A *Caenorhabditis elegans* Model of Age-dependent Neurodegeneration

Purpose

The probability of humans developing neurodegenerative diseases increases as one ages. So the purpose of this study is to use the nematode *Caenorhabditis elegans* as a genetic model for determining if they develop age-dependent neuronal changes.

Hypothesis

If wild type *C. elegans* with neuronal GFP markers are observed on Day 5, 8, and 11 of adulthood, then they will exhibit age-dependent neuronal changes.

Materials

•Wild Type *Caenorhabditis elegans* (at least 30) carrying Neuronal GFP Markers
• Zeiss Upright Fluorescence Microscope with a Digital Imaging System
• Dissecting Microscope
• Petri Dishes
• Worm picker and Gas Burner
• Glass Slides and Cover Glass
• Agar and *Escherichia coli*
• 100mM Sodium Azide

Procedures

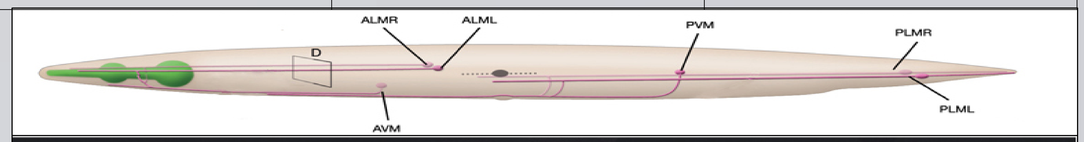
1. Under a dissecting microscope, transfer the *C. elegans* in L4 stage to a Petri dish containing agar with *E. coli* seeded on the agar. Label the dishes with the researcher's initial and the date.
2. Incubate the worms at 20°C.
3. On the fifth day of incubation (Day 5), transfer 10 worms to the agar pad on a glass slide with 5µl 100mM Sodium Azide that will tranquilize the worms for easy observation. (More worms can be used for more accurate information).
4. Cover the glass slide with a cover glass and place it under the fluorescence microscope.
5. Observe and take pictures. Record the number of *C. elegans* that possess degenerated neurons.
6. Calculate the percentage of neurodegenerated *C. elegans* out of the number of worms that were used.
7. Repeat steps 3-6 for Day 8 and Day 11.

Abstract

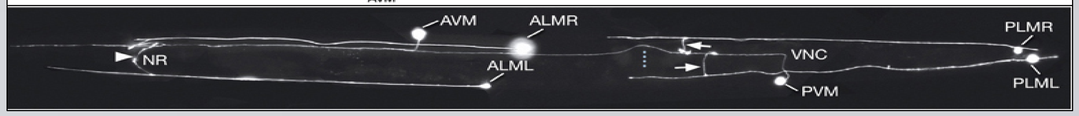
Data

Days of Adulthood	Number of Worms with Degenerated Neurons	Percentage of Worms with Degenerated Neurons
Day 5	0	0%
Day 8	4	40%
Day 11	9	90%

A

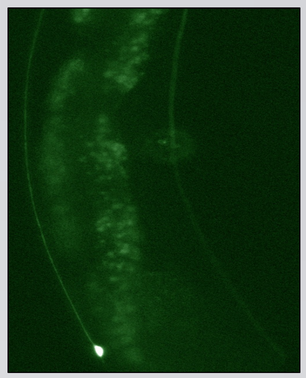


B



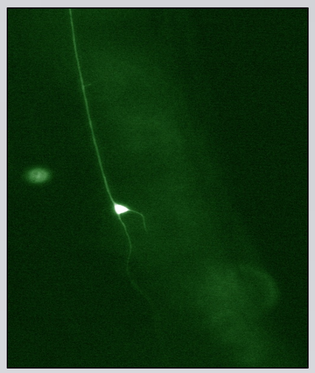
(Picture: A) A diagram of the touch neurons in *C. elegans* (Picture: B) A picture of GFP labeled touch neurons in *C. elegans*
(Left: anterior; Right: posterior)

C



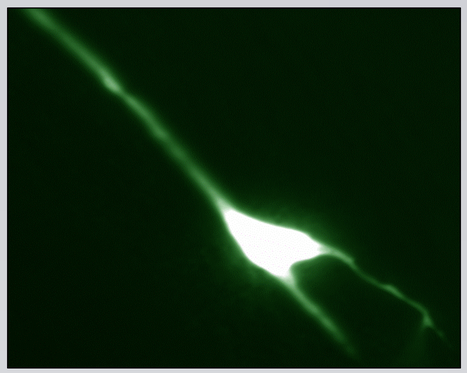
Normal Neuron

D



Branched Neuron

E



Enlarged Branched Neuron

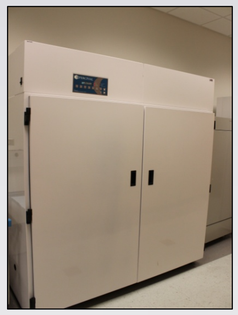
(Picture C-E) Top: Anterior; Bottom: Posterior

