# Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format.

1. **Grantee Institution:** The Pennsylvania State University

2. **Reporting Period (start and end date of grant award period):** 1/1/2010 - 12/31/2013

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** John Anthony, MPA

4. **Grant Contact Person's Telephone Number:** 814 935 1081

5. **Grant SAP Number:** 4100050904

6. **Project Number and Title of Research Project:** 47. Basal Ganglia Neurophysiology During Drug Induced Dyskinesia

7. **Start and End Date of Research Project:** 7/20/2012 - 12/31/2013

8. **Name of Principal Investigator for the Research Project:** Thyagarajan Subramanian, MD

9. **Research Project Expenses**.

   9(A) Please provide the total amount of health research grant funds spent on this project for the entire duration of the grant, including indirect costs and any interest earned that was spent:

   $ 68,850

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

| Last Name, First Name | Position Title | % of Effort on Project | Cost |
|---|---|---|---|
| Handly, Erin | Research Technician | 45% | $17,641 |
| Venkiteswaran, Kala | Asst. Professor | 42% Yr 1 | $13,804 |

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

| Last Name, First Name | Position Title | % of Effort on Project |
|---|---|---|
| Subramanian, Thyagarajan | Professor, PI | 17% |
| Liu, Zhiwen | Assoc Professor | 5% |
| Gilmour, Timothy | Graduate Student | 75% |
| Edwards, Perry | Graduate Student | 75% |
| White, Caitlin | Student Research Assistant | |

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

| Type of Scientific Equipment | Value Derived | Cost |
|---|---|---|
| None | | |

**10. Co-funding of Research Project during Health Research Grant Award Period.** Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No____X_____

If yes, please indicate the source and amount of other funds:

**11. Leveraging of Additional Funds**

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes___X_____ No_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the

application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

| A. Title of research project on grant application | B. Funding agency (check those that apply) | C. Month and Year Submitted | D. Amount of funds requested: | E. Amount of funds to be awarded: |
|---|---|---|---|---|
| Novel optogenetic stimulation | ☐NIH<br>☐ Other federal (specify:_____)<br>X Nonfederal source (specify: Grace Woodward Endowment___) | March 2012 | $ 50,000 | $50,000 |

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes____X_____ No_____

If yes, please describe your plans:

NIH R03 titled Noninvasive optogenetic neuromodulation was submitted in Feb 2014 and is pending review.
NIH R01 NS042402-10 titled "cell transplantation for parkinsonism" was reviewed as an -01 application on 10/25/2012 and a resubmission as an A1 revision is planned July 5, 2014

**12. Future of Research Project.** What are the future plans for this research project?

We will continue to pursue this line of research. The use of optogenetics has provided us with new tools that allow specific dissection of the mechanisms involved in dyskinesias in 3 ways.

1. Evaluating drug induced dyskinesias when there is parkinsonism that is toxin induced (e.g., 6-OHDA lesioning or alpha-synuclein AAV injection)
2. Evaluating drug induced dyskinesias in an optogenetic model where the disease can be induced temporarily and then reversed (see preliminary data in this report).
3. Evaluating both drug induced dyskinesias and graft-induced dyskinesias in parkinsonism with animal model that has optogenetically controlled grafts.

**13. New Investigator Training and Development**. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes___X_____ No_____

If yes, how many students? Please specify in the tables below:

| | Undergraduate | Masters | Pre-doc | Post-doc |
|---|---|---|---|---|
| Male | 4 | | 3 | |
| Female | 5 | | 5 | |
| Unknown | | | | |
| **Total** | **9** | | **8** | |

| | Undergraduate | Masters | Pre-doc | Post-doc |
|---|---|---|---|---|
| Hispanic | | | 1 | |
| Non-Hispanic | 9 | | 7 | |
| Unknown | | | | |
| **Total** | **9** | | **8** | |

| | Undergraduate | Masters | Pre-doc | Post-doc |
|---|---|---|---|---|
| White | 2 | | 3 | |
| Black | | | | |
| Asian | 7 | | 4 | |
| Other | | | 1 | |
| Unknown | | | | |
| **Total** | **9** | | **8** | |

**14. Recruitment of Out-of–State Researchers**. Did you bring researchers into Pennsylvania to carry out this research project?

Yes_____ No_____X_____

If yes, please list the name and degree of each researcher and his/her previous affiliation:

**15. Impact on Research Capacity and Quality**. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes____X_____ No_____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

We were able to bring in new methods for microdialysis (benzoyl chloride derivatization)

and optogenetics that previously did not exist here at Hershey. These are major assets and are used by multiple investigators. Collaboration between Hershey and Penn State Main Campus (University Park) was enhanced by the cooperation of Dr. Liu who brought his expertise in the field of lasers and opportunities to evaluate novel optogenetic tools like the non-invasive ultrashort femtosecond laser pulse system.

**16. Collaboration, business and community involvement.**

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes_____ No_____X____

If yes, please describe the collaborations:

16(B) Did the research project result in commercial development of any research products?

