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:** 26. Transplantation of Human Retinal Pigment Epithelial Cells (RPECs) in the Nucleus Accumbens of Rats

7. **Start and End Date of Research Project:** 9/1/2010 - 6/30/2012

8. **Name of Principal Investigator for the Research Project:** Patricia Sue Grigson, Ph.D.

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:

   $ 61,800

   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
Alexander, Danielle	Research Tech	40% August 2010 – July 2011	$12,386
Rao, Anand	Research Tech	40% August 2010 – July 2011	$ 9,670
Subramanian, Megha	Summer Intern	100% July and August, 2011	$ 1,849.43
St. Pierre, Jessica	Jr. Research Tech	100% August 2011	$ 680

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
Grigson, P.	PI	2%

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:
Cell Transplants into Nucleus Accumbens and Reinstatement of Drug Seeking R21DA029417-01	☒NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	11//2011	$387,750	Not Funded
Cell Transplants into Nucleus Accumbens and Reinstatement of Drug Seeking R21DA029417-01A1	☒NIH ☐ Other federal (specify:__) ☐ Nonfederal source (specify:_)	11//2012	$382,489	Not Funded

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:

If our manuscript describing these data is well received, we will apply for NIH funding to support continuation of the project. Given the cutting edge nature of this cell transplant research, publication of a related manuscript is essential.

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

As described below, completion of this basic study required three full replications. As a result, we did not have time to address many critical follow up questions. For example, we would like to identify the parameters (e.g., the number, timing, and placement of the cell transplant) that most greatly facilitate 'rescue' of drug-addicted rats from relapse. We would also like to challenge the time-course of this effect to determine how enduring the transplant-induced recovery was. In addition, we would test whether the effect was specific to responding for drug (i.e., cocaine or heroin), or whether the transplant also altered

responsiveness to natural rewards such as sucrose, for example. Finally, we have begun to identify a number of brain proteins in the prefrontal cortex that are linked to 'addiction-like' behavior for drug. Thus, we would plan to test whether rescue from drug-induced relapse via RPEC transplant is accompanied by a change in the expression of these genes and proteins in brain.

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_____ No____X_____

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

	Undergraduate	Masters	Pre-doc	Post-doc
Male				
Female				
Unknown				
Total				

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic				
Unknown				
Total				

	Undergraduate	Masters	Pre-doc	Post-doc
White				
Black				
Asian				
Other				
Unknown				
Total				

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 mechanism allowed for a collaboration between two laboratories that, otherwise, could have not occurred. This research effort advanced our knowledge and allowed the students and research technicians in the Grigson laboratory to learn more about the potential benefit (and challenges) of such cell transplants and the Subramanian laboratory to explore how these transplants might impact the development and/or recovery from addiction. These funds offer a great opportunity, particularly in this very challenging fiscal climate where NIH funding for cutting edge work of this nature is exceptionally competitive.

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.

Millions of Americans are addicted to drugs and alcohol. There is no cure and 90% of all addicts will relapse, most more than once. Addiction, however, is not a disease of will, but a disease of the brain. Studies show that chronic exposure to drugs and alcohol leads to damage to dopaminergic neurons in the 'reward pathway' that projects from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) and this damage is thought to contribute to, if not to mediate, the signature symptom of addiction: chronic relapse. In a preliminary study, we found that a single transplant of 20K/side levodopa (L-dopa) producing retinal pigment epithelial cells (fhRPECs) into the medial shell of the NAc rescued drug-experienced rats from reinstatement of cocaine-seeking. This effect was quite striking when tested following 14 days of abstinence. The effect, however, needs to be replicated. In addition, given the chronic nature of the disease, we must examine whether the transplant also is effective at a later time point (e.g., after 30 or 60 days of abstinence). Finally, while we obtained effectiveness with a transplant of 20K RPECs/side, we think it important at this early juncture to examine the effectiveness of a larger transplant (i.e., 30K/side), particularly since we will be challenging the effectiveness of the transplant at longer time points after drug exposure. If a dose response is critical, this would be vital to determine as either underdosing or overdosing could be potentially deleterious to obtaining efficacious results in cocaine addiction. Such a dose response has been seen in many

other transplant situations including Parkinson's Disease where a threshold dose is necessary to see benefits. *Specific Aim 1*, then, will use rats to test whether a transplant of 20K or 30K fhRPECs into the nucleus accumbens will prevent reinstatement following 14, 30, or 60 days of abstinence. *Specific Aim 2* will use instrumental responding for water to rule out a motor impairment and instrumental responding for sucrose to rule out a general motivational deficit. Finally, *Specific Aim 3* will use high performance liquid chromatography (HPLC) and tyrosine hydroylase staining to test the hypothesis that increases in dopamine mediate the protective effects of the transplant. Addiction afflicts millions of Americans, costs hundreds of billions of dollars/year, and is a devastating life-long illness. Rescue of the drug-damaged reward pathway via fhRPEC transplant holds great promise as a novel potential therapeutic intervention.

Anatomically, the dopaminergic 'reward pathway' parallels the dopaminergic motor pathway, Yet, in comparison to the myriad of studies designed to treat parkinsonian symptoms with dopaminergic transplants of one sort or another, we are not aware of any dopaminergic cell transplantation experiments to 'treat' the chronic disease of addiction. In this study (9/1/2010 – 6/30/2012), we show, after three full replications, that bilateral transplants of fhRPEC into the medial shell of the NAc rescue rats with a history of high rates of cocaine self-administration from drug-seeking when returned, after 2 weeks of abstinence, to the drug-associated chamber under extinction conditions (i.e., with no drug available).

Subjects. Across three replications, 123 naïve male Sprague Dawley rats were singly housed and maintained on a 12/12 h light/dark cycle. Ad libitum access to food and water was provided and testing was conducted during the light phase of the cycle.

Phase I – Self-administration Training. All rats were implanted with intrajugular catheters, as described by Grigson and Twining (2002). After recovery, rats were allowed to self-administer cocaine (0.33 mg/infusion) during daily 2-hour sessions on fixed ratio 10 (FR10; Trials 1-6) and then FR20 (Trials 7-13) schedules of reinforcement across 13 once-daily trials.

Phase II -Vehicle/fhRPEC Transplantation. Following 13 trials of cocaine self-administration training, rats were matched on the number of cocaine infusions averaged across terminal Trials 12 and 13 and divided into Low and High drug takers (Low drug-takers averaged 16 or less infusions/2 h, while High drug-takers averaged 17 or more infusions/2h). Low and High drug-takers were then extinction tested for cocaine-seeking after 2 days of abstinence or after 14 days of abstinence. For those tested after 14 days of abstinence, half received bilateral transplantation of fhRPEC (20K cells/hemisphere) and half empty beads (Vehicle) into the medial shell of the NAc 7 days prior to the extinction test on Day 14. Some experimental subjects were lost due to failed catheter patency. Others failed to survive the transplant surgery, or had poorly placed beads (for the vehicle controls) or fhRPEC grafts. The final sample sizes were the following: Day 2 Low: n=12; Day 2 High: n=21; Day 14 Vehicle Low: n=7; Day 14 Vehicle High: n=16; Day 14 fhRPEC Low: n=6; Day 14 fhRPEC High: n=22.

