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. Questions? Contact Health Research Program staff at 717-783-2548.

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

2. **Reporting Period (start and end date of grant award period):** 1/1/2009 – 12/31/2012

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:** 4100047645

6. **Project Number and Title of Research Project:** 12 - Modulation of Basal Ganglia Electrophysiology by Dopaminergic Cell Transplant

7. **Start and End Date of Research Project:** 6/1/2009 – 12/31/2012

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:

   $ 114,663

   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	Position Title	% of Effort on Project	Cost
Gilmour	Graduate Student	74%	$42,742
Lieu	Graduate Student	35%	$9,913
Patel	Student Research Asst.	1%	$162
Handly	Research Technician	7%	$5,700
Venkiteswaran	Asst. Professor	6%	$5,072

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	Position Title	% of Effort on Project
Subramanian	Professor	1%

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____X_____ No_____

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

NIH R01NS42402 - $250,000/year for 5 years beginning 2007 and ending 2011 plus 2 no-cost extensions

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:
Mechanistic Studies of an Ayurvedic Treatment for Parkinson's Disease	xNIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	3/1/1013	$1,506,914	Pending review
Novel optogenetic neuromodulation	☐ NIH ☐ Other federal (specify:_____) ☒Nonfederal source (specify: Grace Woodward Foundation_)	3/1/1012	$50,000	$50,000
Pharmacological neuromodulation for drug induced dyskinesias	xNIH ☐ Other federal (specify:_____) ☐ Nonfederal source specify:___)	10/5/2012	$1,545,750	$0
Transplantation for parkinsonism	xNIH ☐ Other federal (specify:____) ☐ Nonfederal source specify:___)	7/6/2012	$1,914,114	$0
Immunological and anti-teratogenic effects of RPE co-grafts	xNIH ☐ Other federal (specify:___) ☐ Nonfederal source (specify:__)	6/18/2012	$404,134	$0
Water extract of Mucuna	xNIH	11/7/2011	$1,522,293	$0

pruriens for Parkinson's Disease	☐ Other federal (specify:_____) ☐ Nonfederal source (specify:__)			

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:

This line of work has led us to develop strong and robust scientific preliminary data that can be used to leverage new NIH grants. Specifically, beyond the issue of stem cells and their effects that were the original mandate of the specific aims in this funded grant, our techniques to measure and record electrophysiology in the cortex and in the basal ganglia will be useful in many other situations. Similarly, optogenetics and microdialysis techniques that we have developed and incorporated into our research has helped us to examine the effects of *Mucuna pruriens*, a novel Ayurvedic medication in animal models of PD and we have made advances that have led to 2 peer reviewed papers of the highest impact in the field. These studies will be leveraged for grant funding. A new NIH grant application to be submitted on June 5, 2013 is planned. This R01 application titled "Electrophysiological and neurochemical correlates of drug induced dyskinesia" is now under preparation. In addition, we plan to resubmit the grant "Immunological and anti-teratogenic effects of RPE co-grafts" as an A1 revision in July 2013. Finally the NIH R01 NS42402 "transplantation for parkinsonism" that was funded for 10 years will be resubmitted as A1 in November 2013 after the PI has published the 3 pending papers that came out of the funding supported by Tobacco Settlement Funds (CURE program) in the present grant.

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

I plan to resubmit my NIH R01 NS42402 as an A1 resubmission in short order after 3 additional publications that were funded in part by NS42402 and this CURE grant.

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	2		3	
Female	6			
Unknown				
Total	**8**		**3**	

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic	8		3	
Unknown				
Total	**8**		**3**	

	Undergraduate	Masters	Pre-doc	Post-doc
White	2		3	
Black				
Asian	6			
Other				
Unknown				
Total	**8**		**3**	

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.

    This research enabled, for the first time to the best of our knowledge, LPF recordings in behaving animals from the basal ganglia. This research also for the first time allowed us to do non-linear EEG-LFP cross correlation studies for synchrony. This led to the discovery that transfer entropy is the most robust measure and the most sensitive measure for plastic changes in the basal ganglia. Further, we showed that, for the first time, cell transplants can be labeled with a cre dependent dual vector such that synaptic connectivity between the graft

and the host could be confirmed without the use of electron microscopy and we were also able to confirm that protein transfer from the host to the graft could be accomplished artificially. The institutional gain in these areas were: our ability to start optogenetic experiments for the first time at PSU in collaboration with Dr. Karl Diesseroth from Stanford University, tetrode recordings in rats that are awake and behaving using techniques developed by Dr. Burke and Dr. Leventhal from University of Michigan and finally adding *in vivo* microdialysis techniques in awake behaving rats developed by Dr. Kennedy from University of Michigan allowing us to evaluate as many as 17 neurochemicals and neuropeptides from a single microdialysis sample of 5µl taken at every 5 minute intervals. These techniques helped expand research capacity at HMC as my research was the first to develop benzoyl chloride derivatization methods and adopt them to our Mass spectroscopy core. Now this same technique is being used by 2 additional labs (Dr. Grigson and Dr. Hajnal) for their own research. My lab funded through this grant also brought optogenetics to Penn State University-Hershey Medical Center (PSUHMC). We pioneered these techniques with Dr. Zhou from PSU main campus and for the first time PSU used laser activated *in vivo* experiments in awake behaving rodents. These techniques have also been broadly adopted and many investigators have gained experience and advice by visiting our lab and learning techniques from us. We ourselves are poised to make a paradigm shift in our research techniques and our ability to ask fundamental questions in terms of basal ganglia function and pathophysiology of basal ganglia mediated disease like Parkinson's disease.