Yes_____ No____X_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No_____X_____

If yes, please describe involvement with community groups that resulted from the research project:

**17. Progress in Achieving Research Goals, Objectives and Aims.**

List the project goals, objectives and specific aims (as contained in the grant agreement). Summarize the progress made in achieving these goals, objectives and aims for the period that the project was funded (i.e., from project start date through end date). Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

**There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.**

Parkinson's disease (PD) is a neurodegenerative disorder characterized by the loss of dopamine-producing cells in the substantia nigra (SN) and its connections. PD manifests itself with tremor, slowness of movement (bradykinesia) and stiffness (rigidity). Most PD patients develop a complication of therapy called drug induced dyskinesias (DID), 2-5 years after initiation of treatment. DID are difficult to treat and finding the pathophysiological basis of DID is cardinal to finding new treatments. The pathophysiological basis of current treatments for DID are not well understood and in most cases these treatments are short lived. Although loss of continuous dopaminergic stimulation has been implicated as one potential cause of DID, many recent studies indicate that the pathophysiology is inherently related to problems with neuronal circuits in the basal ganglia and surgical stimulation or lesions of specific areas in the basal ganglia can actually mitigate DID. These findings suggest that understanding abnormalities in the electrical properties of the basal ganglia circuits during and around DID will lead to a better understanding of DID pathophysiology and treatments.

The specific aim of this project will be to test the hypothesis that the anti-PD and DID effects of LD/DDCI are mediated by altering the electrophysiology of the basal ganglia in a unique and different manner and that DID has an electrophysiological signature in the basal ganglia. Hemiparkinsonian (HP) rats will be used for these recordings from the subthalamic nucleus (STN), Substantia nigra reticulata (SNr) and the entopeduncular (EP) nucleus. Single cell recordings, local field potentials (LFP) and electroencephalogram (EEG) recordings will be obtained with and without various treatments. Experiments will be performed in awake behaving rats while exhibiting DID and while not exhibiting DID and compared to identical recordings from normal animals.

Our experiments addressed the specific aim listed above. As expected in many situations in science, we had a unique opportunity in the midst of this research study to use novel techniques to address the questions we posed in this research. Hence, as diligent science demands, we leveraged this grant to obtain additional funding to use in parallel these new techniques to help answer the questions we originally posed in this strategic plan.

Results #1 (shown in previous annual report, but updated): Evaluation of microdialysis and electrophysiology in awake behaving HP rats exhibiting Drug induced dyskinesias (DID): To our knowledge, this was the first attempt to evaluate the effects of PD on basal ganglia-cortex-basal ganglia electrical network and the biochemical changes in the brain during DID simultaneously. This issue has contemporary significance as it is increasingly clear that pharmacotherapy by itself is ineffective in providing symptomatic treatment in advanced PD. Many advanced PD patients require surgical interventions like deep brain stimulation (DBS) to get symptomatic relief. The putative effects of DBS on dopaminergic systems are frequently explored in contemporary research but the reverse question of how drug therapy affects basal ganglia electrophysiology and information transfer between the cortex and the basal ganglia has not been thoroughly explored. The approach we have made is novel and innovative and could yield the anatomical, biochemical and pharmacological correlates of electrophysiological abnormalities noted in the basal ganglia – cortex connectome. Figure 1 shows an example of successful recordings and microdialysis using video stills. We used the microdialysis technique described by Song et al. that utilizes benzoyl chloride derivatization, allowing the separation of 17 neurotransmitter analytes from the same sample. Samples were extracted every 5 minutes during DID and without DID. We have obtained technical training from Dr. Robert Kennedy at the University of Michigan and have now reproduced the technique at our Penn State University College of Medicine (PSUCOM) core facility. Figure 2 shows examples of successful recordings in dyskinetic animals and subsequent histology of accurate recordings. The insert shows the construct of the miniature microdrive that permits single cell neuronal recordings from various basal ganglia nuclei. In each animal, the angle of entry permitted recording sessions from the striatum, STN, GP and SNr by adjusting the z-drive and by altering the entry angle into the brain. We have successfully recorded in several animals using this technique and are now in the process of analyzing the data and preparing the manuscripts. This will be to our knowledge the first attempt to evaluate a whole series of basal ganglia neurochemicals in animals exhibiting DID and correlating it to electrophysiology.

