientific advisory board or data safety monitoring board. List specific disclosures in the following format: (1) Commercial or non-profit entity (2) Commercial or non-profit entity... If none, please say "None":

None

2. Gifts (other than travel or compensation for consulting or for educational efforts) worth more than USD $1000. List specific disclosures in the following format: (1) Commercial or non-profit entity, brief description of gift, (2) Commercial or non-profit entity, brief description of gift... If none, please say "None":

None

3. Funding for travel or speaker honoraria to the individual from a commercial or non-profit entity not included in the study funding [Exclude CME activities and Grand Rounds]. List specific disclosures in the following format: (1) Commercial or non-profit entity, type of payment, (2) Commercial or non-profit entity, type of payment... If none, please say “None”:

None

4. Serving as a journal editor, an associate editor, or editorial advisory board member. This may include a journal published by your national medical/scientific organization. Please include regardless of whether you receive compensation. List specific disclosures in the following format: (1) Full journal name, role, year(s), (2) Full journal name... If none, please say “None”:

None

5. Patents issued or pending. List specific disclosures in the following format: (1) Brief description of invention/technology, (2) Brief description of invention/technology... If none, please say “None”:

None

6. Publishing Royalties (do not include honoraria for occasional writing). List specific disclosures in the following format: (1) Full title of work, full name of publisher, year(s) of publication (or receipt of royalties), (2) Full title of work... If none, please say “None”:

None

7. Employment. If you are currently employed by a commercial entity, please disclose below. In addition, if your past employment at a commercial entity is directly related to this manuscript, please disclose below. List specific disclosures in the following format: (1) Commercial entity, position, years (2) Commercial entity, position, years... If none, please say “None”:

None

8. Consultancies. List specific disclosures in the following format: (1) Commercial or non-profit entity, (2) Commercial or non-profit entity... If none, please say “None”:

None

9. Speakers' bureau. List specific disclosures in the following format: (1) Commercial or non-profit entity, (2) Commercial or non-profit entity... If none, please say “None”:

None

10. Other activities not covered in designations above (if in doubt, provide full disclosure). List specific disclosures in the following format: (1) Commercial or non-profit entity, brief description of activity, (2) Commercial or non-profit entity... If none, please say “None”:

None

11. Some studies have potential for financial gain for the project investigators or the sponsor. The following question seeks to provide transparency regarding any financial benefits to investigators or sponsors.

Do you perform clinical procedures or imaging studies in your practice or unit that overlap with the content of your proposed project, practice parameter, or clinical practice guideline and would your sponsor or this part of your practice or unit benefit if the conclusions were widely followed?
Note: This is the only item in this Agreement that applies to an interest that is related specifically to this particular study, practice parameter, or clinical practice guideline.

List specific disclosures in the following format: (1) Name of Practice or Research Unit, Clinical procedure/imaging study, % of effort (e.g. 35%), year(s), (2) Name of Practice or Research Unit, Clinical procedure/imaging study, % of effort (e.g., 35%)... If none, please say “None”:

None

RESEARCH SUPPORT
Within the past two years and during the course of the study under consideration if the study exceeded two years, I or one of my "immediate family members" received financial or material research support or compensation from the following:

All support received during the past two years regardless of the relationship to the study must be disclosed; for the period exceeding two years, only support relevant to the topic of the study needs to be disclosed.

12. Commercial entities. List specific disclosures in the following format: (1) Commercial entity, (2) Commercial entity... If none, please say “None”:

None

13. Government entities. List specific disclosures in the following format: (1) Sponsor/funding source, grant number(s), role, year(s), (2) Sponsor/funding source... If none, please say “None”:

(1) NINDS, R01 NS075222, PI, 07/01/11-06/30/18

14. Academic entities other than those attributed in the manuscript. List specific disclosures in the following format: (1) Academic entity, (2) Academic entity... If none, please say “None”:

None

15. Foundations or societies (include grant number if required by funding agency). List specific disclosures in the following format: (1) Full name of Foundation or Society, (2) Full name of Foundation or Society... If none, please say “None”:

(1) The JPB Foundation, Principle Investigator, 10/15/11-09/30/21

STOCK, STOCK OPTIONS & ROYALTIES

In the past two years and during the course of the study under consideration if the study exceeded two years, I or one of my "immediate family members":

All revenues during the past two years regardless of the relationship to the study must be disclosed; for the period exceeding two years, only revenues relevant to the topic of the study needs to be disclosed.

16. Stock or stock options or expense compensation for serving on a board of directors. List disclosures in the following format: (1) Commercial entity, (2) Commercial entity... If none, please say “None”:

None

17. License fee payments. List specific disclosures in the following format: (1) Invention/technology, source of payment, (2) Invention/technology... If none, please say “None”:

None

18. Royalty payments or have contractual rights for receipt of future royalty payments from technology or inventions (this does not include royalties from publishing). List specific disclosures in the following format: (1) Technology/invention, source of payment, year(s), (2) Technology/invention... If none, please say “None”:

None

19. Stock or stock options in a commercial entity sponsoring research with which the author or "immediate family member" was involved as an investigator (Excludes investments in mutual funds held by the author or dependents). List specific disclosures in the following format: (1) Company, year(s), (2) Company, year... If none, please say “None”:

None

20. Stock or stock options in a commercial entity whose medical equipment or other materials related to the practice of medicine. (Exclude investments in mutual funds held by the author or dependents). List specific disclosures in the following format: (1) Company, year(s), (2) Company, year... If none, please say “None”:

None

LEGAL PROCEEDINGS

In the past two years and during the course of the study under consideration if the study exceeded two years, I or one of my "immediate family members" have (whether or not it pertains to the topic of the current study):

All compensation received during the past two years regardless of the relationship to the study must be disclosed; for the period exceeding two years, only compensation relevant to the topic of the study needs to be disclosed.

