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C. elegans Behavior Kit

Neurology Supplement

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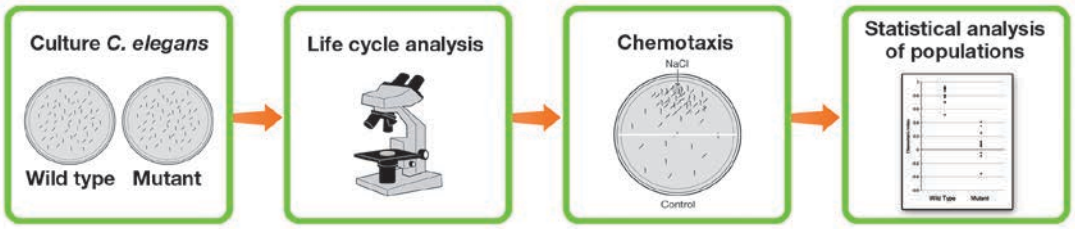

This kit contains temperature-sensitive reagents.
Open immediately and see individual components for storage temperature.

Please see redemption instructions on how to receive your *C. elegans*.

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Introduction

While our understanding of how the human body functions has grown significantly in the past century, much remains to be learned. One of the major gaps in our knowledge lies in our lack of understanding of how the human brain functions. The brain is the most complex organ in the human body and arguably the most remarkable, yet very basic questions remain unanswered. How does the human brain store memories and information? What is cognition? What are emotions? What makes up intelligence? While we do know some details about these questions, the essence of how the brain functions still eludes us. At the time of writing this manual, major scientific endeavors are looking to map all of the neuronal connections (the connectome) in the human brain and to develop technologies that will allow us to see the brain function in real-time in the hope of answering some of these questions.

Ambitious scientific projects, such as the human connectome project and the Brain Research through Advancing Innovative Neurotechnologies (BRAIN) initiative, are looking to answer these very complex questions through direct imaging of human brains. Meanwhile, other researchers are taking a reductionist approach to unlocking the secrets of the brain. A reductionist approach is one that seeks answers to highly complex questions, such as how does the human brain work, in the simplest model available, such as *C. elegans*. The *C. elegans* nervous system is extremely simple when compared to the human brain. A *C. elegans* hermaphrodite has just 302 neurons while the human brain is estimated to be made up of 100 billion neurons.

Notwithstanding this relative simplicity, the 302 neurons that make up the *C. elegans* nervous system mediate highly complex behaviors, including social, mating, and learning behaviors that we typically associate with more complex organisms. In light of this, many researchers have focused their scientific efforts on trying to understand how the simple *C. elegans* neurologic system works and then use the knowledge acquired while studying *C. elegans* to better understand how the very complex human brain works.

Over the last three decades, a lot of information about *C. elegans* neurology has been generated. The *C. elegans* hermaphrodite connectome was fully mapped in 1986 by John G. White, providing a detailed wiring diagram of the neural circuits that make up the *C. elegans* nervous system (White et al. 1986). Using the *C. elegans* connectome, researchers can determine the function of each individual neuron and reveal the contribution of each neuron to the function of individual neural circuits or the entire nervous system. With a neurological wiring diagram in hand and more than 20 years of research invested in studying *C. elegans* neurobiology, researchers are still working hard to understand how information is stored in the *C. elegans* nervous system and how the nematode makes decisions.

This laboratory activity explores learning in *C. elegans*. In this activity we have examined differences in learning that come about due to a deletion of the *daf-18* gene. One of the phenotype of *daf-18*–mutant worms is that they fail to associate environmental conditions with feeding conditions, and thus do not display chemotaxis in response to NaCl, even after conditioning. Even though this experiment demonstrates the importance of *daf-18* in learning associative behaviors, the manner in which this information is stored at the level of the individual neuron or the neuronal circuit remains a mystery. Answering questions such as this, while still challenging, is feasible using a good neurological model organism such as *C. elegans*, and the answers can perhaps be applied to more complex organisms. This section will explore differences and similarities between the *C. elegans* and human nervous systems and explore the significance of the *daf-18* phenotype that we encountered in the laboratory activity in terms of human disease.

C. elegans Neuron Structure and Function

C. elegans neurons share many features with human neurons. Like human neurons, *C. elegans* neurons are composed of a cell body that contains the cell nucleus and the majority of the cellular organelles; dendrites, which are highly branched and receive signals from other neurons or sensory structures; axons, which transmit signals to other neurons or effector cells; and an axon hillock, the point at which the cell body and axon meet that serves as the point for generation of action potentials.

