

6. FRAPPI Assay
 - a. Rhodamine B
 - b. Fluorescein Sodium Salt
 - c. Clear 24-well microplates
 - d. 96-well microplate
 - e. Fluorescence band-pass filters
 1. Rhodamine B
 1. Excitation: 545/40
 2. Emission: 620/40
 - f. Fluorescein
 1. Excitation: 485/20
 2. Emission: 528/20
7. Knockdown Assay
 - a. Inhibitors
 - i. Cylindrical tube (length = 122 cm, diameter = 7.62 cm)
 - ii. Eight 3D-printed diffusers

The purpose of this experiment is to induce various sleep patterns and circadian clock chromosome alterations in *Drosophila melanogaster* in order to see which is most susceptible to Alcohol Use Disorder. In each assay, the negative control is wild type *Drosophila* with an unaltered sleep cycle and the positive control is Ck mutant *Drosophila* with an altered sleep cycle. In the Knockdown, FRAPPE, and Alcohol Dehydrogenase assays, if *Drosophila's* sleep patterns and circadian clock chromosomes are environmentally altered, then the *Drosophila* with the Evening Chromotype will build the most resistance to ethanol, and have the highest Alcohol Dehydrogenase levels; and the *Drosophila* with the Sleep Deprived Chromotype will consume the highest percentages of ethanol; and have the highest odor and taste preferences to ethanol.

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If *Drosophila*'s sleep patterns and circadian clock chromosome are environmentally altered, then the *Drosophila* with the Sleep Deprived Chromotype will build the most resistance to ethanol, and have the highest Alcohol Dehydrogenase levels, and the *Drosophila* with the Sleep Deprived Chromotype will consume the highest percentages of ethanol, and have the highest odor and taste responses to ethanol.

[illegible]

1. Addition of the fluorescent probe to the sample.

2. Addition of the fluorescent probe to the sample.

3. Addition of the fluorescent probe to the sample.

4. Addition of the fluorescent probe to the sample.

5. Addition of the fluorescent probe to the sample.

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II Assay

early anesthetized. *Drosophila melanogaster* are sexually dimorphic, meaning the males and females can be distinguished by appearance, which is important in many scientific experiments. Their life cycles are quite short, which can be beneficial to an experiment as they mature faster, and therefore will multiply quickly.

alipist, or micropipette, however, regardless of how precise the instrument is, there will always be a lack of accuracy. Furthermore, in the CAFE assay, there were some instances in which bubbles formed within the capillary tubes. Since these bubbles can be considered solution that the *Drosophila* consumed, the size of

similar to those of mammals. Another advantage of using *Drosophila* as a model organism would be the fact that around 98% of its genome is known and sequenced. Additionally, the majority of human genes have a *Drosophila* ortholog, which is extremely beneficial when working with genetic mutations, such as the C

assay could be run four more times with a one day recovery period between each of the five runs. This new design would be used to determine which chronotype builds the most resistance to ethanol over the course of 5 exposures. Furthermore, the data from the two versions of the Knockdown assay could be compared to

that of any other sleep cycle. Therefore, this data supported the part of the hypothesis corresponding to it. Knockdown assay, which stated that the *Drosophila* with the Evening chronotype will build the most resistant to ethanol. The knockdown assay was run at the same time for each sleep cycle, approximately 20:00–22:00.

Animals, plants, fungi, and even some prokaryotic organisms all have daily rhythms in behavior, physiology, and molecular activity and gene expression. These rhythms are not determined by external environmental cycles, but rather are determined by endogenous circadian clocks that keep time even without natural time signals. External environmental cycles are, yet, still required to entrain these clocks so they maintain precise circadian time at the proper time of day. In animals, circadian clocks dwell in an assortment of tissues, and in plants and various fungi, circadian clocks are controlled by a circadian oscillator. In some cases, these clocks control autonomous rhythms in physiology and development, while others will structure systems of clock tissues that control rhythms in behavior. There are three essential parts of a circadian clock: an input pathway that gets environmental signals and transmits them to the circadian oscillator, a circadian oscillator that keeps circadian time and minutes output pathways, and output pathways that control numerous metabolic, physiological, and behavioral processes.

There are certain genes that can cause circadian clock disruptions, such as the *CK1ε* mutation. When the circadian clock is mutated, it can cause numerous disastrous effects. One of these effects is insomnia or sleep deprivation. Chronic insomnia is generally defined as difficulty initiating or maintaining sleep for a long period of time. Not only is sleep deprivation a symptom and effect of substance abuse, specifically alcohol abuse, but alcohol use disorder can be an effect of insomnia. People who suffer from insomnia and sleep deprivation tend to take disorders as medicines. In the past, alcohol was widely used as a substitute for inducing sleep and taking away pain, but overuse of alcohol or any other substance can lead to addiction, substance use disorders, and can actually worsen sleep cycles, which was the opposite of what they were intended to do.

Rapid eye movement (REM) and non-REM sleep (NREM) are the two states of sleep. NREM sleep is split into four stages. Stage 1 is the lightest stage, and therefore when in this stage, it is easy to be woken up. Stage 3 is the deepest stage of sleep, and is also referred to as 'slow wave sleep'. The stages of sleep can be monitored by electroencephalogram (EEG). Sleep normally starts with stage 1 sleep, and advances to more profound stages of NREM when primary stages of REM occur. REM and NREM will then interchange in around 90 minute cycles. The states of sleep are homeostatically controlled to such an extent that lack of REM sleep leads to pressure to enter REM and is followed by a rebound increase in REM once started. Similar impacts are seen with lack of deep sleep. Sleep deprivation leads to a reduction in REM sleep, and a corresponding increase in negative effects on not only sleep deprivation and insomnia, but the overall quality of sleep a person obtains decreases enormously. The negative changes in sleep quality is quite evident when alcohol is consumed within an hour before going to sleep, or when taken in high doses, but this side effect, is also apparent when any alcohol is