21. Given expert testimony, acted as a witness or consultant, or prepared an affidavit for any legal proceeding involving a commercial entity (do not include proceedings for individual patients). You may specify role, e.g., 'expert witness for plaintiff' if desired. (Include year only if activity is directly related to the present study.)

List specific disclosures in the following format: (1) Commercial entity, activity, year(s), (2) Commercial entity, activity, year(s)... If none, please say “None”:

None

OPTIONAL: NONFINANCIAL DISCLOSURE

22. I have chosen to declare one or more non-financial competing interests (e.g., special interest groups you represent or others that may be affected if your paper is published or that could be perceived as biasing the study; the corresponding author should be aware of conflicts of interest that Co-investigators or Contributors may have). Non-financial disclosures will not be published.

List specific disclosures, if none, please say “None”:

None

☑ I have completed this Disclosure Statement fully and to the best of my ability. I understand that all Applicants must complete this Disclosure Statement and that the information disclosed may be published if their project is accepted for crowdfunding.

By my electronic signature, I verify the completeness and accuracy of the contents of this form.

Click in the box above to add your electronic signature

10/18/2018

Date [MM/DD/YYYY]

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Mosharov, Eugene V.

eRA COMMONS USER NAME (credential, e.g., agency login): EM706X

POSITION TITLE: Research Scientist

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Moscow State University, Russia	M.S.	05/1994	Chemistry
National Hematology Research Center, Moscow, Russia	Ph.D.	06/1998	Biology
University of Nebraska, Dept of Biochemistry	Postdoctoral	04/2000	Biochemistry
Columbia University Medical Center, Dept of Neurology	Postdoctoral	04/2003	Neuroscience

A. Personal Statement

During my career, I have worked on various metabolic systems, including energy metabolism, redox homeostasis, autophagic protein degradation and neurotransmitter synthesis and release machinery. Over the last 16 years my research was focused on molecular mechanisms of dopaminergic transmission and neurotoxicity in genetic and neurotoxin-induced models of Parkinson's disease (PD). In this proposal, we will investigate the difference in the regulation of Ca2+ currents between SN and VTA dopaminergic neurons under normal and stress conditions using patch-clamp electrophysiology and fluorescence microscopy. The results will provide unique information about the interaction between two well-known PD risk factors and will help to better design strategies aimed to rescue susceptible neuronal populations in PD and other neurodegenerative and psychiatric disorders. The current application builds logically on my prior work and I have a record of successful and productive research projects in areas of synaptic transmission, dopamine biochemistry and neurodegeneration. As a result of my previous experiences, I am aware of the importance of frequent communication among project members and of constructing a realistic research plan, timeline, and budget. In summary, I have the expertise, leadership, expertise and motivation necessary to successfully carry out the proposed research project.

B. Positions and Honors

Positions and Employment

2003-2012 Associate Research Scientist, Dpt of Neurology, Columbia University Medical Center, NY.
2012-2016 Research Scientist, Dpt. Neurology, Columbia University Medical Center, NY.
2016- Research Scientist, NYSPI, Columbia University, NY.

Other Experience and Professional Memberships

1995- Member, Russian Biochemical Society
2000- Member, Society for Neuroscience
2011 NINDS, Cellular and Molecular Neuroscience, ad hoc reviewer
2011-2012 Italian Ministry of Health, ad hoc reviewer
2011- Invited lecturer, UMDNJ/SOM, Department of Cell Biology, NJ.
2014 Dynasty Foundation, ad hoc reviewer

2011-2018 Parkinson's Disease Foundation, ad hoc reviewer

C. Contribution to Science

1. **Regulation of energy metabolism and homocystein homeostasis.** For my PH.D thesis I used a combination of biochemical and mathematical approaches to study the regulation of energy metabolism in human erythrocytes and the role of AMP synthesis and degradation on the stabilization of cellular volume. In 1998, I joined the laboratory of Prof. Ruma Banerjee in the Department of Biochemistry at the University of Nebraska, Lincoln as a Postdoctoral Fellow where I studied the metabolism of homocysteine, an amino acid, high serum levels of which are linked to an increased incidence of cardiovascular disease, neurodegenerative disorders and certain cancers. In particular, I studied the involvement of homocystein homeostasis in the modulation of the cellular concentrations of S-adenosylmethionine, the major cellular methyl donor for methylation reactions, and glutathione, the cell's most abundant antioxidant.
   a. Mosharov EV, Vitvitsky VM, and Ataullakhanov FI. Product activation of human erythrocytes AMP deaminase. *FEBS Letters* (1998) 27, 440(1-2):64-66.
   b. Martinov MV, Vitvitsky VM, Mosharov EV, Banerjee R, and Ataullakhanov FI. A substrate switch: A new mode of regulation in the methionine metabolic pathway. *Journal of Theoretical Biology* (2000) 204(4):521-532.
   c. Mosharov EV, Cranford MR, Banerjee R. The quantitatively important relationship between homocysteine metabolism and glutathione synthesis by the transsulfuration pathway and its regulation by redox changes. *Biochemistry* (2000) 39(42):13005-13011.
   d. Vitvitsky VM, Mosharov E, Tritt M, Ataullakhanov FI, Banerjee R, Redox regulation of homocysteine-dependent glutathione synthesis. *Redox Reports* (2003) 8(1):57-63.

