SOAR Research Proposal – Summer 2014

Submitted March 11, 2014

Title of Proposed Project: Intranasal Administration of DNSP-11 in a Chronic 6-Hydroxydopamine Model of Parkinson's disease

Faculty Advisor: Cecilia M. Fox, Associate Professor of Biological Sciences

Name of Student: Adam Ghoweri (GPA: 3.31)

Purpose of Project: To determine whether intranasal administration of DNSP-11, a biological active synthetic peptide derived from the human pro-sequence of glial cell line-derived neurotrophic factor (GDNF) is protective of substantia nigra dopamine neurons against the striatal 6-hydroxydopmine (6-OHDA) rat model of Parkinson's disease.

Background and Relevance of Project:

Parkinson's disease is a progressive neurodegenerative disorder in which resting tremor, muscular rigidity, bradykinesia (slowness of movement) and impaired postural reflexes predominate. It is observed in approximately 1 % of the American population over the age of 55. Within ten years of onset, 60 % of patients diagnosed with Parkinson's disease are severely disabled or deceased.

The primary pathology of this disease is degeneration of the nigrostriatal pathway. This pathway originates in the substantia nigra of the midbrain and projects anteriorly to the striatum. As degeneration of this pathway progresses, there is a loss of substantia nigra dopamine neurons as well as depletion of dopamine and dopamine metabolite levels in the striatum. Current available therapy relieves many of the symptoms in the early to middle stages of the disease but does not arrest the advancement of the disease. Therefore, it is of significant benefit to identify possible alternative therapies in alleviating or inhibiting the progression of this debilitating neurodegeneration.

The Rat Model of Parkinson's Disease:

Many advances in our understanding of the cause of Parkinson's disease as well as insights into its treatment (for example, L-dopa therapy) have been derived from animal studies. The discovery of neurotoxins that selectively destroy dopamine neurons, such as 6-OHDA has played an important role in the study of this disorder. The discriminating effects of 6-OHDA in the rat midbrain are a result of its structural similarity to dopamine and its ability to efficiently bind to receptors on the dopamine cell membrane for subsequent entry into the neuron. Once inside the dopamine neuron, 6-OHDA undergoes a rapid auto-oxidative process resulting in the formation of several highly reactive oxygen species such as hydrogen peroxide, the superoxide radical and the hydroxyl radical. These oxidative products initiate a series of events that lead to the destruction of DNA and proteins as well as deterioration of cell membranes. In the past, my lab has focused on an intranigral 6-OHDA lesion which is capable of destroying neurons within minutes. For this study, we intend to use the more chronic model of an intrastriatal lesion. 6-OHDA would be administered in the striatum (also known as the target tissue for substantia nigra neurons) which would lead to a slowly progressive type of cell death that is analogous to the human condition.

DNSP-11:

The neurotrophic factor known as Glial Cell Line-Derived Neurotrophic Factor (GDNF) has been shown to be protective of dopamine neurons *in vitro* and *in vivo* when challenged with a wide array of

neurotoxins. However, it is a very large protein that makes it difficult to deliver into a human brain. The proprotein version of GDNF can be alternatively post-translationally processed into three independently acting peptides stretching 5, 11, or 17 amino acids in length. These dopamine neuron stimulating peptides (DNSPs) are termed DNSP-5, DNSP-11, and DNSP-17, respectively. All three propeptides are highly stable over a broad range of temperatures (-81C-37°C) and both DNSP-5 and DNSP-11 are transported into the brain regions of interest without binding heparin. This is important because GDNF binding to heparin has been shown to limit its biodistribution. While DNSP-5 and DNSP-17 have shown only limited protective properties at the cellular level, DNSP-11 significantly protects human embryonic kidney 293 (HEK 293) cells from staurosporine, a general cytotoxin that induces apoptosis, and 3-NP, a mitochondrial toxin. In addition, unlike GDNF, DNSP-11 is protective of B65 cells exposed to gramicidin, which acts to destroy the ion gradient essential to mitochondrial function. This suggests that the protective mechanism of DNSP-11 is related to preventing mitochondrial inhibition. In addition, DNSP-11 is highly homologous to rat and mouse proGDNF sequences which suggest an evolutionary conservation of DNSP-11's function and hints at its biological importance.