Methods and research details: Forty-two (updated from 16 reported in the last annual report) stable HP rats were comprehensively evaluated with a rodent behavioral battery of tests (RBBT tests: stepping test, vibrissae evoked forelimb placement (VEFP) test, cylinder test and elevated body swing test (EBST), apomorphine and amphetamine rotational tests). These HP rats underwent *in vivo* microdialysis in the awake behaving freely moving state at baseline and post-treatment for 100 minutes with L-dopa/BZ administered IP (see Figure 1). All microdialysis was performed from the dorsal neostriatum after animals received a surgically implanted stainless steel microdialysis cannula that was cemented in acrylic under deep general anesthesia. Stereotaxic coordinates were (all in reference to Bregma): AP = +0.7, ML = +2.5, DV = -7.0 (tip of 3mm probe and dialysis occurred within this whole length). On the 5th day post-surgery, rats were moved into microdialysis chambers, hooked into the chamber tether system to allow them to acclimate to the weight of the tether now attached to the headcap, as well as to their new

environment. They were given ad lib food and water. On 7th post-op day, the rats were anesthetized briefly and the microdialysis probes were implanted into the cannulas and connected to the tubing via the tether after checking for good flow of the aCSF through the tubing and through the probe itself. A slow infusion of aCSF was started after implantation and continued overnight until the sample collection began the following morning. Prior to beginning sample collection each day the rats' food and water bottles were removed from the chambers. At the end of sample collection on the first day, food and water were returned. At the end of sample collection on the second day, the rats were unhooked from the system and returned to their home cages. Samples were collected for 20-minute time blocks. The flow rate of aCSF was 1µl/minute. Samples were derivatized using the benzoyl chloride method using methods described techniques by Song et al., with active consultation of Dr. Kennedy (see Figure 3 for preliminary results of 5 major neurotransmitters). Note that we have now confirmed the results of the microdialysis both at PSUHMC and at University of Michigan and shown that the results are reproducible.

Results #2 (New experiments not previously reported): *Optogenetic techniques: targeting the nigrostriatal pathways and development of implantable fiberoptics for laser light delivery*. During the course of the current project, we had the opportunity to develop and use a novel technique to evaluate DID. This involves the hypothesis that DID does not occur in HP animals and in PD patients with hemiparkinsonism (HP, stage I disease) do not develop DID or motor fluctuations. The "lack of CDS hypothesis" would predict that HP patients and HP animals would develop unilateral DID if they had sufficient nigrostriatal degeneration and exposure to intermittent high doses of L-dopa. Our studies show that HP animals with profound unilateral nigrostriatal loss (>90%) do not develop DID despite high intermittent doses of L-dopa [7] and suggest a role for interhemispheric connections for the genesis of DID [2]. This hypothesis is not testable using conventional model systems available to study PD or DID and is highly relevant to our original proposal in this grant period – i.e., study the pathophysiology of DID in PD. Therefore, taking advantage of newly emergent technology and a small pilot grant that we leveraged from this grant – we were able to use optogenetics to begin the study of this question. This opportunity also gave us the chance to bring state of the art techniques to Hershey Medical Center that did not exist in terms of optogenetics.

We have recently completed a dual viral vector injection system into normal rats to label interhemispheric nigrostriatal neurons and the nigrostriatal pathway completely on one side (see Figure 5). Rats received 3 injections of AAV recombinant gene vector that expressed the transynaptic WGA-Cre ("switch gene") into the left striatum and one injection of AAV recombinant gene vector that expressed eNpHR 3.0 (opsin driven chloride channel, halorhodopsin) and eYFP (enhanced yellow fluorescent protein) into the left nigra and 1 injection into the right nigra (Cartoon shown in Figure 4). After 3 weeks, we implanted fiberoptic implants targeted to the left SNpc and right SNpc and cemented it in place. After surgical recovery, animals were habituated to the tethering systems for several days (Fig 5). Laser activation of eNpHR was performed 1 month after the implantation of the laser fiberoptic into the brain. Following a mean of 4.5 hours of laser activation, these animals developed right hemiparkinsonism that persisted despite turning off the laser light source. This right HP state remained in place for a mean duration of 14 days after laser activation was stopped and then showed complete recovery to the normal baseline state without any residual parkinsonism. This

laser activation experiment was repeated 6 times in the same set of animals (N=5) with the same results and without any permanent residual effects. The opposite SN (that received eNpHR 3.0 to label the interhemispheric SN neurons was separately inactivated using laser light application at the same lengths of time). As expected, the solo activation of the interhemispheric right to left nigrostriatal interhemispheric fibers that represent a very small population of cells (see figure 5) did not elicit any behavioral effects or significant parkinsonism. This finding is exactly as we anticipated from the inhibition of the interhemispheric fibers. At the end of the experiment, we examined the entire brain including the nigral brain sections. We found a large number of eNpHR+ cells (yellow) within the left SNc and a small number of interhemispheric nigral neurons on the right SNc, much as expected (Fig 5). No evidence of phototoxicity was seen. Stereological estimate of eNpHR+ cells in the nigra indicated that virtually 100% of the SNpc TH +ve dopaminergic neurons were successfully expressing eNpHR. No evidence of tyrosine hydroxylase (TH) neuronal loss or local evidence of phototoxicity was noted in the SNpc, its vicinity or along the tract of the optic fiber. These preliminary results demonstrate the capability to accurately label the nigrostriatal pathways using optogenetics labeling techniques, and the ability to develop a tethering system for laser fiberoptic experiments to cause reversible hemiparkinsonism. Additional tests are ongoing to evaluate DID in this system and to perform electrophysiology and microdialysis in this new animal model system that has the ability to completely reverse to the normal behavioral state (see Figure 5 for details). This is an important new discovery in the field of PD experimental therapeutics.

