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TOWARDS THE ROOTS OF HIGHER FUNCTION

MOLECULAR, CELLULAR, AND GENETIC UNDERPINNINGS OF BEHAVIOR IN *C. ELEGANS*

By Pushan Dasgupta

Have you ever wondered where complex higher functions originate from and how they actually work? This has puzzled intellectuals and scientists for eons. Only recently has humankind developed the tools and knowledge necessary to start probing this vast ocean of mystery. The heart of this enigma is that evidently complex higher functions like behavior are the result of biological phenomena.

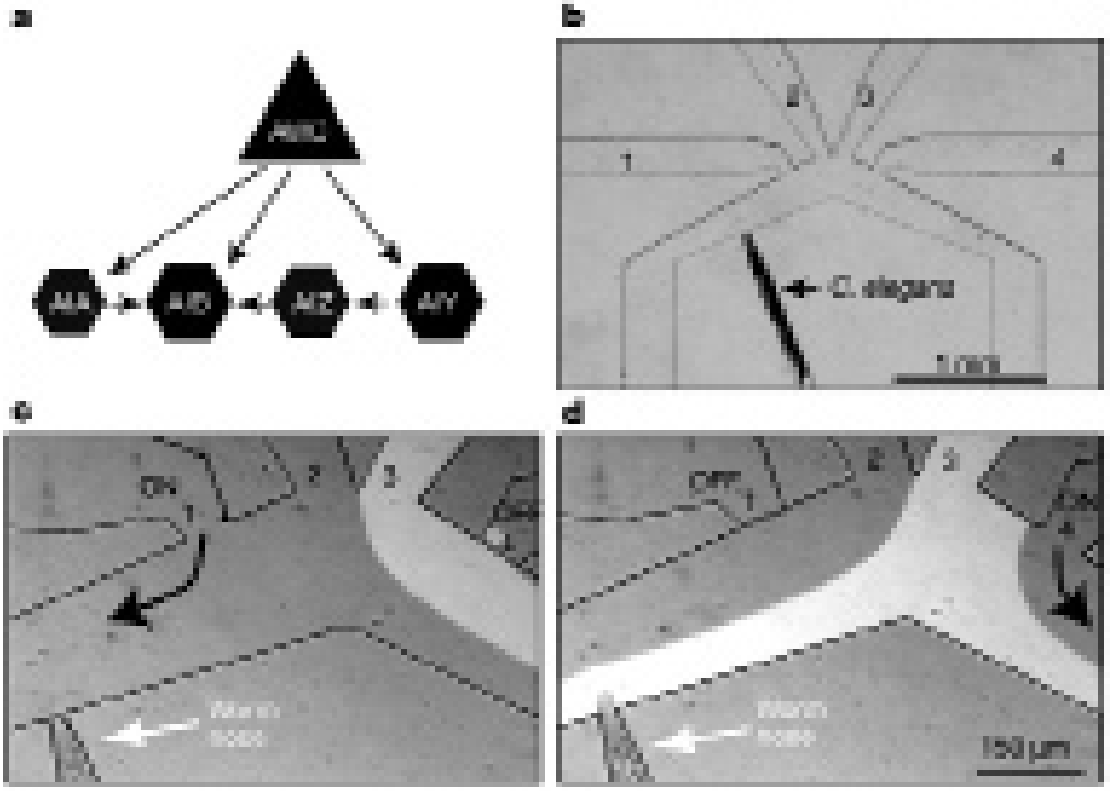
The primary question here is: how do events at the cellular and protein level result in something as complex and multifaceted as behavior? Evidence shows that these higher functions do arise from genetic, molecular, and cellular phenomena. Scientists are able to use genetic, molecular, and cell biological techniques in order to learn more about these higher functions. However, before we are able to elucidate these higher functions in humans, it is important to understand them in simpler model organisms in which it is easier to study such complex traits. This is exactly what scientists have been doing; their findings are gradually unveiling the beauty behind these higher functions.

Behavior in the worm *Caenorhabditis elegans*

Neurons are the core components of the brain and the nervous system of all animals. Therefore, it is very important to understand the neural circuitry behind behaviors. It is known that behavior is a result of the environment interacting with special cells called neurons, which use electrochemical signaling to process and transmit information. A prominent model organism that has been used in the study of the neural circuitry behind behavior is the worm *Caenorhabditis elegans* (*C. elegans*). It is a small animal of about 1 mm in length. *C. elegans* is an ideal model organism for neural circuit function analysis at single-cell resolution because it contains only 302 neurons, the synaptic connections between all of which have been mapped (1, 2). This is a miniscule number of neurons compared to the 100 billion neurons the human brain contains.