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___x_____ No_____

If yes, please describe the collaborations:

The entire grant was a major success in fostering collaboration across campuses and across institutions. Mr. Gilmour (now Dr. Gilmour) was a student at PSU main campus in their electrical engineering department (EE). He chose as his project to be with Dr. Subramanian (PI) at the Hershey COM campus. This was the beginning of an outstanding collaborative effort between EE and medicine across campuses. The PI made several visits to EE campus and presented at their conferences findings from this funded research. The graduate student, Mr. Gilmour also made several presentations. Several papers came out of this collaboration with multiple members of the EE department including Drs. Lagoa, Monga and Jenkins. In addition, through contacts that the PI developed, a new collaboration grew with Dr. Zhiwen Lou also of the EE department and this led to the successful adoption of optogenetics into the PI's laboratory. These techniques are part of the annual report submitted this year showing the feasibility of performing optogenetic experiments. This research work also led to an inter-university

collaboration with University of Michigan. Two separate labs, one is of Dr. Joshua Burke, a leading expert in recording the brain from awake behaving rats.

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___X_____ No_____

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

We have run a highly successful summer internship program and the Central PA Brain Bee every year under the auspices of my laboratory. The research that was supported by Tobacco Settlement Funds in the present grant enabled my graduate student, Tim Gilmour, my colleague, Dr. Venkiteswaran and my technician to participate in the internship that allows both local and nationwide high school, undergraduate and medical students to spend 10 weeks in the summer engaged in neuroscience research. This highly successful program has been going on for 6 years since I started at PSUHMC. But, the highest success of this program came after the TSF funding came through in 2009 and we had our largest class of student interns (9) past summer. This internship provides local high school students a taste of neuroscience research. Specifically, this past summer we had 3 high school students from central PA who participated in our internship program and 2 of these students presented their research at local science fair competitions winning first award and placement into state competition. These 2 science fair presentations attracted a lot of community attention and involvement of local school teachers and students in our research. The annual Brain Bee competition was also a great way to get community involvement with our research. Again, my graduate student Timothy Gilmour, Dr. Venkiteswaran, Ms. Erin Handly were all actively involved in the Brain Bee contest 2012 and over 20 high school students participated, similar to 2011 and 2010.

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.

The broad research objective is to better understand the normal electrical properties of the brain, how they are altered in Parkinson's disease and whether "normalizing" the aberrant electrical patterns is critical to restore normal behavior. Part of this overall objective is to better understand the mechanisms by which cell transplants ameliorate Parkinson's disease symptoms.

The specific research aim is to answer the question: Do dopaminergic cell grafts modulate basal ganglia (BG) electrophysiology in the anesthetized and unanesthetized hemiparkinsonian (HP) rat? Planned subquestions are: Are the changes different in asleep versus awake conditions, do fetal ventral mesencephalic (FVM) cell grafts cause different changes than retinal pigment epithelium (RPE) cell grafts, and is there a difference between striatal-only grafts versus combined striatal-nigral grafts?

The methods involve comparing the recorded electrical patterns from the brains of rats. Rats are divided into five groups: Normal, Control, and Transplanted (3 different transplant paradigms: FVM-striatal, FVM-striatal/nigral, and (RPE)-striatal). After baseline testing, and induction of hemiparkinsonism via the 6OHDA neurotoxin-injection paradigm, the rats in the chronic group will be transplanted with cells (or vehicle) and implanted with electroencephalogram (EEG) screw electrodes and chronic electrodes for recording local field potentials (LFPs) from the subthalamic nucleus (STN), whereas rats for urethane recordings will receive transplants but no chronic electrodes. After recovery, rats in the chronic group will have EEG and LFP signals recorded weekly for three months under both asleep and awake conditions, whereas rats in the anesthetized-recordings group will undergo electrophysiology solely at the end of the three months. Afterward the brains will be histologically examined. The recorded signals will be examined for differences in specific frequency bands between the different groups.

Results:
Broadly, two sets of experiments were performed and they could be classified as anesthetized recordings and chronic awake recordings.

All rats were tested for baseline apomorphine-induced rotations (APIRs). Group 1 was implanted with electroencephalogram (EEG) screw electrodes and chronic electrodes for recording local field potentials (LFPs) from the subthalamic nucleus (STN) (cf. Sharott et al. 2005, "Dopamine depletion increases the power and coherence of beta-oscillations in the cerebral cortex and subthalamic nucleus of the awake rat", *Eur J Neurosci* 21(5): 1413-22).

Group 2 was lesioned with 6OHDA by Charles River Laboratories prior to transfer to our lab. Then in our lab the rats underwent surgery to implant the EEG screws and LFP chronic electrode.

Groups 3-5 were initially lesioned with 6OHDA by Charles River Laboratories. After verification of hemiparkinsonism, Groups 3-5 underwent cell transplant surgery and were implanted with the EEG screws, EMG electrode, and chronic LFP electrode (cf. Subramanian et al 2002, "Striatal xenotransplantation of human retinal pigment epithelial cells attached to microcarriers in hemiparkinsonian rats ameliorates behavioral deficits without provoking a host immune response", *Cell Transplant* 11(3): 207-14). Figure 1 shows a sample cresyl violet stain showing accurate electrode targeting into the STN.

After recovery, EEG and LFP signals were recorded at least once per month in each rat during both daytime (for sleep) and nighttime (for awake) sessions. Sleep was verified using EEG and electromyogram (EMG) recordings and video monitoring. The signals recorded were bipolar STN electrode, two EEG channels, one EMG channel, and a cage accelerometer signal to measure activity. Figure 2 shows sample EEG and EMG activity with both awake and asleep periods.

Parkinsonism was assessed monthly by APIR tests and by other standard behavioral tests such as the forelimb stepping test, vibrissae stimulation/forelimb placement test, extended body-axis test,

and cylinder test. After three months, the rats were perfused and the brains were histologically examined for graft viability and verification of recording locations.

