EDELMAN, HAILEY

Exploring the Role of Cannabidiol in a *Caenorhabditis elegans* Epilepsy Model

Research Plan/Project Summary Instructions

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.

Epilepsy affects over three million people in the United States. Due to the high mortality associated with drug resistant epilepsy, new modalities are being explored for treatment. Cannabidiol has been found to be effective for Dravet syndrome, a drug resistant form of epilepsy. The mechanisms of cannabidiol in mitigating seizure activity are not understood. Further research in elucidating the mechanisms of action of cannabidiol in epilepsy could expand the potential therapeutic uses for other forms of resistant seizures.

b. **RESEARCH QUESTION(S), HYPOTHESIS(ES), ENGINEERING GOAL(S), EXPECTED OUTCOMES:** How is this based on the rationale described above?

Cannabidiol will prevent or lessen seizure activity in seizure prone unc-49(e407) *C. elegans*. A tumor necrosis factor alpha antagonist (etanercept) and the adenosine antagonist (ZM241385) will mitigate the protective effects of cannabidiol and increase epileptiform activity in unc-49(e407) *C. elegans*. The adenosine agonist (methotrexate) will restore the protective effects of cannabidiol and lessen or prevent seizure activity.

c. Describe the following in detail:

- **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. Include details regarding chemical concentrations and drug dosages.

***C. elegans* Culture**

C.elegans unc-49(e407) will be obtained from the Caenorhabditis Genetics Center, University of Minnesota.

Age Synchronization

1. Allow worms to grow until adult stage.
2. Recover adults in 15 ml tubes by washing plates with 1ml of M9 buffer (2X).
3. Pellet worms by centrifuging for 1 minute at 15,000 rpm in a microcentrifuge.
4. Remove and discard supernatant.
5. Add bleaching solution (800µl bleach/200µl dH20) and agitate tubes. Destruction of the adult tissue should be monitored under the dissecting microscope.
6. Centrifuge for 4 minute at 3,500 rpm and discard supernatant.
7. Wash pellet three more times by filling the tube with 1ml of M9 buffer.
8. Centrifuge for 1 minute at 1000 rpm between each wash.

9. Add 1 ml of M9 buffer to the pellet and place in 20 °C shaking water bath overnight.

100µl of age synchronized *C. elegans* will be transferred to standard (60 mm diameter) NGM agar plates seeded with 0.2 ml of *E. coli* OP50 liquid culture at 17 °C.

Liquid based administration

Liquid based administration of PTZ will be performed by incubating 1-3 days old individual young adult nematodes in 50µl liquid droplets of PTZ(7 mg/ml) dissolved in Dent's Ringer solution. Droplets will be applied to empty plastic well plates and observed with microscopy and videography.

Control

1. *C. elegans unc-49*(e407) will be cultured and maintained on standard NGM agar plates seeded with OP50 *E. coli.*
2. 1- 3 days old *C. elegans unc-49*(e407) will be washed with 100µl of PTZ (7 mg/ml) dissolved in Dent's Ringer solution and 50µl will be transferred to plastic well plates.
3. The nematodes will be incubated for 15 minutes and observed under the microscope.
4. Record seizure duration, frequency, and intensity of convulsions under light microscopy
    a. Duration of seizure to be recorded with timer by direct observation via microscopy of induced seizure activity
        i. Videography will be utilized to record seizure induced *C. elegans*
    b. Frequency of seizures to be detected via microscopy and recorded as non occurrence(0) versus occurrence (1)
        i. Convulsions will be identified as "head bobbing" where the posterior half of the animal is immobile and the anterior muscle contraction occur repeatedly. The worms also display continuous shaking where the anterior and posterior half of the animal is aligned.
    c. Intensity recorded on a scale of 0-2, being nonconvulsive, mild convulsions, major convulsions, respectively. Data to be charted numerically for each nematode in table in logbook.