Transplantation into the NAc: fhRPECs were grown in tissue culture as we have previously described (Subramanian et al., 2002). On the day before transplantation, the monolayer was scraped and cells were allowed to attach to pre-hydrated microcarriers as we have previously

described (Subramanian et al., 2002). Once anesthetized with Sodium Pentobarbital, rats were placed in the stereotax with the skull level and the tooth bar set at -5.0. All NAc transplants were delivered at a rate of 1µl/min using a 10µl Hamilton syringe attached to a 27gauge needle, with an inner diameter of 0.21mm. Empty beads or fhRPEC (20K/hemisphere in 1µl) were transplanted bilaterally at the following coordinates: AP = +2.7, ML = +/-1.2, DV = -6.8 from dura. Twenty thousand cells/hemisphere appeared a good starting point, given that 20,000 VTA neurons reportedly project into the NAc (Nair-Roberts et al., 2008). The wound was sutured and the rats were returned to their home cages.

Phase III – Extinction Testing. During the drug-seeking test, which occurred either 2 or 14 days after the final drug self-administration trial, all rats were placed in the experimental chamber for a 2 h extinction session and testing proceeded as described in self-administration training, with the exception that no drug was delivered. The number of infusion attempts, then, served as the dependent measure.

Results. Terminal number of infusions of cocaine/2h. As shown in Figure 1, High drug-takers took more infusions of cocaine during the terminal trials (i.e., averaged across Trials 12 and 13) than did Low drug-takers and this was true for all three groups (i.e., for subjects for whom extinction, ultimately, would be tested following 2 (group Day 2) or 14 days of abstinence, with vehicle (group Day 14 Veh) or fhRPEC (group Day 14 fhRPEC) transplant, ps < .05. That said, High drug-takers who later would serve in the Day 14 fhRPEC condition did take significantly fewer infusions than did high drug-takers who later would be extinction tested following only 2 days of abstinence. Although these groups initially were matched on this behavior, this group difference is now evident due to the post hoc elimination of a number of subjects due to lost catheter patency, surgery, or poorly placed transplants.

Mean number of Infusion Attempts/2h. Figure 2 shows the mean number of infusion attempts/2 h extinction session for rats with a history of low and high drug-taking when tested following 2 or 14 days of abstinence. Rats in the 14 day group were transplanted, in the interim, with either empty beads (Veh) or fhRPECs. As shown in the figure, greater cocaine seeking was evidenced by rats with a history of high vs. low drug-taking when tested after either 2 (group Day 2) or 14 days of abstinence (group Day 14 Veh), ps < .05. This contrasts with findings for the rats having received the fhRPEC bilateral transplants into the medial shell of the NAc where the mean number of infusions attempts/2 h did not differ between rats with a history of low or high drug-taking, p > .05. Indeed, fhRPEC transplanted rats with a history of high drug-taking exhibited significantly less seeking than did high drug-takers in either the Day 2 or the Day 14 Vehicle condition, ps < .05. Finally, although fhRPEC transplanted rats with a history of low drug-taking tended to make more infusion attempts than low drug-takers in the other conditions, these differences did not attain statistical signficance, ps > .05. Bilateral transplant of the L-Dopa-producing fhRPECs into the NAc, then, serve to rescue rats with a history of high cocaine self-administration from cue/context-induced reinstatement when tested following a 2 week abstinence period. Indeed, these fhRPEC transplanted subjects perform like low drug-takers.

Histology. Day 2 animals were not subject to detailed histology as they did not have grafts (N=33). However, they were used as controls for all the histological analysis for immunohistochemistry. Histology was conducted on 45 out of 51 (88%) of the rats that received

RPEC or Vehicle transplants and 12% (N=6) were saved for dopamine analysis via HPLC. Transplants were found to be located in the shell and/or the core of the NAc in both sides in all transplanted animals that had behavioral recovery from cocaine seeking. A representative coronal section stained with cresyl violet through the NAc is shown in Figure 3 below. Rats that received only unilateral accurate grafts did not exhibit behavioral benefits and were excluded from the analysis. Quantitative estimation of fhRPEC graft locations in each animal using unbiased stereology revealed each graft site to have 19433+/- 421 fhRPEC cells. In this context, it is important to note that these human origin cells were transplanted into outbred rats without any immunosuppression.

Immunohistochemistry for Tyrosine hydroxylase (TH) and Dopa decarboxylase (DDC). TH is the rate limiting enzyme in the synthesis of dopamine and, as such, is indicative of dopamine positive cells. Examination of the graft sites using TH showed the presence of TH positive fhRPEC cells attached to beads located within the NAc. In contrast, the empty beads in vehicle treated animals located in the NAc had no TH positive profiles (Figure 5). Design based unbiased stereology of TH positive cell bodies in the VTA was performed. Total counts for TH in the VTA of cocaine inexperienced rats was as predicted a mean 18,098 cells/hemisphere (similar to what has been reported in the literature, N=2). In comparison cocaine experienced rats that received the empty microcarrier vehicle transplant (Vehicle Only), showed a significant reduction (73%) in dopaminergic neurons in the VTA. Cocaine experienced rats that received bilateral fhRPEC transplants showed better preservation of VTA TH cell counts (40% reduction) that was statistically significant, Student t test at $p<0.035$, N=10 each, fhRPEC and vehicle treated animals (Figure 4). These data suggest that there are benefits to fhRPEC graft placement into the NAc in causing corresponding VTA dopaminergic neurons to have improved survival. No differences were noted in the TH staining between the low drug takers and the high drug takers among the fhRPEC transplanted animals or in the vehicle treated animals.

DDC staining demonstrated no differences at the level of the VTA. However, in the NAc there was a clear difference in DDC immunostaining. Compared to Day 2 animals that did not receive any transplants, both fhRPEC and vehicle treated animals had increased expression of DDC in the NAc. More importantly, the vehicle treated animals had a greater increase in DDC expression compared to fhRPEC transplanted animals that was statistically significant, $p<0.0003$, N=10 each for fhRPEC and vehicle groups (Figures 6 and 7).

The most likely mechanistic explanation of the beneficial effects of NAc grafts on the TH positive neurons in the VTA is via the retrograde transport of beneficial trophic factors secreted by the fhRPEC grafts that are transported via the remaining intact VTA-NAc axons and their neuritic processes. The relative down regulation of DDC expression in animals that received bilateral fhRPEC grafts compared to vehicle grafted animals suggests that functional fhRPEC grafts may be causing behavioral benefits that we observed via ameliorating the dopamine deficit in cocaine experienced rats. Whether the observed behavioral benefits are mediated by the improved survival of dopaminergic neurons in the VTA-NAc pathway alone or whether there is a contribution from the exogenous fhRPEC graft derived dopamine secretion cannot be distinguished in the present study. The finding of improved TH cell survival in the VTA combined with reduced DDC expression in the NAc supports this dual mechanism. The planned measurement of dopamine and its metabolites in the tissue samples that we have procured from

these experiments are likely to provide some preliminary supportive data to determine the extent of dopamine production in vivo by these fhRPEC grafts in cocaine treated rats.