2. **Interaction between dopamine, calcium and alpha-synuclein.** During my postdoctoral training in David Sulzer laboratory, I worked on various aspects of dopamine neurons homeostatic regulation under physiologically normal and pathological conditions. It has been hypothesized that the buildup of cytosolic dopamine and its interaction with α-synuclein and other PD-related proteins underlie neurotoxicity in dopaminergic neurons affected in Parkinson's disease, which raises a possibility that L-DOPA therapy may accelerate the progression of the disease. To address these questions we developed the only currently available technique to measure cytosolic dopamine, intracellular patch electrochemistry. Using various cellular models I directly studied the effect of various pharmacological and genetic factors on neurotoxicity, mitochondrial oxidation, Ca2+ homeostasis, CMA activity and intracellular dopamine turnover, with the focus on assessing the specificity of these effects for neurons from the substantia nigra (SN), the area most affected in PD. My work generated a hypothesis that selective death of SN neurons results from a combination of "multiple hits", including the presence of pacemaking activity of the L-type Ca2+ channels, an upregulation of dopamine synthesis by Ca2+, the presence of α-synuclein, and the blockade of chaperone-mediated autophagy (CMA).This work has changed our understanding of how Parkinson's disease develops and provided novel avenues for the treatment of the disease.
   a. Mosharov EV, Staal RGW, Bové J, Prou D, Hananiya A**,** Markov D, Poulsen N, Larsen KE, Moore CMH, Troyer MD**,** Edwards RH, Przedborski S, Sulzer D. α-Synuclein overexpression increases cytosolic catecholamine concentration. *Journal of Neuroscience* (2006) 6;26(36):9304-9311.
   b. Mosharov EV, Larsen KE., Kanter E, Phillips KA, Wilson K, Schmitz Y, Krantz DE, Kobayashi K, Edwards RH, Sulzer D. Interplay Between Cytosolic Dopamine, Calcium and alpha-Synuclein Causes Selective Death of Substantia Nigra Neurons. *Neuron* (2009), 62(2):218-229
   c. Choi SJ, Panhelainen A, Schmitz Y, Larsen KE, Kanter E, Wu M, Sulzer D, Mosharov EV. Changes in Neuronal Dopamine Homeostasis Following 1-Methyl-4-Phenylpyridinium (MPP+) Exposure. *J Biol Chem.* (2015) Mar 13;290(11):6799-809.
   d. Lieberman OJ, Choi SJ, Kanter E, Saverchenko A, Frier MD, Fiore GM, Wu M, Kondapalli J, Zampese E, Surmeier DJ, Sulzer D, Mosharov EV. α-Synuclein-Dependent Calcium Entry Underlies Differential Sensitivity of Cultured SN and VTA Dopaminergic Neurons to a Parkinsonian Neurotoxin. eNeuro. 2017 Nov 21;4(6). doi: 10.1523/ENEURO.0167-17.2017.

3. **Mechanism of exocytotic release of dopamine.** The most important aspect of a normal function of a neurons is its ability to release neurotransmitter in response to stimulation. Mechanisms that govern this neuronal property include regulation of cytosolic transmitter levels by synthesis and degradation, vesicular neurotransmitter uptake, and Ca^{2+}-dependent vesicle fusion with the plasma membrane. I studied different

characteristics of dopamine neurotransmission in various cells, including adrenal chromaffin cells, PC12 cells, primary cultures of midbrain dopaminergic neurons, and striatal brain slices. One of my major contributions to the field was the development of algorithms for automated analysis of amperometrically recorded exocytotic dopamine release events, including the freely available data analysis program widely used by the laboratories studying catecholamine release.

a. Staal RGW, Mosharov EV, Sulzer D. Dopamine neurons release transmitter via a flickering fusion pore. *Nature Neuroscience* (2003) 7(4):341-346.
b. Mosharov EV, Analysis of single-vesicle exocytotic events recorded by amperometry. (2008) *In* Exocytosis and Endocytosis (*Methods in Molecular Biology*), Ed. A.I. Ivanov. 440:315-327.
c. Kempadoo KA, Mosharov EV, Choi SJ, Sulzer D, Kandel ER. Dopamine release from the locus coeruleus to the dorsal hippocampus promotes spatial learning and memory. Proc Natl Acad Sci U S A. 2016 Dec 20;113(51):14835-14840. PMID: 27930324;
d. Aguilar JI, Dunn M, Mingote S, Karam CS, Farino ZJ, Sonders MS, Choi SJ, Grygoruk A, Zhang Y, Cela C, Choi BJ, Flores J, Freyberg RJ, McCabe BD, Mosharov EV, Krantz DE, Javitch JA, Sulzer D, Sames D, Rayport S, Freyberg Z. Neuronal Depolarization Drives Increased Dopamine Synaptic Vesicle Loading via VGLUT. Neuron. 2017 Aug 30;95(5):1074-1088. PMID: 28823729.

