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Towards an Animal Model to Study Sporadic ALS

Cell biology

Research Plan

3. *The Research Plan/Project Summary should include the following:*

   a. **RATIONALE**: *Include a brief synopsis of the background that supports your research problem and explain why this research is important and if applicable, explain any societal impact of your research.*

   Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease that has no known cure. In order to study such a disease, researchers may use animal models of study that genetically inherit the disease. The issue arises in that with ALS, ten percent of cases are attributed to genetic factors, while the remaining ninety percent are sporadic, caused by unknown factors. This distinction means animal models of study created with genetics cannot be used for sporadic ALS. In order to create a model that can apply to sporadic ALS, it is imperative to find a way to induce ALS without reliance on genetic inheritance.

   One possible way of accomplishing this is by causing oxidative damage in *Drosophila melanogaster*. Mito-killer red is a protein that when exposed to green light will release hydrogen peroxide, a toxic reactive oxygen species that causes oxidative damage. The neuromuscular junction is the meeting point between motor neurons and muscles, so it follows that damage here could cause ALS symptoms.

   b. **OBJECTIVE**: *How is this based on the rationale described above?*

   To determine the effect of Mito-killer activation on motor behavior and the structure of the neuromuscular junctions.

   c. *Describe the following in detail:*

      i. **Procedures**: *Detail all procedures and experimental design including methods for data collection. Describe only your project. Do not include work done by mentor or others.*

We will resort to crosses of fly lines that where either previously generated in the laboratory or purchased from the Bloomington Stock Center (BDSC, Indiana), taking advantage of the UAS/Gal4 system, which allows targeted expression of the Mito-killer proteins. To accomplish Mito-Killer expression in motor neuron mitochondria, we will cross the Mito-killer fly line (w+; UAS-Hsp70-Mito-KillerRed; +) with a D42-Gal4 driver line (D42 fly) and confirm expression by sorting wandering L3 larvae with red fluorescent motor neurons in the ventral nerve cord. Green light exposure is anticipated to release hydrogen peroxide (H_2O_2) in motor- neuron mitochondria.

The overall procedure will involve monitoring the motor function of the *Drosophila melanogaster* larvae before and after a green light treatment. Motor impairment will be determined using flipping assays during which the instar 3 larva is flipped into a "belly up" position and the time until it flips back over is recorded. After the final flipping assay, the larvae will be dissected into larval fillets and immunohistochemistry will be utilized to prepare the fillets for fluorescence microscopy to image the neuromuscular junctions of both the Mito-killer and D42 lines. This process will allow for the analysis of motor impairment and changes in morphology of the neuromuscular junction in an effort to induce ALS symptoms and create a disease model for sporadic ALS.

Four transgenic (UAS-Hsp70-Mito-KillerRed / D42-Gal4) and four wildtype (D42- Gal4/D42-Gal4) stage 3 larvae will be collected and individually separated into apple juice agar plates. Their phenotypes will be confirmed using brief exposure to green light to test for red fluorescence in the motor neurons. The larvae will then be allowed to acclimate to the agar plates for a minimum of 30 seconds.

The flipping assay procedure will then be carried out on each larva and repeated for a total of four assays: while in a "belly-down" position, the larva will be rotated to a "belly-up" position and a stopwatch will be started. Once the larva flipped back the timer will be stopped.

At this point in experimentation, the larvae will be subjected to green light treatment (see below). At the 90-minute mark, the flipping assay will be repeated four times again for each larva. The assay will be repeated another four times at the 120-minute mark.

Following the final flipping assay, the larvae will be dissected along abdominal walls using a stereomicroscope, fine tweezers, scissors, and dissection pins. They will be immediately treated in 4% paraformaldehyde in PBS for 15 minutes to prevent decay. The larval fillets will then be washed three times in 100 µL PBT.

Each fillet will then be incubated with 100 µL (1:500 FITC to PBT), refrigerated overnight. The next day, the fillets will be washed and treated with 100 µL of a 1:500 dilution of anti- horseradish peroxidase in PBT and a 1:500 dilution of anti-discs large in PBT at room temperature for 2 hours, after which they will be washed again.

The fillets will then be mounted on slides using 50 µL mounting solution to be imaged under 40X immersion oil lens of the NIKON TE2000 automated fluorescence microscope. Images of 1398p x 1040p will be taken of neuromuscular junctions in the 3rd muscle segment (if possible). Both red and green fluorescence will be captured. ImageJ will be used to measure the area of both green and red fluorescence.

