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:** 50. Characterizing a New Mouse Model to Study Diabetes and Stroke

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

8. **Name of Principal Investigator for the Research Project:** Ian Simpson, PhD

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:

   $ 85,680

   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
Kumari, Rashmi	Director of Research Projects	50%	$21,504
Willing, Lisa	Research Technician	20%	$10,456

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
Simpson, Ian	Principal Investigator	3%
Andrea Canales	Research Intern- Summer only	100%

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
Computer Software-Anymaze System	Essential for the project. This software is now available for use by all College of Medicine personnel.	$6,804

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:

Received $60,000 - Research sub-award from Georgia Health Sciences University (DCC) Grant #12GHSU204.
Used to establish the RCS10 animal colony.

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:
Neuronal regeneration following stroke	☐NIH ☐ Other federal (specify:_____) ■ Nonfederal source (specify: American Heart Association	01/2014	$154,000	Submitted
Regenerating functional neurons in situ to treat stroke	■NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	10/2013	$554,624	Submitted

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:

We intend to resubmit our R01renewal award, R01DK075130-06 entitled "Diabetes & Stroke: Impaired wound healing in the brain" and our Amer. Diabetes Association Grant, entitled "Stroke and the Blood-Barrier" for renewal.

In both cases we propose to use RCS10 mice and will use data obtained during completion of this project to support the submission.

We will submit a grant application to the American Diabetes Assn. in July, 2014 and a revised renewal of the original R01when we have completed the control NON animal studies.

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

As indicated in response to question 11, we intend to submit two grant renewals to continue our work on Diabetes and Stoke. The original ADA grant focused on the mechanistic aspects of the impaired stroke recovery in the *db/db* mouse, which we intend to continue in the RCS10 mice. We will also incorporate such studies in our NIH renewal together with the studies looking at potential interventions for which we have tested the feasibility in the current study.

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				
Female	1			
Unknown				
Total	**1**			

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic	1			
Non-Hispanic				
Unknown				
Total	**1**			

	Undergraduate	Masters	Pre-doc	Post-doc
White	1			
Black				
Asian				
Other				
Unknown				
Total	**1**			

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.

The purchase of the Anymaze software and the assembly of the behavioral apparatus, which are now available to all personnel in the College of Medicine, provides a significant improvement in the ability of investigators here to perform and analyze behavioral experiments.

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:

We have established an active collaboration with Dr. Gong Chen who is located on the University Park Campus of Penn State to investigate the effects of a retrovirus that converts activated astrocytes to neurons on stroke recovery using the technology developed during this project.

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.

The overall objective of this project was to address specific deficiencies identified in our R01 renewal submission in which we proposed to determine the role of anti-diabetic agents on stroke outcome in a new mouse model of type II diabetes. The reviewers of the renewal application raised three significant reservations, two of which were actively addressed in this project: 1) the viability of the RCS10 mice to serve as a better model to investigate stroke outcomes than the other rodent models of Type II diabetes, 2) the failure to provide any mechanistic approaches to investigate what underlies the impaired recovery in the diabetic mice, and 3) the absence of behavioral studies to assess the distinctions in stroke recovery in the diabetic and non-diabetic mice and the ability to determine the efficacy of the proposed therapeutic interventions. Reservation 1 was addressed in Specific Aim 1 in which we proposed to obtain more preliminary data characterizing the RCS10 mouse response to stroke with a specific focus on the extent to which the inflammatory response is compromised in the diabetic mice, how this leads to a

breakdown in the blood-brain barrier (BBB), and that underlies these changes and how they are modulated by various anti-diabetic agents. In Specific Aim 2 we propose to develop a battery of behavioral tests that will enable a non-invasive assessment of stroke damage and subsequent recovery and the efficacy of therapeutic interventions.

Specific Aim 1. To investigate the inflammatory responses initiated in male RCS10 mice following a hypoxic/ischemic (H/I) insult. The hypothesis to be tested is that the increased cell loss observed in the diabetic RCS10 mice compared to their non-diabetic littermates and closely related NON mice controls following an H/I insult will result from an impairment in inflammatory responses that mediate recovery, resulting in a breakdown in the BBB.