4. **Fluorescent False Neurotransmitters (FFNs)**. Monoamine neurons (dopamine, norepinephrine, and serotonin) project their axons throughout the brain and regulate diverse brain functions including arousal, stress, emotion, reward, learning, and cognition, whereas aberrant monoamine neurotransmission have been implicated in numerous neurological and neuropsychiatric disorders including Parkinson's disease, schizophrenia, ADHD, drug addiction, depression, and anxiety. Due to methodological limitations, monoamine neurotransmission have only been studied on the bulk level of large ensembles of monoaminergic synapses. When working in David Sulzer lab and later in my own lab, I was involved in the development of novel optical tools to examine the monoamine release characteristics at individual presynaptic boutons (in collaboration with Dalibor Sames). As a result of this work, "Fluorescent False Neurotransmitters" (FFNs) were designed as tracers of dopamine, enabling for the first time to visualize synaptic vesicles release at individual presynaptic terminals in several brain areas (striatum, somatosensory cortex, hippocampus). These new probes and associated imaging methods unlocked the possibility of addressing many long-standing questions about release properties of single synapses and their physiological regulation and malfunction in various disease models.
   a. Gubernator NG, Zhang H, Staal RG, Mosharov EV, Pereira DB, Yue M, Balsanek V, Vadola PA, Mukherjee B, Edwards RH, Sulzer D, Sames D. Fluorescent false neurotransmitters visualize dopamine release from individual presynaptic terminals. *Science* (2009) Jun 12;324(5933):1441-4.
   b. Wong MY, Borgkvist A, Choi SJ, Mosharov EV, Bamford NS, Sulzer D. Dopamine-dependent corticostriatal synaptic filtering regulates sensorimotor behavior. *Neuroscience* (2015) Apr 2;290:594-607.
   c. Pereira DB, Schmitz Y, Mészáros J, Merchant P, Hu G, Li S, Henke A, Lizardi-Ortiz JE, Karpowicz RJ Jr, Morgenstern TJ, Sonders MS, Kanter E, Rodriguez PC, Mosharov EV, Sames D, Sulzer D. Fluorescent false neurotransmitter reveals functionally silent dopamine vesicle clusters in the striatum. *Nat Neurosci*. (2016)

5. **Human dopamine neurons.** Technical advances in differentiating induced pluripotent stem cells (iPSCs) provide access to human dopamine neurons derived from fibroblasts of healthy volunteers and PD patients thereby allowing to compare their function in vitro. We have recently initiated a series of collaborative projects with Lorenz Studer at Sloan Kettering University, NY and New York Stem Cells Foundation, NY to 1) characterize biochemical and electrophysiological properties of iPSC-derived DA neurons from control and monogenetic PD patients, and 2) to investigate the therapeutic potency and the mechanism of action of DA neurons grafted in a parkinsonian brain.
   a. Woodard CM, Campos BA, Kuo S-H, Nirenberg MJ, Nestor MW, Zimmer M, Mosharov EV, Sulzer D, Zhou H, Paull D, Clark L, Schadt EE, Sardi SP, Rubin L, Eggan K, Brock M, Lipnick S, Rao M, Chang S, Li A, Noggle SA. iPSC-Derived Dopamine Neurons Reveal Differences between Monozygotic Twins Discordant for Parkinson's Disease. *Cell Report* (2014) 9, 1173–1182.
   b. Steinbeck JA, Choi SJ, Mrejeru A, Ganat Y, Deisseroth K, Sulzer D, Mosharov EV*, Studer L* (*-corresponding authors, equal contribution). Optogenetic dissection of human ESC graft function in a preclinical model of Parkinson's disease. *Nat Biotechnol.* (2015) Feb;33(2):204-9.

c. Chung SY, Kishinevsky S, Mazzulli JR, Graziotto J, Mrejeru A, Mosharov EV, Puspita L, Valiulahi P, Sulzer D, Milner TA, Taldone T, Krainc D, Studer L, Shim JW. Parkin and PINK1 Patient iPSC-Derived Midbrain Dopamine Neurons Exhibit Mitochondrial Dysfunction and α-Synuclein Accumulation. Stem Cell Reports. 2016 Oct 11;7(4):664-677. PubMed PMID: 27641647

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/1n5HTZjL5e95w/bibliography/42104410/public/?sort=date&direction=descending

D. Additional Information: Research Support and/or Scholastic Performance

Current:

The JPB Foundation Sulzer (PI) 10/15/11-09/30/21
Mechanisms of Parkinson's Disease Pathogenesis
This study is aimed at investigation of the effects of deregulated cytosolic dopamine homeostasis and abnormal chaperone-mediated autophagy in the progression of PD
Role: Co-Investigator