Another finding in these studies is the successful survival of fhRPEC in this xenograft paradigm without the need for any immunosuppression. This finding suggests that fhRPEC secretes immunomodulators that suppress the host immune response to these grafts into the brain. Such secreted immunomodulators may also play a role in the observed behavioral benefits in these rats.

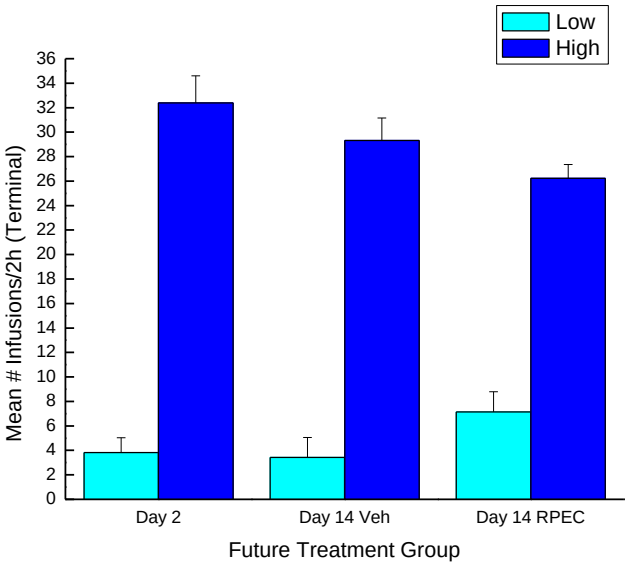

Figure 1. Mean number of cocaine infusions/2 h averaged across terminal self-administration trials 12 and 13 for Low and High drug-takers as a function of later group assignment for extinction testing (Day 2, Day 14 Veh, Day 14 RPEC).

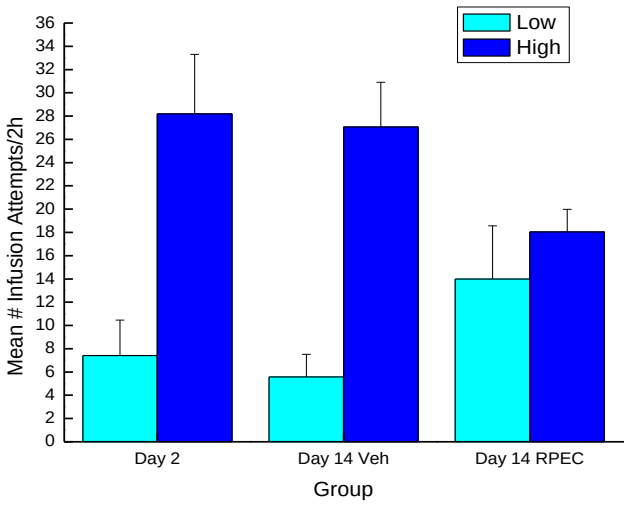

Figure 2. Mean number of cocaine infusion attempts/2 h for Low and High drug-takers for rats that received extinction testing after 2 (group Day 2) or 14 days of abstinence. Approximately half of the subjects in the Day 14 group received a bilateral empty bead vehicle transplant (group Day 14 Veh), and half a bilateral RPEC transplant (group Day 14 RPEC) into the medial shell of the NAc approximately 7 days prior to test.

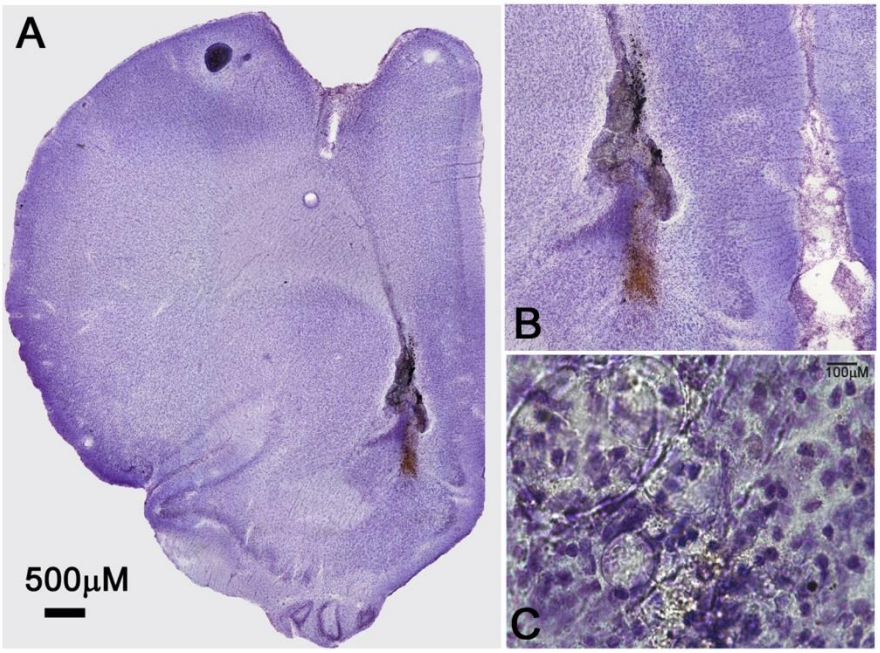

Figure 3. A. Representative coronal section stained with cresyl violet through the NAc in a rat that received RPEC transplants showing accurate placement of the graft into the NAc shell. Note the needle tract and the entry wound in the cortex. B. High power view of graft site with the collagen beads. C. Details of the graft showing viable large brownish purple cells attached to the bead matrix (round profiles). The brown color is due to the melanin pigment in these fhRPEC cells.