Specific Aim 2. To develop a battery of behavioral tests to assess the extent of neurological impairment in diabetic and non-diabetic RCS10 mice following a hypoxic/ischemic insult. The hypothesis to be tested is that behavioral changes will accurately reflect differences in ischemic damage between diabetic and non-diabetic RCS10 mice following a stroke.

Our initial studies focused on drawing comparisons between the RCS10 mice that did develop diabetes with those that did not. These comparisons, illustrated in Figure 1, clearly demonstrate that the extent of neural cell loss was greater in the diabetic mice when exposed to the same duration of hypoxia as their non-diabetic counterparts and an additional two minutes of hypoxia is required to elicit a comparable level of ischemic damage. These experiments were performed on mice of between 18-20 weeks of age that had experienced a duration of diabetes of 3-5 weeks. The Stroke community has been forcefully promoting the use of adult rather than young adult animals to be used in stroke studies and we have attempted to comply by using animals that are at least 20 weeks old and diabetic animals that have 4 weeks of sustained diabetes. Unfortunately, as our RCS10 mouse colony evolved, it became increasingly apparent that the numbers of mice that remained as non-diabetic controls at 20 weeks of age and beyond were significantly reduced (our initial breeding data suggested approximately 30-40% would be diabetic) and there were too few non-diabetic controls available to be able to conduct meaningful comparative experiments. This is effectively illustrated in Figure 2, which depicts the developmental time course for the onset of hyperglycemia in the RCS10 mice and the narrow error bars reflect that 18/19 out of twenty mice became diabetic by week 20. Faced with this complication, we accepted the suggestion made by one of our R01 reviewers to use a related mouse strain, the NONShiLtJ (NON) that has an 88% genomic identity to the NONcNSO10/LtJ (RCS10) mice. These mice become equally obese as the RCS10 mice, but do not develop diabetes. In collaboration with Dr. Sarah Bronson, we have been able to establish a breeding colony for these mice and have now begun to complete the necessary comparative experiments with the RCS10 mice. These experiments are ongoing although it should be noted that it takes 6-7 months to generate each set of experimental animals and the number of successful matings, pups /litter and the ratio of males to females are variables that are far from predictable. We currently anticipate that sufficient experimental animals will be generated within the next 4-6 months to complete our characterization experiments.

In separate experiments, we did investigate the effects of normalizing blood glucose with the widely used diabetic agent Metformin on stroke recovery. In earlier experiments using the *ob/ob* mouse model of Type II diabetes we were able to demonstrate that the *ppar* gamma agonist

Darglitazone was able to restore euglycemia in the diabetic *ob/ob* mice and in doing so dramatically reduced the infarct volume (Kumari et al., 2010). As discussed extensively in the resubmission of the R01 proposal and the basis for the current proposal, these experiments could not be extended due to a change in the mouse phenotype and thus it was never clarified whether the effects were due to the restoration of euglycemia or the anti-inflammatory actions of the Darglitazone. In the current study we have investigated the actions of Metformin, an extensively prescribed agent to treat Type II diabetes. Figure 3 illustrates the ability of Metformin to establish and sustain euglycemia in the RCS10 mice over a period of four weeks, which addresses a concern raised by the original reviewers. The Metformin was administered to the mice in their water to which 0.15% saccharin was added to ensure that mice consumed sufficient metformin/water to normalize their blood sugar (0.8-1.3 g/kg/D). The saccharin levels in the water of control mice were diluted to normalize saccharin consumption. The effect of the normalization of the blood sugar on the outcome following the stroke is illustrated in Figure 4. The infarct areas were measured from H/E images as described in (Kumari et al., 2011). In contrast to the darglitazone data described above no significant difference in the extent of the infarct was observed between the uncontrolled diabetic RCS10 mice and those receiving the Metformin despite the four weeks of euglycemia. These results, while in some respects are disappointing, are comparable to those obtained by (Tureyen et al., 2007) who found no difference in outcome following metformin treatment in the *db/db* mouse. However, in those studies, the *db/db* mice were too insulin resistant to restore euglycemia and thus the observations were always equivocal. It should be noted that the levels of Metformin used in both studies is higher on a mg/kg basis than used in patients, suggesting marked difference in sensitivity between rodents and humans that was not seen with the *ppar* gamma agonist darglitazone. The studies performed in Aim 1 have addressed several concerns raised by the reviewers. We have been able to demonstrate that we are able to conduct experiments on age appropriate diabetic mice. We have also been able to maintain euglycemia over a period of 4 weeks. We are now able to breed the corresponding NON control mice and we will soon complete the necessary comparison with the RCS10 mice required to confirm that the RCS10 mice are an excellent mouse model to study Stroke and other complications associated with Type II diabetes.