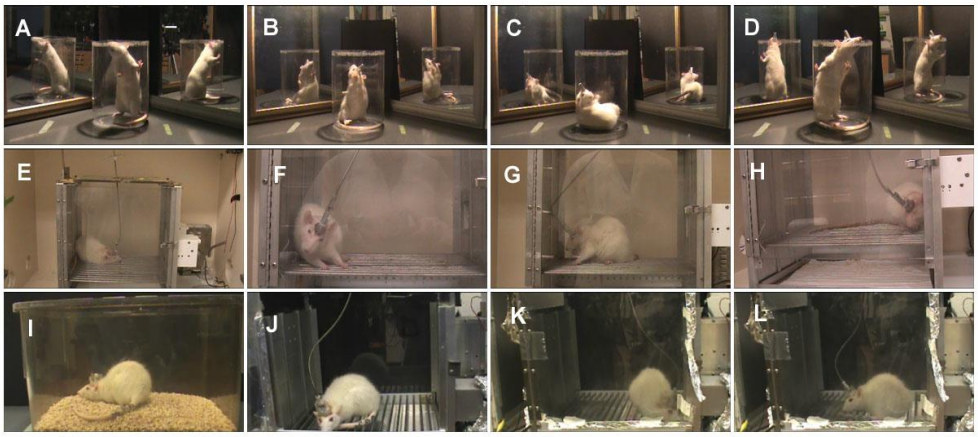



**Figure 1:** Stills from video files demonstrating **(A)** HP rat baseline behavior in cylinder test, **(B)** Post L-dopa/Benserazide(BZ) - neck and trunk DID in a rat with a microdialysis implant, **(C)** Post L-dopa/BZ – neck, trunk and limb DID, **(D)** L-dopa/BZ treated HP rat showing anti-PD effects and normal exploration after DID has subsided, **(E)** Baseline behavior in a microdialysis chamber, **(F)** L-dopa/BZ treated rat expressing neck and trunk DID, **(G)** L-dopa/BZ treated rat expressing limb DID, **(H)** L-dopa/BZ treated rat after DID has subsided, (see figure 3 for microdialysis data), **(I)** Rat implanted with microdrive (see figure 2), freely moving in transfer cage, **(J)** Rat connected in chamber prior to beginning recording, **(K)** & **(L)** Rat connected and freely moving during recording.

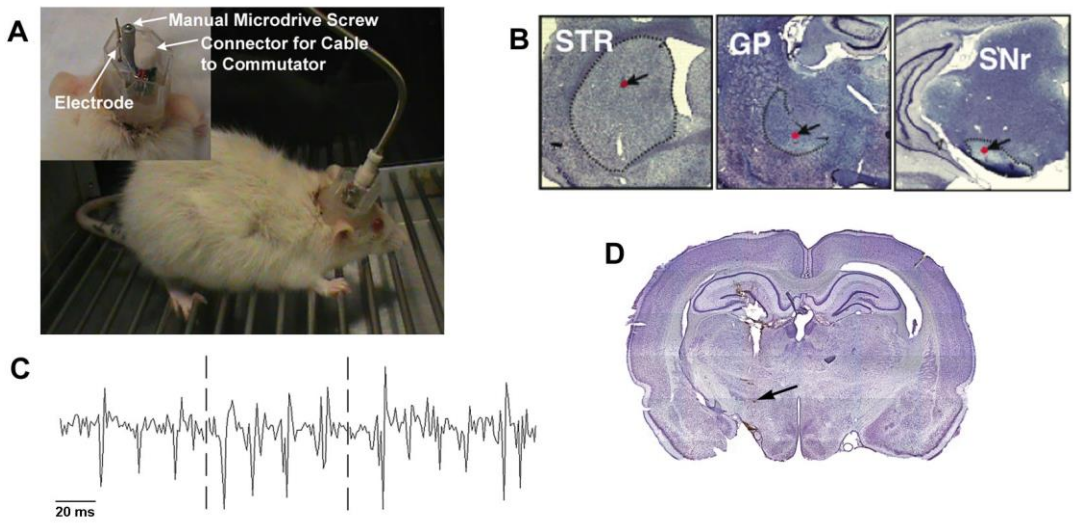



**Figure 2:** **(A)** Rat implanted with a microdrive and connected in recording chamber; inset shows the implant apparatus, **(B)** Simultaneous recording sites throughout the basal ganglia, **(C)** Average spike waveforms from the microdrive electrode, **(D)** CV-stained section of a rat implanted with the microdrive assembly shown in A; arrow shows electrode targeting in the subthalamic nucleus (STN). This is a novel new recording system that we have developed and successfully tested.

