How do changes in neural dynamics influence behavior in *Caenorhabditis elegans* during periods of sleep-like quiescence?

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SUMMARY

Sleep is crucial for allowing the nervous system to consolidate past experiences into memories. To achieve this goal, I focus on understanding changes in neural activity and behaviors in C. elegans before, during, and after periods of quiescence. To analyze quiescence, Day 1 adult C. elegans undergo an odor training paradigm consisting of three 80-minute odor cycles of S Basal (buffer control) or Butanone (innate attractive odor) with two 30-minute food cycles in between. C. elegans are imaged via confocal microscopy, and annotations on behaviors are used to compare behavioral and neuronal activity. While Butanone-trained C. elegans are known to show more quiescent bouts, I found that their bodies are not completely immobile, displaying nose twitches and head bends but a still body. By focusing on the transitions in behavior during sleep-like quiescent bouts, we can learn about which potential neurons are active during sleep-like quiescent bouts and drive these micro behaviors.

BACKGROUND

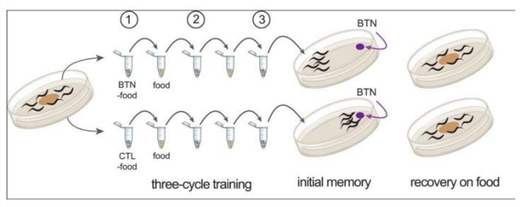
Day 2 butanone C. elegans showed a new behavior and similar sleep-like quiescent length to Day 1 adults. Chronic sleep deprivation is the most common symptom in neurodegenerative diseases, impairing an organism's cognitive ability. Yet, current research has not found the mechanisms responsible for protecting our neurons from deteriorating. *Caenorhabditis elegans* is the ideal organism to study sleep and its effect on the nervous system due to the simplicity of its nervous system, rapid aging (develops from egg to adult in three days), easy genetic manipulation, and visible neural circuitry due to its transparent body.

The JZ3068 mutant (ltSi915 [osm-6p::zif-1::operon-linker::mCherry::histone::tbb-2 3'UTR + Cbr-unc-119(+)] II; unc-119(ed3) III x pan-neuronal GCaMP in lite-1 mutant background (lite-1 is on X chromosome)) carries the GCaMP protein on each neuron, showing single neuron activity and whole brain neural activity and is used to assess how age influences neyral dynamics during sleep. To explore if reversal neurons play a role in these sleep-like quiescent bouts, the JZ3110 mutant (mzmEx337[Pnpr-9::mCherry (pHK105) 50 ng/µL Pinx-1::GCaMP5K (pHK55) 50 ng/µL]; lite-1(xu7); AIB calcium indicator) has the AIB AIB neuron tagged by red fluorescence protein (RFP), allowing for tracking of the single neuron and GCaMP protein in AIB. The interest lies in understanding if AIB could predict sleep-like quiescent bouts, and how age affects this mutant's circuitry post-conditioning.

Two-channel, spinning disk confocal microscopy will be used to record behavior in of freely moving c. elegans after conditioning in the JZ3110 mutant. The AIB neuron will be tracked throughout the video via mCherry and GCaMP. The mCherry tagged protein allows for visualization of the neuron, while GCaMP tracks the changes in activity of this neuron. The neuron will be manually tracked. The JZ3068 mutant has GCaMP in all neurons, allowing for extraction of all neural traces.

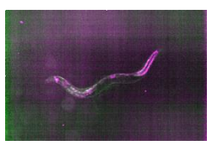
Behavioral annotations will allow for comparison between behavior and neural activity. After behavioral annotations are done, a machine learning algorithm will take 10 folds of the data and extract most active neurons. These neurons will allow us to explore predictors of sleep.

KEY METHODS



Long-Term Memory (LTM) Assay

Training paradigm consisting of three 80- minute odor training cycles and two 30- minute food cycles, with buffer washes in between. After the third training cycle, C. elegans are plated on a recovery plate with food to allow for recovery and sleep. If no food is present, c. elegans will not sleep.