During the project, a need arose to separate the awake and asleep portions of the recordings. After surveying the literature on available segmentation methods, a new method was devised based on the principal component decomposition of the spectral features. This method was tested, submitted for peer review, and published in Neuroscience Letters (Gilmour, T. P., J. Fang, Z. Guan and T. Subramanian (2009). "Manual rat sleep classification in principal component space." Neurosci Lett 469(1): 97-101).

Figure 1 – Coronal cresyl violet-stained rat brain slice from rat implanted with chronic STN electrode and euthanized after three months, showing the electrolytic lesion from the tip of the electrode localized in the STN.

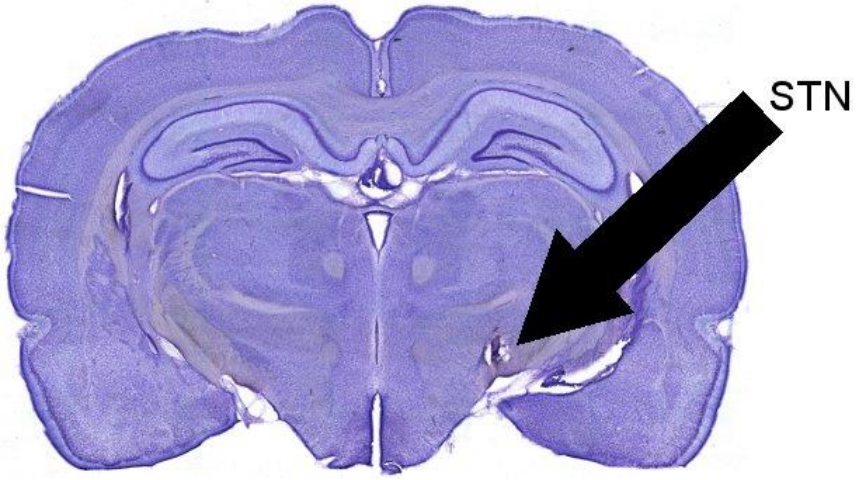


Figure 2 – Spectrogram of EEG signal and concomitant nuchal EMG signal showing periods of wakefulness and drowsiness.

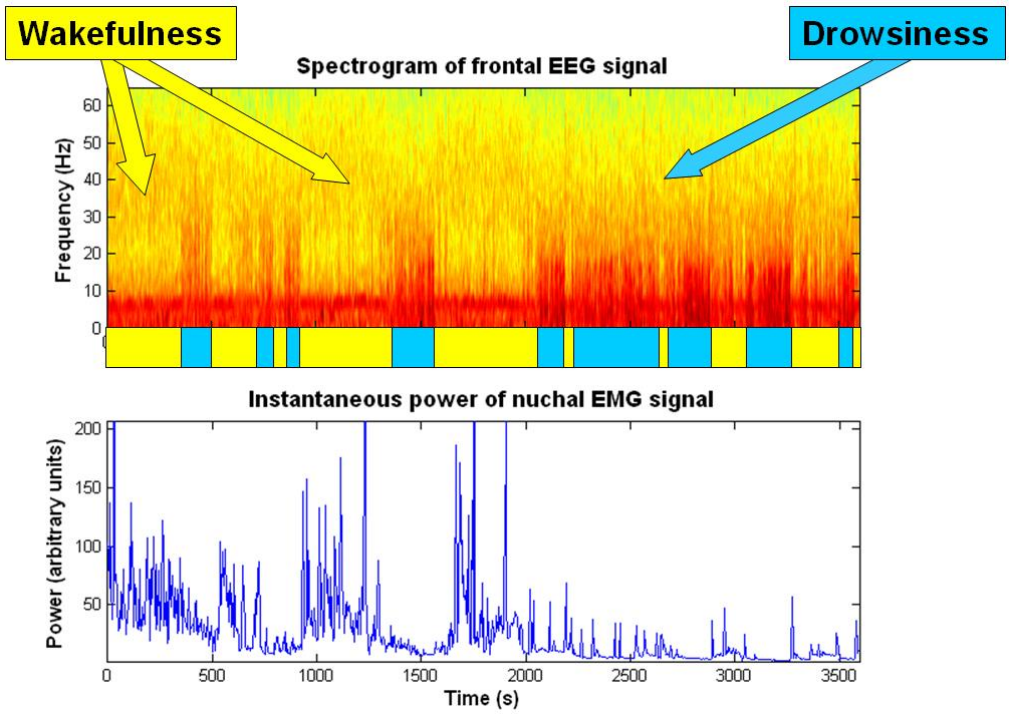


Figure 3 – Histology from anesthetized-recordings project. (A) Sample tyrosine hydroxylase (TH) stained section of HP rat showing lesioned left striatum, (B) sample TH stained section of transplanted rat showing lesioned left striatum with surviving TH-positive cell transplant, (C) Higher magnification of boxed area in (B), (D) TH stained section confirming denervation of left substantia nigra pars compacta, (E) Immunofluorescent staining showing typical graft derived TH-positive cells and their neurites, (F) Immunofluorescent staining of the same area as (E) showing DAT-positive graft cells and their neurites, (G) Composite overlay of (E) and (F), (H) Cresyl violet stained section showing electrode track into subthalamic nucleus (STN), (I) Cresyl violet stained section showing electrode track into substantia nigra pars reticulata (SNR),. Scalebars: 1mm for A, B, D, H, I; 250um for C; 100um for E, F, G.