Specific Aim 2.

In this aim, we proposed to establish an array of behavioral tasks to analyze the outcome of the hypoxic /ischemic insult that would ultimately be used to assess differences in outcome between the diabetic RCS10 and control NON mice. This Aim required the purchase of the Anymaze computer program video camera and designing and manufacturing the respective behavioral tests that have been reported to provide an assessment of various neural functions that are affected following a stroke. The primary objective of this Aim was to establish the methodology and viability of a battery of behavioral tests to assess stroke outcome. We elected to use C57Bl6 mice to conduct these initial experiments as these mice are substantially less expensive than RCS10 mice and eliminated the complications associated with diabetes. The data presented in Figure 5 indicates the outcomes from four such tests Corner, Cylinder, Foot Fault and SNAP that were performed on the C57Bl6 mice before they received a stroke in their right hemisphere and at various days after the insult. In the Corner test, which depends upon whisker sensitivity and the sensory motor cortex, the corticostriatal tract and the striatum, the mice enter a corner and the way they turn to come out of the corner is evaluated. In the Cylinder test, which assesses motor function, the way they support themselves (one or both paws) when they stand on hind legs is

evaluated. In the Foot Fault test, the mice walk across a grid and the frequency of specific foot faults is determined. The SNAP test consists of 7 simple assessments of the mice that include interaction, cage grasp, circling, visual placement, gait and posture, baton, and visual field, each of which are scored from 0-5 with 0 indicating best performance and 5 most impaired. The major objective of each test is to indicate the level of impairment in the mouse performance in general and the specific loss of control in the movements in the limbs contralateral to the hemisphere that has undergone the stroke. In each case the performances were markedly impaired at 2 days of recovery and a measure of recovery is seen with each task at 7 days of recovery. These tests have revealed that the foot-fault and SNAP tests were the most effective in identifying functional deficits in C57BLK/6 mice that appeared to reflect the degree of cerebral infarct. However, many more animals must be tested over longer recovery periods to firmly establish such relationships. The corner test effectively reflects severe damage and may serve as a sensitive test for therapeutic intervention and longer recovery and may be effective in delineating differences in diabetic vs non-diabetic animals. The cylinder test does not appear to reflect the extent of neurological damage. We are continuing to establish the Barnes maze and the novel object and location tests to assess memory loss following stroke. Having established methodologies for all the tests proposed in the grant with C57Bl6 mice, we will now perform the comparable experiments in the RCS10 and NON mice to assess the deficits that are attributable to the diabetes. These data will then allow us to evaluate the efficacy of therapeutic interventions that restore euglycemia to promote stroke recovery. Overall we consider that Specific Aim 2 was highly successful, as we have established the behavioral paradigms to allow us to answer the R01 reviewers concerns about the absence of behavioral studies and have successfully mastered and adopted techniques that were previously absent in our laboratory. Moreover we have effectively harnessed these same procedures to investigate the role of a retrovirus to promote stroke recovery, in C57 Bl6 mice which have been submitted to the NIH and AHA.

Kumari R, Willing LB, Patel SD, Baskerville KA, Simpson IA (2011) Increased cerebral matrix metalloprotease-9 activity is associated with compromised recovery in the diabetic db/db mouse following a stroke. J Neurochem 119:1029-1040.

Kumari R, Willing LB, Patel SD, Krady JK, Zavadoski WJ, Gibbs EM, Vannucci SJ, Simpson IA (2010) The PPAR-gamma agonist, darglitazone, restores acute inflammatory responses to cerebral hypoxia-ischemia in the diabetic ob/ob mouse. J Cereb Blood Flow Metab 30:352-360

Tureyen K, Kapadia R, Bowen KK, Satriotomo I, Liang J, Feinstein DL, Vemuganti R (2007) Peroxisome proliferator-activated receptor-gamma agonists induce neuroprotectionfollowing transient focal ischemia in normotensive, normoglycemic as well as hypertensive and type-2 diabetic rodents. J Neurochem 101:41-56.