Completed

R01 NS075222 Mosharov (PI) 07/01/11-06/30/18
National Institute of Neuronal Disorders and Stroke
Interaction between DA, alpha-Synuclein and Chaperone-Mediated Autophagy
This study is aimed at the examination of the synergistic effects of different cellular stress factors and their role in neurodegeneration of dopaminergic neurons in PD.
Role: PI

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: Choi, Se Joon

eRA COMMONS USER NAME (credential, e.g., agency login): CHOISJ

POSITION TITLE: Associate research scientist

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Kyunghee University, Seoul	BS	02/2002	Genetic engineering
Catholic University of Korea, Seoul	MS	02/2004	Pharmacology
Catholic University of Korea, Seoul	PHD	02/2007	Neurobiology
University of Manchester, Manchester	Postdoctoral Fellow	10/2009	Neuroscience
Southern Illinois University, Sprinfield, IL	Postdoctoral Fellow	05/2010	Neuroscience
Columbia University, New York, NY	Postdoctoral Fellow	08/2013	Neuroscience

A. Personal Statement

During the last >10 years I have been working on various studies of dysfunctions in the basal ganglia, including the effect of drugs of abuse and PD mutations on the electrophysiological and electrochemical properties of various neuronal populations. Throughout my career, I have demonstrated a record of productive research and acquired training and expertise in key research techniques required for the ongoing project. This study will investigate the mechanisms of vulnerability of SN DA neuron to toxin-induced stress. Specifically, we will study the interaction between L-type calcium channels and alpha-synuclein using patch clamp recordings from SN and VTA neurons in cultures and acute brain slices. These results will help to better design strategies aimed to rescue susceptible neuronal populations in PD and other neurodegenerative and psychiatric disorders.

1. Choi SJ, Panhelainen A, Schmitz Y, Larsen KE, Kanter E, Wu M, Sulzer D, Mosharov EV. Changes in neuronal dopamine homeostasis following 1-methyl-4-phenylpyridinium (MPP+) exposure. J Biol Chem. 2015 Mar 13;290(11):6799-809. PMID: 25596531.
2. Steinbeck JA, Choi SJ, Mrejeru A, Ganat Y, Deisseroth K, Sulzer D, Mosharov EV, Studer L. Optogenetics enables functional analysis of human embryonic stem cell-derived grafts in a Parkinson's disease model. Nat Biotechnol. 2015 Feb;33(2):204-9. PMID: 25580598.
3. Baxter AW, Choi SJ, Sim JA, North RA. Role of P2X4 receptors in synaptic strengthening in mouse CA1 hippocampal neurons. Eur J Neurosci. 2011 Jul;34(2):213-20. PMID: 21749490.
4. Choi SJ, Kim KJ, Cho HS, Kim SY, Cho YJ, Hahn SJ, Sung KW. Acute inhibition of corticostriatal synaptic transmission in the rat dorsal striatum by ethanol. Alcohol. 2006 Oct;40(2):95-101. PMID: 17307645.

B. Positions and Honors

Positions and Employment

2013 - Associate research scientist, Columbia University, New York, NY

Other Experience and Professional Memberships

2002 - Member, Society for Neuroscience

Honors

C. Contribution to Science

1. During my PhD training at the Catholic University of Korea, I addressed the mechanism of ethanol intoxication on glutamatergic synaptic transmission in the dorsal striatum. Using whole cell patch clamp

recording from medium spiny neurons in the striatal slices, I was able to demonstrate that acute ethanol directly inhibits glutamatergic components that may induce a change of locomotion.

a. Choi SJ, Kim KJ, Cho HS, Kim SY, Cho YJ, Hahn SJ, Sung KW. Acute inhibition of corticostriatal synaptic transmission in the rat dorsal striatum by ethanol. Alcohol. 2006 Oct;40(2):95-101. PMID: 17307645.
b. Cho HS, Jeun SH, Li QZ, Kim KJ, Choi SJ, Sung KW. Involvement of the endocannabinoid system in ethanol-induced corticostriatal synaptic depression. J Pharmacol Sci. 2012;120(1):45-9. PMID: 22971846.

2. As a member of the laboratory of Dr. Ki-Wug Sung at the Catholic University of Korea, I used whole cell patch clamp recordings on NCB20 primary cell culture to study pharmacological modulation of 5-HT3 receptors-induced currents.
   a. Yang HS, Kim SY, Choi SJ, Kim KJ, Kim ON, Lee SB, Sung KW. Effect of 5-hydroxyindole on ethanol potentiation of 5-hydroxytryptamine (5-HT)3 receptor-activated ion current in NCB-20 neuroblastoma cells. Neurosci Lett. 2003 Feb 20;338(1):72-6. PMID: 12565143.
   b. Kim KJ, Cho HS, Choi SJ, Jeun SH, Kim SY, Sung KW. Direct effects of riluzole on 5-hydroxytryptamine (5-HT)3 receptor-activated ion currents in NCB-20 neuroblastoma cells. J Pharmacol Sci. 2008 May;107(1):57-65. PMID: 18460823.
   c. Cho HS, Lee HH, Choi SJ, Kim KJ, Jeun SH, Li QZ, Sung KW. Forskolin Enhances Synaptic Transmission in Rat Dorsal Striatum through NMDA Receptors and PKA in Different Phases. Korean J Physiol Pharmacol. 2008 Dec;12(6):293-7. PMID: 19967070.
   d. Li QZ, Cho HS, Jeun SH, Kim KJ, Choi SJ, Sung KW. Effects of grape seed proanthocyanidin on 5-hydroxytryptamine(3) receptors in NCB-20 neuroblastoma cells. Biol Pharm Bull. 2011;34(7):1109-15. PMID: 21720021.