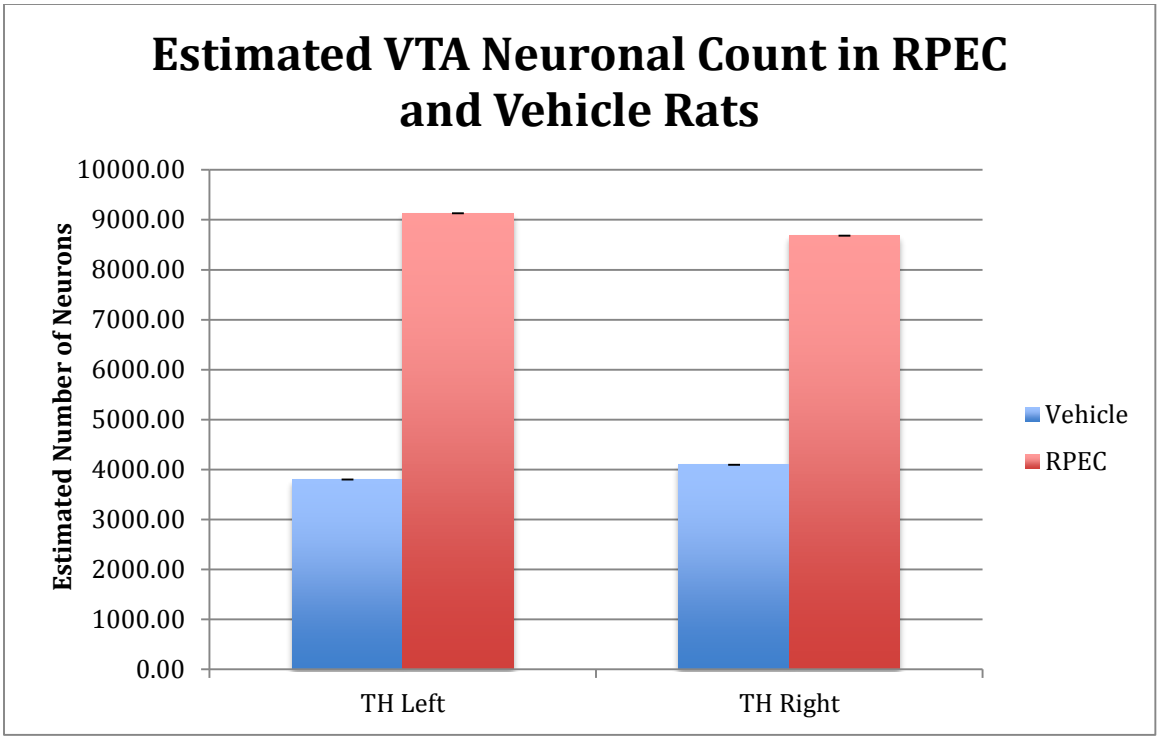

Figure 4. Design based unbiased stereology of tyrosine hydroxylase (TH) positive cell bodies in the left and right VTA. TH is the rate limiting enzyme in the synthesis of dopamine and, as such, is indicative of dopamine positive cells. Cocaine experienced rats that received the empty microcarrier vehicle transplant (Vehicle Only), showed a significant reduction (79%) in dopaminergic neurons in the VTA. Cocaine experienced rats that received bilateral RPEC transplants, on the other hand, showed 51% reduction relative to naïve controls that was statistically significant, p=0.03. Error bars represent CE of 0.15 and 0.18 respectively for these estimates.

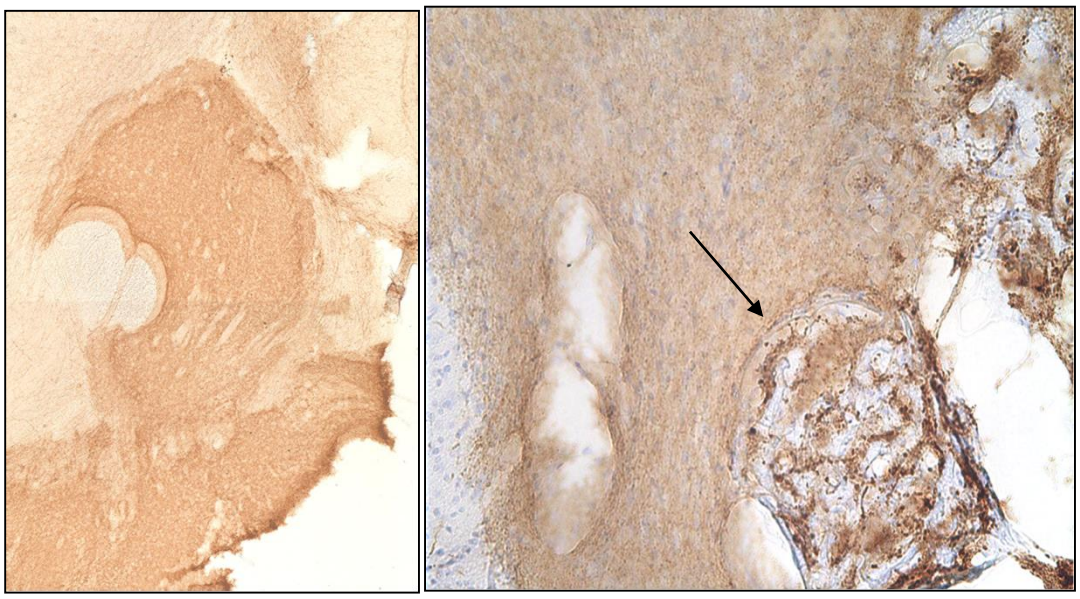

Figure 5: Left. A representative coronal photomicrograph of TH staining in the NAc of a vehicle treated rat. Right. A representative photomicrograph of TH staining from a RPEC grafted rat showing grafted beads with TH positive cells (arrow).

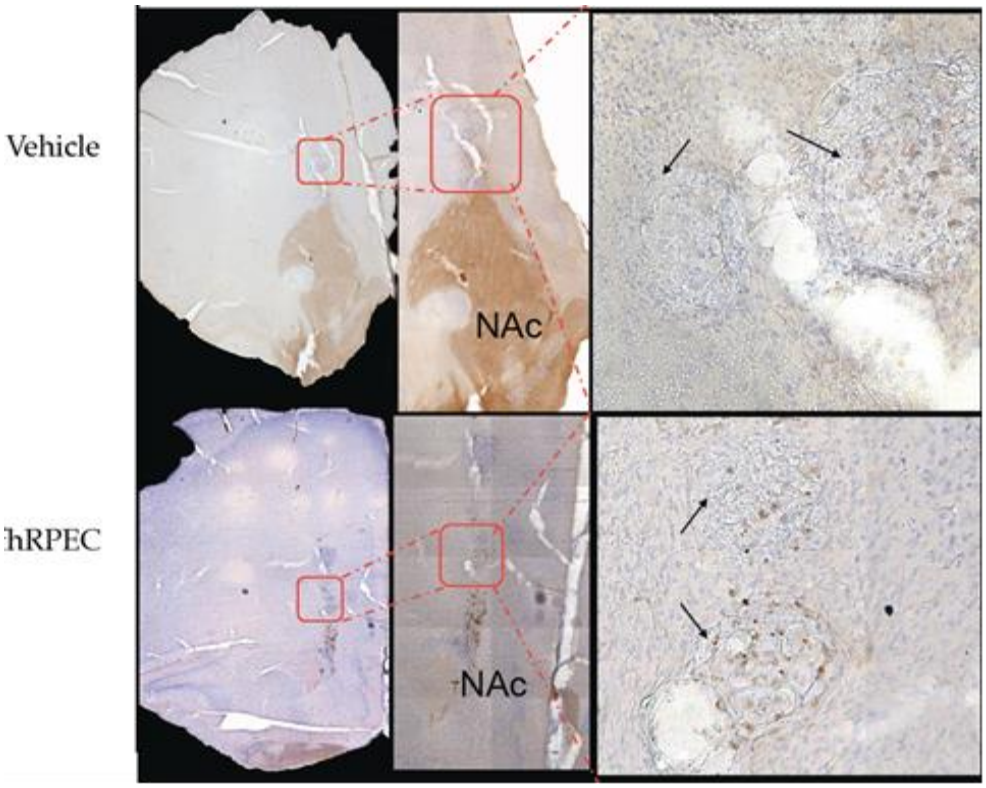

Figure 6. DDC staining in the NAc and adjacent needle tract in representative examples counter stained with cresyl violet. Note the difference in DDC staining in the vehicle treated animal versus the fhRPEC treated animal