C. elegans exhibits two major forms of olfactory behavior: chemotaxis and local search behavior, produced by regulation of the movement of the worms. These little nematodes exhibit two types of movement, called runs and turns. Runs refer to a long series of sinusoidal swimming movements (3). These runs are interrupted by turns. Turns are produced in two main ways: by an "omega turn" and a reversal. In an omega turn a worm's head curl's

back, touching or crossing the tail, as the animal continues to move forward. In a reversal a worm moves backward for several seconds and then moves forward again in a new direction (3). Regulation of turns leads to chemotaxis and local search behavior. In chemotaxis the worms move towards food by sensing food odors or tastes and turning their body in response to them. During local search behavior, the animal spends about 15 minutes exploring a restricted area by increasing turning probability when food has been recently removed. Turns refer to one of the two movements the worm engages in which are runs and turns.



▲ a) Heirarchy of interneurons downstream of the AWC sensory neurons. b) Low magnification view of nematode in the PDMS imaging chip. c) High magnification view depicting the worm nose exposed to buffer. d) High magnification view of the worm nose exposed to the odor coming from channel 3.

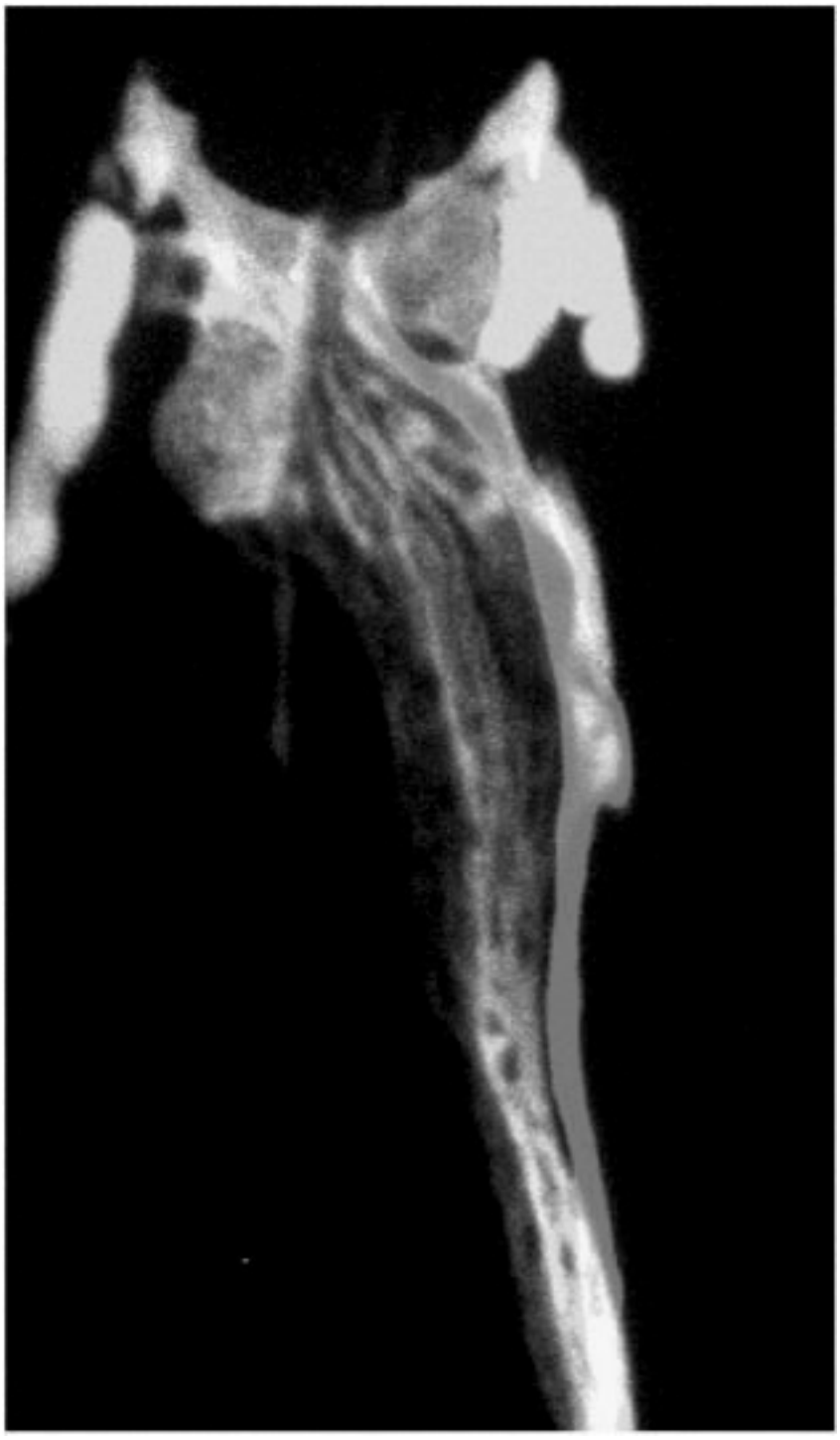
Understanding the Neural Circuitry: An example of How *C. Elegans* are used in the Lab

