Establishing Evidence for the Development of CPG in Caenorhabditis Elegans

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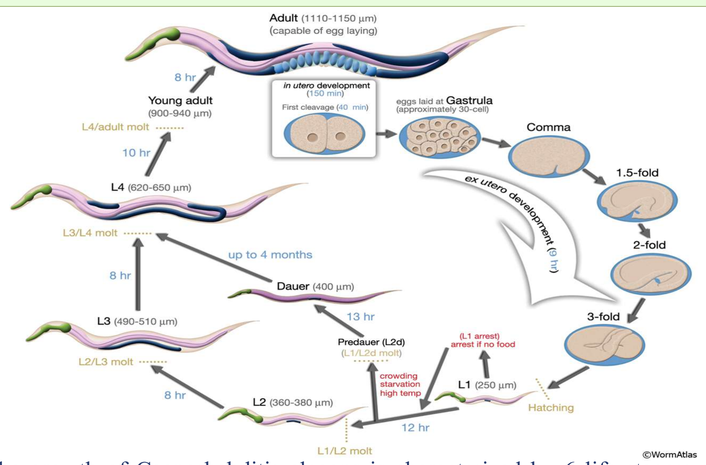
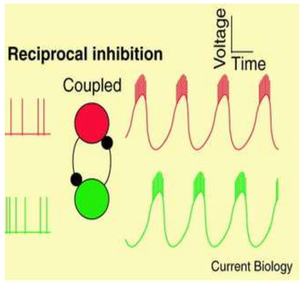
ABSTRACT

- The connectome of *Caenorhabditis elegans* is known and of great interest to those in the neuroscience community, but how this connectome is established and functions in the early stages of development is not yet fully understood.
- During the L1 life stage, *C. elegans* does not have a fully differentiated nervous system. Undifferentiated neurons are more likely than differentiated cells to respond similarly and simultaneously to stimuli, which should result in widespread synchronized neuronal firing.
- We studied the dynamical firing of L1 *C. elegans* in order to determine whether their neurons indeed fire in a synchronized and rhythmic pattern.
- If we find synchronized rhythmic firing to be prominent during the L1 life stage that would provide support for the hypothesis that a Central Pattern Generator (CPG) is one of the first connectomes to be established during nervous system development.

BACKGROUND



The growth of Caenorhabditis elegans is characterized by 6 life stages; egg, L1, L2, L3, L4, and adult (egg-laying) stage. After hatching, the transition between each stage is marked by a period of lethargus (sleeping) during which molting occurs. At the L1 life stage, the neural system is not yet fully developed, and consists mainly of undifferentiated neurons. It is believed that during each period of lethargus synaptogenesis and neural differentiation occur. As a nematode progresses through the life stages, its neural systems become exponentially more differentiated; and by the time it is a young adult, most of the neurons of its neural network should be differentiated.



Central pattern generators (CPG) are a type of neuronal circuit that can produce rhythmic motor patterns. It contains no sensory or descending input but does contain temporal information. A pair of neurons reciprocally connected by inhibition forms CPG, as can be seen by the diagram below. In C. Elegans, we believe a CPG is present and controls the rhythmic motion of the nematode's head moving from side to side.