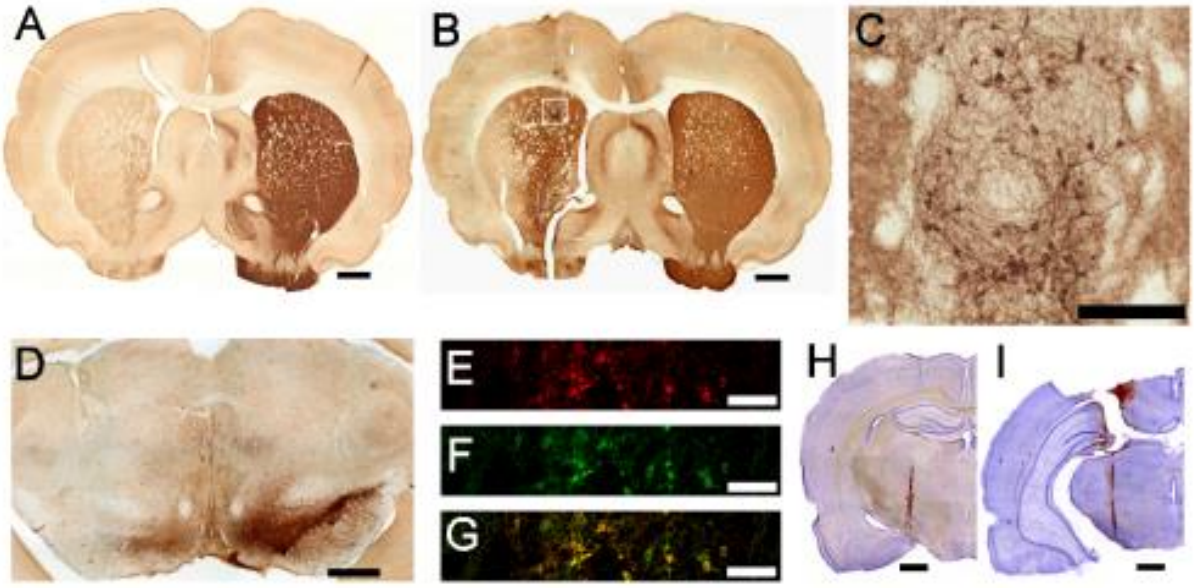
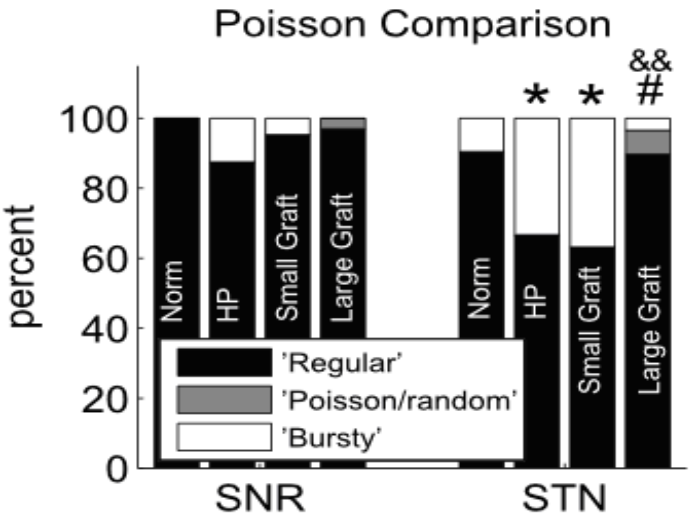


Figure 4 - Slow wave (SWS/SWA) firing rates and patterns (* $p < 0.05$, *** $p < 0.001$ compared to normal control; # $p < 0.05$ compared to HP control; & $p < 0.05$, && $p < 0.01$, &&& $p < 0.001$ compared to small graft group). Legend in E also applies to A, B, C, F, and



G.

The project had two major branches. The first branch compared anesthetized recordings from grafted and control rats, and the second branch examined chronic awake recordings from identical groups.

Anesthetized Recordings

The anesthetized recording project achieved significant discoveries and was published.

> Gilmour, TP., Piallat, B., Lieu, CA., Venkiteswaran, K., Ramachandra, R., Rao, AN., Petticoffer, AC., Berk, M., *Subramanian*, **T**., The effect of striatal dopaminergic grafts on the neuronal activity in the substantia nigra reticulata and subthalamic nucleus in hemiparkinsonian rats, *Brain*, 134(Pt 11):3276-89, 2011. PMID: 21911417

This seminal paper in a journal of impact factor of >10, showed that cell transplants have a unique and powerful effect on the basal ganglia circuit that had hitherto never been predicted in that continuous dopamine replacement locally into the striatum evokes neuronal plasticity. This plasticity appears to effect the local striatum-GP-thalamus pathways more than the direct cortex to basal ganglia pathways.

> Gilmour, TP., *Subramanian***, T.,** Lagoa, C., Jenkins, KW., Multiscale autoregressive identification of neuro-electrophysiological systems, *Computational and Mathematical Methods in Medicine*, 2012;2012:580795 PMID: 22400052

This paper identified yet another and completely different approach to basal ganglia modeling. We showed that MS-ARX is a powerful new tool to evaluate the cortex to basal ganglia

communication and that the hemiparkinsonian state could very well be modeled by this novel tool.

Chronic Recordings

The chronic-recordings project led to the successful defense of a PhD thesis that contains all the data from acute and chronic recordings from these animals.

> Gilmour, TP. (Thesis co-supervised by Jenkins, KW. and Subramanian, T.) Signal Processing Applications to Electrophysiology of Continuous Dopaminergic Stimulation Therapy in Preclinical Parkinson's Disease Models, PSU Press, May 2012

Several additional manuscripts were submitted and a few are currently in review. Among the reviews that are back with peer scientific comments, one paper with acute recordings that compared RPEC grafted animals to FVM grafted animals to L-dopa treated animals was criticized for the small sample size of the l-dopa treated control group. Hence, we asked for an extension of time for this project and have now completed the additional experiments. The data from this manuscript that is back from peer review is shown in figures 5 and 6.

