Katherine St George

Research Plan: 6/8/19

The Effect of Caffeine Intake and Dietary Restriction on Seizure Intensity in *Drosophila melanogaster*

a. Rationale

Epilepsy is a neurological disease involving spontaneous and chronic seizures. Seizure susceptibility and intensity are affected by a multitude of factors, including caffeine exposure and dietary restrictions such as low-protein, high-carbohydrate dietary restriction (DR) and the ketogenic diet (KD). Caffeine exposure increases seizure susceptibility, whereas dietary restriction (KD) decreases it. DR causes a decrease in target of rapamycin (TOR) signaling, a nutrient pathway which mitigates the negative physiological effects of caffeine. Studies concerning dietary restriction, caffeine, and epilepsy have each used Drosophila melanogaster as a model organism. Nutrition and lifestyle have huge impacts on seizure intensity in epileptic patients, so research elucidating the role that certain nutritional patterns have on these patients' seizures is invaluable in improving the quality of life for people with epilepsy.

b. Objective & Hypothesis

Because the effect of caffeine *and* KD, jointly, on seizures, is unknown, this study seeks to determine the effect. Additionally, this study pioneers DR as an alternative to KD as an anticonvulsant diet. It is hypothesized that dietary will mitigate the effect of caffeine on seizure intensity because it moderates the negative effects of caffeine and seizure susceptibility individually.

c. Plan

- Procedures

There will be 27 experimental groups, comprised of three different factors each with three different levels. The first factor is *Drosophila* genotype. There will be a wild type strain as a control (w^{1118}), a partial loss of function seizure-susceptible strain (eas^{alaE13}), and a complete loss of function/knock-out seizure-susceptible strain (eas^{KO}). The second factor is level of caffeine exposure. There will be a control group that is exposed to no caffeine, a group that is exposed chronically to caffeine by being fed media with .1mg/mL of caffeine for five days prior to experimentation, and a group that is exposed acutely to caffeine by being placed in a vial with an agar plug composed of .3mg/mL caffeine concentration sucrose solution for three hours prior to experimentation. The third factor is diet. There will be a control group that is fed a standard fly media with a 1:1 ratio of protein to carbohydrates (in the form of yeast and sucrose,

respectively), a ketogenic group that is fed a 2:1 ratio of fats to carbohydrates (in the form of coconut oil and sucrose, respectively), and a group that is raised on a low-protein form of dietary restriction designed to have the same effects as a reduced-signaling TOR pathway by being fed a 1:7.7 ratio of protein to carbohydrates. Each experimental group will be made up of 30 flies, yielding a 810 total experimental units.

Each group will undergo the nutritional manipulations detailed above, and will then have seizures induced. Seizures will be induced by vortexing individual flies in empty vials for ten seconds each on maximum speed, and then tapping them into a petri dish with a piece of white paper underneath (for visibility). An iPhone will be mounted over the experimental set-up to record induced seizures. The percentage of flies paralyzed, the percentage of flies exhibiting seizure-like activity (SLA), and the seizure durations in seconds will be determined for each experimental group using the video footage.

- Risk and Safety

  When handling methylparaben, used as an antifungal for fly media, plastic gloves and protective eyewear will be worn to prevent contact with skin or eyes. When removing beakers of fly media from the microwave, oven mitts will be used and the beaker will be tilted towards the back of the microwave to prevent burns.

- Data Analysis

  EthoVision XT software will be used to confirm SLA. An ANOVA will be performed on seizure durations to test for significance. Percent of flies paralyzed and percent of flies exhibiting SLA will be tested for significance using a binary logistic regression model. If the regression model(s) prove(s) significant, individual t-tests will be done between groups of interest. Additionally, a linear regression model will be generated to inspect the relationship between seizure durations and percent paralyzed and percent exhibiting SLA, and a linear regression t-test will be performed on both regression models. All statistics will be performed on SPSS, and graphs will be generated on Excel.

- Hazardous Chemicals, Activities, & Devices

  When using caffeine and dilute acids, protective eyewear and gloves will always be worn. Caffeine and dilute acids will not be handled in the absence of the Qualified Scientist. Caffeine and dilute acids will always be transported in a tray to lessen the chances of spillage. Because caffeine and dilute acids are used for *Drosophila* media in this study, and thus will be incredibly diluted in the process of making the media, the media that contains caffeine and/or dilute acids can be disposed of by simply emptying the food into the garbage (again, because of the extent of the dilution).

d. Bibliography

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Project Summary (ADDENDUM): 12/14/19

The project was renamed "The Ketogenic Diet Ameliorates the Effects of Caffeine in Seizure Susceptible Drosophila melanogaster" to better reflect the study's novel findings.