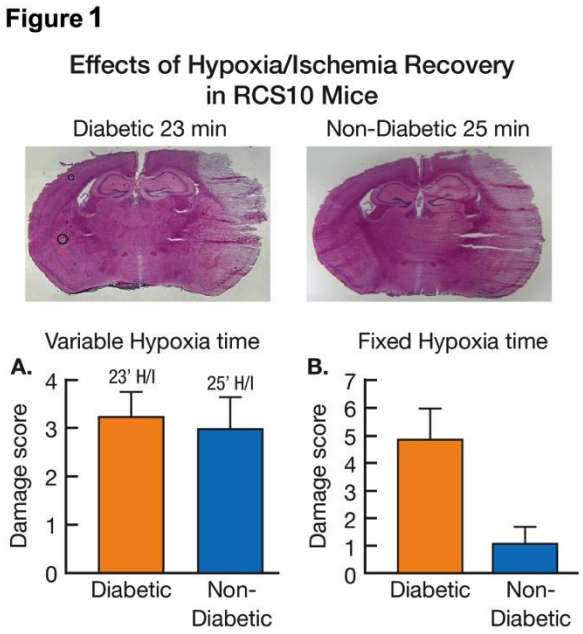

Figure 1: Upper panels illustrate typical H/E staining of diabetic and non-diabetic brains at 48 hours after H/I at a fixed hypoxia time of 23 min. (9% O2). Panel A indicates the quantification of the corresponding infarct volumes. Panel B illustrates a comparison between non- diabetic mice exposed 25 min. hypoxia (n=13) with diabetic min (n=20) exposed to 23 min. hypoxia.

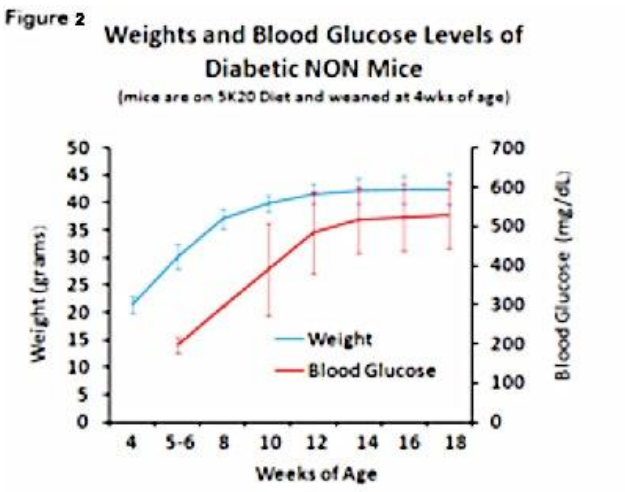

Figure 2 illustrates the relationship between the weight of the RCS10 mice and the onset of overt diabetes. Upon attaining a weight of 40 g the mice all become diabetic which occurs between 10 and 14 weeks of age.

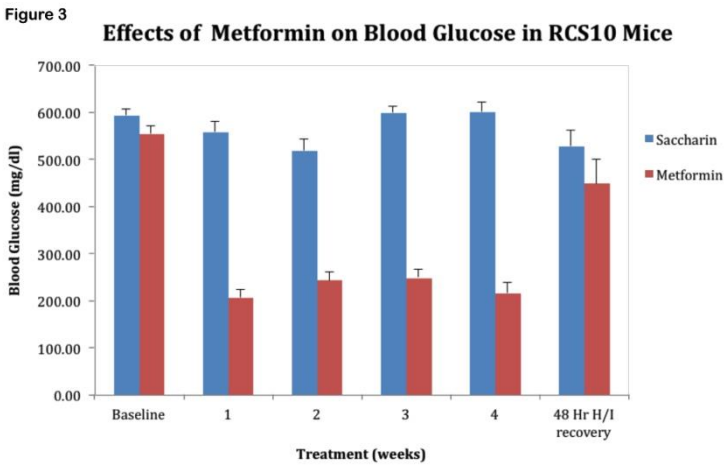

Figure 3 describes the time course for the normalization of blood glucose in the RCS10 mice. To maintain euglycemia the Metformin concentration in the drinking water was increased from 100 -120 mg/ml over the course of the 4 weeks. 0.15% saccharin was added to mask Metformin and the concentrations were adjusted to ensure equal saccharin intake in the control RCS10 mice. The Metformin was unable to maintain euglycemia following the stroke presumably due to the increased insulin resistance. The RCS10 mice were 20 weeks old when Metformin treatment was initiated and had been diabetic for at least 4 weeks.

