SOAR Research Proposal – Summer 2016

Submitted by March 14, 2016

Title of Proposed Project: The Neuroprotective Potential of Curcumin in the 6-Hydroxydopamine Model of Parkinson's Disease

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

Name of Student: Loukya Kanakamedala

Purpose of Project: To determine whether intraperitoneal administration of cucumin, a biphenolic compound derived from turmeric and known to have antioxidant/anti-inflammatory properties, may protect substantia nigra dopamine neurons in 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 (Yokoyama, Uchida, Kuroiwa, Kasahara, and Araki, 2010).

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) that would lead to a slowly progressive type of cell death that is analogous to the human condition (Sauer and Oertel,1994).

Curcumin:

Curcumin, a biphenolic compound derived from turmeric, has been shown to have antioxidant and anti-inflammatory properties in animal models of neurodegeneration. It is known that inflammation is

present in the parkinsonian brain, which may be the result of enhanced glial cell responses that contribute to the destruction of dopamine neurons (Chinta and Anderson, 2004). The activation of microglia and astrocytes (types of glial cells) results in an increase production of toxic proteins, such as chemokines and cytokines. Research has shown that activated microglia also produce reactive oxygen species (ROS) and nitric oxide (NO), which are inflammatory mediators (Peterson and Flood, 2012).

Through extensive research it has been found that curcumin displays unique antioxidant and anti-inflammatory properties in treating a multitude of disorders ranging from brain cancer to Alzheimer's disease to traumatic brain injury (Hu et al, 2015). As an antioxidant, curcumin effectively protects against DNA oxidative damage in the Tg2576 mouse and lipid peroxidation in an Alzheimer rat model. It conserves glutathione (an antioxidant) levels and reduces the concentration of free radicals within Alzheimer mouse models as well as humans (Hu et al, 2015). Through a reduction of neuroinflammation, curcumin has been identified as a novel protectant.

However, there is not much research focusing on the protective effects of curcumin in parkinsonian animal models. A study examining the administration of curcumin in a mouse model of Parkinson's disease has shown to not only provide protection against a 6-hydroxyopamine lesion, but also reduce the response of astrocytes and microglia within the brain (Tripanichkul and Jaroensuppaperch, 2011). There was a significant correlation between the loss of dopamine neurons and the increased amount of glial response. At this time, there are no documented studies examining whether curcumin provides neuroprotection through the anti-inflammatory properties as a consequence of a diminished glial cell response in a rat parkinsonian model. Therefore, this study aims to investigate this mechanism of protection. It is hypothesized that curcumin will function in protecting dopaminergic neurons by specifically inhibiting glial cell responses that will, in turn, provide a reduction in neuroinflammation.

Proposed Project:

Design:

Animals- 20 Sprague Dawley young adult male rats will be divided equally into the following 2 groups:

a) DMSO (vehicle) + intrastriatal 6-OHDA lesion
b) Curcumin (20mg/kg dissolved in DMSO/day) + intrastriatal 6-OHDA lesion

Curcumin and DMSO – Animals will receive an intraperitoneal injection for 45 days

Behavior – All animals will be evaluated for motor function using the pellet-reaching task and footfault every week for 10 weeks post-surgery. Cylinder test will be administered pre-surgery and twice post-surgery.

Tissue Processing- Animals will be euthanized at 11 weeks post-surgery and then the brain tissue will be processed for tyrosine hydroxylase (TH)-immunocytochemistry (to identify the remaining dopamine neurons, glial fibrillary acidic protein (GFAP, a marker for activated astrocytes) and ionized calcium binding adapter molecule 1 (a marker for reactive microglia).

Cell Counting and Sizing- Immunoreactive neurons in the substantia nigra pars compacta will be counted using stereology and measured via the BIOQUANT Imaging System.

Due to the intense nature of this project, this SOAR study will focus on the surgical procedures and behavior testing of these experimental animals. When the fall 2016 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.

In the past, the Moravian College Institutional Animal Care and Use Committee has approved my research protocols. We will be sure to file to appropriate paperwork for project approval.

Student Involvement and Faculty Responsibilities:

Louyka is a very 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. Loukya will learn stereotaxic brain surgery, animal care, behavior testing, euthanasia, brain removal, immunocytochemistry (a specialized brain tissue staining procedure) and cell counting/measuring. Since many of these techniques are not commonplace in our teaching laboratories, I will assist her in every stage of this research process. As she becomes more comfortable, Loukya will perform each of the procedures described in this proposal.

Proposed Project Timetable: *Due to the expected length of this project, we would begin our work on May 16th.*

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 curcumin/DMSO administration

Week 3-13: Post lesion behavior testing and continued curcumin/DMSO administration

Week 13: Euthanasia via intracardiac perfusion

Future Time Commitment:

Loukya is aware that this project will take the length of her summer. Yet, due to the timing of procedures, I will ensure she does not exceed the "10 week" limit. There will be some weeks when she 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.