Figure 5: Behavioral tests from L-dopa treated animals, showing behaviorally effective dose (*p<0.05, **p<0.01, ***p<0.0001)

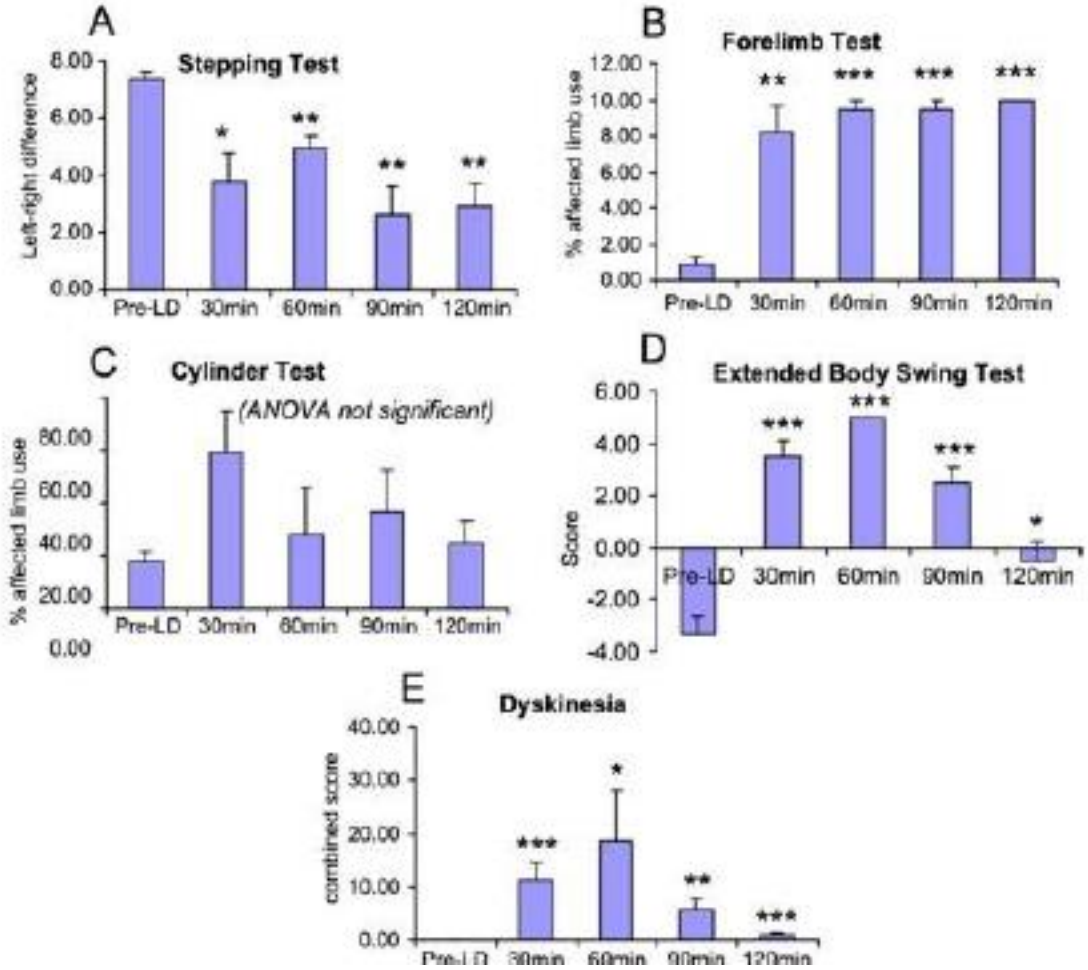
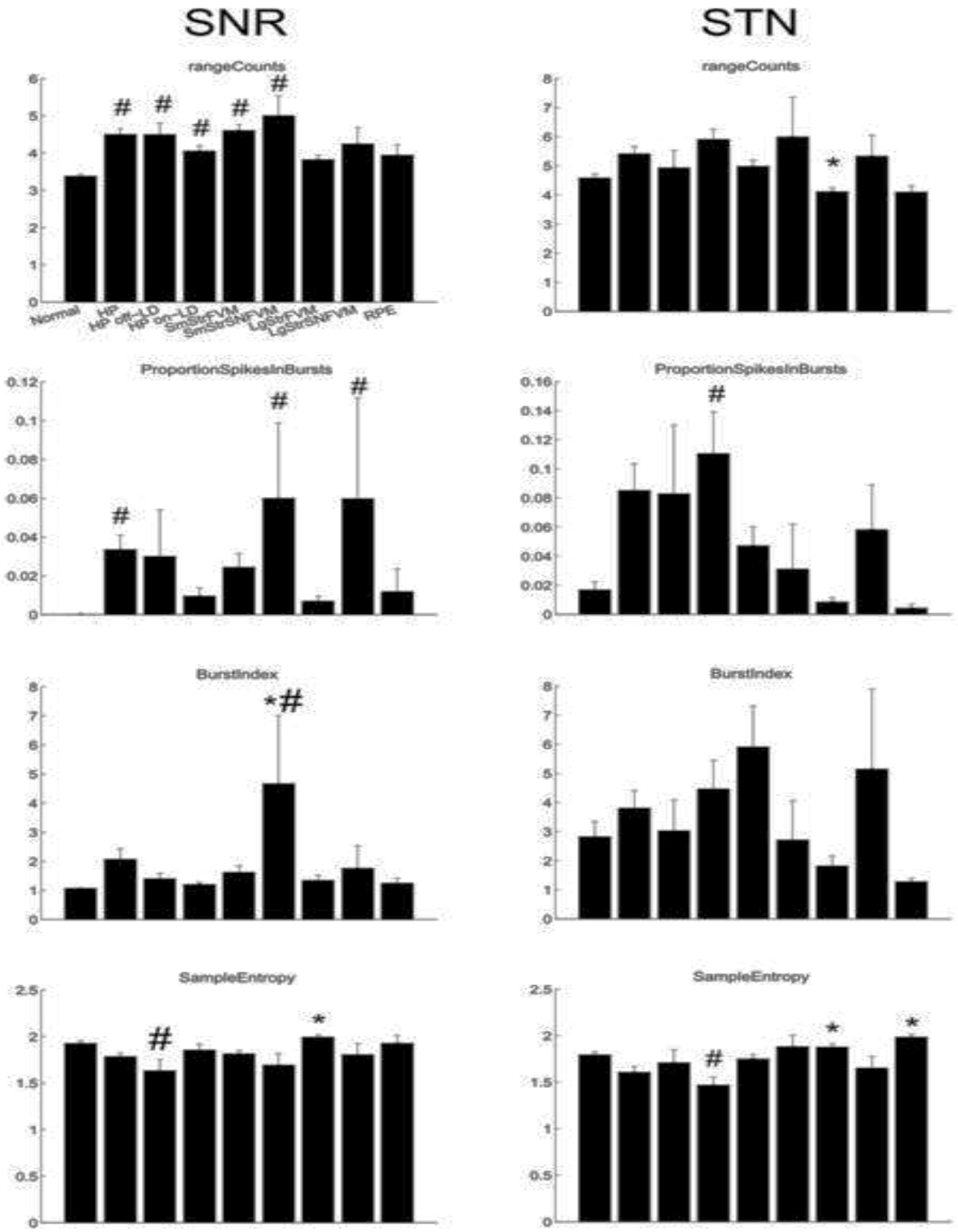