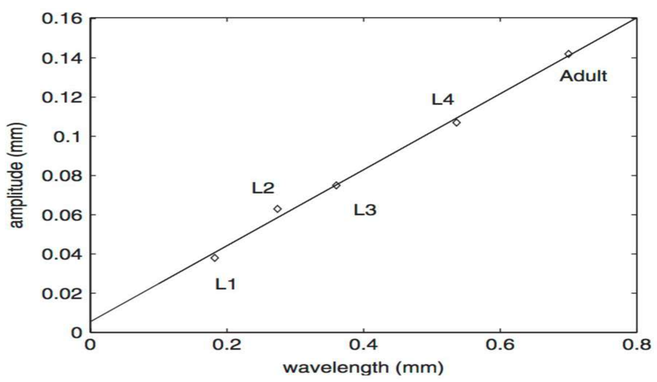


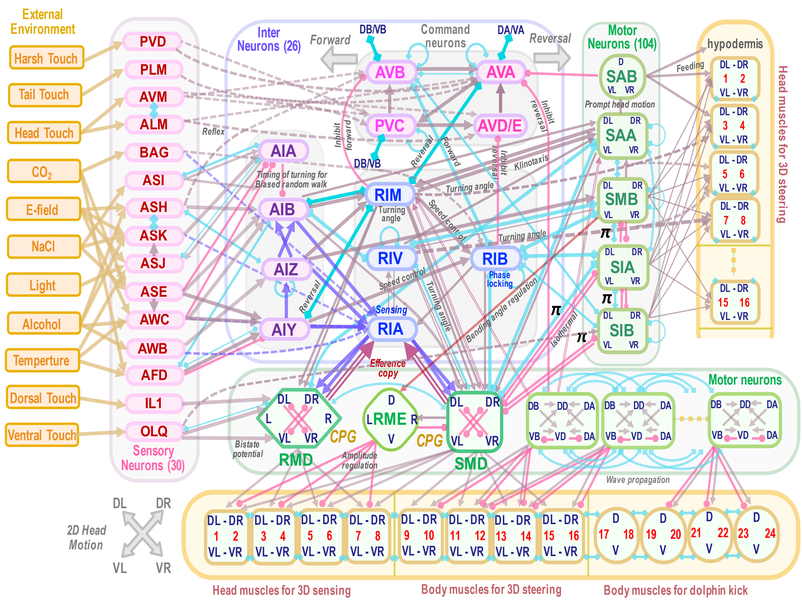
Fig. 6. Linear scaling of the amplitude of undulations with the wavelength during different developmental stages. The least-square fit to the data points yields regression line $A_0 = 0.194\lambda + 0.006$ with $R^2 = 0.98$.

Central pattern generators have no descending or sensory input, control rhythmic motor movements, and carry specific timing information. The rhythmic motion of the C. elegans undulating head movement lacks descending or sensory inputs, and thus parallels the neuronal circuit of CPG. This movement operates like a clock allowing the nematode to interpret its surroundings in a time domain. More evidence for the early development of a CPG in C. elegans comes from free-motion data taken throughout the different stages of its life. The nematode exhibits obvious rhythmic undulations of its head region that can be described with a wave function over time, with an amplitude that increases over time as it grows in size and an approximate frequency of 3 /s.

HYPOTHESIS

- Because the neural systems of an L1 *C. elegans* are not fully differentiated, we expect its neurons to fire at the same time, in response to the same stimuli.
- We expect to see a higher ratio of synchronized rhythmically flashing neurons to randomly flashing neurons at the L1 life stage than at the adult life stage.
- This would provide evidence for the development of a Central Pattern Generator at the L1 life stage, and a reliance on the CPG for movement.

PROPOSED DYNAMIC CONNECTOME



Using the QW1217 strain of *C. elegans* and knowledge of the nematode's full dynamic connectome, this pattern of signaling is hypothesized. The neurons RMD and SMD behave very much as would be expected of a CPG: they fire in synchronized rhythm, and are cross-inhibited via acetylcholine channels. We propose that RMD and SMD together constitute the CPG of *C. elegans.*

MATERIALS & METHODS

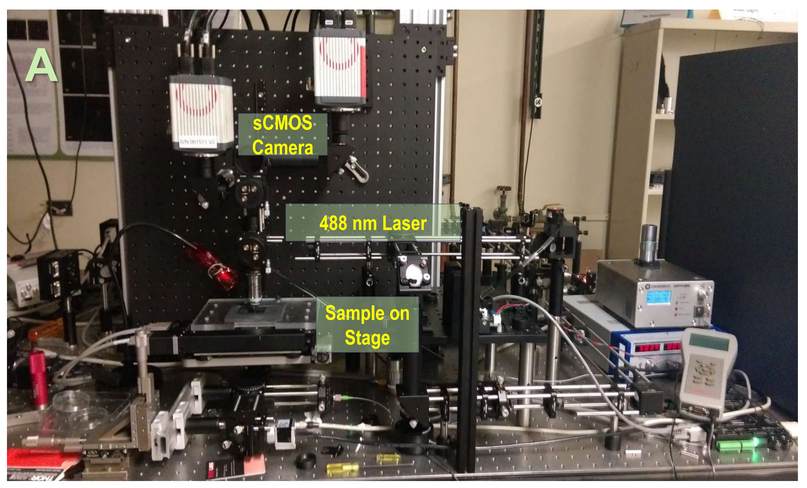


Image A: *2-Dimensional Line Confocal Microscope*
This microscope is designed to convert 2-dimensional information into a 3-dimensional representation of a C. eleganss. The image is produced by a 406nm laser and is magnified 40x.