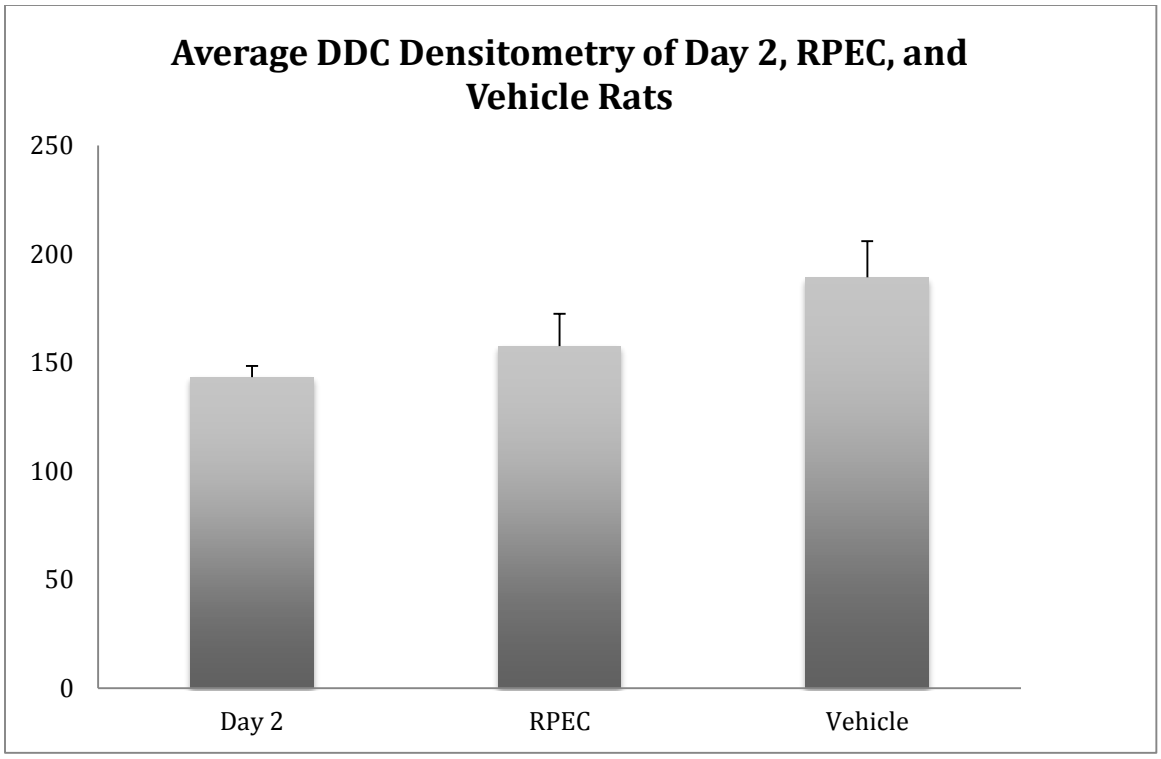

Figure 7: Quantitative DDC densitometry using NIH J image program and unbiased raters who were unaware of the tranplant status of each set of tissue sections show objective differences between the day 2 animals (no transplants or surgery) versus fhRPEC transplanted animals versus vehicle treated animals, N = 10 each, $p<0.0003$ for fhRPEC treated animal versus vehicle treated animals.

References

Grigson, P.S. and R.C. Twining, Cocaine-induced suppression of saccharin intake: a model of drug-induced devaluation of natural rewards. Behav Neurosci, 2002. 116(2): p. 321-33.

Nair-Roberts, R.G., Chatelain-Badie, S.D., Benson, E., White-Cooper, H., Bolam, J.P., and Unglass, M.A., Stereological estimates of dopaminergic, gabaergic and glutamatergic neurons in the ventral tegmenatal area, substantia nigra and retrorubral field in the rat. Neuroscience, 2008. 152: p. 1024-1031.

Subramanian, T., D. Marchionini, E.M. Potter, and M.L. Cornfeldt, Striatal xenotransplantation of human retinal pigment epithelial cells attached to microcarriers in hemiparkinsonian rats ameliorates behavioral deficits without provoking a host immune response.
Cell Transplantation, 2002. 11(3): p. 207-14.

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):
None				☐Submitted ☐Accepted ☐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:

Dr. Grigson is on sabbatical for the 13/14 academic year and Dr. Subramanian is just to begin a short sabbatical. This should allow for the final analysis of the data from all three replications and for the writing of the manuscript for publication. We would hope to submit a manuscript by spring of 2014.

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 major discovery here is that bilateral transplantation of fhRPECs, L-dopa producing cells, into the nucleus accumbens can significantly reduce cocaine-seeking behavior in high drug-taking rats following a 2 week period of abstinence. This is the first study of its kind, as such transplants have never been employed in an effort to treat drug addiction. Additionally, and as discussed, the graft was effective without the use of immunosuppressive agents. This important finding likely occurs because fhRPECs secrete immunomodulators into the brain that suppress the host immune response to these grafts.

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_____ No____X_____

If yes, please describe your plans:

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 Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Patricia Sue Grigson	Professor
eRA COMMONS USER NAME (credential, e.g., agency login) psgrigson	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Elizabethtown College, Elizabethtown, PA	B.A.	1984	Psychology
Rutgers University, New Brunswick, NJ	M.S.	1987	Psychology
Rutgers University, New Brunswick, NJ	Ph.D.	1990	Psychology
Penn State College of Medicine, Hershey, PA	Postdoc	1990-93	Behavioral Neuroscience

For the most part, scientists study either drugs of abuse or natural rewards. Dr. Grigson has decades of experience studying intake, learning, memory, and motivation as it relates to natural rewards. Over the last decade or so, she has begun to use her knowledge of natural reward systems to understand substance abuse and addiction. Given that drugs of abuse are thought to 'hijack' natural reward systems, this knowledge base is a great advantage. More than studying the responsiveness of the rat to absolute properties of natural rewards, Dr. Grigson has been trained in the study of the comparison of disparate levels of a given natural reward over time. The study of these contrast effects, as they are termed, uniquely prepared Dr. Grigson to hypothesize that rats might avoid intake of a natural reward taste cue following daily pairings with a drug of abuse because the natural reward pales in comparison to the much anticipated drug. This was a novel hypothesis that allowed for the development of a first animal model for the systematic study of drug-induced devaluation of natural rewards – a devastating consequence of addiction in humans. The natural reward, however, is not just devalued by the drug, it also serves as the most reliable and salient cue for the opportunity to take drug. As such, with the incorporation of drug self-administration into the model, Dr. Grigson has begun to use this model to study how cues might gain control of behavior to elicit drug-seeking and relapse. One primary means by which cues elicit relapse is by inducing, through experience with drug, the onset of withdrawal, and evidence suggests withdrawal is accompanied by the onset of an aversive state. Rats, it turns out, make orofacial responses following the intraoral delivery of palatable and aversive sapid stimuli and these orofacial responses can be used to test whether a stimulus is palatable or aversive (i.e., positive or negative). Dr. Grigson reported on these faces in 1997 with Drs. Grill and Norgren – the scientists who first described the behaviors in 1978. Dr. Grigson, then, was prepared to discover, along with her former students and Dr. Regina Carelli, that aversive faces (i.e., gapes) are elicited by the intraoral infusion of a drug-paired taste cue and that greater aversion predicts greater responding for drug. In this case, however, the taste reactivity behavior was measured at the end of training, following multiple taste-drug pairings. Presently, the Grigson laboratory has been examining this behavior on a trial by trial basis, beginning with the first exposure to drug. Results show that the conditioned aversive affective response occurs immediately and predicts who will take drug, when, and how much. A parallel effort has been to begin to determine the underlying neurocircuitry and, in the present case, an assessment of whether rats can be rescued from relapse for drug-taking by

transplantation of the RPECs into the nucleus accumbens.