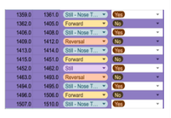


Confocal Microscopy

C. elegans are freely moving on an agar pad with liquid food on a glass slide. AIB neurons is tagged with mCherry & GCaMP. mCherry channel will show red flurouescnce on AIB neuron. GCaMP channel shows changes in calcium (neural acticity) of AIB. Manual tracking of AIB tagged neuron

Behavioral Annotations

Recordings of the beginning and end of behaviors showed during imaging. Allows for comparison to neural activity

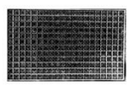


Cross Validation

Fragments from recorded data are inputted into training algorithm and most active neurons are extracted.

Wormotel

Quantifies movement of c. e elegans over time Used to assess sleep.

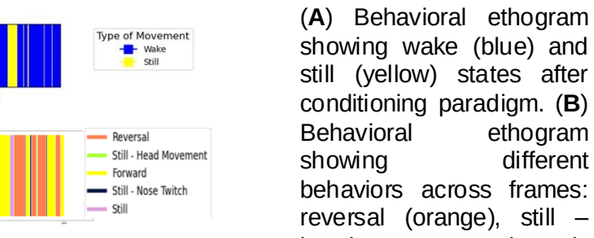


NEURAL ACTIVITY DURING SLEEP

Information travels through a human's brain during sleep but little is known about what happens to this information as it travels through the brain. Using C. elegans, scientists can observe the changes in neural activity across the brain and on an individual neuron level, during quiescence. The activity traces can be extracted from the file and plotted to show the change in activity throughout the recording. The AVA neuron showed slower and longer patterns of activity during quiescence.

WHY AVA?
AVA is a reversal command neuron and shows more activity in naïve c. elegans than in the trained group. AVA has shown neural activity during sleep. AVA is also an easily identifiable neuron via whole brain imaging.

WHY AIB?
AIB is a reversal interneuron and shows similar activity patterns to AVA. AIB is also easily identifiable via whole brain imaging.



OBJECTIVES

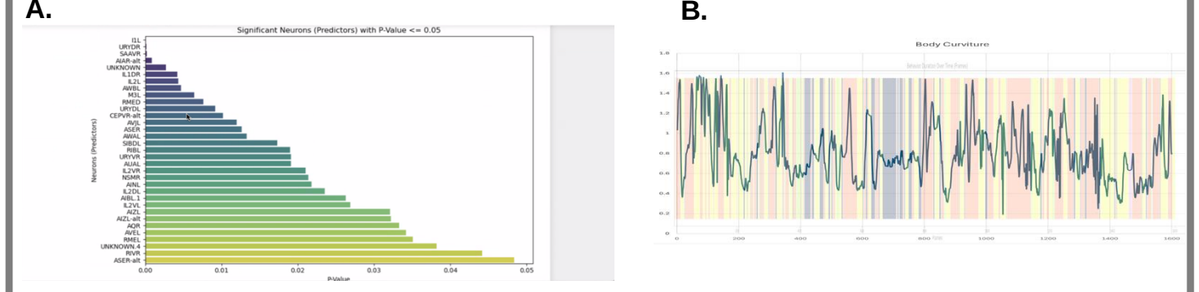
Q1: Which neuron's activity predicts sleep?
Q2: How does c. elegans age influence sleep and behavior post-conditioning paradigm?
Q3: How does memory consolidation correlate with sleep quality?

I. Explore the neurons that show activity during sleep.
II. Explore if aging in C. elegans affects quiescence behavior after a conditioning paradigm.

