Towards Identifying the Genetic Pathways That Affect Stress-Induced Sleep in C. Elegans

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Introduction

While the importance of sleep has been highly documented, the evolution of sleep and the genetic pathways that regulate it remain poorly understood. This project aimed to gain further insights into the genetic pathways of sleep regulation using the nematode *Caenorhabditis elegans*. We studied mutagenized lines with reduced sleep and fatigue behavior after exposure to conditions which normally cause **stress-induced sleep (SIS)** behaviors. SIS behaviors are induced by viral infection (Orsay virus), prolonged heat exposure, and UV exposure.[1]

ALA is a neuron important to Orsay-induced stress-induced sleep.[2] We used ceh-17 mutants, which are ALA deficient[3], as a positive control which will not exhibit increased quiescence, or sleep behavior, due to Orsay infection. We also used rde-1 mutants, which are more susceptible to being infected by Orsay[4], as a negative control that should significantly increase quiescent activity after Orsay infection. The mutagenized lines screened: B3.1, B3.2, B3.3, and D3.1, will be compared to these control strains, with the null hypothesis being that these mutagenized lines will display increased quiescence after exposure to Orsay virus, compared to the control groups. These mutagenized lines are deficient in both ceh-17 as well as aptf-13, a gene that is responsible for another important neuron in the C. elegans SIS pathway, RIS.[5] Previous screenings of these mutagenized lines indicated that they outperformed the ceh-17 mutants by showing increased activity post-Orsay infection. However, before performing any genetic sequencing of these mutagenized lines, the robustness of the phenotype must be replicated and stress-tested.

Methods

To assess the reliability and durability of the isolated mutagenized phenotypes, all strains were screened using a Motivated Displacement Assay (MDA) and a machine learning analysis procedure named WorMotel.

Egg prep: To ensure that the worms were all the same age during these assays, an egg prep is conducted, where a plate filled with adult worms and eggs are washed with a buffer known as M9, bleach, and sodium hydroxide to eliminate existing worms and to leave behind eggs that will all hatch nearly simultaneously after a 24-hour incubation, after which they will be plated into a 3 cm radial agar plate. This prevents any behavioral differences resulting from the worms being in different life stages. The experimental worms are plated onto a plate with Orsay virus pipetted into their bacterial-lawn food supply, thus infecting them as they grow up and ingest the virus. The control worms are plated onto non-Orsay contaminated plates.

Motivated Displacement Assay:
After 2-3 days, these egg-prepped worms should all simultaneously reach young adulthood, where they were washed with M9 and pipetted into the center of a large, 10 cm radial plate, with food plated on the periphery. If worms display higher levels of stress-induced sleep, they will be less likely to travel from the center to the periphery; inversely, worms that display lower levels of stress-induced sleep will be more likely to travel to the periphery with less time. In intervals of 15 minutes, count and remove worms from the periphery and normalize the data to see the rates of worms that actively seek food.

WorMotel: 48 young adult worms are placed into individual wells in a 6x8 chip and then placed underneath a camera for 4-8 hours, with individual frames being taken every 10 seconds. Afterwards, image tracking analysis is used to determine the rates of quiescence for the worms. High rates of quiescence would indicate that the mutant phenotypes are not reducing stress-induced sleep (SIS) behaviors.

MDA and Imaging Analysis Results

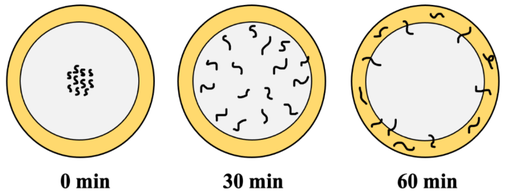

Figure 1. Illustration of an MDA from Carlos Chavez Perez.[4] Worms are pipetted to the center of the plate and make their way to the food at the periphery. Worms that display quiescent behavior and greater affinity to stress-induced sleep will spend more time in the center of the plate than in the periphery.