B. Positions and Honors

Positions and Employment

1990-1993 Postdoctoral Fellow - Penn State University, College of Medicine, Hershey, PA
1993-1995 Sr. Research Associate -Penn State University, College of Medicine, Hershey, PA
1995-2000 Assistant Professor - Penn State University, College of Medicine, Hershey, PA
2000-2007 Associate Professor - Penn State University, College of Medicine, Hershey, PA
2007-present Professor – Penn State University, College of Medicine, Hershey, PA

Honors and Awards

1990-1993 Postdoctoral National Research Service Award
May, 2000 Recipient of the Annual Hinkle Society Junior Investigator Award
July, 2004 Recipient of the Alan N. Epstein Research Award, Society for the Study of Ingestive Behavior
2011 Recipient of a MERIT Award for R01 DA009815, NIDA
2013 Recipient of a Dean's Award for Excellence in Teaching

C. Selected peer-reviewed publications (out of 70)

Grigson, P.S. (1997). Conditioned taste aversions and drugs of abuse: A reinterpretation. Behav Neurosci. 111: 129-136. PMID: 9109631

Grigson, P. S., & Twining, R. C. (2002). Cocaine-induced suppression of saccharin intake: A model of drug-induced devaluation of natural rewards. Behav Neurosci. 116, 321-333. PMID: 11996317

Jones, B. C., Wheeler, D. S., Beard, J. L., & Grigson, P. S. (2002). Iron deficiency in rats decreases acquisition of and suppresses responding for cocaine. Pharmacol Biochem Behav. 73: 813-819. PMID: 12213526

Kuntz, K.L., Twining, R.C., Baldwin, A.E., Vrana, K.E., & Grigson, P.S. (2008). Heroin self-administration: I. Incubation of goal-directed behavior in rats. Pharmacol Biochem Behav. 90(3): 344-348. PMCID: PMC3636717

Kuntz, K. L., Patel, K. M., Grigson, P. S., Freeman, W. M., & Vrana, K. E. (2008). Heroin self-administration: II. CNS gene expression following withdrawal and cue-induced drug-seeking behavior. Pharmacol Biochem Behav. 90(3): 349-356. PMID: 18466961

Wheeler, R.A., Twining, R.C., Jones, J.L., Slater, J.M., Grigson, P.S., and Carelli, R.M. (2008). Cue-induced negative affect: A behavioral and neural mechanism of cocaine seeking. Neuron 57(5): 774-785. PMID: 18341996

Freet, C.S., Steffen, C., Nestler, E.J., & Grigson, P.S. (2009). Over expression of ΔFosB is associated with attenuated cocaine-induced suppression of saccharin intake in mice. Behav Neurosci. 123(2): 397-407. PMCID: PMC2819926

Grigson, P.S. (2009). Reward Comparison: The Achilles' Heel and hope for addiction. "Animal models of addiction" Drug Discovery Today: Disease Models 5: 227-233. PMCID: PMC2794208

Puhl, M. D., Fang, J., & Grigson, P. S. (2009). Acute sleep deprivation increase the rate and efficiency of cocaine self-administration, but not the perceived value of cocaine reward in rats. Pharmacol Biochem Behav. 94:262-270. PMCID: PMC2778345

Twining, R.C., Bolan, M., & Grigson, P.S.(2009). Yoked delivery of cocaine is aversive and protects against motivation for drug in rats. Behav Neurosci. 123: 913-925. PMID: 19634952 NIHMSID # 154391

Puhl, M.D., Cason, A.M., Wojnicki, F.H.E., Corwin, R.L., & Grigson, P.S. (2011). A History of Bingeing on Fat Enhances Cocaine Seeking and Taking. Behav Neurosci. 125: 930-942. PMCID: PMC3226865

Nyland, J., & Grigson, P.S. (2012). A drug-paired taste cue elicits withdrawal and predicts cocaine self- administration. Behav Brain Res 240: 87-90. PMCID: PMC3538898

Puhl, M. D., Blum, J. S., Acosta-Torres, S., & Grigson, P. S. (2012). Environmental enrichment protects against the acquisition of cocaine taking and seeking in adult male rats, but does not attenuate avoidance of a drug-associated saccharin cue. Behav Pharmacol. 23(1): 43-53. PMCID: PMC3650841

Cason AM, Grigson PS. (2013). Prior access to a sweet is more protective against cocaine self-administration in female rats than in male rats. Physiol Behav. 112-113: 96-103. PMCID: PMC3665359

Puhl MD, Boisvert M, Guan Z, Fang J, Grigson PS. (2013). A novel model of chronic sleep restriction reveals an increase in the perceived incentive reward value of cocaine in high drug-taking rats. Pharmacol Biochem Behav. 109: 8-15. PMCID: PMC3740787

D. Other Support

Active

Sponsor: NIH/NIDA R37 DA09815-09 (Renewal) Period: 5/1/11-4/30/16
Title: Drugs of Abuse and Learned Aversions: Solving a Paradox
Current DC: $192,000
Role: Grigson (PI) 40% effort
There is no overlap with the studies proposed in the Pilot application.

Sponsor: CURE Grant, PA Department of Health, SAP#4100055573 Period: 6/01/11-5/31/15
Title: A Multidisciplinary Research Paradigm for Assessing & Guiding Addiction Treatment.
Current DC: $237,914
Role: Grigson (PI) 25% effort
There is no overlap with the studies proposed in the Pilot application.

Sponsor: PA Department of Health, SAP#4100050904 Period: 7/20/12 – 12/31/13
Title: Determining the neurochemical profile of addiction in near-real-time.
Current DC: $24,001
Role: Grigson (Co-I) 2% unfunded effort
There is no overlap with the studies proposed in the Pilot application.

Sponsor: National Institute on Drug Abuse Fellowship Grant Period: 08/01/2013-07/31/2016
Title: Substance abuse: Individual differences in behavior and epigentetics
Current DC: $36,677
Role: Grigson (Mentor)
There is no overlap with the studies proposed in the Pilot application.

Pending

Sponsor: National Institutes of Health Project Period: 4/1/2014-03/31/2016
Title: Heroin Addiction: Predicting Vulnerability & Identification of a Novel Treatment
Year 1 DC: $125,000
Role: Grigson (Co-I) 5% 0.6 calendar months
There is no overlap with the studies proposed in the Pilot application.

