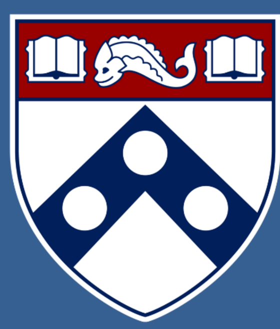
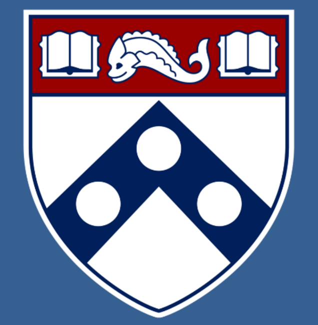


The Discovery of Drugs Reducing Stress-Induced-Sleep Using High-Throughput Screening



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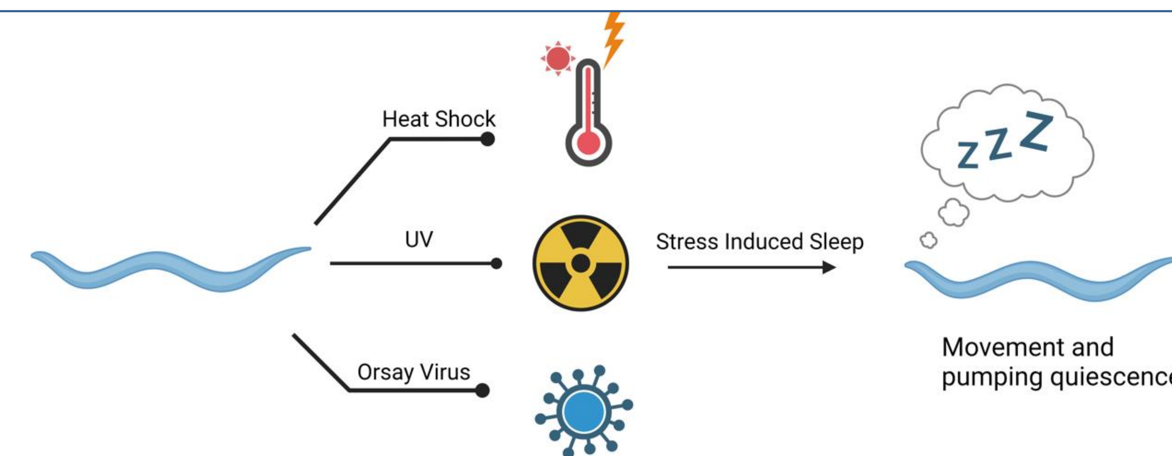
Introduction

Caenorhabditis *elegans* is a nematode that is an ideal model organism because many of its genes have functional counterparts to the human genome. C. *elegans* displays a rudimentary version of sleep, where locomotion and digestive quiescence (inactivity or dormancy) is observed. This quiescence can occur in between molting stages of their life cycle and is called lethargus. We are interested in the other form of quiescence: stress-induced-sleep (SIS). As the name implies, this type of quiescence occurs after the animal undergoes a significant stressor such as illness, UV, and heat-shock. SIS behavior mimics human behavior after stressors like sickness. We are interested in what drugs or chemical compounds with known pharmacological effects help reduce stress-induced quiescence in C. *elegans*.