One major difference between *C. elegans* neurons and human neurons is that *C. elegans* neurons are never wrapped in myelin. While many of the neurons in humans, particularly in the brain, are not wrapped in myelin, most of the neurons in the human peripheral nervous system are myelinated to preserve action potential intensity over long distances and to speed up signal transmission by serving as an insulator. *C. elegans* neurons do not transmit signals across large distances, since *C. elegans* grow to be only about 1 mm long, and thus do not have a need for myelin.

The neuronal system is the most complex organ system in the *C. elegans*, making up just under one third (302 of 959) of the cells of an adult hermaphrodite. The *C. elegans* neuronal system is made of two independent nervous systems; the pharyngeal and the somatic nervous systems. The pharynx is a tube-like muscle pump that concentrates food, grinds it, and transports it to the intestine. The pharyngeal nervous system is made up of 20 neurons responsible for the coordination of pharyngeal (feeding) activities and interacts with the somatic nervous system through just two interneurons. The remaining 282 neurons in *C. elegans* make up the more complex somatic nervous system that is in charge of coordinating all other *C. elegans* functions.

The *C. elegans* nervous system is made up of three basic types of neurons: sensory neurons, interneurons, and motor neurons. *C. elegans* has 68 sensory neurons, which are specialized neural cells that possess the capability of detecting external stimuli such as soluble and volatile chemicals, tactile stimuli, and temperature. Sensory neuron and interneuron cell bodies make part of a cluster of nerve cells, or ganglia, in the head that can be loosely interpreted as the “brain” of *C. elegans*. Sensory neurons send dendrites from the head ganglia to the tip of the nose and transmit sensory information to interneurons in the head ganglia that are responsible for analyzing and interpreting it. Once the interneuron network has determined what action *C. elegans* should take based on the sensory neuron input, interneurons then stimulate motor neurons to produce the desired outcomes. Motor neurons interface with effector cells, such as muscle cells, to control their activity, thus allowing the organism to move in response to the directions of the interneurons.

While the human nervous system is far more complex than the nervous system of *C. elegans*, the human nervous system can be categorized in a similar manner. Humans also have sensory neurons (rod and cone cells in the eye, taste buds in the tongue), which transmit information to interneurons in the central nervous system (brain) or the spine, which in turn stimulate motor neurons. The motor neurons then stimulate muscle or endocrine cells or the spine, and motor neurons, which stimulate muscle or endocrine cells to produce a desired outcome. The human nervous system is much larger than the *C. elegans* nervous system, with an estimated 100 billion neurons making up the human brain alone. Yet the basic structural organization of neuron subtypes into sensory neuron, interneuron, and motor neuron is conserved between humans and *C. elegans*.

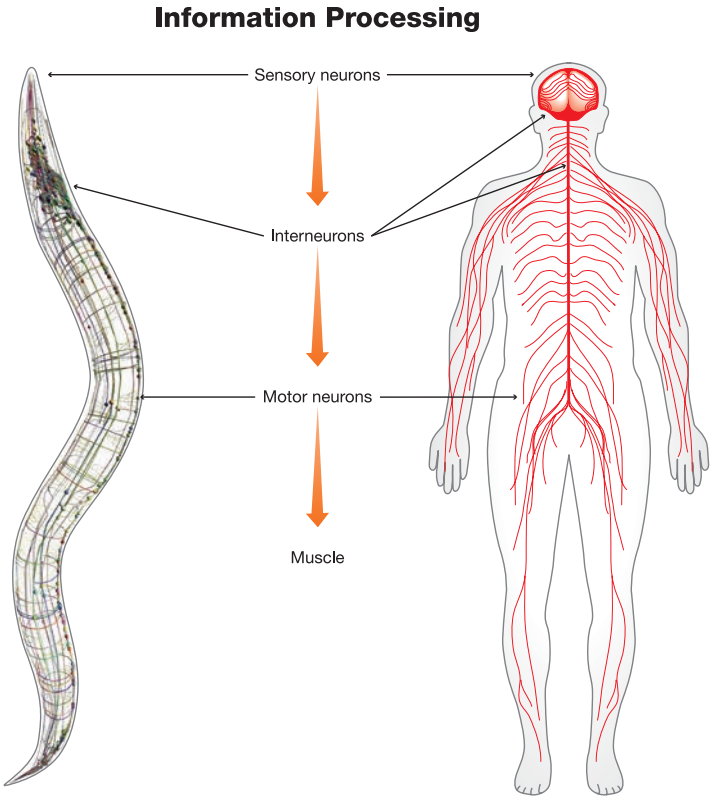


Fig. 1. Similarities in information processing between *C. elegans* and humans. Although the complexity of the human connectome is far greater than that of *C. elegans*, the basic transfer of information — from the sensory neurons to the interneurons for processing, and then to motor neurons to complete an action — is conserved between both species.