In order to trigger the Mito-Killer protein, the larvae will be individually placed into wells of a 96-well plate with 100 µL HL3.1 buffer and positioned under the green LED such that all larvae would be directly underneath the strongest light. The LED will then be activated in pulses of 100 ms at 5Hz at an intensity of 10 V. This light activation will be administered for 5 minutes, after which the larvae will rest for 15 minutes in the same well plate but with no green light exposure. This process will be repeated for a total of five exposures each lasting 5 minutes. After the last exposure, the larvae will rest for 90 minutes in apple agar plates before assaying continues.

1. non-applicable
2. non-applicable
3. non-applicable
4. **Hazardous chemicals, activities & devices:** *Describe Risk Assessment process, supervision, safety precautions and methods of disposal.*

   During this experiment, the toxic chemical paraformaldehyde will be used to preserve the dissections and stop decay.

Paraformaldehyde can cause damage when it comes into contact with human skin, so caution will be exercised during use. When handling this chemical, gloves, goggles, and a lab coat will always be worn. The chemical will be disposed of in labelled containers designated for paraformaldehyde and pipette tips will be placed in sharps containers. Skin will be washed after handling.

ii. **Risk and Safety**: *Identify any potential risks and safety precautions needed.* During this experiment, the toxic chemical paraformaldehyde will be used to preserve the dissections and stop decay. When handling this chemical, gloves, goggles, and a lab coat will always be worn. The chemical will be disposed of in labelled containers and pipette tips will be placed in sharps containers.

iii. **Data Analysis**: *Describe the procedures you will use to analyze the data/results.* The results from the flipping assays will be analyzed using two-sample T tests to investigate significance between the two lines of *Drosophila*. The fluorescence images of the larval dissections will be analyzed in the software ImageJ. The area of both green and red fluorescence will be measured and divided to find the ratio between them, which should give an indication of the structural integrity of the neuromuscular junction.

d. **BIBLIOGRAPHY**: *List major references (e.g. science journal articles, books, internet sites) from your literature review. If you plan to use vertebrate animals, one of these references must be an animal care reference.*

1. Arthur, Karissa C, et al. "Projected Increase in Amyotrophic Lateral Sclerosis from 2015 to 2040." *Nature Communications*, Nature Publishing Group, 11 Aug. 2016.

2. Swinnen, Bart, and Wim Robberecht. "The Phenotypic Variability of Amyotrophic Lateral Sclerosis." *Nature Reviews Neurology*, vol. 10, no. 11, 2014, pp. 661–670.

3. Hall, Z W, and J R Sanes. "Synaptic Structure and Development: the Neuromuscular Junction." *Cell*, U.S. National Library of Medicine, Jan. 1993.

4. Pulver, Stefan R., et al. "Optogenetics in the Teaching Laboratory: Using Channelrhodopsin-2 to Study the Neural Basis of Behavior and Synaptic Physiology in Drosophila." *Advances in Physiology Education*, 1 Mar. 2011.

5. Bulina, Maria E, et al. "A Genetically Encoded Photosensitizer." *Nature News*, Nature

Publishing Group, 20 Dec. 2005.

6. Bandyopadhyay, Uday, Dipak Das, and Ranajit K. Banerjee. "Reactive oxygen species: oxidative damage and pathogenesis." *Current Science-Bangalore*- 77 (1999): 658-666.

7. Barber, Siân C., et al. "Oxidative Stress in ALS: A Mechanism of Neurodegeneration and a Therapeutic Target." *Biochimica Et Biophysica Acta (BBA) - Molecular Basis of Disease*, Elsevier, 4 Apr. 2006.

8. Ataman, Bulent, et al. "Rapid Activity-Dependent Modifications in Synaptic Structure and Function Require Bidirectional Wnt Signaling." *Neuron*, U.S. National Library of Medicine, 13 Mar. 2008.

9. Baldwin, Katie R., et al. "Axonal Transport Defects Are a Common Phenotype in Drosophila models of ALS." Human Molecular Genetics, July 2016, doi:10.1093/hmg/ddw105.

10. Casci, Ian, and Udai Bhan Pandey. "A Fruitful Endeavor: Modeling ALS in the Fruit Fly." Brain Research, vol. 1607, 2015, pp. 47–74., doi:10.1016/j.brainres.2014.09.064.

11. Pandey, Udai Bhan, and Charles D. Nichols. "Human Disease Models in Drosophila Melanogaster and the Role of the Fly in Therapeutic Drug Discovery." Pharmacological Reviews, vol. 63, no. 2, 2011, pp. 411–436., doi:10.1124/pr.110.003293.

Research Plan Addendum

NO ADDENDUMS EXIST