BIOGRAPHICAL SKETCH

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

NAME Thyagarajan Subramanian, MD	POSITION TITLE Professor of Neurology and Neural and Behavioral Sciences Director, Central PA APDA Information and Referral Center and Movement Disorders Program
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 neurologist and a neuroscientist. I am the director of the movement disorders program at Penn State University that consists of 2 clinical faculty and 2 PhD scientists and several graduate students, post-doctoral fellows, research nurses and staff. I see patients in my movement disorders clinic 2 days a week.
My major area of interest is neural cell transplantation, gene therapy, basal ganglia electrophysiology, RPEC and stem cell biology and experimental therapeutics in Parkinson's Disease (PD). We routinely use brain imaging using multiple modalities, electrophysiology, stereotactic surgery, histology, microdialysis, protein chemistry, molecular biology and immunohistochemistry in my laboratory using rodent and nonhuman primate models of PD. In addition, I am involved in clinical trials for a variety of movement disorders including PD. This research project came about after a chance conversation I had with Dr. Grigson and Dr. Venkiteswaran. This has lead to a series of very interesting experiments that have lead to exciting results. We anticipate pursuing this line of research and exploring mechanistic basis for cell mediated therapies for drug addiction and other neuropsychiatric disorders.

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

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

C. Selected Peer-reviewed Publications

Wichmann T, H Bergman, PA Starr, **T Subramanian**, RL Watts, and MR DeLong. Comparison of MPTP-induced changes in spontaneous neuronal discharge in the internal pallidal segment and in the substantia nigra pars reticulata in primates. *Exp. Brain Res.,* 125 (4): 397-409, 1999

Starr, P, **T Subramanian**, RAE Bakay, T Wichmann. Electrophysiological localization of the substantia nigra in the parkinsonian primate. *J Neurosurg*. 93:704-710, 2000

Subramanian T, D Marchionini, E Potter, M Cornfeldt, Striatal xenotransplantation of human retinal pigment epithelial cells attached to microcarriers in hemiparkinsonian rats ameliorates behavior deficits without provoking a host immune response. *Cell Transplantation*, 11 (3): 207-14, 2002

Bakay, RAE, Stover, NP, Raiser, CD, **Subramanian,** T., Cornfeldt, ML, Schweikert. AW., Allen, RC, Watts, RL, Implantation of Spheramine in advanced Parkinson's disease (PD). *Frontiers in Bioscience 9:592-602, 2004*

Subramanian T., Deogaonkar MS., Brummer ME, Bakay RAE. MRI guidance improves accuracy of stereotaxic targeting of cell transplantation in parkinsonian monkeys *Exp. Neurology, 193 (1): 172-180, 2005*. PMID: 15817276

Stover SP, Bakay RAE, **Subramanian T**, Raiser CD, Cornfeldt ML, Schweikert AW, Allen, RC, Watts, RL, Intrastriatal Implantation of human retinal pigment epithelial cells attached to microcarriers in advanced Parkinson's Disease, *Arch Neurol., 62* (12): 1833-1837, 2005. PMID: 16344341

Gilmour TP, Fang J, Guan Z, **Subramanian T.** Manual rat sleep classification in principal component space. *Neurosci Lett*., 469 (1) :97-101, 2010. PMID:19944737

Lieu CA, Kunselman AR, Manyam BV, Venkiteswaran K, **Subramanian T.** A water extract of Mucuna pruriens provides long-term amelioration of parkinsonism with reduced risk for dyskinesias. *Parkinsonism Relat Disord*., Aug; 16 (7) :458-65, 2010. PMID:20570206.

Lieu, CA, Deogaonkar, M., Bakay, RAE, **Subramanian, T**., Dyskinesias do not develop after chronic intermittent levodopa therapy in clinically hemiparkinsonian rhesus monkeys, *Parkinsonism Relat Disord.,*17(1):34-9, 2011*.* PMID 21074478

Gilmour, T, Lieu, CA, Nolt, MJ, Piallat, B, Deogaonkar, M, **Subramanian, T,** The effects of chronic levodopa treatments on the neuronal firing properties of the subthalamic nucleus and substantia nigra reticulata in hemiparkinsonian rhesus monkeys*, Exp Neurol.,* 228(1):53-8, 2011. PMID 21146527

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

Lieu CA, **Subramanian T.** The interhemispheric connections of the striatum: Implications for Parkinson's disease and drug-induced dyskinesias*. Brain Res Bull.,* 4;87(1):1-9, 2012. PMID: 21963946

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

Piquet, AL., Venkiteswaran, K., Marupudi, NI, Berk, M., **Subramanian, T.** Immunological Challenges to Cell Transplantation in Parkinson's disease. *Brain Res Bull.,* 88(4): p.320-331 *2012* PMID: 22521427. PMC3376210

Lieu, CA., Venkiteswaran, K., Gilmour, TP., Rao, AN., Petticoffer, AC., Gilbert, EV., Deogaonkar, M., Manyam, BV., **Subramanian, T.,** The anti-parkinsonisn and anti-dyskinetic mechanisms of *Mucuna pruriens* in MPTP-treated non-human primate*, Evid Based Complement Alternat Med*. 2012; 2012: 840247. PMCID: PMC3445014

Research Support

Ongoing Research Support

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)

Grace Woodward Collaborative Engineering- Medicine Grant 7/1/12-6/30/14
Novel optogenetic stimulation
Co-PI: Thyagarajan Subramanian and Zhiwen Liu
No overlap

Dean's Bridge Grant: Alternatives to Primate Research. 7/1/12-12/31/13
PI: Thyagarajan Subramanian
No overlap

Ibsen 169 study Clinical trial – no overlap 7/1/13-12/31/14
Sponsor: Ibsen
Site PI: *Thyagarajan Subramanian*

Ibsen 170 study Clinical trial – no overlap 7/1/13-12/31/14
Sponsor: Ibsen
Site PI: *Thyagarajan Subramanian*

Completed Research in past 3 years

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)

NIH **RO1** NS42402 Thyagarajan Subramanian (PI)
4/01/2006-1/31/2013 (no cost extn year 2)
National Institutes of Health (NINDS)
"Intranigral transplantation in parkinsonian monkeys"
This grant will evaluate the effects of dual transplants into the striatum and into the nigra on parkinsonism and to understand the pathophysiology of cell transplants using neurophysiological assessments in the basal ganglia circuits.
Role: Principal Investigator

PSIN Pilot Grant Patricia Sue Grigson/ Thyagarajan Subramanian (Co-PI's)
10/01/2012-09/30/12
Transplantation of Human Retinal Pigment Epithelial Cells (RPECs) in the Nucleus Accumbens of Rats
This pilot project will examine the effects of RPEC grafts in cocaine addicted rats
Role: Co-PI

Clinical Trial Thyagarajan Subramanian (site PI) 09/01/2005 – 12/31/2010
PostCEPT: A long term follow up study of PRECEPT study patients (LABS-PD)
Parkinson's disease study group (PSG) and Cephalon Pharmaceuticals
A clinical study of PD patients that are longitudinally followed from early diagnosis over a period of 7 years using clinical and imaging tests
Role: Penn State Milton S. Hershey College of Medicine- Principal Investigator

Clinical Trial Thyagarajan Subramanian (site PI) 12/01/2008 – 11/30/2011
Effects of Coenzyme Q10 in Parkinson's disease-Phase 3 (QE3), PSG and NIH Thyagarajan Subramanian
A blinded placebo controlled study of co-Q10 in early PD patients
Role: Penn State Milton S. Hershey College of Medicine- Principal Investigator