Figure 4

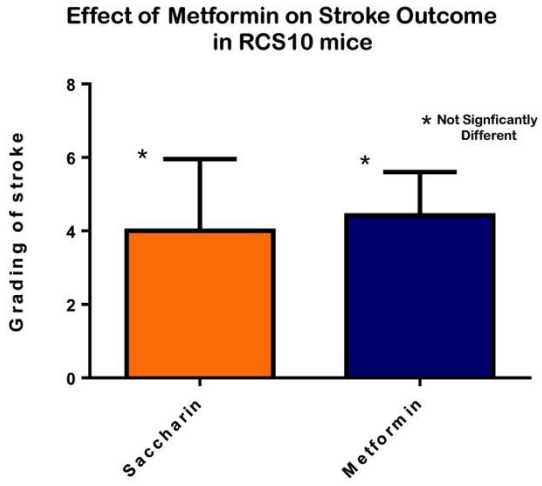

Figure 4 illustrates the effects of maintaining RCS10 mice euglycemic for four weeks on the subsequent outcome following stroke in the mice maintained as described in Figure 3. Both sets of animals were exposed to 22 min of 9% oxygen. The extent of the stroke damage was determined at 48 h post stroke infarct from H&E sections as illustrated in Figure 1. (n=8 Saccharin control mice and 15 Metformin mice). There was no significant difference in the extent of the insult between the diabetic and euglycemic mice.

Figure 5

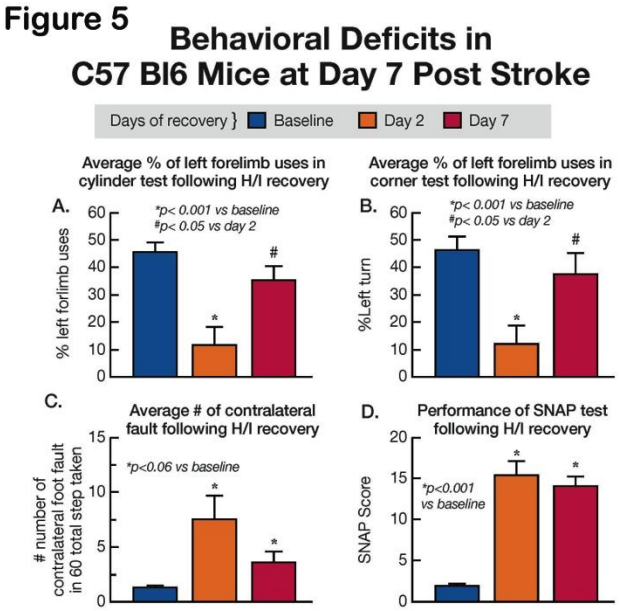

Figure 5. The studies depicted in Figure 5 were performed on 16 week-old C57 Bl6 mice (n=20). For the cylinder test a 5 min. trial period was used to observe each mouse at each time point. 5 trials at each time point were observed for the foot fault test and the corner test. The SNAP tests were conducted at each time point.

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

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

_____Yes
___X__No

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

_____Yes
__X___No

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

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

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

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

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

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

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

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

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

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

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

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

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

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

20. Articles Submitted to Peer-Reviewed Publications.

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

publication for Zhang (PI for Project 03), and one publication for Bates (PI for Project 04), the filenames would be:

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

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

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

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

We intend to submit a manuscript entitled, "A characterization of RCS10 mice as model for stroke research" to the journal Diabetes.

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.

This project has established that the RCS10 mice will serve as a better rodent model of Type 2 diabetes than currently exists and thus opens up many possibilities for future studies addressing therapeutic interventions that were hitherto unavailable with the other widely used mouse model-the db/db mouse.