Figure 6: (Below) Electrophysiological measures of firing pattern activity in the SNr and STN comparing normal rats to hemiparkinsonian (HP) rats with and without L-dopa (LD) treatments, small fetal ventral mesencephalic (FVM) grafts and large FVM grafts in either the substantia nigra (SN) and the striatum (STR) or both, and compared to RPE grafts in the striatum alone. (# p<0.05 compared to normal control, * p<0.05 compared to HP control)


SNR
STN
rangeCounts
Normal
HP
HP off-LD
HP on-LD
SmStrFVM
SmStrSNFVM
LgStrFVM
LgStrSNFVM
RPE
ProportionSpikesInBursts
BurstIndex
SampleEntropy

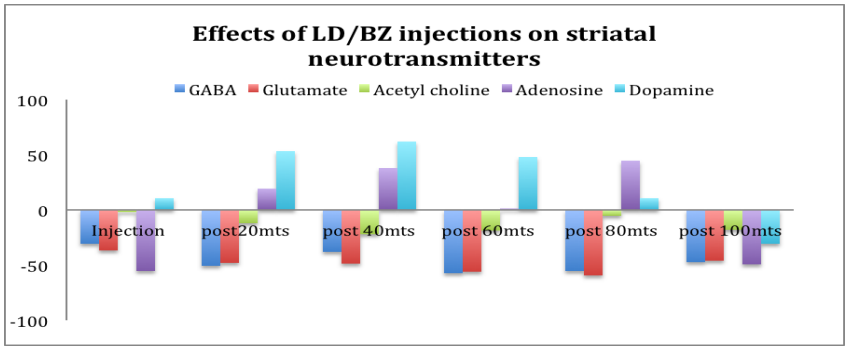


Figure 7: Panels shows % change from baseline for 9 out of 17 neurochemicals. All 17 compounds listed by Song, P., O.S. Mabrouk, N.D. Hershey, and R.T. Kennedy, *In vivo neurochemical monitoring using benzoyl chloride derivatization and liquid chromatography-mass spectrometry.* Anal Chem. 84(1): p. 412-9. were detected, we are only showing 5 out of the 17 for lack of space.

Several additional papers and abstracts resulted from this line of research. This included a new multiscale autoregression modeling of basal ganglia functioning that provided a new perspective to modeling in the basal ganglia. These ideas are discussed in our paper that is attached. Another paper examined the electrophysiological effects of L-dopa treatments in

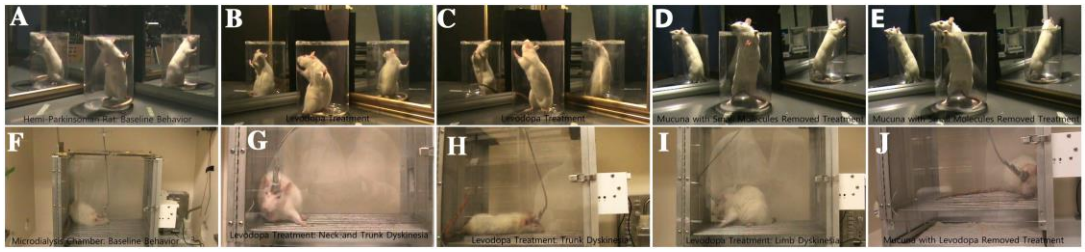


Figure 8: Stills from video files demonstrating **A.** HP rat baseline behavior in cylinder test, **B.** post LD/BZ - Neck and trunk dyskinesias, **C.** post LD/BZ - trunk dyskinesia, **D.** & **E.** *Mucuna pruriens* treated rats showing anti-PD effects and normal exploration, **F.** Baseline behavior in a microdialysis chamber, **G.** LD/BZ treated rat expressing neck and trunk dyskinesia, **H.** LD/BZ treated rat expressing trunk dyskinesia, **I.** LD/BZ treated rat expressing limb dyskinesia and **J.** *Mucuna pruriens* treated rats does not exhibit any dyskinesias for the entire duration of the experiment and for up to 24 hrs that they were videotaped. No deleterious behavioral or systemic effects noted in any animal. Body weight, behavior, fur, eating, drinking, all bodily functions remained completely normal for the entire duration of experiment and a complete GLP necropsy in all animals showed no evidence of systemic toxicity.

parkinsonian monkeys. This work came about using several of the techniques that we used in the rat model. This was published in the highly regarded experimental neurology journal. Ancillary to this line of research and the rat experiments that are described above, we were able to adopt the electrophysiology and behavior to test the effects of an anti-dyskinetic medication, *Mucuna pruriens* that has been used for over 3000 years in Ayurvedic medicine.

