Section II: Methodology

Role of Student vs. Mentor

During the 3 months of which this project was worked upon, my mentor and I took upon different responsibilities. I conducted the experiment, data gathering, data analysis, background research, and all logging associated with the project. My mentor, Dr. Kevin Crowthers, provided me with the materials I need and guided me throughout the research process. Dr. Kevin Crowthers also made the later stage media which involved boiling.

Equipment and Materials

For this project, the equipment used includes: bifidobacterium, inulin, levodopa, water, drosophila media, drosophila vials, solution vials, pipettes, scale, and a hood. The levodopa used was of a concentration of 10mM and the bifidobacterium and inulin were both of a concentration of 10%.

Technique 1

A technique used in this study was the creation of fly media, which during the first pre-experimental flies, was composed of potato flakes and water (50 mL of each). During the experimental groups, the media was composed of 50mL potato flakes, 50mL water, and the according treatment concentration (10% bifidobacterium, 10% inulin, or both). For the second round of this study (10mM levodopa concentration) new media was used. This new media involved the use of corn syrup, water, and starch. After mixing the many different materials it is composed of, it is then boiled until it becomes a thick liquid and then used to feed the flies.

Technique 2

Many different concentrations of substances were used in this study, levodopa, bifidobacterium, and inulin. Both bifidobacterium and inulin were used at 10%. Initially, levodopa was used at 10muM,

however, the levodopa could not depress the flies at that concentration. As a result, for the rest of the study the levodopa concentration increased to 10mM.

Technique 3

Depending on the time available for a certain step, two types of transferring techniques were used to transfer the flies. The first technique involved simply allowing the flies to perform negative geotaxis from one container to the other. The second technique involved using carbon dioxide to anesthetize the flies. Thereafter, they would be collected into a piece of paper and then transferred into the new container. The first method would take longer, however, would require much less effort, and as a result, was more effective for situations in which time was not a concern. On the other hand, carbon dioxide would be used when there is a time constraint regarding transferring the flies.

Technique 4

A technique employed in this study is the culturing of bifidobacterium, which involves using LB broth and culturing 10% bifidobacterium in it. Thereafter, it is put into an incushaker for three days and then subsequently placed in the freezer.

Technique 5

A technique used in my project is the Negative Geotaxis Assay. The protocol which is used for this procedure in this study involves the use of a clear tube and drosophila. A marking is made at the 8cm mark from the bottom of the tube. Thereafter, flies are allowed to fly up the tube and this process is recorded on video tape to count the number of flies at each time point measurement. This technique is used to determine depression levels as it has been proven by previous studies that it accurately measures depression levels (Moulin et. al).

Technique 6

Another technique used in this project is the Forced Swim Test. The materials for the Forced Swim Test involve a clear tube, drosophila, water, and SDS (0.08%). The protocol for this method involved adding SDS to the water to form the concentration and pouring it into a clear tube until half full. Thereafter, the tube is turned horizontal, and the flies are placed into the tube. Thereafter, the activity of the flies for the next five minutes is recorded on camera. This film is then analyzed to find how many flies are immobile at a certain timepoint. This technique is used to determine depression levels as it has been proven by previous studies that it accurately measures depression levels (Moulin et. al).

Statistical Tests

The statistical test used for this study is a difference in slopes test along with a linear regression test. Since this study will be measuring the rate of change in both assays, the slope is necessary for this. Thereafter, to determine if there is a significant difference in the values of the different slopes a difference in slopes test is conducted.