CBD Dose Response

1. Age synchronized *C. elegans unc-49*(e407) were placed on standard NGM agar plates seeded with OP50 *E. coli* cultured with CBD at varying concentrations (10%, 25%, 50%,100%(1 mg/ml)) to determine optimal concentration and exposure times without inducing death or other motility impairments. Observation of the *C. elegans* by microscopy will be made at 24, 48, and 72 hour interval to determine optimal exposure duration and worm maturation.
    a. Experiment was modified after initial trial and repeated on plates containing *E. coli* cultured with CBD at 100% (1 mg/ml) and with an additional 100µl of CBD (1 mg/ml) spread directly onto the plate.

Experimental Variable 1

1. *C. elegans unc-49*(e407) will be cultured on standard NGM agar plates seeded with OP50 *E. coli* cultured with CBD at 100% (1 mg/ml) and with an additional 100µl of CBD (1 mg/ml) spread directly onto the plate at 17 °C for 24 hours.
2. 1- 3 days old *C. elegans unc-49*(e407) will be washed with 100µl of PTZ (7 mg/ml) dissolved in Dent's Ringer solution and 50µl will be transferred to plastic well plates.
3. The nematodes will be incubated for 15 minutes and observed under the microscope.
4. Record seizure duration, frequency, and intensity of convulsions under light microscopy as in control procedure a, b and c.

Experimental Variable 2

1. *C. elegans unc-49*(e407) will be cultured on standard NGM agar plates seeded with OP50 *E. coli* cultured with CBD at 100% (1 mg/ml) and with an additional 100µl of CBD (1 mg/ml) and 100µl of 1mM adenosine antagonist (ZM241385) spread directly onto the plate at 17 °C for 24 hours.
2. 1- 3 days old *C. elegans unc-49*(e407) will be washed with 100µl of PTZ (7 mg/ml) dissolved in Dent's Ringer solution and 50µl will be transferred to plastic well plates.
3. The nematodes will be incubated for 15 minutes and observed under the microscope.
4. Record seizure duration, frequency, and intensity of convulsions under light microscopy as in control procedure a, b and c.

Experimental Variable 3

1. *C. elegans unc-49*(e407) will be cultured on standard NGM agar plates seeded with OP50 *E. coli* cultured with CBD at 100% (1 mg/ml) and with an additional 100µl of CBD (1 mg/ml) and 100µl of TNF alpha antagonist (etanercept) (50mg/ml) spread directly onto the plate at 17 °C for 24 hours.
2. 1- 3 days old *C. elegans unc-49*(e407) will be washed with 100µl of PTZ (7 mg/ml) dissolved in Dent's Ringer solution and 50µl will be transferred to plastic well plates.
3. The nematodes will be incubated for 15 minutes and observed under the microscope.
4. Record seizure duration, frequency, and intensity of convulsions under light microscopy as in control procedure a, b and c.

Experimental Variable 4

1. *C. elegans unc-49*(e407) will be cultured on standard NGM agar plates seeded with OP50 *E. coli* cultured with CBD at 100% (1 mg/ml) and with an additional 100µl of CBD (1 mg/ml) and 100µl of 1mM adenosine antagonist (ZM241385) and adenosine agonist methotrexate (100µl) spread directly onto the plate at 17 °C for 24 hours.
2. 1- 3 days old *C. elegans unc-49*(e407) will be washed with 100µl of PTZ (7 mg/ml) dissolved in Dent's Ringer solution and 50µl will be transferred to plastic well plates.

3. The nematodes will be incubated for 15 minutes and observed under the microscope.
4. Record seizure duration, frequency, and intensity of convulsions under light microscopy as in control procedure a, b and c.

- **Risk and Safety:** Identify any potential risks and safety precautions needed.

Chemicals

1. Methotrexate is teratogenic, harmful to unborn children and can cause infertility. Cautious handling requires use of impervious disposable gloves (double recommended), safety goggles with indirect vents, and impervious disposable protective clothing. Must be used under the supervision of a qualified scientist.
    a. Solutions will be prepared by Qualified Scientist and will supervise the handling of hazardous materials.
2. ZM241385 should be handled with the use of eye and face protection with face shield and safety glasses. Skin should be protected with gloves and a complete suit protecting against chemicals. The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.
3. Pentylenetetrazole is toxic if swallowed and causes skin and eye irritation. Handle with gloves and wear complete suit protecting against chemicals.