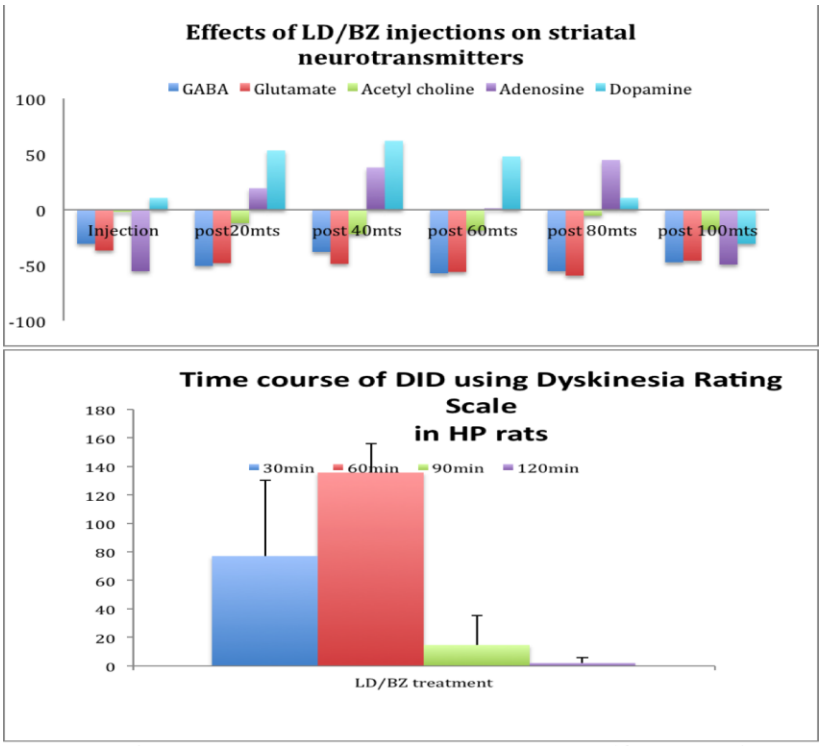
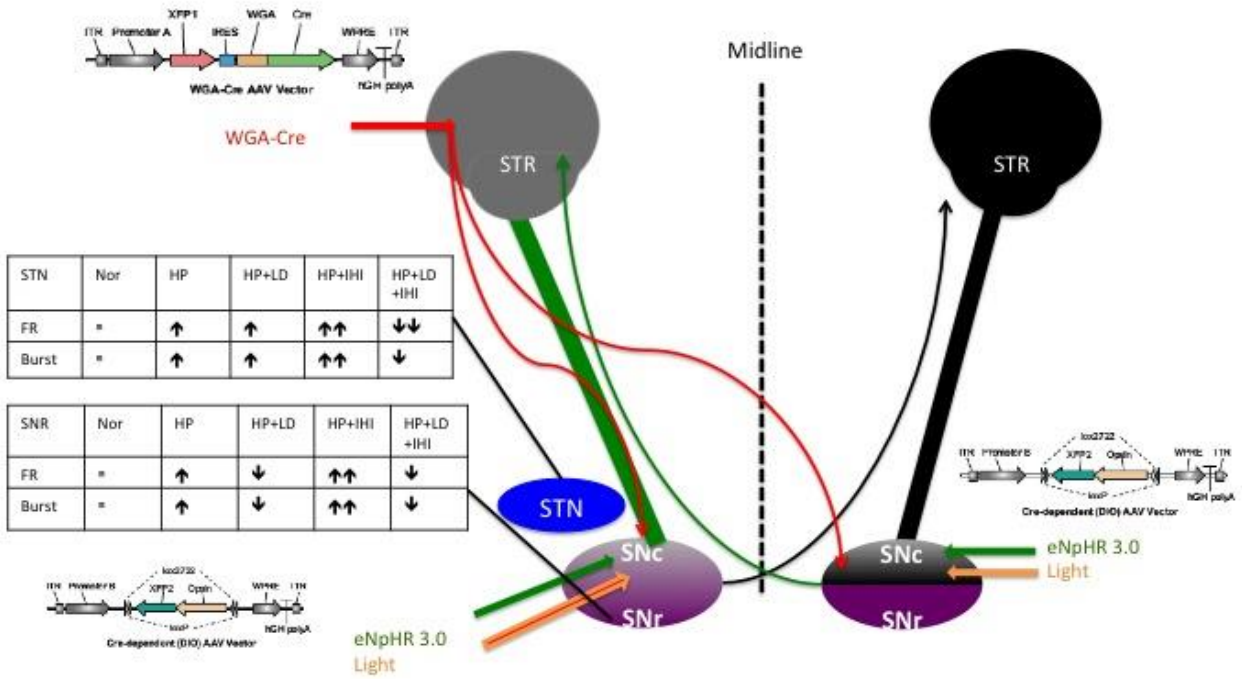



**Figure 3 :** Striatal microdialysis in HP rats showing % change from baseline for 5 out of 17 neurochemicals measured using methods described by Song, et. al.,. LD/BZ treatment had earlier positive change in dopamine levels in the striatum accompanied by DID in HP rats. Note changes in other neurotransmitters including GABA, glutamate and acetyl choline that are reduced compared to baseline during DID. Sensitivities were in the pM to nM range. All 17 compounds listed by Song et. al., were detected, we are only showing 5 out of the 17 for lack of space.