The neural circuit responsible for sensing and responding to chemical stimuli in the environment in *C. elegans* is made up of just four sensory neuron pairs and ten interneuron pairs. Each neuron pair comprises one neuron that moves along the left side and one neuron that moves along the right side of *C. elegans*, thus bringing the total number of neurons involved in the *C. elegans* chemical-sensing neural circuit to 28 individual neurons. These 28 neurons make up a highly interconnected neural network in which a single neuron interacts with multiple other neurons via two distinct types of synapses, electrical and chemical.

Synapses that pass information between two neurons via gap junctions connecting the cytoplasms of two neurons, allowing for electrical impulses to move directly from the cytoplasm of one neuron to the next, are called electrical synapses. Electrical synapses conduct nerve impulses faster than chemical synapses. In humans, electrical synapses are most commonly observed in neural circuits involved in defensive or avoidance reflexes that require the fastest possible response. The vast majority of synapses, however, are chemical. Chemical synapses involve the release of a chemical neurotransmitter from the presynaptic cell that travels between the two interfacing neurons to chemically stimulate the postsynaptic cell.

Making sense of the relatively simple *C. elegans* neural network may seem like a daunting challenge, but it pales in comparison with the challenge of understanding the far more complex human neural network. Whereas the 302 neurons of a hermaphrodite *C. elegans* have approximately 5,000 total synaptic connections, the current estimate is that each of the human brain's 100 billion neurons has, on average, 7,000 synaptic connections to other neurons (Drachman 2005). Simply put, a single human neuron averages more synaptic connections than are found in the entire *C. elegans* nervous system.

C. elegans is, to date, the only organism for which we have a connectome, or complete map of the neural synapses. Having this blueprint of neural system wiring is incredibly valuable, but many questions still remain. How is information stored in *C. elegans*? Why does *C. elegans* behave the way it does in response to NaCl? To answer questions such as these, researchers must first understand the role of each individual neuron in governing behaviors, as well as the role of individual proteins in the function of individual neurons.

To understand the role of individual neurons in governing specific behaviors researchers can eliminate the specific neuron in question using lasers, and then assay, or test, whether the phenotype, or behavior, is changed. Similarly, to understand whether a particular gene or protein is important to a phenotype such as learning, researchers can use a mutation in a gene that will impair its function or create a genetic knockout that eliminates the gene altogether and assay whether the phenotype is altered. Experiments such as these, which seek to mechanistically understand the function of proteins or cells, cannot be performed without model organisms.

In this experiment we have utilized a functional *daf-18* knockout *C. elegans* mutation to understand whether the *daf-18* gene is important to *C. elegans* in learning associative behaviors. Nine hundred and fifty-six nucleotide base pairs have been removed from the *daf 18* gene in our mutant *C. elegans*, essentially eliminating the expression of the DAF-18 protein in all tissues. While the DAF-18 protein is expressed in many of the cells that make up *C. elegans*, research has shown that it is essential for proper functioning of the ASE neurons, one of the neuron types that make up the *C. elegans* chemical-sensing neural circuit, the ASE neurons.

The ASE neurons are the major NaCl-sensing neurons in *C. elegans*. Despite being sensory neurons, the ASE neuron pair has been demonstrated to mediate learning associative behaviors toward or away from NaCl, a role that is typically reserved for interneurons. The DAF-18 protein is a protein phosphatase that is homologous to the human PTEN protein. In *C. elegans* the DAF-18 protein phosphatase functions as part of a signal transduction pathway that receives signals via a cell membrane receptor and transduces the signal via a kinase cascade, which is made up of protein kinases and protein phosphatases and culminates in the activation or deactivation of a transcription factor that alters gene expression. The DAF-18 protein phosphatase in the *C. elegans* ASE neuron functions as part of a signal transduction pathway homologous to the human insulin receptor pathway. Is there evidence in humans that PTEN or the insulin receptor pathway is important for learning and memory?