- **Data Analysis:** Describe the procedures you will use to analyze the data/results.

A t-test and ANOVA test will be employed to determine statistical significance. Data will be represented in graph format for experimental and control variables and each condition.

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.

Perucca, E. (2017). Cannabinoids in the treatment of epilepsy: Hard evidence at last? *Journal of Epilepsy Research*, *7*(2), 61–76.

Risley, M. G., Kelly, S. P., Jia, K., Grill, B., & Dawson-Scully, K. (2016). Modulating behavior in *C. elegans* using electroshock and antiepileptic drugs. *PloS One*, *11*(9), e0163786.

Sengupta, P., & Samuel, A. D. T. (2009). Caenorhabditis elegans: a model system for systems neuroscience. *Current Opinion in Neurobiology*, *19*(6), 637–643.

Williams, S. N., Locke, C. J., Braden, A. L., Caldwell, K. A., & Caldwell, G. A. (2004). Epileptic-like convulsions associated with LIS-1 in the cytoskeletal control of neurotransmitter signaling in *Caenorhabditis elegans*. *Human Molecular Genetics*, *13*(18), 2043–2059.

Devinsky, O., Helen Cross, J., Laux, L., Marsh, E., Miller, I., Nabbout, R., … Wright, S. (2017). Trial of cannabidiol for drug-resistant seizures in the Dravet syndrome. *The New England Journal of Medicine*, *376*(21), 2011–2020.

1. **N/A**
2. **N/A**
3. **N/A**
4. **Hazardous chemicals, activities & devices:**

- Include details regarding chemical concentrations and drug dosages
- Describe the risk assessment process
- Supervision
- Safety precautions
- Methods of disposal

Methotrexate: 10mg/ml
Chemical concentrations- 100µl
Risk Assessment- SDS forms (Sigma Aldrich), Methotrexate is teratogenic, harmful to unborn children and can cause infertility.
Supervision- Solutions will be prepared by qualified scientist, Dr. Mary Hendrickson, who will also supervise handling.
Safety Precautions- Cautious handling requires use of impervious disposable gloves (double recommended), safety goggles with indirect vents, and impervious disposable protective clothing. Must be used under the supervision of a qualified scientist.
Disposal- Offer surplus and non-recyclable solutions to a licensed disposal company. Contact a licensed professional waste disposal service to dispose of this material. Dissolve or mix the material with a combustible solvent and burn in a chemical incinerator equipped with an afterburner and scrubber.

ZM 241385:
Chemical Concentrations- 1mM
Risk Assessment- SDS forms (Sigma Aldrich)
Supervision- Solutions will be prepared by qualified scientist, Dr. Mary Hendrickson, who will also supervise handling.
Safety Precautions- Handle with the use of eye and face protection with face shield and safety glasses. Skin should be protected with gloves and a complete suit protecting against chemicals. The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.
Disposal- Offer surplus and non-recyclable solutions to a licensed disposal company. Contact a licensed professional waste disposal service to dispose of this material. Dissolve or mix the material with a combustible solvent and burn in a chemical incinerator equipped with an afterburner and scrubber.

Pentylenetetrazole:
Chemical Concentration- 7 mg/ml
Risk Assessment- SDS forms (Sigma Aldrich)
Supervision- Qualified scientist Dr. Mary Hendrickson will supervise handling.
Safety Precautions- Handle with gloves and wear complete suit protecting against chemicals.
Disposal-

Etanercept:
Chemical Concentration- 50 mg/ml
Risk Assessment- SDS forms (AMGEN)
Supervision- Solutions will be prepared by qualified scientist, Dr. Mary Hendrickson, who will also supervise handling.
Safety Precautions- Handle with gloves and wear complete suit protecting against chemicals.
Disposal- Contact a licensed professional waste disposal service to dispose of this material.