Macuna pruriens endocarp for dyskinesias, 2005-2007 (no cost extension until 2010 March), National Institutes of Health (NCCAM) **R21** AT001607,
PI: **Thyagarajan Subramanian**
This grant tested the effects of the Indian ayurvedic plant product, mucuna pruriens in animal models of Parkinson's disease. These experiments tested the notion that mucuna pruriens has additional anti dyskinetic properties besides anti-parkinsonian properties.
Role: Principal Investigator

BIOGRAPHICAL SKETCH

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

NAME Kala Venkiteswaran, PhD	POSITION TITLE Assistant Professor of Neurology and Neural and Behavioral Sciences
eRA COMMONS USER NAME (credential, e.g., agency login) kvenkiteswaran	

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	B.Sc	1986	Zoology
Cochin University of Science and Technology, Kochi, India	M.Sc	1989	Biotechnology
University of Calicut, Calicut, India	Ph.D.	1999	Biochemistry
Emory University, Atlanta, GA, USA	Res Associate	2000	Exp. Pathology
Emory University, Atlanta, GA, USA	Post-doc	2001	Cell Biology
Cleveland Clinic Lerner Research Institute	Visiting Fellow	2001-05	Neuroscience
Penn State Hershey Medical Center and Hershey College of Medicine	Research Associate	2005-2006	Neuroscience

A. <u>RESEARCH EXPERIENCES (PERSONAL STATEMENT):</u>

I am a biochemist with experience and expertise in molecular biology, biochemistry and morphological quantization. My current area of interest is the neurochemistry of basal ganglia and how the chemistry modulates behavior in Parkinson's disease (PD). To answer such questions, my laboratory utilizes microdialysis, protein chemistry, behavior testing, serum and CSF chemistry, immunohistochemistry and trageted proteomics using Tandem Ms/Ms combined with HPLC. In this project, I have been intimately involved in the cell culture of fhRPEC and their preparation and characterization before transplantation and after transplantation. This work has been a valuable set of experiments that expand the application of fhRPEC beyond PD and retinal research for me and has allowed me to expand into the realm of neuropsychiatric disorders including drug addiction.

B. <u>PROFESSIONAL EXPERIENCE</u> (all full time)

1990-1994 Graduate Research Fellow, Biochemistry, University of Calicut, India 680 581.

1994-1998 Research Fellow, Department of Pathology, Emory University, Atlanta, GA 30322. (Drs. Periasamy Selvaraj Ph.D. and Demitreous Sgoutas Ph.D).

1999-2000 Postdoctoral fellow, Department of Dermatology, Emory University, Atlanta, GA 30322 (Dr. Andrew Kowalczyk, Ph.D).

2001-2005 Research Associate, Departments of Neurology and Neurosciences, Cleveland Clinic Foundation, Cleveland, OH 44195.

2005-2006 Research Associate, Department of Neurology, Penn State Hershey Med Center, PA

2006-current Assistant Professor, Department of Neurology and Neural and Behavioral Sciences, Penn State Hershey Medical Center and College of Medicine, Hershey, PA

HONORS AND AWARDS

NIH (NINDS) Travel Award for "best paper presentations by junior members" at American Society for Neural Transplantation and Repair (ASNTR), Clearwater Beach, Clearwater, FL, 2005

Albert Kligman Memorial Award, Society for Investigative Dermatology, 2000.

University Grants Commission Research Fellowship (India), 1990-1994

Valedictorian, Graduate School (Biotechnology), Cochin University of Science and Technology, Cochin, India, 1989

Valedictorian, Undergraduate Class of 1986 (Major in Zoology), University of Calicut, India, 1986

C. PUBLICATIONS RELEVANT TO APPLICATION

1. Shanavas KR, **Venkiteswaran K**, Vasudevan DM, Vijayakumar T, Yadav M. Anti-HHV-6 antibodies in normal population and in cancer patients in India. *J Exp Pathol*. 1992; 6(1-2): 95-105. PMID: 1320669

2. McHugh, R. S., **Venkiteswaran, K**., Wang Y-C. and Selvaraj P. Cells modified with lipid-anchored proteins: A novel approach to cancer vaccines. Proceedings of the Symposium on: *Frontiers in Biology and Biotechnology*, Ed. A. Oommen, pp. 26-27, 1997.

3. Bruce Sundstrom, **Kala Venkiteswaran,** Periasamy Selvaraj and Demetrious Sgoutas Oxidized lipoproteins enhance the *in vitro* tube formation by endothelial cells cultured on Matrigel. *Angiogenesis: Models, Modulators and Applications*, Edited by Maragoudakis, Plenum Press, New York, 367-375, 1998.

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PEER REVIEWED ABSTRACTS

1 K. Venkiteswaran, M. Deogaonkar, D. Dluzen, T. Subramanian, Xenotransplantation of human retinal pigment epithelial (RPE) cells into the striatum of 6-OHDA lesioned hemiparkinsonian rats causes a 10 fold increase in dopamine level, *Experimental Neurology, 198 (2): 592, 2006*

2 Lieu CA, Nyland JE, Wiley NJ, Stull AM, Venkiteswaran K, Manyam BV, Subramanian T. (2007). Effects of *Mucuna pruriens* Extract on Drug-Induced Dyskinesias in Hemiparkinsonian Rats. The 14th Annual Meeting of the American Society for Neural Therapy and Repair (ASNTR). *Cell Transplantation* (16)3: 333.

D. RESEARCH SUPPORT (Ongoing)

PSU HMC Dean's Bridge Grant: Alternatives to Primate Research. July 1, 2011 - Jan 1, 2014 (no cost extn).
PI: Thyagarajan Subramanian
This bridge grant is to develop suitable alternatives to the non-human primate for the study of LID using other species like rodents with reduced mobidity.
Role: Co-I

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: Collaborator

COMPLETED RESEARCH

1. **NIH RO1** NS42402 National Institutes of Health (NINDS) 4/01/01-1/31/2013. "Intranigral transplantation in parkinsonian monkeys" Thyagarajan Subramanian (PI). The goal of this study is to understand the effects of intranigral dopaminergic cell transplantation in parkinsonism. Using MER, microdialysis and behavioral testing in MPTP treated hemiparkinsonian monkeys we are testing the effects of transplanting fetal ventral mesencephalic dopaminergic tissue and retinal pigment epithelial cells into the striatum and the nigra. **Role: Co-investigator**

2. **Barsumian Trust Grant** (Jan 2009 – Dec. 2009) awarded by the Barsumian Trust of South Central Pennsylvania. "Understanding the immune privilege of human retinal pigment epithelial cell (hRPEC) transplants in Parkinson's disease". **Role: Principal Investigator**

3. **APDA young investigator grant** (2008 – 2009). *"Transplantation of transdifferentiated dopaminergic neurons derived from human retinal pigment epithelial cells (hRPEC)".* **PI:**.**Kala Venkiteswaran, PhD** (no salary support). The goal of this study is to test feasibility of transplantation of transdifferentiated neurons derived from hRPECs, into an in vitro slice culture model of the rat brain and to use microdialysis to study the effects of RPE transplants on basal ganglia neurochemistry in association with Dr. Andars Hajnal. **Role: Principal Investigator.**