Figure 2. Stacked column visualization of representative Motivated Displacement. Worm lines that have the fastest worms will have a greater representation of "blue stack" worms since this stack represents the normalized number of worms that were found in the periphery at 15 minutes, the earliest checked time interval.

Imaging Analysis

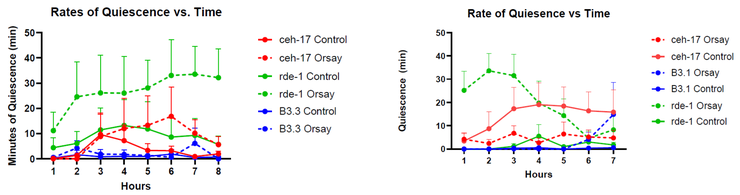

Figure 3. MATLAB generated analysis on quantified rates of sleep, represented by PRISM. The y-axis determines the average minutes of detected quiescent behavior (quantified by lack of movement from the worms) over the x-axis, the number of hours that the worms were under observation. The higher the y-axis value, the stronger the quiescent behavior from the worms at that given hour.

Discussion

From the data collected in the MDAs, two mutagenized lines stand out as rejecting the null hypothesis: B3.1 and B3.3. Worms from these mutagenized lines do not increase quiescent activity when exposed to Orsay Virus, compared to their uninfected analogues. For B3.1, we see that the average percentage of worms found at the periphery was approximately 5.75% greater for Orsay-infected worms than non-infected worms. For B3.3, that difference was much more drastic with a nearly 23% increase in periphery presence for the experimental group. With D3.1, however, we see a marked decrease in the percentage of worms that make it to the periphery with a nearly 36% drop between the experimental and the control worms. This rules out D3.1 as a potential candidate for genomic sequencing. From these preliminary screenings, B3.1 and B3.3 were then selected to undergo a more thorough screening utilizing WorMotel. From the WorMotel data, we see that quiescence activity from the mutagenized worms are, on average, lower than the quiescence activity of the ceh-17 and rde-1 mutants. That is, the mutagenized worms display decreased sleep behavior, both when healthy and sick, compared to the positive and negative controls. This behavior is interesting because the mutagenized lines of worms seem to be more active than wild-type worms, regardless of the health of the worms. This could indicate that disruptions in RIS and ALA simultaneously may decrease the overall sleep-drive of the worms.

Future Directions

With the findings of the MDAs and WorMotels, we have two candidates of mutagenized worm lines that we hope to sequence. Our next steps are to enrich our stock of mutagenized worms, making sure that we have worms that are homozygous in their mutations. We plan to do this by undergoing a series of back-crosses, selectively breeding worms that display the desired behavior: the ability to carry out normal feeding and movement behaviors after SIS-inducing stressors. We will cross these mutation-enriched worms with wild-type and analyze the resulting progeny to determine the genetic makeup of the parents. We will also subject the worms to different types of stressors to determine whether the phenomenon displayed with previous MDAs are applicable in generic contexts, not just specific to Orsay infection. Once the genetic sequencing is complete, there will be a thorough comparison between the mutant worms and the wild type, in hopes to illuminate which specific gene pathways appear to control the SIS pathway.

C. elegans has always been a strong model organism for scientific research because of its applicability to human genetic studies. We believe that exploring the genetic pathways of sleep within C. elegans will elucidate some reasons why sleep behavior has been so well-conserved throughout evolution. Studying these SIS-deficient worm lines may help us gain an understanding of why sleep can be circumvented, despite the physical need for it. We believe that this knowledge will be useful to understanding some potential genetic roots for sleep disorders such as insomnia, and it may help further our understanding of fatigue and the need for sleep during illness. Especially under the pretext of COVID-19, where major symptoms include brain fog and fatigue, understanding the genetic and epigenetic factors that trigger sleep behaviors will be crucial in public health. We hope that these worms will be a positive step in the ever-growing field of sleep science and research.

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