5-1-2021

The Effect of Circadian Phase Shifts on cellular respiration in wild type and flies with mutant circadian genes

Shannon Maguire

Michael Yarrows

Kathryn Donohue

Over the past semester, our team's research has focused on circadian rhythms in wild type and mutant flies. Circadian rhythms, our body's internal clock, are the biological processes which regulate the sleep/wake cycle as well as other physiological rhythms in all living organisms. Following a 24-hour light/dark cycle, circadian rhythms influence eating habits, physical activity, and metabolism. An organism's circadian rhythms can be disrupted by an offset light/dark cycle, food availability, or changes in sleep patterns. In mammals, the region of the brain that controls circadian rhythms is known as the suprachiasmatic nuclei or SCN, while in flies, there is a clock neuron network we will call the master clock.

It might be possible to measure cell activity in the neurons of the master clock by measuring cell respiration. Through cell respiration, cells produce energy (ATP) in order to carry out metabolic processes. An active cell would need more ATP and therefore carry out more cell respiration. Because oxygen is consumed in this process, measuring oxygen consumption is one way to follow cell respiration. The Seahorse extracellular flux analyzer is a novel way of measuring this in whole living fly brains. By sampling the brains at different times of day, we will be able to measure the rhythm of cell respiration over a circadian day.

Our team has met, over the course of the semester, to discuss papers, plan experiments, order materials, and develop the skills to measure the circadian cycle of cell respiration in the fly brain. We plan to measure what happens when you disturb the molecular clock with light or with mutations to the master clock. Following the pandemic's closing of the lab, we needed to grow up a colony of flies to run our experiments and optimize data collection. Over the semester, each student learned how to maintain a fly colony by learning how to make food and flip and sort flies to rapidly expand a fly colony. We have practiced dissection techniques under the microscope to learn how to dissect out fly brains and maintain viable tissue. We will use these techniques to collect the fly brains and will measure cellular respiration using the flux analyzer. Measurements will be taken at different times during the 24-hour cycle and in wild type flies as well as flies with circadian mutations. We are currently entraining the flies to two different light dark cycles to make 24-hour collections easier. This type of data has never been collected before. Once we understand how cell respiration fluctuates over the circadian day, we will correlate that data with the oscillation of known circadian clock genes in these flies using quantitative PCR. This summer we plan to disrupt the molecular clock using shifts in the light dark cycle, mimicking jet lag, to see how it alters the cell respiration cycles.

Circadian rhythms affect humans in a similar way as in flies. When human circadian rhythm patterns are disrupted, such as in night-shift workers, adverse effects are observed in glucose levels and weight patterns. Specifically, shift workers have a high incidence of obesity and Type-2 diabetes. By analyzing metabolic changes in the master clock neurons of flies following altered light/dark cycles, we hope to gain insight into the harmful effects that disrupted circadian rhythms have on humans. Since circadian research using the Seahorse machine has never been published, we anticipate that our team's novel research will aid in understanding human metabolism at both a cellular and organismal level and the implications of irregular sleep/wake cycles on shift workers as well as the shifting light cycles associated with jet lag.