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

NAME Ian A. Simpson, Ph.D.	POSITION TITLE Professor
eRA COMMONS USER NAME ISIMPSON	

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Hull College, Hull, G.B.	B.Sc.	1971	Biochemistry
University College, London, G.B.	Ph.D	1975	Biochemistry

Personal Statement

Dr. Simpson has been engaged in stroke research for the last 15 years and was instrumental in the original adaptation of the Levine hypoxia/ischemia stroke model to the diabetic *db/db* mouse. For the last twelve years has been actively engaged in investigating the role diabetes plays in stroke recovery in both rat and mouse models of diabetes. These studies have been supported by both the NIH, American Diabetes Association and Tobacco Settlement funds have utilized a variety of techniques that have included monitoring the stroke evolution by MRI and immuno-histochemistry, measurement of inflammatory cytokines mRNA and protein, investigating the role circulating leukocytes play in stroke outcome and more recently measuring behavioral outcomes following diabetic interventions. His primary responsibilities in the proposal that was submitted with Dr. Chen will be to determine the efficacy of introducing the retro-viral vector pCAG in which the Neuro D1 transcription factor has been inserted. Studies by Dr. Chen at Penn State University has extensive studies demonstrating that this retro-viral vector is able to infect activated astrocytes that are undergoing proliferation following a cerebral injury and to promote the transformation of the infected astrocytes to neurons. This study will investigate whether such transformations of activated astrocytes to neurons will: 1) survive, 2) form functional synaptic connections with indigenous neurons and 3) improve functional deficits that result from the hypoxic/ischemic stroke. The studies proposed in the study will harness many of the technologies that have been acquired by Dr. Simpson and if successful will have the potential to revolutionize approaches not only to stroke therapy but provide insights into the potential treatment of other CNS injuries such as traumatic brain injury and Parkinson's disease.

Positions and Employment

1967	Junior Research Chemist, Rentokill, East Grinstead, G.B.
1968	Hospital Technician, Huddersfield Royal Infirmary, 1968
1974-1977	Research Assoc., Muscular Dystrophy of Great Britain, Guy's Hospital Med. School, London, G.B.
1977-1979	Research Assoc., Physiologisch-Chemisches Institut, Univ. of Wuerzburg, Wuerzburg, W.Germany
1979-1982	Visiting Associate, Cellular Metabolism and Obesity Section, National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases, National Institutes of Health
1982-1985	Visiting Scientist, Cellular Metabolism and Obesity Section, National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases, National Institutes of Health

1985-1999 Visiting Scientist (Intent to Tenure), Associate Chief, Experimental Diabetes, Metabolism and Nutrition Section, formerly Cellular Metabolism and Obesity Section, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health

1999-present Professor, Dept. of Neural & Behavioral Sciences, Penn State University, College of Medicine

Honors and Awards

H.N.D. with distinction, thereby receiving membership to the Royal Institute of Chemistry, Huddersfield

Polytechnic, 1969 B.Sc. Honors in Biochemistry, Class 2, Division I, Hull University, 1971

Selected Peer-reviewed Publications

1. Vannucci, S. J., L. B. Willing, S, Goto, N. J. Alkayed, R. M. Brucklackler, T. L. Wood, J. Towfighi, P. D. Hurn and I. A. Simpson. Cerebral hypoxia-ischemia in the diabetic db/db mouse: interaction between gender and diabetes. J. Cereb. Blood Flow Metab., 21: 52-60, 2001
2. Kumari R., L.B. Willing, J.K. Krady, S.J. Vannucci, I.A. Simpson. Impaired wound healing after cerebral hypoxia-ischemia in the diabetic mouse. J Cereb Blood Flow Metab. 27: 710-718, 2007. PMID:16926846
3. Kumari R, Willing LB, Patel SD, Krady JK, Savadoski WJ, Gibbs EM, Vannucci SJ, Simpson IA The PPAR-gamma agonist, darglitazone, restores acute inflammatory responses to cerebral hypoxia-ischemia in the diabetic ob/ob mouse. J Cereb Blood Flow Metab.; 30(2): 352-60, 2010. PMCID:PMC2949120
4. Kumari R, Willing LB, Patel SD, Baskerville KA, Simpson IA. Increased Cerebral Matrix Metalloprotease -9 Activity is associated with Compromised Recovery in the Diabetic db/db Mouse Following a Stroke. J Neurochem. 119(5): 1029-40 2011 PMCID:PMC3217107
5. Kumari R, Willing LB, Jefferson LS, Simpson IA, Kimball SR. REDD1 (regulated in development and DNA damage response 1) expression in skeletal muscle as a surrogate biomarker of the efficiency of glucocorticoid receptor blockade. Biochem Biophys Res Commun. 412(4): 644-7, 2011. PMCID:PMC3175593

Ongoing Research Support

Grant 1-11-BS-146 -Simpson (PI) 01/01/2011-03/31/2014
American Diabetes Association
Diabetes, Stroke and the Blood-Brain Barrier
The overall objective of this proposal is to determine the role that matrix metalloproteinase MMP-9 plays in the increased cellular damage observed in the diabetic *db/db* mouse brain following stroke.