Two important papers, one in *parkinsonism and related disorders* that deals with the water extract of *Mucuna pruriens* that eliminates all GI toxicity and proof that this compound has ingredients other than L-dopa to produce its anti-PD and anti-dyskinetic effects. The second paper published in *evidence based alternate and comparative medicine*, the highest impact factor paper in the field of Complementary and Alternate Medicine, discussed the effects of *Mucuna pruriens* in the MPTP treated monkey model of PD and its specific effects on electrophysiological recordings.

Additional research findings that came out as publications from the funding of this grant include a state of the art review of interhemispheric connections of the basal ganglia and the immunology of cell transplantation in the brain. Finally as result of these funded research projects we were able to show that the hemiparkinsonian monkey does not develop dyskinesias, which was again published as a comprehensive and highly cited paper in parkinsonism and related disorders and we came up with a new magnetic coil that was custom built to obtain good images from parkinsonian monkeys. Since research using non-human primates has been discontinued at PSUHMC, we have focused our research in developing techniques in the 6-OHDA rat that simulate the same techniques that were used in the primate model. We have had excellent success in adopting the behavioral modeling to assess dyskinesias, perform electrophysiology in such dyskinetic animals and to perform in vivo microdialysis while the animal exhibits dyskinesias. These preliminary experiments have shown excellent success and have set the stage for us to pursue external funding from other sources.

Note: Work on non-human primates was hand in hand, as the NIH funded grant NS42402 titled "Cell transplantation in parkinsonian moneys" was parallel to the TSF funded grant for a large part of funding period. However, in the summer of 2011, the primate facility was closed due to lack of manpower at PSU to support its operation. This resulted in the PI having to transition from the dual use of rodent and primate models to exclusively rodent models of PD. As noted in many locations throughout the report, the scientific work performed within the scope of the TSF funded grant had direct impact on the parallel primate work and was synergistic to the success of both grants and this is reflected in this report. Specifically, behavioral techniques that we developed in the primates to characterize levodopa induced dyskinesias and the seminal work that led to the notion that hemiparkinsonian monkeys do not develop dyskinesias no-matter how hard one tries to induce them, significantly impacted the research at hand for the TSF grant. This led to many novel hypotheses including the notion that brain plasticity is critical to the genesis of dyskinesias and that such plastic change is not exclusive to alterations in the dopaminergic striatal receptors (D1 and D2 receptor supersensitivity) or loss of dopamine storage and release capabilities. Our studies in the rat model when combined with the insights from the monkey model provided the most interesting scientific data and conclusions from this research: i.e., that dyskinesias in PD is not a phenomenon that can be neatly isolated to nigrostriatal pathology and that network wide changes in the cortex and its connections with the basal ganglia are cardinal to the genesis of dyskinesias. The implications are that such network wide changes require at the present time the use of electrical neuromodulation as we

see in clinical practice with deep brain stimulation, but, additional work using novel pharmacological agents may allow us to add on or substitute agents that alter electrical properties of neural networks to the therapeutic armamentarium for PD.

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, the number of the publication and an abbreviated research project title. For example, if you submit two publications for PI Smith for the "Cognition and MRI in Older Adults" research project (Project 1), and two publications for PI Zhang for the "Lung Cancer" research project (Project 3), the filenames should be:

Project 1 – Smith – Publication 1 – Cognition and MRI
Project 1 – Smith – Publication 2 – Cognition and MRI
Project 3 – Zhang – Publication 1 – Lung Cancer
Project 3 – Zhang – Publication 2 – Lung Cancer

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. Multiscale autoregressive identification of neuro-electrophysiological systems.	Gilmour, TP., Subramanian, T., Lagoa, C., Jenkins, KW.,	Computational and Mathematical Methods in Medicine	September 2011	☐Submitted ☐Accepted X Published
2. The effect of striatal dopaminergic grafts on the neuronal activity in the substantia nigra reticulata and subthalamic nucleus in hemiparkinsonian rats.	Gilmour, TP., Piallat, B., Lieu, CA., Venkiteswaran, K., Ramachandra, R., Rao, AN., Petticoffer, AC., Berk, M., Subramanian, T.,	Brain	March 2011	☐Submitted ☐Accepted X Published
3. The interhemispheric connections of the	Lieu CA, Subramanian T.	Brain Res Bulletin	July 2011	☐Submitted ☐Accepted X Published

striatum: Implications for Parkinson's disease and drug-induced dyskinesias.				
4. The effects of chronic levodopa treatments on the neuronal firing properties of the subthalamic nucleus and substantia nigra reticulata in hemiparkinsonian rhesus monkeys.	Gilmour, T, Lieu, CA, Nolt, MJ, Piallat, B, Deogaonkar, M, Subramanian, T,	Experimental Neurology	August 2010	☐Submitted ☐Accepted X Published
5. Quadrature RF coil for in vivo brain MRI of a macaque monkey in a stereotaxic head frame	Roopnariane, CA., Ryu, Y-C., Tofighi, M-R, Miller, PA., Oh, S., Wang, J., Park, BS, Ansel, L., Lieu, CA., Subramanian, T., Yang, QX., Collins, CM.	Concepts in Magnetic Resonance Part B	September 2011	☐Submitted ☐Accepted X Published
6. The anti-parkinsonism and anti-dyskinetic mechanisms of Mucuna pruriens in MPTP-treated non-human primate	Lieu, CA., Venkiteswaran, K., Gilmour, TP., Rao, AN., Petticoffer, AC., Gilbert, EV., Deogaonkar, M., Manyam, BV., Subramanian, T	Evidence-based Complementary and Alternate Medicine	May 2012	☐Submitted ☐Accepted X Published
7. Immunological Challenges to Cell Transplantation in Parkinson’s disease.	Piquet, AL., Venkiteswaran, K., Marupudi, NI, Berk, M., Subramanian, T.	Brain Res Bull.,	January 2012	☐Submitted ☐Accepted X Published
8. Manual rat sleep classification in principal component space.	Gilmour TP, Fang J, Guan Z, Subramanian T.	Neurosci Lett.	September 2009	☐Submitted ☐Accepted X Published
9. A water extract of Mucuna pruriens provides long-term amelioration of	Lieu CA, Kunselman AR, Manyam BV, Venkiteswaran K,	Parkinsonism Relat Disord.	August 2009	☐Submitted ☐Accepted X Published