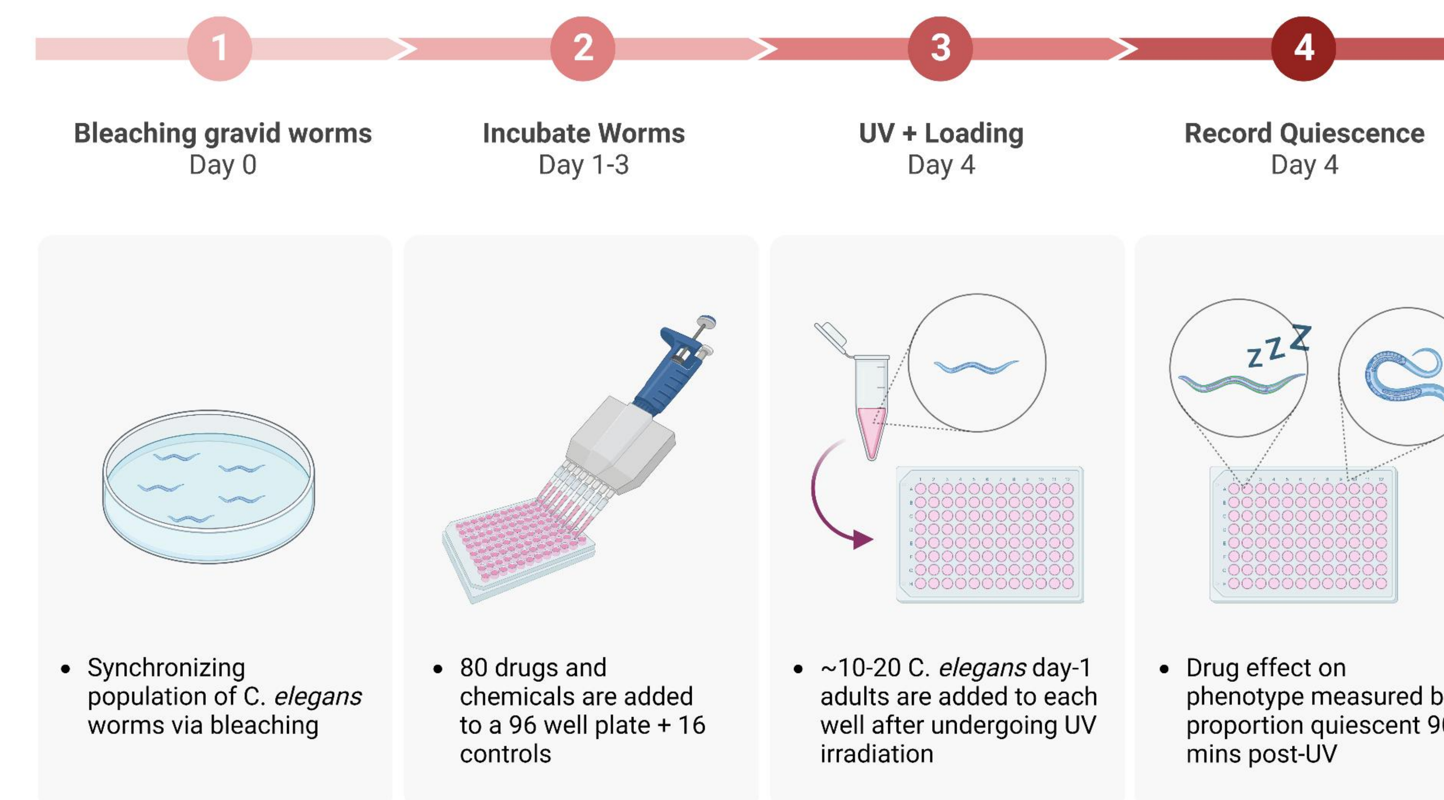


High-Throughput Screen Method

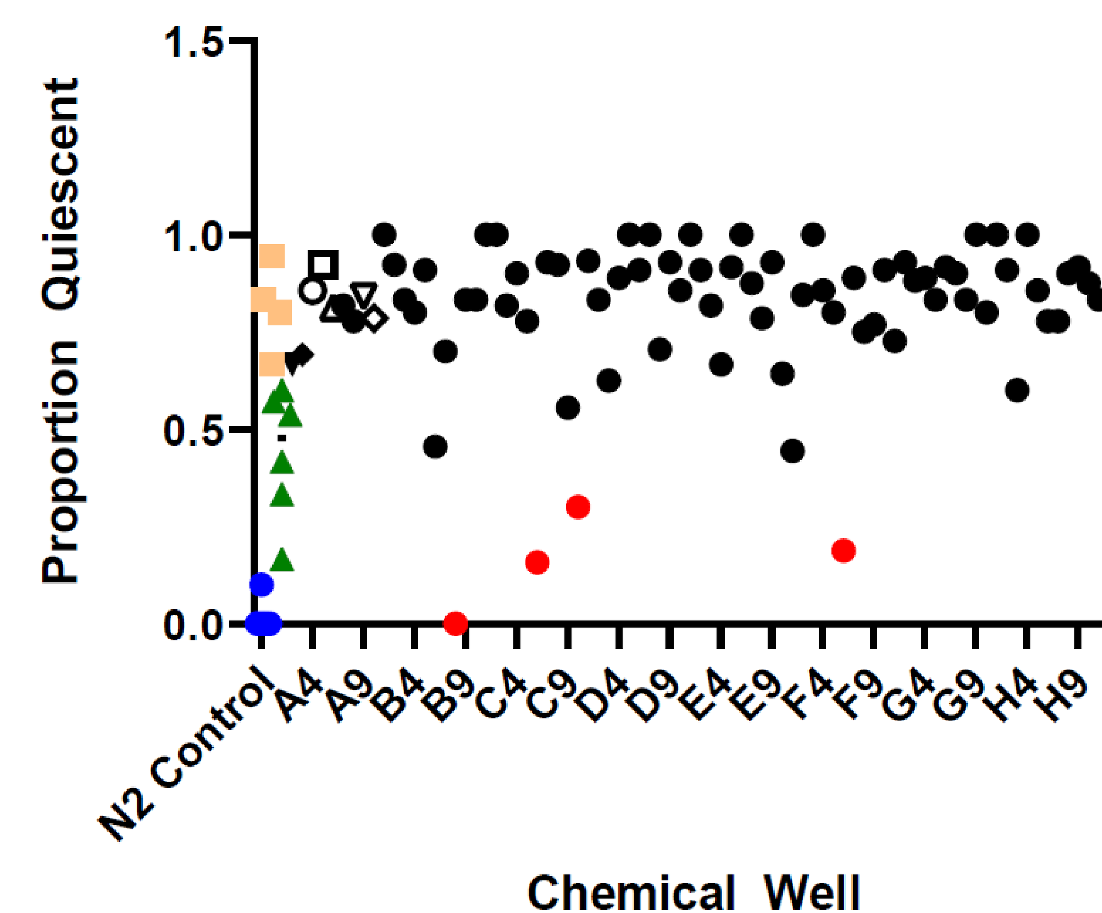
We utilized a high-throughput-screening (HTS) assay to effectively screen through thousands of chemicals in the Perelman HTS Core Facility Library. First, we synchronize the population of worms by bleaching gravid worms. We do this to ensure the worms are all the same age by the time of the screen: the bleach and NaOH solution kills the animals, leaving behind only the eggs which are then suspended in M9 buffer overnight, resulting in a synchronized population of L1 worms the next morning. These worms are plated on agar plates with an E. *coli* bacterial lawn for food. After 3-4 days the worms will be young adults. Once the population reaches young adulthood, we begin the assay by irradiating the worms under UV light to induce SIS.

