Analysis of Two Neurons Underlying Sickness Sleep Behavior in C. Elegans

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Background

- The nematode C. elegans enters a state of quiescence following exposure to noxious stimuli such as UV radiation, high temperatures, toxic bacterial lipopolysaccharides as well as mechanical stimuli. This behavior is known as sickness-induced sleep (SIS).
- In worms, the ALA neuron, functionally known as the stress encoding neuron, is believed to be correlated with SIS. The sleep active neuron RIS lies downstream of the ALA in the SIS pathway and is depolarized by ALA activation.
- Past studies have suggested that the ALA but not the RIS is responsible for recovery cellular stress.
- Given that sickness behavior is observed across species including humans, it is of interest to explore the relation between how stress influences sleep.

Methods

Strains:

- N2 males: Wild-type (WT) males
- NQ1416: RIS::GCAMP
- NQ1417: ALA::GCAMP
- KG1180: lite-1 mutants

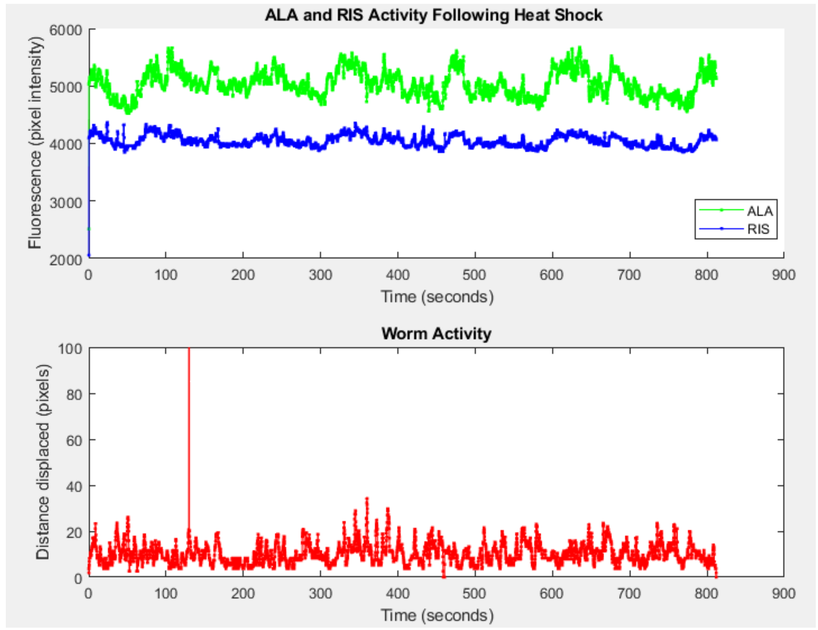
Creating the Dual-Expressing Strain: To generate a strain co-expressing GCAMP in both the ALA and RIS neurons, I crossed between strains expressing GCAMP from each neuron. I then crossed in the lite-1 background to nullify the natural blue light aversion exhibited by worms.

Heat Shock Assay and Microscopy:

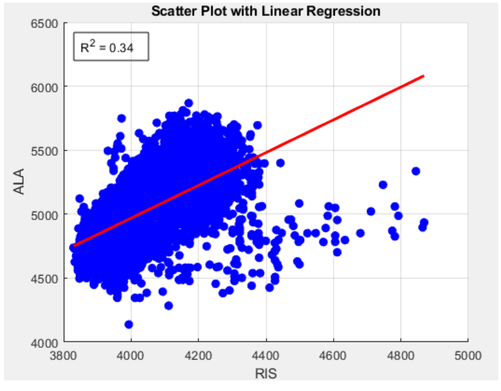
- Adult worms at the fourth larval stage of development (L4s) were carefully selected the day before the experiment.
- Individual adult worms were placed into miniature agarose chambers (Bringmann et al. 2015) for sequential imaging under a compound microscope. The worms were exposed to blue excitation frequency to induce GCAMP emission.
- Before applying heat shock treatment, imaging sessions lasting 5-10 minutes were conducted to establish baseline neural activity.
- Worms were enclosed in Petri dishes and wrapped twice with Parafilm before being subjected to heat shock via immersion in a water bath set at 37°C for a duration of 10 minutes.
- Following heat shock exposure, the worms were promptly returned to the compound microscope for immediate post-treatment imaging.

Single-Organism Neural Profiling Approach: For optimal temporal resolution of the ALA and RIS neurons and the capture of individual-specific neural-behavior correlations, I adopted an individual-level analysis approach rather than a population-level method. This approach aligns with the goals of my project, enabling a more nuanced understanding of neural responses and their connection to sickness sleep behavior.

Calcium transients of the ALA and RIS are correlated



- The top recording of ALA and RIS GCAMP signals suggests that an increase in ALA activity is correlated with an increase in RIS activity.
- Linear regression analysis of the two datasets offers and R-squared value of 0.34, indicating that the two neurons are modestly correlated.
- However, it's crucial to acknowledge the potential influence of other variables that might contribute to the synchronized neuronal activity. These factors could encompass aspects such as video recording quality, the orientation of the worm on the recording plane, and the movement of the worm itself.

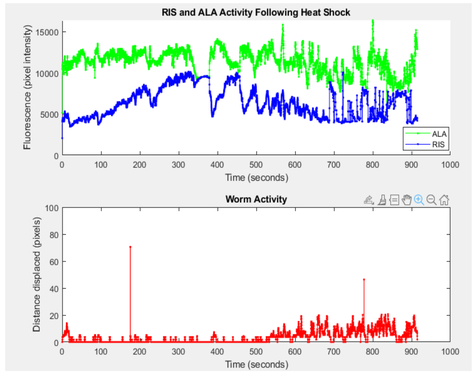


Study limitations and obstacles

- **Low Sample Size (n):** My study's relatively small sample size limits the generalizability of the findings. I am limited in detecting meaningful effects and making robust conclusions.
- **Time-Consuming Progeny Generation:** The process of generating progeny through crosses takes several weeks, which plays a part in obtaining a substantial dataset within a reasonable timeframe.
- **Uncertain Behavior of Worm SIS:** The SIS behavior of worms following a heat shock is not consistently guaranteed. Variability in the response could arise from a range of factors, including differences in individual worm sensitivity or variations in experimental conditions.
- **High Noise-to-Signal Ratio in GCAMP Fluorescence:** The GCAMP fluorescence signal exhibits a high noise-to-signal ratio, posing challenges for accurately capturing and quantifying subtle changes in neuronal activity. This noise can introduce uncertainty into the recorded ratiometric data, potentially impacting the precision of the measurements.
- **Challenges in Replicating Calcium Transients:** The mean fluorescence values did not exhibit significant changes before and after heat shock in the collected data. This discrepancy might be attributed to the worms' initial state, which could have been influenced by environmental conditions or prior disturbances.
- **Insignificant Magnitude of Change:** In addition to the challenge of replication, even when changes were observed, the magnitude of these changes were minor. This limited magnitude of change might affect the clinical relevance of the findings, especially if the observed effects are not practically meaningful in the context of neuronal dynamics.

Discussion and future directions

- The SIS phenotype of worms has been widely studied in C. elegans research. In the recording on the right, it is known that depolarization of ALA as well as RIS lead to quiescence, indicated by the reduced worm activity.
- While my results are insufficient in determining a relation between the ALA, RIS, and sleep, a future step could be to use optogenetics to manipulate for causation.



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