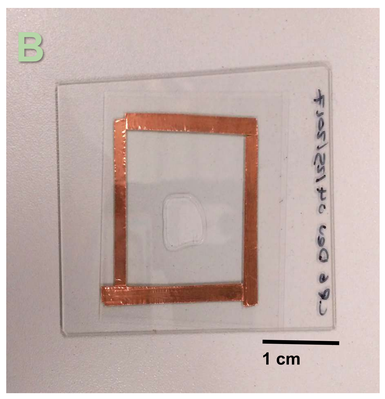
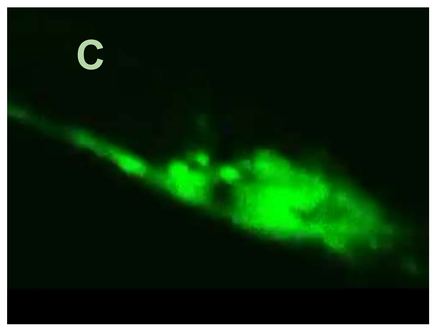


Image B: *Thin Gelatin Samples*
The 2-dimensional microscope requires a thin gelatin sample preparation. Copper tape is placed in a square on a thin glass plate. In the middle of this copper square, we place worms covered in levamisole surrounded by a 10% gelatin solution. We chose a 10% gelatin because it hardens quickly and is more transparent than agar. A cover sheet is placed over the solution and can then be imaged.

RESULTS

Image C: *QW1217 Strain*
We used the QW1217 strain because it expresses cGAMP6, a pan neuronal expresser. This strain is particularly useful for observing CPG, as it allows us to visualize neuronal activity in real time.



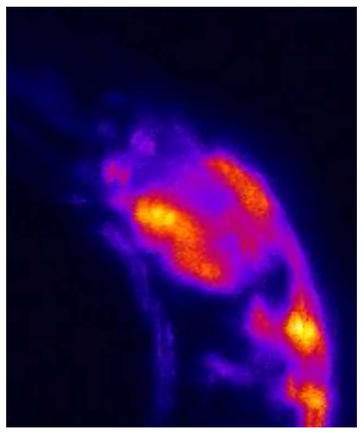
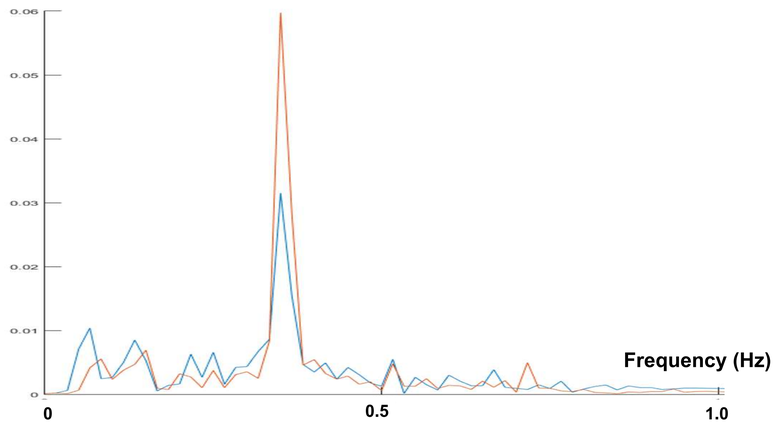


Image from a real time video of a young adult *C. elegans* immobilized with levamisole. This image illustrates the most active neurons at one particular instant. To determine the presence of a CPG pattern, we plot the intensity of the expressed QW1217 signal versus time, and look for regions that exhibit a rhythmic pattern of firing. In the past, we have found that the SMD and RMD neurons exhibit a rhythmic pattern of firing, with a frequency one spike of activity every three seconds.



This pattern of rhythmic firing seems promising, as it aligns with the observable rhythmic undulation of *C. elegans* undergoing free-motion. However, we do not yet have conclusive evidence that this frequency is due solely to the activity of RMD and SMD, and not the result of noise caused by equipment or unrelated neuronal activity.

FUTURE DIRECTIONS

In light of the lack of conclusive evidence to either prove or disprove the presence of a CPG in *C. elegans*, we will continue our investigation, focusing on nematodes in the L1 life stage. We expect young, undeveloped *C. elegans* to exhibit more obvious CPG behavior due to their undifferentiated neural system. Additionally, we will explore imaging both adult and L1 nematodes undergoing free-motion, and determine whether the practice of using levamisole to immobilize *C. elegans* might obscure the detection of a CPG pattern.

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