Loukya is committed to completing her project and preparing a poster for presentation at the spring 2017 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 Loukya hopes to pursue a career in medicine, this SOAR experience will help her develop skills of surgical care, critical thinking, 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 she needs to be a competitive candidate for medical school. I intend to have him present his work at the Lehigh Valley SfN conference as well as the NCUR conference.

Faculty:

This SOAR project will have a positive impact on my professional development as a neuroscientist as well as a professor at Moravian College. I have been studying the neuroprotection of dopamine neurons in animal models of Parkinson's disease for over twenty years. Though I have focused on growth factors and certain antioxidants, this study could take my research into a new exciting area. 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, it is my hope to publish this work 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/neuroscience 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
$ 500.00	Supplies and expenses - $400 for rats - $100 for Isoflurane anesthesia

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

On-campus housing for research student is not needed.

Works Cited:

Chinta, S., & Andersen, J. (2004). Cell in focus: Dopaminergic Neurons. The International Journal of Biochemistry & Cell Biology, 37, 942-946.

Hu, S., Maiti, P., Ma, Q., Zuo, X., Jones, M., Cole, G., & Frautschy, S. (2015). Clinical development of curcumin in neurodegenerative disease. Expert Review of Neurotherapeutics, 15(6), 629.

Peterson, L., & Flood, P. (2011). Oxidative Stress and Microglial Cells in Parkinson's Disease. Mediators of Inflammation, 1, 1-12.

Sauer, H. and Oertel, W.H. (1994). Progressive degeneration of nigrostriatal dopamine neurons following intrastriatal terminal lesions with 6-hydroxydopamine: a combined retrograde tracing and immunocytochemical study in the rat. Neuroscience, 59 (2), 401-415.

Tripanichkul, W., & Jaroensuppaperch, E. (2011). Curcumin Protects Nigrostriatal Dopaminergic Neurons and Reduces Glial Activation in 6-Hydroxydopamine Hemiparkinsonian Mice Model. International Journal of Neuroscience, 122, 263-270.

Yokoyama, H., Uchida, H., Kuroiwa, H., Kasahara, J., & Araki, T. (2010). Role of glial cells in neurotoxin-induced animal models of Parkinson's disease. Neurological Sciences, 32(1), 1-7.

Student Statement of Purpose

Student name: Loukya Kanakamedala

Major: Neuroscience-Cellular Track

Graduation Date: May 2017

Faculty Mentor: Dr. Cecilia Fox

Campus Housing: No

Participation Rationale & Expected Outcomes:

Throughout my undergraduate studies I have been given the opportunity to learn many of the techniques required in research, more specifically neuroscience research. I have always strived for the goal of medical school, however the research techniques I have learned, opened my eyes to a new path I would be able to take with me into medical school. Performing a SOAR this year will allow me to understand what it means to engage in neuroscience research. The techniques I have learned have provided me with the foundation I need, and actually engaging in the scientific research will allow me to learn how to apply the knowledge I have acquired to solve real world problems.

This SOAR project will specifically look at the mechanism of protection of curcumin, a compound found in turmeric, in parkinsonian animal models. Researchers have found the benefit curcumin has provided in protecting dopaminergic neurons, however there has not been any studies observing the method of protection it provides. Not only will this research allow me to further my understanding of performing research in a field I am fascinated by, but it will also provide me with a chance to make my grandmother proud. My grandmother was diagnosed with Parkinson's disease when I was 14 years old. At that age, I was not fully aware of the disease, but through my own research I came to understand what causes it and the methods in relieving the symptoms. Through my curiosity, I acquired a love for neuroscience and it has allowed me to find a passion within the field. To be able to work on a project that pursues my passion and can add to a search for treating the disease is personally rewarding.

Since I was a freshman, I have had my eyes set on medical school. This opportunity will allow me to understand if I would like to add research into my future career goals. I have always been fascinated with the idea of using my knowledge and skills to find a solution to a question that has been posed, however I was never given a chance to engage in a study that allows me to test my skills and challenge myself in solving the particular problem at hand. This endeavor will allow me to understand if I would like to shift my academic focus to not only include medicine, but also the innovation that lies behind research.

This experience is further improved by working with Dr. Fox, who has always pushed me to be the best I can be. She will ensure that I gain as much as I can from this experience and continuously challenge

myself while improving my knowledge and skills within the subject. This opportunity will allow me to have a greater appreciation of science and engage in a study that enhances my knowledge about the mechanism of protection by curcumin. Granted the chance, I will be able to expand my experiences in a field that I am very passionate about. The major outcome I would like to take from this project is experience within the field of neuroscience research. I want to know how it feels to be a part of community that strives to answer the many scientific problems plaguing the world. I expect to improve the skills I would have acquired from the study and understand what it means to apply my knowledge within a real world setting. Taking apart of this study will open my eyes to a field that I have learned so much about and have acquired a sense of respect for.