Elena Grajales

1. Research Plan/Project Summary

    1. Rationale-

        Despite Parkinson's disease (PD) being the second most common neurodegenerative disorder, it still predominately remains an idiopathic disease of largely unknown pathogenesis. Understanding the underlying disease mechanisms in PD epidemiology will enable the identification of new target treatments and disease-modifying strategies for this debilitating neurodegenerative condition. Cognitive dysfunction is one of the most prevalent non-motor symptoms of PD with the incidence of dementia in PD patients at about 40%, up to six times higher than that of healthy people, with the greatest risk factor being advanced age (Emre 2003). Often times, this cognitive decline is seen as more debilitating to patients and caregivers than the characteristic motor symptoms, such as tremors, slowed movement, and rigid muscles, seen in PD patients. Therefore, in attempt to further understand the processes and molecular mechanisms of cognitive decline associated with PD, the objective of my experiment was to investigate the role of neuronal pentraxin II (NPTX2) in the development of PD in post-mortem cerebral tissue of healthy and PD patients.

    2. Research Question- How in NPTX2 regulated in Parkinson's Disease Progression?

        Hypothesis- NPTX2 is suggested to be a novel player, likely to be involved in the neurodegenerative pathway underlying PD due to its upregulation in the substantia nigra in a previous study done by Moran et al. As dementia progresses in Parkinson's disease patients, there may be a downregulation of NPTX2, which was found to be directly linked to cognitive decline in Alzheimer's Disease.

    3. Procedures-

        Tissue Preparation

        Brain tissue from the posterior cingulate gyrus, superior temporal gyrus, and frontal cortex of both healthy (n=18; mean age=76.4) and PD patients (n=18; mean age=72.1) will be dissected into pieces approximately 5 mm^3 in size by a

qualified supervisor. 1 mL of lysis will be added to each tissue samples and then homogenized using a micro pestle. After loose homogenization the sample will be passed through sterile 18G needle 10 times. Homogenized samples will be sonicated 3 times for 1.5 minutes. After sonication, samples will be centrifuged at 14,000 rpm for 10 min at 4°C. The supernatant will then be separated. Protein concentration will be quantified and appropriately normalized. 10 mL 5x SDS loading buffer will be added to 40 mL of samples, then boiled for approximately 5 minutes to prepare for Western blot analysis. All tissue preparation will be performed by lab members with the appropriate qualifications.

Western blot

Tissue will be lysed in RIPA buffer. 30 μg of the total protein will be separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to 0.2 μm nitrocellulose membrane. The membranes will then be blocked with 3% bovine serum albumin (BSA) in tris-buffered saline (TBS) for 1 h at room temperature and then incubated with primary antibodies 3% BSA in TBS overnight. Following the incubation with primary antibodies, the membranes will be incubated with the HRP-linked secondary antibodies at 1:5000 in 3% BSA in TBS for 1 h at room temperature. The membranes will be developed using a ChemiDoc Gel Imaging System after being incubated in ECL Western Blotting Detection Reagent for one minute. Patient numbers will be used to refer to the appropriate deidentified sample.

Risk and Safety-

When working with any potentially hazardous gloves, lab coats, and safety goggles will be used. All tissue preparation will be completed by a supervising scientist with proper qualifications.

Data Analysis-

ImageJ Analysis

ImageJ software will be used to compare the density of bands. The area of the frame for each protein will be established to be the area surrounding the largest band in each row and will be held constant across the given row. The band will be centered inside the frame and the measurement tool will be used to find the intensity of the given band. Data will then be exported to GraphPad Prism-6 for statistical analysis

Statistical Analysis

All statistical analyses will be performed using GraphPad Prism-6 provided by New York University School of Medicine. NPTX2 intensity will be normalized compared to the intensity of its loading control. Variables between groups will be determined by unpaired Student's *t*-test. Values of $p < 0.05$ were considered statistically significant. Data is presented as mean ± SEM.

4. Bibliography-

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1. Potentially hazardous biological agents research:

Tissue was received by the Stephen Ginsberg Lab and the Nathan Klein Institute. BSL determination was based on the pathogenicity of the agent, modes of transmission, availability of preventive measure, and activities in the experiment. BSL-2 was determined for this experiment. All tissue preparation will be completed by a supervising scientist with proper qualifications. Student will always be supervised by a qualified researcher. All substances and biological substances were disposed of according to EPA standards.

2. Hazardous chemicals, activities & devices:

When working with any potentially hazardous gloves, lab coats, and safety goggles will be used. Student will always be supervised by a qualified researcher. All substances and biological substances were disposed of according to EPA standards.

No changes have been made to the previous research plan.