parkinsonism with reduced risk for dyskinesias.	Subramanian T.			
10. Dyskinesias do not develop after chronic intermittent levodopa therapy in clinically hemiparkinsonian rhesus monkeys.	Lieu, CA, Deogaonkar, M., Bakay, RAE, Subramanian, T.	Parkinsonism Relat Disord.	June 2010	☐Submitted ☐Accepted X Published

Note: The publications listed above include all research results that came out from the funding via TSF. These include publications that benefited by the symbiotic co-existence of NIH funded work and since each grant (TSF and the NIH) had research elements that complemented each other, separate publications and combined publications resulted. Needless to say, the TSF and the NIH funding were equally important for the successful publication of these results and the ongoing attempts to get additional publications out.

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 additional papers are in revision or preparation. Specifically 1 paper dealing with the results from the awake rat recordings, paper 2 dealing with transfer entropy as the key measure of neural plasticity, paper 3 dealing with dual grafting into the SNr and striatum, paper 4 dealing with the sleep effects on neuronal recordings before and after transplantation and finally paper 5 dealing with the immunology of CNS transplantation and the effects of co-grafting RPE cells are in preparation or revision. Paper 1 came back with request for new data, which we have acquired in the last 6 months of this grant. This data is being analyzed now.

4. **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.

Our seminal paper in Brain has for the first time provided a unique suggestion for the notion that neuronal plasticity in the brain may be critical for the prevention and amelioration of drug induced dyskinesias. Clinical trials to test this notion are being contemplated now.

5. **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.

Although this was not the primary goal of the research funded in this grant, the techniques we were able to develop have led to the further characterization and fractionation of *Mucuna pruriens* water extract. Our paper on evaluating the electrophysiological consequences of using Mucuna water extract in the parkinsonian rat has provided compelling data to test Mucuna in PD patients as an anti-dyskinetic agent. This is now underway.

6. **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_____ No_____X____

If yes, please describe your plans:

7. **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	POSITION TITLE
Thyagarajan Subramanian, MD	Professor of Neurology and Neural and Behavioral Sciences. Director, APDA Movement Disorders Center
eRA COMMONS USER NAME (credential, e.g., agency login) 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. 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 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, perform electrophysiology experiments, behaviorally assess using operant conditioned tests, and 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. 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 both animal models of PD and in patients with PD. I also have the advantage of a joint appointment in the department of electrical engineering at Penn State University (main campus) and a close collaboration with the former EE chairman, and signal-processing expert, Dr. Ken Jenkins and many others in the EE department. Through this collaboration, I co-supervised a EE graduate student in my laboratory to develop novel signal processing techniques from neuronal brain recordings. We have also recently discovered ways to perform *in vivo* microdialysis in parkinsonian rats and to simultaneously measure from a single 5µl sample 17 different neurotransmitters. This method is fully incorporated in our lab along with the use of optogenetics to selectively inhibit or stimulate various basal ganglia nuclei and to assess the effects of cell transplants, drug treatments and electrophysiological interventions in animal models of PD.

B. Positions and Honors

Positions and Employment

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

Other Experience and Professional Memberships

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

ELECTED OR NOMINATED POSITIONS

2005-2009 Executive Committee Member, Interventional Neurology Section, American Academy of Neurology (AAN)
2004-2006 Educational Committee Member, American Society for Neural Therapy and Repair (ASNTR)
2006-2008 Chairman, Educational Committee American Society for Neural Therapy and Repair (ASNTR)
2004-2005 Executive Committee Member, Association of Indian Neurologists in America (AINA)
2006-2009 Scientific Advisory Board Member, Penn State Neuroscience Institute, Hershey, PA
2006-2008 President, Central Pennsylvania Chapter of the Society for Neuroscience
2006-2008 Member, Institutional Review Board A, Penn State Hershey Medical Center, Hershey, PA
2007- Member, Institutional Biosafety Committee, Penn State Hershey Medical Center
2008- Member, Scientific Review Committee, PSUHMC, Penn State College of Medicine, Hershey, PA,
2010 Elected Member, Credentials Committee (2010-2013), Parkinson's Study Group (PSG).
2010 Full Member, Neurology Department Promotion and Tenure Committee, PSUHMC
2010-2012 President, Central Pennsylvania Chapter of the Society for Neuroscience
2011 Co-Chair, Parkinson's disease and Huntington's Disease Platform Presentation, INTR-11 Conference, Clearwater Beach, Clearwater, FL, May 9, 2011

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

Clinical Trial Thyagarajan Subramanian (site PI)
05/10/2009 – 5/09/2013
CD Probe: a study of the effects of botulinium toxin injections in cervical dystonia, techniques and outcomes.
Role: (site PI)

The Charles A. Dana Foundation (Wang, Jianli - PI)
10/01/09 – present
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)