Last year, my lab was able to demonstrate that intranigral administration of DNSP-11 was protective of dopamine neurons in the substantia nigra (the area of the midbrain that degenerates in Parkinson's disease) against two different mitochondrial toxins (MPP+ and TaClo). In this study, we would like to examine the efficacy of administering DNSP-11 using an intranasal approach. Dr. Don Gash from the University of Kentucky has had some success with this method in acute models of degeneration. In this study, we intend to examine the protective effects of intranasal DNSP-11 in a chronic lesion model. When 6-OHDA is administered into the striatum, neurons retrogradely transport the toxin toward their cell bodies. Cell death begins one week post lesion and may continue until 20 weeks later. This approach more accurately reflects the type of degeneration experienced in the human condition of Parkinson's disease.

Proposed Project:

Design:

Animals- 20 Fisher 344 young adult male rats divided equally into the following 2 groups:

a) Intranasal saline followed by intrastriatal 6-OHDA lesion
b) Intranasal DNSP-11 (30μg) followed by intrastriatal 6-OHDA lesion

DNSP-11 – the intranasal spray will need to take place 3 times a week for the duration of the study

Behavior – All animals will be evaluated for motor function using the footfault and rotametry tests every week for 12 weeks post-surgery.

Tissue Processing- Animals will be euthanized at 13 weeks post-surgery and then the brain tissue will be processed for tyrosine hydroxylase (TH)-immunocytochemistry.

Cell Counting - TH-immunoreactive neurons in the substantia nigra pars compacta will be counted using stereology.

Due to the intense nature of this project, this SOAR study will focus on the behavior data from these experimental animals. When the fall 2014 term begins, this study will then be continued as an Honors project. Brain tissue processing, immunocytochemistry staining, cell counting via stereology and data analysis will take place in the second half of this project.

The Moravian College Institutional Animal Care and Use Committee has approved my research protocols.

Student Involvement and Faculty Responsibilities:

Adam is a strong student who will become more familiar with the implementation of the scientific method, acquisition and interpretation of relevant primary literature and data analysis through this SOAR experience. As with all my research students, I will work with them as colleagues. I will be responsible for personally teaching them the background information expressed in this proposal as well as the techniques for successfully completing this project. Adam will learn stereotaxic brain surgery, animal care, behavior testing, euthanasia, brain removal, immunocytochemistry (a specialized brain tissue staining procedure) and cell counting. Since many of these techniques are not commonplace in our teaching laboratories, I will assist them in every stage of this research process. As he becomes more comfortable, Adam will perform each of the procedures described in this proposal.

Proposed Project Timetable:

Week 1: Literature searches, familiarization with neuroanatomy and techniques of study
Tour of animal facility, introduction to guide for care and use of animals in research
Baseline behavior testing

Week 2: 6-OHDA Surgery and DNSP-11 administration

Week 3-15: Post lesion behavior testing and continued DNSP-11 administration

Week 16: Euthanasia: Intracardiac perfusion

Future Time Commitment:

Adam is aware that this project will take the length of his summer. Yet, due to the timing of procedures, I will ensure he does not exceed the "10 week" limit. There will be some weeks when he will not work 40 hours. Yet, the overall amount of time working on this project will be the equivalent of what is expected in this program.

Adam is committed to completing his project and preparing a poster for presentation at the spring 2015 Lehigh Valley Society for Neuroscience Research Symposium and Moravian College Scholars Day.

Impact of the Project – Benefits for Student, Faculty and Moravian College:

Student:

Since Adam hopes to pursue a career in biomedical research, this SOAR experience will help him develop skills of critical thinking, accurate data analysis and research presentation. I am confident this research endeavor as well as the academic opportunities at Moravian College will offer this young scholar the foundation he needs to be a competitive candidate for graduate school. I intend to have him present his work at the Lehigh Valley SfN conference as well as the NCUR conference.

