1. Title: The effect of exercise in Parkinson's Disease
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2. Abstract: Parkinson's disease (PD) is the most common neurodegenerative movement disorder and is characterized by the loss of dopaminergic neurons in the substantia nigra and the development of alpha-synuclein positive lewy bodies and lewy neurites in the brain and periphery. The cardinal motor symptoms in PD (rigidity, resting tremor, bradykinesia, postural instability) are well studied and can be managed to a certain extent with dopamine replacing therapies. However, there are also a host of non-motor symptoms that can negatively impact the quality of life for people with PD. Cognitive dysfunction is one of those symptoms. It is common and can progress to dementia over time. Unfortunately, how dementia develops in PD is unclear. In this project we are working to develop a new model of PD that develops dementia. In addition, we will examine whether exercise therapy using treadmill running can protect against the development of cognitive dysfunction and dementia in our new rat model.

3. Background and Rationale: Cognitive dysfunction is a common and potentially debilitating non-motor symptom in Parkinson's disease (PD) that profoundly reduces quality of life for patients and caregivers. Although much is understood about the underlying pathology associated with motor symptoms in PD, less is known about the pathophysiology and mechanisms associated with cognitive impairment in PD. The estimated prevalence of cognitive impairment in PD ranges from ~20 to 40% in the early to mid-stages and is reported to dramatically increase in up to 80% of patients who have had PD for 20 or more years. PD patients typically develop executive dysfunction early in the disease that can affect attention, strategy switching, and cognitive flexibility and then dementia dominates in the later stages. Studies show midbrain dopaminergic (DA) dysfunction can contribute to executive function impairments in PD, however, dysfunction in other neurotransmitter systems and circuitry likely drive the progression to dementia. Indeed, cholinergic structures within the basal forebrain have been linked to the development of dementia in PD. In this project we will develop a new animal model of PD with dementia in order to identify novel mechanisms, pathways, and targets for therapy. In addition, we will test the effect of exercise on cognitive function in this model.

4. Goals and Objectives: The goal of these studies is to develop a novel rat model of PD that recapitulates the development of dementia seen in patients. In addition, we will then determine the therapeutic potential of exercise on cognitive function. The objective is to develop this novel model in order to better understand the pathology and circuits involved in dementia in PD so that we begin to identify novel targets for therapy.

5. Investigative Methods: A combination of behavioral, cellular, molecular, and genetic methods will be employed to determine the effect of exercise in the alpha-synuclein preformed fibril model of Parkinson's disease.

Development of a rat model of Parkinson's disease with dementia. Rats will be injected with alpha-synuclein preformed fibrils in the substantia nigra, striatum, or in the basal forebrain. At six or nine months post injection all rats will undergo a battery of cognitive testing to determine the impact of synuclein pathology on executive function, spatial memory, working memory, and habit learning.

Treadmill Training. Separate cohorts of alpha-synuclein monomer control or PFF-injected rats will be exposed to treadmill training. Rats will be tested during the dark cycle (rats are housed in a reverse light/dark cycle) and will build up to a running rate of 10 meters/min for 20 minutes per day, 3x per week. Rats will then be behaviorally tested to determine the effect of the exercise on cognitive function. In the brain alpha-synuclein accumulation and neurodegeneration of the nigrostriatal dopaminergic system will be determined.

Alpha-Synuclein Accumulation (brain). Soluble and insoluble alpha-synuclein protein will be measured using both immunoblot and immunohistochemistry techniques. For immunoblot fresh frozen tissues will be homogenized and subjected to successive freeze-thaw cycles. Lysates will then be centrifuged and supernatants will be collected as the soluble fraction. The remaining pellet will be resuspended in a SDS-based lysis buffer, boiled and sonicated. Lysates will be centrifuged and the supernatants collected as the insoluble fraction. Protein from each sample is fractionated on gels and then transferred to membranes. The membranes are incubated with primary antibodies for alpha-synuclein. The membranes are developed using ECL Plus Western Blot Detection Kit. For immunohistochemistry, free-floating coronal sections will be collected for analysis. Sections will be processed with primary antibodies and for controls, sections will be incubated with the corresponding IgG at the same concentration as the primary antibody. The avidin-biotin complex method will be used to detect the secondary antibody and the reaction product visualized by DAB.

6. Proposed Method of Data Analysis: A combination of parametric and non-parametric statistics will be used to analyze the behavior and tissue data. For parametric statistics, ANOVA will be used to compare Monomer and alpha-synuclein preformed fibril injected groups on each of the cognitive tests. Similarly, for the exercise study rats receiving treadmill training or stationary on treadmill will be compared. Post hoc comparisons will use the Bonferroni corrected factor when multiple comparisons are being made. For scores that do not meet the assumptions of ANOVA nonparametric statistics will be used to compare genotypes and treatment.

7. Significance of Anticipated Findings We will investigate the biological basis of cognitive dysfunction in PD combined with the impact of exercise on the same outcomes. Results from these experiments will yield essential data on the biological mechanisms contributing to cognitive decline in PD and the therapeutic potential of exercise as a disease-modifying intervention. In addition, this work has high translatability and will help to inform clinical trials and identify optimal intervention strategies for PD patients and at-risk populations.

Student Fellow Training/Mentoring Plan:

Plan. This is a large project that is ongoing in the lab. The PI will work with the student to determine what aspect of the project best suits his/her interests, abilities, and goals. The student would have the option to work mainly on one aspect of the project (such as behavioral testing and analysis or tissue processing and immunohistochemistry) or multiple aspects of the study. The student will meet with the PI on a weekly basis to discuss project progress and literature in the field. In addition to individual meetings the student will attend regular lab meetings where each person in the lab discusses the project they are working on and the progress or setbacks they have encountered. Short PowerPoint presentations are encouraged during these meetings as they will keep the student on track for the final poster session at the end of the summer.

*Resource*s. This project is funded by the Department of Defense and the lab has all resources necessary for the student to complete a summer project. The rats and treadmills are available and behavioral testing protocols are already established. Supplies and space for tissue processing are also available.

Location. The experiments will be conducted primarily in the laboratory area in RGE-400. There is behavioral testing space in C-133 where motor and cognitive testing will take place. The student will have a desk and access to a computer in the write-up area for data analysis and presentation.