Pictures

F

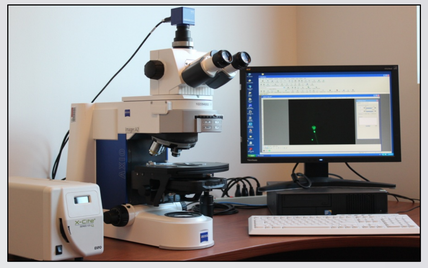
(Picture F) Dissecting Microscope with a gas burner, Petri dishes, and a worm pick

G



(Picture G) Incubator (20°C)

H



(Picture H) Fluorescence Microscope with an Imaging System

Discussion

The researcher was very intrigued with the mechanisms behind neural degeneration after studying psychological disorders. After a great deal of research, this specific topic was chosen due to the fact that *C. elegans* are readily and easily accessible, and that the researcher had access to a specific lab equipment.

The genetic and cellular processes that help perpetuate neuronal integrity during normal aging remains elusive. In this study, we show that *Caenorhabditis elegans* touch neurons display age-dependent morphological deficiencies. These may include cytoskeletal disorganization and defasciculation.

But what exactly is neurodegeneration? It's the process where neurons progressively lose their structure and/or function. This includes death of neurons and usually occurs as one ages. In the nematode *Caenorhabditis elegans*, age-dependent morphological changes are widespread in somatic tissues. However, there is very little that is known about the changes in neurons during aging. Despite the inadequacy of definite evidence, there is a clear age-dependent behavioral decline in *C. elegans,* including decreases in locomotion and chemotaxis.

In order to carry out this experiment, a fluorescence microscope was used. A fluorescence microscope works by emitting light of an excitation frequency which is absorbed by fluorophores (a chemical compound that can re-emit light upon light excitation). The fluorophores then emit fluorescent light which is focused by an objective lens and filtered into the detector. This is why the *C. elegans* carrying a neuronal expressed GFP marker was used. GFP stands for green fluorescent protein, a natural fluorophore.

It is believed that as the *C. elegans* aged, they would develop morphological changes in the neurons in relation to the age. The wild type *C. elegans* were observed on Day 5, Day 8, and Day 11. On Day 5, no worms with degenerated neurons were observed, so there was 0% neurodegeneration. However, (as the *C. elegans* aged) on Day 8, 4 worms were observed that possessed degenerated neurons, meaning 40% of the worms developed neurodegeneration. And by Day 11, 9 worms were observed with degenerated neurons. This corresponds with the hypothesis, as there were age-dependent changes. However, in this experiment, only the number of degenerated neurons was observed. The functions of the neurons as the aged were not determined. Therefore, it is plausible that although they deteriorated, they can still function albeit impaired.

In this experiment, the only thing tested was what percentage of a certain population of *C. elegans* would develop degenerated neurons. This project could be adapted further do determine causes, certain loss of functions, and drug testing to slow down or reverse the effects of neurodegeneration.

Conclusion

In conclusion, the expected outcomes were confirmed. The *C. elegans* did have age-dependent neuronal changes because, according to the results recorded, as the *C. elegans* aged, more and more of the worms began to experience neurodegeneration. On Day 5 of adulthood, the worms experienced no neurodegeneration. But as their age increased, so did the number of worms with degenerated neurons with 40% of the worms on Day 8 and 90% of the worms on Day 11.

This information can be very helpful to researchers studying neurodegeneration, and neurodegenerative diseases. The data proves that *C. elegans* develop age-dependent neural changes which can parallel neural changes in humans making *C. elegans* an ideal choice as a genetic model.

Applications

Also, this information can be applicable to the real world in a variety of different scenarios. Because *C. elegans* exhibit a neural deterioration that nearly parallels that which has been observed in humans, *C. elegans* can be used as a genetic model to conduct further research on neurodegenerative diseases. Furthermore, this information can also apply to humans. For example, if a *C. elegans* lifespan to human lifespan ratio was developed, you would be able to theoretically see at what age would most people start developing degenerated neurons. After calculating a ratio of the average, non-mutated *C. elegans* lifespan (18 days) to the average human lifespan (79 years), it was found that approximately 90% of people at age 48 will begin to have degenerated neurons. Humans at age 22 will have no degenerated neurons, and 40% of humans at age 35 will have degenerated neurons. This suggests that neurodegenerative diseases can begin to develop at age 30-50 and can progressively deteriorate as one ages.