In model organisms, as in humans, researchers often discover the function of a particular protein only when that protein malfunctions or when an individual who has inherited or developed a mutation in the gene that encodes the protein develops symptoms. That is, oftentimes in humans we learn about the function of a protein only when a problem with the protein causes a patient to get sick. While PTEN defects in humans are most commonly associated with cancer formation, such defects have also been associated with learning defects and autism.

Since the mechanism by which memories and information are stored at the neuron or neural circuit levels have not yet been discovered, it remains unclear whether learning disorders in humans due to PTEN defects and learning disorders in *C. elegans* due to DAF-18 defects share the same origin. However, it is known that PTEN functions as part of the insulin receptor signaling transduction pathway in humans in a manner similar to that observed for DAF-18 in *C. elegans* (Gupta and Dey 2012). Although the pathologies most commonly associated with insulin receptor defects in humans are metabolic diseases such as type II diabetes, defects in insulin receptor signaling in the human brain have also been associated with problems in learning and memory (Talbot et al. 2012, Schiöth et al. 2012). Many researchers even believe that defects in the insulin receptor signal transduction pathway in human neurons may be one of the underlying causes of Alzheimer's disease.

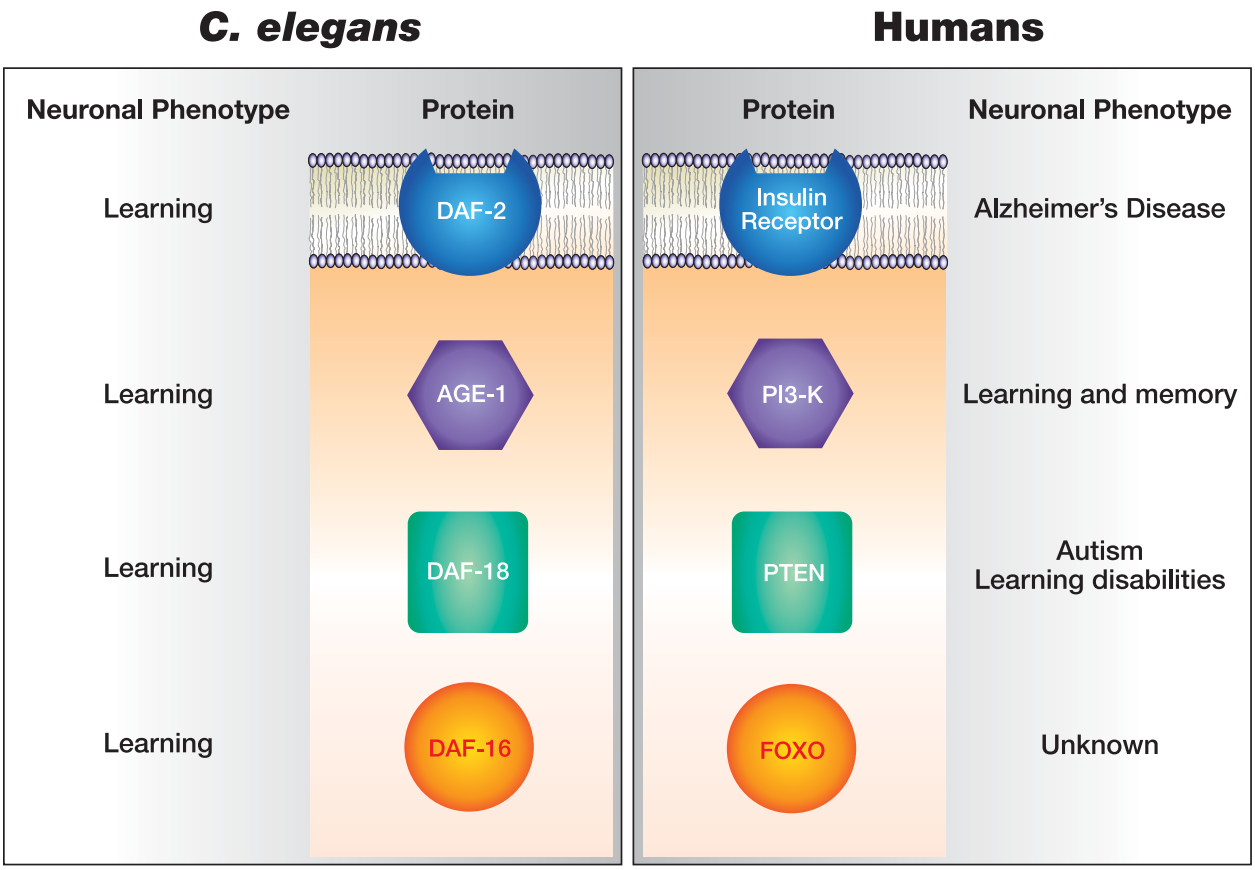
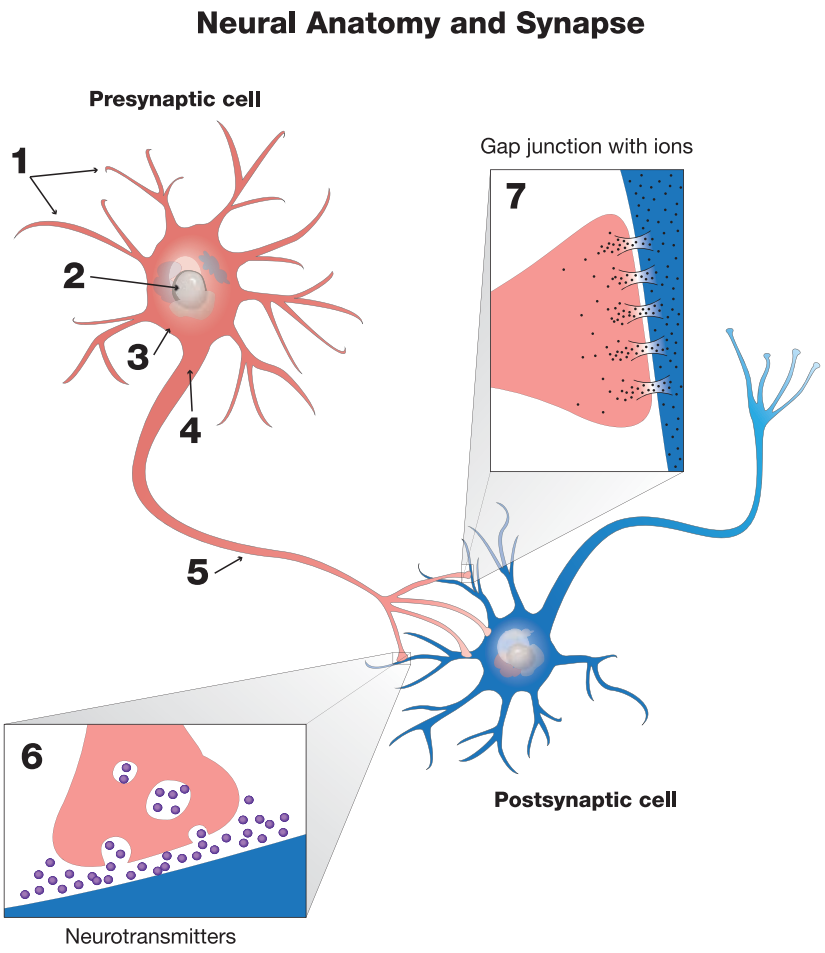


Fig. 2. *C. elegans* DAF-2 signal transduction is homologous to human insulin receptor signal transduction. Protein function and phenotypes can be correlated between *C. elegans* and humans. Can *C. elegans* studies help us treat diseases of the human brain?

What are the implications for human disease of identifying DAF-18 as a protein necessary for associative learning in *C. elegans*? Even though we still do not fully understand how information is stored in neurons, we do know that insulin receptor signaling is important in *C. elegans* learning in a manner that may be similar to its importance in human learning (Chen et al. 2013, Sasakura and Mori 2013).

The information acquired in the *C. elegans* experiment gives us a good clue as to how the human system may work, but we remain uncertain as to whether the mechanism of *C. elegans* learning is conserved in humans. We are also unsure of how signaling that is downstream of DAF-18 leads to learning. If we can answer this question in *C. elegans*, perhaps we can help unlock mysteries surrounding some basic functions of the human brain.

Focus Questions



Match the number on the image with the correct label below:

Label	Number
Axon	
Axon hillock	
Cell body	
Chemical synapse	
Dendrite	
Electrical synapse	
Nucleus	

Glossary

Ganglia

Pharynx

Sensory neuron

Interneuron

Motor neuron

Dendrite

Axon

Axon hillock

Action potential

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