| STN | Nor | HP | HP+LD | HP+IHI | HP+LD +IHI |
|---|---|---|---|---|---|
| FR | = | ↑ | ↑ | ↑↑ | ↓↓ |
| Burst | = | ↑ | ↑ | ↑↑ | ↓ |

| SNR | Nor | HP | HP+LD | HP+IHI | HP+LD +IHI |
|---|---|---|---|---|---|
| FR | = | ↑ | ↓ | ↑↑ | ↓ |
| Burst | = | ↑ | ↓ | ↑↑ | ↓ |

**Figure 4:** Cartoon explaining the dual vector optogenetic inhibition experiments. The grey striatum and substantia nigra pars compacta (SNc) ‘s represents the lesioning caused by the chloride channel activation by the application of light (orange) after the animal has been previously injected with the dual vectors. WGA-Cre is represented in red as it co expresses the marker mCherry which is red and all halorhodopsin eNpHR 3.0 expressing cells and axons will coexpress enhanced yellow fluorescent protein (eYFP) which is shown here in green. The axon density is represented by the thickness of the arrows. So the left nigrostriatal pathway is shown as a thinner arrow in green compared to the interhemispheric right to left nigrostriatal pathway. The Substantia nigra reticulate (SNr) is shown in purple and the subthalamic nucleus (STN) is shown in blue. Also note the predicted changes in firing rates (FR) and firing patterns (burst) are shown as possible outcomes with interhemispheric inhibition (IHI) and without IHI inhibition. Possible effects of LD treatments and its predicted effects on the SNr and on the STN are also shown. These experiments are ongoing.

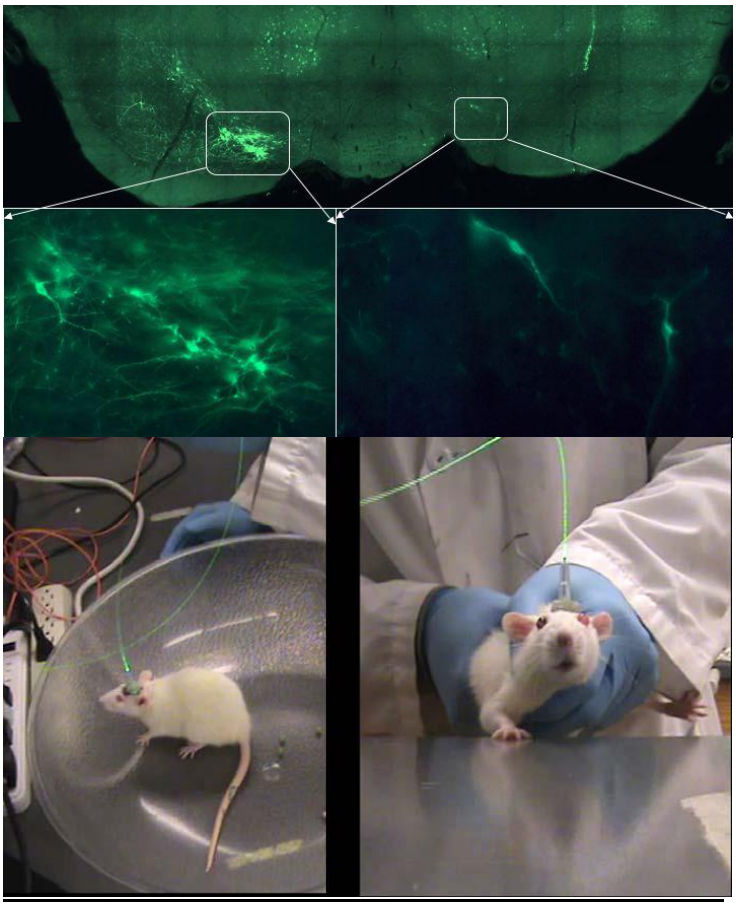


**Figure 5:** Optogenetic labeling and activation using tethered laser system. Top shows details of eYFP positive SNc neurons in the ipsilateral side and a smaller but definite population of eYFP labeled cells in the contralateral SNc after labeling with dual vector systems that requires transynaptic "Cre" activation of eYFP expression and eNpHR 3.0.

Presentations:

1. Ding Ma, Baigang Zhang, Perry Edwards, Yizhu Chen, Zhiwen Liu, Thyagarajan Subramanian, Kala Venkiteswaran, Timothy Gilmour, Megan Dawson, Caitlin White, Chris Lieu, Erin Handly, Megha Subramanian, Charu Ramakrishnan, Karl Deisseroth, "Towards non-invasive optogenetic neuromodulation", Poster Presentation, Penn State Neuroscience Institute Retreat, Hershey, PA, USA March 27-28, 2014.
2. Thyagarajan Subramanian, Kala Venkiteswaran, Zhiwen Liu, Timothy Gilmour, Baigang Zhang, Megan Dawson, Caitlin White, Chris Lieu, Erin Handly, Megha Subramanian, Charu Ramakrishnan, Karl Deisseroth, "Functional optogenetic modulation of striatal dopaminergic transplants in parkinsonian rats", Data Blitz Slide Presentation, Penn State Neuroscience Institute Retreat, Hershey, PA, USA March 27-28, 2014.