For the actual screen, we use a 96 well-plate with 200 µL of M9 buffer, setting 80 of the wells to be experimental and reserving 16 to be for our controls. Our positive controls are N2 (wild type) and IB16 (ceh-17) worms. Post-UV, the N2 quiescence rate is roughly 85-100%. IB16 mutants lack RIS neuron function which is a major component of stress-induced sleep. Thus, it has ~30% quiescence rate. In both controls, the worms are placed in untreated M9 buffer. Our experimental worms are N2 worms that are placed in M9 buffer containing 100 µM of one of the chemicals in the screen. If the drug or chemical compound is pharmacologically active in the SIS pathway, we would expect the quiescence rate of the worms to be lower than the wildtype, and more in line with the IB16 phenotype (or lower). For both the experimental and control wells, we aim to have at least 10 worms per well to reduce variation in the data. Quiescence is measured by locomotive activity by the worms in the well. A lack of activity indicates quiescence whereas any thrashing or turning is noted as reduced quiescence.

HTS Overview



Example of Single Assay Data



Discussion

Only 1/3 of the library has been fully screened. From the ~1,200 chemicals that were screened, we have found around 7 "hard hits", which we define wells with 0% quiescence (i.e., all the worms are moving). We also have a variety of "soft-hits" which we define as wells that have worms displaying IB16 behavior (i.e., major reduction in quiescence tracking with the RIS mutant worms, but not as drastic as our hard hits). Unfortunately, due to backlog at the core facility, we are unable to know the chemical identities of these hits currently, except for Amitriptyline HCl, which is a "dirty drug" that affects both serotonin and norepinephrine receptors. As such, all further experiments and replications were done exclusively with Amitriptyline HCl. The first step was to reproduce the experimental results, which was done with a sample size of ~2000 animals. Nearly every animal lost quiescence behavior when treated with Amitriptyline HCl. We then conducted a dose-response curve to determine the effective concentrations of Amitriptyline HCl. The animal was highly responsive to Amitriptyline HCl even up to 5 µM indicating that C. *elegans* is highly sensitive to Amitriptyline HCl. It is still unclear whether the reduction in quiescence can be attributed to Amitriptyline's effects on serotonin levels or octopamine (OA), the invertebrate equivalent to norepinephrine. We will delve deeper into the next steps and future directions to elucidate this information in the next section.

Future Directions

We plan to finish the remainder of the screen and replicate hard hits to ensure that the results are reproducible. Then we will further conduct dose-response curves to determine animal sensitivity to the particular drug and further test the effects of the drug on pumping quiescence and developmentally-timed sleep. With each hard hit, we will conduct a reverse-genetics search to isolate the mechanistic pathway of the drug. For example, in the case of Amitriptyline HCl, we will utilize the tph-1 mutant which is deficient in serotonin, to determine which receptor Amitriptyline is targeting in the worm (serotonin or octopamine). By introducing several loss-of-function mutations we can see which neuron or genetic pathway is essential for drug interaction and hopefully elucidate new information about the SIS pathway. We also hope to categorize the drugs that have pharmacological effects. For example, we know that Amitriptyline HCl is a tricyclene, so we may potentially see that other tricyclenes also have similar effect sizes reducing quiescence.

While these mechanistic pathways are being explored, we will coordinate with mice and fly labs to determine whether these compounds also reduce stress-induced-sleep in those organisms as well. Should any hard-hits result from those experiments, then we would hope to send these up for further trials in primates, with the ultimate goal resulting in a clinical trial for human use. We believe that this method of high-throughput analysis can help expedite knowledge of which compounds can pharmacologically reduce fatigue in patients suffering from conditions such as chronic fatigue syndrome.

Acknowledgements

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