3. As a postdoctoral fellow in the laboratory of Dr. R. Alan North, I studied the role of purinergic receptors in the hippocampal synaptic transmission and plasticity. Using P2X4 knockout mice, I documented that P2X4 receptors strenthen synaptic transmission and plasticity through upregulation of NMDA NR2B subunits in the hippocampal pyramidal neurons. Apart of P2X4 function, I collaborated with Dr. Shin Hee Yoon at the Catholic University of Korea to address antidepressant effects on hippocampal neurons. This work suggested that fluoxetine suppresses excitotoxicity through the inhibition of AMPA receptors.
   a. Baxter AW, Choi SJ, Sim JA, North RA. Role of P2X4 receptors in synaptic strengthening in mouse CA1 hippocampal neurons. Eur J Neurosci. 2011 Jul;34(2):213-20. PMID: 21749490.
   b. Kim HJ, Kim TH, Choi SJ, Hong YJ, Yang JS, Sung KW, Rhie DJ, Hahn SJ, Yoon SH. Fluoxetine suppresses synaptically induced $[Ca^{2+}]i$ spikes and excitotoxicity in cultured rat hippocampal neurons. Brain Res. 2013 Jan 15;1490:23-34. PMID: 23131584.

4. Since I joined Dr. Mosharov's lab as an Associate Research Scientist, I studied various aspects of brain neurotransmission, including post- and presynaptic modulation of MSN neurotransmission by forskolin and dopamine, changes in MSN connectivity in 6-OHDA lesioned striatum following optogenetic excitation of grafted DA neurons, and co-release of dopamine and norepinephrine from the locus coeruleus to the dorsal hippocampus.
   a. Wong MY, Borgkvist A, Choi SJ, Mosharov EV, Bamford NS, Sulzer D. Dopamine-dependent corticostriatal synaptic filtering regulates sensorimotor behavior. Neuroscience. 2015 Apr 2;290:594-607. PMID: 25637802.
   b. Steinbeck JA, Choi SJ, Mrejeru A, Ganat Y, Deisseroth K, Sulzer D, Mosharov EV, Studer L. Optogenetics enables functional analysis of human embryonic stem cell-derived grafts in a Parkinson's disease model. Nat Biotechnol. 2015 Feb;33(2):204-9. PMID: 25580598.
   c. Kempadoo KA, Mosharov EV, Choi SJ, Sulzer D, Kandel ER. Dopamine release from the locus coeruleus to the dorsal hippocampus promotes spatial learning and memory. Proc Natl Acad Sci U S A. 2016 Dec 20;113(51):14835-14840. PMID: 27930324.

5. Another project in Dr. Mosharov's laboratory involved an investigation of the selective vulnerability of SN DA neurons to stress which laid ground for the current proposal. We found that cytosolic DA and intracellular Ca^{2+} level of SN DA neurons were greater than those in neurons from the VTA and that this leads to mitochondria oxidation and neurotoxicity. We will now investigate the mechanisms by which parkinsonian neurotoxin MPP+ and alpha-synuclein regulate plasma membrane Ca^{2+} currents.

a. Choi SJ, Panhelainen A, Schmitz Y, Larsen KE, Kanter E, Wu M, Sulzer D, Mosharov EV. Changes in neuronal dopamine homeostasis following 1-methyl-4-phenylpyridinium (MPP+) exposure. J Biol Chem. 2015 Mar 13;290(11):6799-809. PMID: 25596531.
b. Lieberman OJ, Choi SJ, Kanter E, Saverchenko A, Frier MD, Fiore GM, Wu M, Kondapalli J, Zampese E, Surmeier DJ, Sulzer D, Mosharov EV. α-Synuclein-Dependent Calcium Entry Underlies Differential Sensitivity of Cultured SN and VTA Dopaminergic Neurons to a Parkinsonian Neurotoxin. eNeuro. 2017 Nov 21;4(6). doi: 10.1523/ENEURO.0167-17.2017.