**18. Extent of Clinical Activities Initiated and Completed**. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___X__No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___X__No

**If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed.** (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

**Note**: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

**19. Human Embryonic Stem Cell Research.** Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
___X_ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
_____ No

19(C) Please describe how this project involved human embryonic stem cells:

**20. Articles Submitted to Peer-Reviewed Publications**.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, and an abbreviated title of the publication. For example, if you submit two publications for Smith (PI for Project 01), one publication for Zhang (PI for Project 03), and one publication for Bates (PI for Project 04),

the filenames would be:

Project 01 – Smith – Three cases of isolated
Project 01 – Smith – Investigation of NEB1 deletions
Project 03 – Zhang – Molecular profiling of aromatase
Project 04 – Bates – Neonatal intensive care

If the publication is not available electronically, provide 5 paper copies of the publication.

**Note:** The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

| Title of Journal Article: | Authors: | Name of Peer-reviewed Publication: | Month and Year Submitted: | Publication Status (check appropriate box below): |
|---|---|---|---|---|
| 1. None | | | | ☐Submitted<br>☐Accepted<br>☐Published |

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes____X_____ No_____

If yes, please describe your plans:

Several peer reviewed papers are in preparation. These include the data shown in this report upon completion of all analysis and histology and listed as 2 recent presentations. We expect both of these presentations to be submitted for peer review. In addition, a new manuscript that describes our work on the interhemispheric nigrostriatal pathways and their effect on drug induced dyskinesias is expected to be submitted in the next few weeks and will include our microdialysis and electrophysiology data that are shown in this report.

**21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project.** Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

**22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment**. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

The optogenetic model of reversible parkinsonism is a major new finding.

**23. Inventions, Patents and Commercial Development Opportunities.**

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No___X_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No____

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No____

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes____X_____ No_____

If yes, please describe your plans:

We plan to patent the optogenetic reversible model of Parkinson's disease in collaboration with our colleagues from Stanford University.

**24. Key Investigator Qualifications.** Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages.

**BIOGRAPHICAL SKETCH**

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.

Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

| NAME<br>Thyagarajan Subramanian, MD | POSITION TITLE<br>Professor of Neurology and Neural and Behavioral Sciences<br>Director, Movement Disorders Program<br>Director, Central PA APDA Information and Referral Center |
|---|---|
| eRA COMMONS USER NAME (credential, e.g., agency login)<br>subramanian | |

EDUCATION/TRAINING (*Begin with baccalaureate or other initial professional education, such as nursing,*

| INSTITUTION AND LOCATION | DEGREE *(if applicable)* | YEAR(s) | FIELD OF STUDY |
|---|---|---|---|
| University of Calicut, Calicut, India | MBBS | 1987 | Medicine |
| University of Calicut, Calicut, India | residency | 1987-88 | Surgery |
| University of Pittsburgh, Pittsburgh, PA, USA | Grad. fellow | 1988-90 | Neuroscience |
| University of Pittsburgh, Pittsburgh, PA, USA | residency | 1990-94 | Neurology |
| Emory University, Atlanta, GA, USA | Post-doc | 1994-97 | Movement Disorders |

**A. Personal Statement**

I am a board certified practicing neurologist and a neuroscientist. I am the director of the APDA Central PA Movement Disorders Center at Penn State University. I see patients in my movement disorders clinic 2 days a week and I participate in DBS intraoperative neurophysiology. Over the past 20 years I have been involved in numerous experimental therapeutic studies using animal models of Parkinson's disease (PD) and in PD patients. I have been involved in many investigator initiated therapeutic studies for DID and as a member of the Parkinson Study Group, I have served as site PI on several PD experimental therapeutic studies for DID and motor fluctuations. As such I am intimately aware of the clinical problems faced by PD patients and their risk for LID. I have worked with the 6-OHDA lesioned rat and the MPTP treated nonhuman primate models of PD and am familiar with all aspects of these models. We have developed detailed techniques to create stable models, behaviorally assess them using operant conditioned tests, measure their response to anti-dopaminergic therapy using methods that simulate how patients with PD are treated in the clinic. I was trained as a general surgeon in India before I came to the United States and I have kept up my surgical skills. As such I am very comfortable working with cranial surgery, electrophysiology, *in vivo* microdialysis, histology and stereology. My research team has expertise in cell transplantation, gene therapy, biochemistry, plant product chemistry, biosynthesis and experimental pharmacology in animal models of PD and in patients with PD. We routinely use brain imaging using multiple modalities, electrophysiology, stereotactic surgery, histology, microdialysis, protein chemistry, molecular biology and immunohistochemistry. I share an electrical engineering (EE) graduate student with the former chair of the Department of EE, PSU Main Campus, State College, PA, Dr. Kenneth Jenkins and this senior graduate student has been involved in advanced signal analysis of electrical recordings from the basal ganglia in the 6-OHDA lesioned rat. We have also gained expertise in optogenetics with Dr. Liu and in in vivo microdialysis and analysis using the benzoyl chloride derivatization techniques to evaluate multiple neurotransmitters simultaneously from the brain.