Faculty:

I am currently writing the results of my earlier DNSP-11 studies for publication. I have developed a strong collaboration with Drs. Don Gash and Luke Bradley in this area of research. I also intend to write a

grant proposal to secure funding for future DNSP-11 work with Luke Bradley. This SOAR project will have a positive impact on my professional development as a neuroscientist as well as a professor at Moravian College. Successful completion of this project will ensure the presentation of the results at the annual Society for Neuroscience Conference as well as the Lehigh Valley Society for Neuroscience Research Symposium. Furthermore, as stated earlier, it is my hope to publish this and any future meaningful data gathered from my lab in peer reviewed neuroscience journals. Finally, as a college professor, I look forward to incorporating the results of this research into my Neuroscience and Physiology courses.

Moravian College:

The benefits to the college are the following –

- Increased biology faculty participation in research programs
- More opportunities for science majors to engage in scientific research
- Enhanced student interest in my Neuroscience and Human Physiology courses
- Continued growth of the Neuroscience research program
- Future opportunities for collaborative research with other institutions
- Acquisition of preliminary data for NSF/NIH funded grant proposals
- Publication of research findings in peer reviewed journals

Budget Request:

$3000.00	Student stipend
$1000.00	Faculty stipend
$ 650.00	Supplies and expenses - $500 for rats - $150 for anesthesia

Additional expenses for the project will be covered by the Department of Biological Sciences.

On-campus housing for research student is needed.

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Student Statement of Purpose

Project title: Intranasal Administration of DNSP-11 in a Chronic 6-Hydroxydopamine Model of Parkinson's disease

Student name: Adam Ghoweri

Major: Neuroscience (Cellular Neurobiology track)

Graduation Date: May 2015

Faculty Mentor: Dr. Cecilia M. Fox

On-Campus Housing: Requested

Participation Rationale:

While I have long had a strong interest in research, the opportunity to work intensely on a project has yet to present itself. If accepted to conduct this SOAR project, it will serve as an enlightening experience to prepare me as I apply to graduate programs. Throughout my undergraduate years I have studied an array of techniques to be applied when conducting research. If granted the opportunity to utilize these skills and concepts in a hands-on manner, I will hone in on what it truly means to engage in neuroscience research.

This particular research project will involve using a rodent model of Parkinson's disease. There is much that remains unknown about this degenerative disorder of the central nervous system, but through experimentation we are slowly unraveling the mysteries that underlie it. Studying Parkinson's not only appeals to me as a fascinating disease, but on a personal level as well. My grandfather suffered from the disorder until his passing in 2009. It was clear how much the condition took a toll on his motor functions and how it affected his livelihood. To work on a project that could potentially revolutionize a disease that has plagued many would make him quite proud. Additionally, the benefit of engaging in this project also comes from the experience of working with Dr. Fox.

Since transferring to Moravian College in the fall of 2012, Dr. Fox has consistently challenged my intellect and perception of what it means to be a neuroscientist. Learning from her transcends the traditional roles of student and professor. Her lectures are lively, interactive, and require students to actively think critically. In the laboratory she conducts herself in a manner to which she understands the intricate nature of each component needed to facilitate an

experiment successfully. Concurrently, she maintains an indescribable control of the lab with an ease that's seemingly effortless.

That type of control is far from accidental or luck. It's from years of hard work, dedication to a field that she's truly passionate about, and the experience she has gained through it all. She was one of my primary reasons for choosing Moravian College- I knew I would get a top-of-the-line education from not only an educator, but a role-model for whom I aspire to be like. By working alongside Dr. Fox on this project I know I will be focused, engaged, and most importantly challenged. She exudes a type of energy in her work that will only drive me to try my hardest and gain the most from this experience.

Expected Outcomes:

Ultimately, I hope to achieve what this program is offering: an experience. As stated previously, I have never engaged in a true research project before. Prior to entering graduate school, conducting research, and then questioning if research is truly the right field for me; this offers insight as to what I should expect. Perhaps I'll loathe this experience and realize that this isn't what I aspire to do. Contrary to that notion, it may solidify the idea that research is my passion. Of course I hope the latter scenario to be true. Furthermore, I want this to be a learning experience in which I refine my own skills. By the end of the program, I want to understand my project inside and out and have a comfort with my newly acquired techniques. My hope is to also present this SOAR project at Moravian's Scholar's Day, the Lehigh Valley Society for Neuroscience, or the National Conference for Undergraduate Research. Alas, the opportunity to engage in this research experience would be a privilege, to say the least.