Complete List of Published Work in MyBibliography:

https://www.ncbi.nlm.nih.gov/myncbi/browse/collection/47473910/?sort=date&direction=ascending

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

R01 NS101982 Kang (PI) 04/01/17-03/31/21
NIH/NINDS
Striatal cholinergic interneurons in Parkinson's disease and treatment
This study examines the biochemical and physiological properties of striatal cholinergic interneurons (ChI) in mouse models of L-DOPA-induced dyskinesia.
Role: KP

Completed Research Support

R01 NS075222-01 Mosharov (PI) 09/30/11-07/31/18
National Institute of Neuronal Disorders and Stroke
Interaction between DA, alpha-Synuclein and Chaperone-Mediated Autophagy
This study is aimed at the examination of the synergistic effects of different cellular stress factors and their role in neurodegeneration of dopaminergic neurons in PD.
Role: KP

PDF-CEI-1362 Choi (PI) 07/01/13-06/30/14
Parkinson's disease foundation
Amperometric measurment of quantal dopamine release from primary dopamine neurons#
Quantitate measurement of dopamine release from mice dopamine neurons and human dopamine neurons derived from human iPS cells.
Role: PI

Program Director/Principal Investigator *(Last, First, Middle)* : Mosharov, Eugene Eugene Mosharov

DETAILED BUDGET FOR INITIAL BUDGET PERIOD
DIRECT COSTS ONLY

FROM: September 1, 2018 THROUGH: August 31, 2019

List PERSONNEL *(Applicant organization only)*

Use Cal, Acad, or Summer to Enter Months Devoted to Project

Enter Dollar Amounts Requested (omit cents) for Salary Requested and Fringe Benefits

NAME	ROLE ON PROJECT	SALAR	Cal. Mnths	Acad. Mnths	Sum. Mnths	INST. BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
Eugene Mosharov	PI	#####	6.00			86,642	43,321	15,162	58,483
Sejoon Choi	research scientist	#####	6.00			61,557	30,779	10,772	41,551
SUBTOTALS →							**74,100**	**25,934**	**100,034**

CONSULTANT COSTS		
EQUIPMENT *(Itemize)*		
SUPPLIES *(Itemize by category)*		
TRAVEL		
INPATIENT PATIENT CARE COSTS		
OUTPATIENT PATIENT CARE COSTS		
ALTERATIONS AND RENOVATIONS *(Itemize by category)*		
OTHER EXPENSES *(Itemize by category)*		
Animal Housing	20,000	
		20,000
CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS	
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD (Item 7a, Face Page)		**$120,034**
	FACILITIES AND ADMINISTRATIVE COSTS	
TOTAL COSTS FOR INITIAL BUDGET PERIOD		**$120,034**

YEAR 2

*** FOR REFERENCE USE ONLY ***

Program Director/Principal Investigator *(Last, First, Middle)* :

DETAILED BUDGET FOR SECOND BUDGET PERIOD
DIRECT COSTS ONLY

FROM: September 1, 2019 THROUGH: August 31, 2020

PERSONNEL *(Applicant organization only)*

Use Cal, Acad, or Summer to Enter Months Devoted to Project

Enter Dollar Amounts Requested (omit cents) for Salary Requested and Fringe Benefits

NAME	ROLE ON PROJECT	Cal. Mnths	Acad. Mnths	Sum. Mnths	INST. BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
SUBTOTALS →								

CONSULTANT COSTS

EQUIPMENT *(Itemize)*

SUPPLIES *(Itemize by category)*

TRAVEL

INPATIENT PATIENT CARE COSTS

OUTPATIENT PATIENT CARE COSTS

ALTERATIONS AND RENOVATIONS *(Itemize by category)*

OTHER EXPENSES *(Itemize by category)*

CONSORTIUM/CONTRACTUAL COSTS

DIRECT COSTS

SUBTOTAL DIRECT COSTS FOR SECOND BUDGET PERIOD

FACILITIES AND ADMINISTRATIVE COSTS

TOTAL COSTS FOR SECOND BUDGET PERIOD

YEAR 3

*** FOR REFERENCE USE ONLY ***

Program Director/Principal Investigator *(Last, First, Middle)*:

DETAILED BUDGET FOR THIRD BUDGET PERIOD DIRECT COSTS ONLY	FROM	THROUGH

PERSONNEL *(Applicant organization only)*

Use Cal, Acad, or Summer to Enter Months Devoted to Project

Enter Dollar Amounts Requested (omit cents) for Salary Requested and Fringe Benefits

NAME	ROLE ON PROJECT	Cal. Mnths	Acad. Mnths	Sum. Mnths	INST. BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
SUBTOTALS →								

CONSULTANT COSTS	
EQUIPMENT *(Itemize)*	
SUPPLIES *(Itemize by category)*	

YEAR 4

*** FOR REFERENCE USE ONLY ***

Program Director/Principal Investigator *(Last, First, Middle)*:

DETAILED BUDGET FOR FOURTH BUDGET PERIOD DIRECT COSTS ONLY	FROM	THROUGH

PERSONNEL *(Applicant organization only)*

Use Cal, Acad, or Summer to Enter Months Devoted to Project

Enter Dollar Amounts Requested (omit cents) for Salary Requested and Fringe Benefits

NAME	ROLE ON PROJECT	Cal. Mnths	Acad. Mnths	Sum. Mnths	INST. BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
SUBTOTALS →								

CONSULTANT COSTS

EQUIPMENT *(Itemize)*

SUPPLIES *(Itemize by category)*

YEAR 5

*** FOR REFERENCE USE ONLY ***

Program Director/Principal Investigator *(Last, First, Middle)*:

DETAILED BUDGET FOR FIFTH BUDGET PERIOD DIRECT COSTS ONLY	FROM	THROUGH

PERSONNEL *(Applicant organization only)*

Use Cal, Acad, or Summer to Enter Months Devoted to Project

Enter Dollar Amounts Requested (omit cents) for Salary Requested and Fringe Benefits

NAME	ROLE ON PROJECT	Cal. Mnths	Acad. Mnths	Sum. Mnths	INST. BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
SUBTOTALS →								

CONSULTANT COSTS

EQUIPMENT *(Itemize)*

SUPPLIES *(Itemize by category)*

Program Director/Principal Investigator *(Last, first, middle)*: Eugene Mosharov

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS	INITIAL BUDGET PERIOD *(from Form Page 4)*	2nd ADDITIONAL YEAR OF SUPPORT REQUESTED	3rd ADDITIONAL YEAR OF SUPPORT REQUESTED	4th ADDITIONAL YEAR OF SUPPORT REQUESTED	5th ADDITIONAL YEAR OF SUPPORT REQUESTED
PERSONNEL: *Salary and fringe benefits. Application organization only.*	100,034				
CONSULTANT COSTS					
EQUIPMENT					
SUPPLIES					
TRAVEL					
INPATIENT CARE COSTS					
OUTPATIENT CARE COSTS					
ALTERATIONS AND RENOVATIONS					
OTHER EXPENSES	20,000				
DIRECT CONSORTIUM/ CONTRACTUAL COSTS					
SUBTOTAL DIRECT COSTS (Sum = Item 8a, Face Page)	120,034				
F&A CONSORTIUM/ CONTRACTUAL COSTS					
TOTAL DIRECT COSTS	120,034				
TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD					**$120,034**

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

TOTAL COST FIGURES FOR FACE PAGE

7. COSTS REQUESTED FOR INITIAL BUDGET PERIOD		8. COSTS REQUESTED FOR ENTIRE PROPOSED PROJECT PERIOD	
7a. Direct Costs ($)	7b. Total Costs ($	8a. Direct Costs ($)	8b. Total Costs ($)
120,034	194,455	120,034	194,455

• Program Director/Principal Investigator *(Last, First, Middle)*: Eugene Mosharov

CHECKLIST

TYPE OF APPLICATION *(Check all that apply.)*

☐ NEW application. *(This application is being submitted to the PHS for the first time.)*

☐ RESUBMISSION of application number: _____
(This application replaces a prior unfunded version of a new, renewal, or revision application.)

☐ RENEWAL of grant number: _____
(This application is to extend a funded grant beyond its current project period.)

☐ REVISION to grant number: _____
(This application is for additional funds to supplement a currently funded grant.)

☐ CHANGE of program director/principal investigator.

Name of former program director/principal investigator: _____

☐ CHANGE of Grantee Institution. Name of former institution: _____

☐ FOREIGN application. ☐ Domestic Grant with foreign involvement List Country(ies) Involved

INVENTIONS AND PATENTS (Renewal appl. Only) ☐ No ☐ Yes

If "Yes", ☐ Previously reported ☐ Not previously reported

1. PROGRAM INCOME *(See instructions.)*

All applications must indicate whether program income is anticipated during the period(s) for which grant support is request. If program income is anticipated, use the format below to reflect the amount and source(s).

Budget Period	Anticipated Amount	Source(s)

2. ASSURANCES/CERTIFICATIONS (See instructions.)

In signing the application Face Page, the authorized organizational representative agrees to comply with the policies, assurances and/or certifications listed in the application instructions when applicable. Descriptions of individual assurances/certifications are provided in Part III and listed in Part I, 4.1 under Item 14. If unable to certify compliance, where applicable, provide an explanation and place it after this page.

3. FACILITIES AND ADMINISTRATIVE COSTS (F&A)/ INDIRECT COSTS. See specific instructions.

☒ DHHS Agreement dated: 02/09/16 ☐ No Facilities and Administrative Costs Requested

☐ DHHS Agreement being negotiated with _____ Regional Office.

☐ No DHHS Agreement, but rate established with _____ Date _____

CALCULATION* *(The entire grant application, including the Checklist, will be reproduced and provided to peer reviewers as confidential information.)*

a. Initial budget period:	Amount of base $	120,034	x Rate applied	62.0%	= F&A Costs	$	74,421
b. 02 year	Amount of base $	0	x Rate applied	62.0%	= F&A Costs	$	0
c. 03 year	Amount of base $	0	x Rate applied	62.0%	= F&A Costs	$	0
d. 04 year	Amount of base $	0	x Rate applied	62.0%	= F&A Costs	$	0
e. 05 year	Amount of base $	0	x Rate applied	62.0%	= F&A Costs	$	0
					TOTAL F&A Costs	$	74,421

*Check appropriate box(es):

☐ Salary and wages base ☒ Modified total direct cost base ☐ Other base *(Explain)*

☐ Off-site, other special rate, or more than one rate involved *(Explain)*

Explanation *(Attach separate sheet, if necessary.)*:

4. DISCLOSURE PERMISSION STATEMENT: If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)? ☐ Yes ☒ No