**B. Positions and Honors**

| | |
|---|---|
| 1994 -1997 | Movement Disorders Fellowship, Emory University, Atlanta, GA 30322. (Drs. Mahlon R. DeLong, Ray L. Watts and Roy A.E. Bakay, Preceptors) |
| 1997 – 2000 | Asst Professor of Neurology, Department of Neurology, Emory University, Atlanta, GA 30322. |
| 2000 – 2001 | Clinical Associate Staff, Cleveland Clinic Foundation, Cleveland, OH 44195 |
| 2001 – 2005 | Staff Neurologist and Neuroscientist, Cleveland Clinic Foundation, Cleveland, OH 44195 |
| 2005 - 2008 | Professor of Neurology, Penn State Milton S. Hershey College of Medicine, Hershey, PA |

2008 - Tenured Professor of Neurology and Neural and Behavioral Sciences, Penn State University, Director, Movement Disorders Program, PSUHMC

AD-HOC MANUSCRIPT REVIEWER: Experimental Neurology, Journal of Comparative Neurology, Journal of Neuroscience Methods, Brain Research, Pharmacology, Biochemistry and Behavior, Neuroscience and Behavioral Reviews, Brain, Neurology, Experimental Eye Research, Annals of Neurology, Cell Transplantation, Neuroscience Research.

GRANT REVIEWER

2006-2008 Member, Institutional Review Board A, PSUHMC, Hershey, PA
2008- Member, Scientific Review Committee, PSUHMC, Hershey, PA
2008- Member, Biosafety Committee, PSUHMC, Hershey, PA
2009 NIH study section ZRG1 CBQ30 special emphasis panel (reviewed 11 grants)
2011 NIH study section ZRG1 ZRG1 F05-A (20) L

**Honors**

1988 – 1989 Deans Fellowship, University of Pittsburgh School of Medicine, Pittsburgh
1997 Fellow, American Society for Neural Transplantation and Repair
2000 Member, Parkinsons Study Group
2002 First, Dr. S. Kalyanaraman Oration Award, Neurological Institute of MMC, Chennai, India

**Ongoing Research Support**

The Charles A. Dana Foundation (Wang, Jian-li - PI) 10/01/09 – 10/01/14
Quantitative MRI Evaluation of Nigrostriatal Pathway Damage in Early Yong Onset Parkinson's Disease
*The major goal of this project is to uncover the underlying pathophysiology of PD progression from Stage I to Stage II using novel MRI techniques. All patient enrollment into the study is complete.*
Role: Co-PI and mentor (all patients are evaluated and enrolled by Thyagarajan Subramanian from his practice) No overlap

Grace Woodward Medicine-Engineering Collaborative Grant: Novel optogenetic neuromodulation. 6/12-6/14
Co-PIs: Thyagarajan Subramanian and Zhiwen Liu
This pilot grant is to evaluate novel optogenetic techniques overlap is discussed

A PHASE IIIb, MULTICENTRE, RANDOMISED, DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY EVALUATING THE EFFICACY AND SAFETY OF DYSPORT USING 2 mL DILUTION IN ADULTS WITH CERVICAL DYSTONIA (Ibsen/Dysport 169) Sponsor: Ibsen
Site PI: Thyagarajan Subramanian No overlap

A PHASE IIIb, MULTICENTRE, OPEN-LABEL EXTENSION STUDY TO ASSESS LONG TERM SAFETY AND EFFECTIVENESS OF DYSPORT USING 2 mL DILUTION IN ADULTS WITH CERVICAL DYSTONIA (Ibsen/Dysport 170) Sponsor: Ibsen
Site PI: Thyagarajan Subramanian No overlap

CD Flex: An Open-Label, Non-Inferiority Study Evaluating the Efficacy and Safety of Two Injection Schedules of Xeomin® (incobotulinumtoxinA) [Short Flex vs. Long Flex] in Subjects with Cervical Dystonia < 10 Weeks of Benefit from OnabotulinumtoxinA Treatment
Sponsor: Ibsen
Site PI: Thyagarajan Subramanian No overlap

**Completed Research in past 3 years**

NIH **RO1** NS42402 Thyagarajan Subramanian (PI) 4/01/2006-1/31/2013
National Institutes of Health (NINDS) "Intranigral transplantation in parkinsonian monkeys"
*This grant will evaluate the effects of dual transplants to understand the pathophysiology of cell transplants using neurophysiological assessments in the basal ganglia circuits.*