5R01NS077678-02 -Connor/Simpson (Multiple PI's)) 07/01/2013-06/30/2014
NIH/NINDS
Mechanisms and Regulation of Brain Iron Uptake
The goals of this project are to elucidate the mechanisms and establish that the mechanisms are regulated by signaling peptides in the CSF using cell culture and animal models.

Grant #12GHSU204 01/10/2012-03/31/2014
DCC Pilot & Feasibility Program
Stroke outcome in a new mouse model of type II diabetes: therapeutic intervention
The objective of this proposal is to determine the responses of RCS10 mice to anti-diabetic agents.

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

Dr. Grigson has several decades of experience studying intake, learning, memory, and motivation for natural rewards using rodent models. Over the last decade or so she has developed an animal model where brief access to a palatable sweet cue is followed by the opportunity to self-administer a drug, such as cocaine or heroin. In this paradigm, rats are found to greatly avoid intake of an otherwise palatable saccharin cue as it comes to predict the opportunity to self-administer cocaine. Individual differences are evident in this paradigm such that greater avoidance of the taste cue is associated with greater drug-self-administration. When infused directly into the oral cavity, the taste cue also can elicit aversive taste reactivity behaviors (i.e., gapes) and, again, a greater number of aversive faces (i.e., greater negative affect) predicts a shorter latency to take drug, greater load-up on cocaine, and faster acquisition of drug self-administration. Cues, then, can elicit an aversive state and subjects for whom this state is most aversive exhibit the greatest responding for drug. Evidence suggests that cues also can elicit the onset of an aversive state in human smokers and this aversive affective state strongly predicts relapse during a quit attempt. The present pilot study is designed to test whether random, rather than steady state, delivery of nicotine will serve to dissociate nicotine-paired cues from nicotine and thereby reduce nicotine seeking and taking behavior in humans. Dr. Grigson has reported that random delivery of cocaine greatly reduces the willingness to work for cocaine in preclinical studies. Completion of this pilot project seeks to translate this work by testing the merits of this novel random delivery hypothesis in human smokers. If accurate, random delivery will lead to reduced pleasantness with smoking, a reduction in the urge to smoke and, ultimately, a reduction in smoking. Pilot data of this nature will allow for a highly competitive resubmission of a recently reviewed Eureka grant with Drs. Wilson and Tan that was designed to test whether random nicotine delivery also would block cigarette-induced devaluation of monetary reward using fMRI. Indeed, inclusion of Dr. Jonathan Foulds, a clinical trials expert, directly addresses the primary criticism noted in that review, and the collection of much needed pilot data addresses the other. If the random delivery hypothesis proves correct, it will impact not only the treatment of smokers, but the treatment of those addicted to other substances of abuse as well.

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,
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

Selected peer-reviewed publications (out of 70)

1. 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
2. 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
3. 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
4. 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
5. 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

Ongoing Research Support:

Grant #R01DA09815-Grigson (PI) 05/01/2011-04/30/2016
Agency: NIH/NIDA
Drugs of Abuse and Learned Aversions: Solving a Paradox
Research Objective: Use a behavioral analysis to determine the mechanisms by which rats compare the rewarding properties of drugs of abuse with those of natural rewards.

Grant #SAP#4100055573-Grigson (PI) 06/01/2011-05/31/2015
CURE Grant, PA Department of Health,
A Multidisciplinary Research Paradigm for Assessing & Guiding Addiction Treatment.
Research Objective: To evaluate the role of conditioned changes in brain, behavior, and physiology in the development of addiction to heroin and to test whether these indices can be reversed following the administration of depot naltrexone.

F31 Kirschstein Grant-Grigson (Mentor) 08/01/2013-07/31/2016
NIH – NIDA Fellowship Grant
Substance abuse: Individual differences in behavior and epigenetics

Completed:

SAP#4100050904 – Grigson 07/20/2012-12/31/2013
Determining the neurochemical profile of addiction in near-real-time