TRAINED C. ELEGANS SLEEP MORE

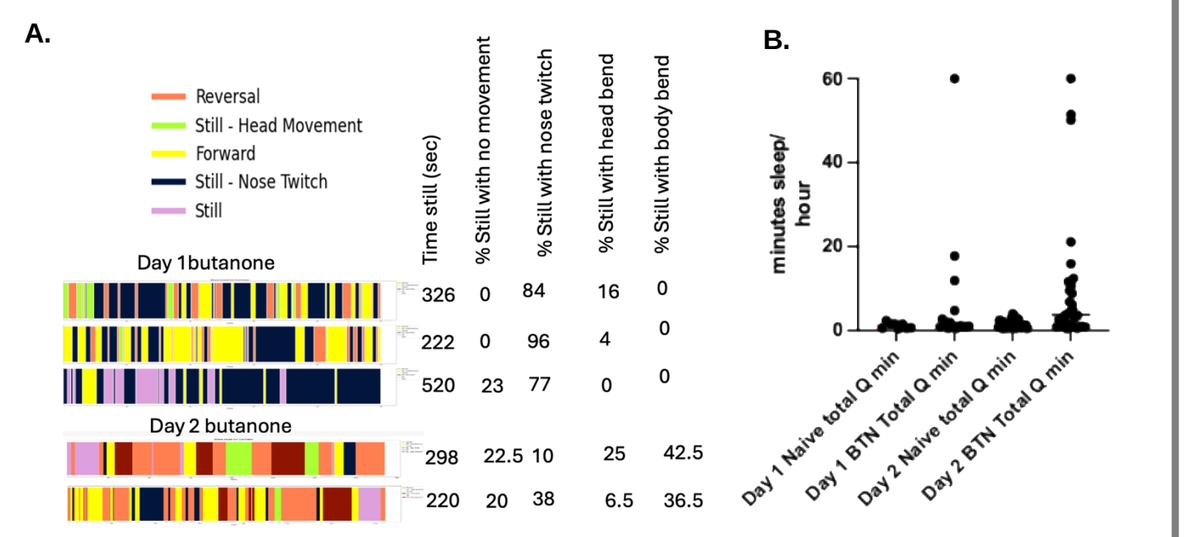
(**A**) Behavioral ethogram showing wake (blue) and still (yellow) states after conditioning paradigm. (**B**) Behavioral ethogram showing different behaviors across frames: reversal (orange), still – head movement (green), forward (yellow), still – nose twitch (navy blue), and still (lavender).
(**C**) Behavioral ethogram comparisons between conditions: buffer, naïve, butanone. Total quiescent time and percentage of behavior type from total quiescent time

I. AIB NEURON SHOWS ACTIVITY DURING SLEEP



(**A**) Bar graph displaying most active neurons during periods of quiescence. (**B**) Neural trace of AIBL.1's (AIB neuron) body curvature is aligned to behavioral ethogram comparing neural trace to observed behavior.

II. DAY 2 ADULTS SHOW NEW BEHAVIOR POST CONDITIONING



(**A**) Behavioral ethogram showing different behaviors across frames comparing behaviors in Day 1 vs Day 2 conditioned C. elegans (**B**) Graph comparing Day 1 and Day 2 naive and butanone trained C. elegans showing similar quiescent bouts.

CONCLUSIONS

- Day 1 butanone-trained C. elegans showed a preference for doing a nose twitch while being still.
- Day 1 butanone-trained C. elegans do not often remain completely still.
- AIB neuron shows activity during quiescence.
- Day 2 butanone C. elegans showed a new behavior and similar quiescent length to Day 1 adults.

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NEXT STEPS

- •To better understand transitions in neural dynamics, exploring nested behaviors during periods of quiescence is essential.
- •To assess whether the AIB-tagged strain's learning and memory are similar to those of the JZ3068 mutant, a chemotaxis assay will be conducted.
- •To improve confidence in neuron identifications, more training of the machine learning algorithm is needed.
- •To better understand the mechanisms that occur during sleep, exploring whole brain dynamics to find potential predictors of sleep-like quiescent bouts is required.

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