In order to study the neural circuitry behind these behaviors in *C. elegans,* genetic and cell biological techniques were applied. It was known that a pair of olfactory neurons called AWC direct chemotaxis to many attractive odors, and also increases turning probability during local search. AWC is also known to synapse onto several interneurons including AIB and AIY which enhance and suppress turning respectively (4). In order to monitor neural activity and depolarization in C. elegans, scientists at the Rockefeller University and Stanford University used AWC specific gene promoters to express G-CaMP which is a genetically encoded calcium sensor (2). Since their expression was driven by gene promoters specific to the AWC cells, the calcium sensor would only be present in AWC cells since their expression was driven by gene promoters specific to AWC cells. The concentration of calcium increases when a neuron is activated and depolarizes. Thus when the neuron is activated the calcium sensor would give off greater fluorescence. It was found that when the odor was taken away, there was an increase in AWC activity, but when the odor was added, there was a decrease in activity. AIB cells showed the same response pattern, while AIY showed the opposite, just as expected. Another method used in *C. elegans* studies is ablation studies, in which organismal function is examined after particular neurons are killed by laser or removed surgically. Ablation of the AWC neurons eliminated the AIY response, which indicates that AIY is inhibited by AWC. Release from this inhibition on odor presentation results in AIY activation. AIB on the other hand was found to be activated by AWC (2).

Another commonly used genetic approach is the "rescue experiment." In a rescue experiment, scientists take a mutant organism with some defective gene. They rescue the phenotype lost due to the mutation by expressing the proper version of the mutated gene in a subset of cells using cell-specific promoters (2). Previously, scientists had identified a type of mutant called "eat-4(ky5)," which were defective in a vesicular glutamate transporter, Eat-4, which concentrates glutamate into synaptic vesicles (5). Glutamate is a neurotransmitter and needs to be released into the synapse in order to activate the next neuron. So, these eat-4(ky5) mutant worms are, defective in olfactory chemotaxis, local search and numerous other behaviors (5). In order to rescue Eat-4 expression in eat-4(ky5) mutants, scientists expressed Eat-4 complementary DNA via an AWC selective promoter (2). This rescued chemotaxis in odor gradients, which suggests that AWC uses glutamate as its transmitter (2).

AIB interneurons on the other hand express GLR-1, another type of glutamate receptor. GLR-1 (n2461) mutant

credit: ref. 2



credit: Shaham S. and Bargmann C. Control of neuronal subtype identity by the *C. elegans* ARID protein CFI-1. Genes Dev. 2002 April 15; 16(8): 972-983.

▲ Staining of AWC and AWB interneurons of *C. elegans*.

animals have diminished local search behavior (4). In another rescue experiment, scientists expressed GLR-1 using an AIB specific promoter and rescued the local search defect. This indicates that the AWC neuron releases glutamate to stimulate GLR-1 on AIB interneurons. While the AWV cells have an excitatory GLR channel, AIY neurons were known to express the glutamate-gated chloride channel, GLC-3, which reduces the action potential firing activity of the cell (6). Worms with a mutation in the GLC-3 channel had diminished local search behavior. Interestingly, killing AIY interneurons was found to result in the opposite effect which is an amplified and long-lasting local search behavior (2). These results indicated that AWC inhibits AIY during local search behavior by glutamate release onto GLC-3.

By utilizing these genetic approaches the scientists were able to understand the neural circuit behind this olfactory behavior in *C. elegans*. In broader terms, the findings of all of these studies can be summarized as when food is around and the odor is present, the AWC neuron will not be very active and turning will be inhibited. However, when food is no longer present and the odor is gone, the AWC neuron will be active and turning will be promoted leading to local search. Although this is just a simple circuit in *C. elegans*, understanding its intricacies brings us one step closer to understanding the nature of higher function in our own brains.

Understanding Adaptation

A very interesting aspect of animal behavior is adaptation. Adaptation occurs when neurons modulate their excitability as a function of experience. In adaptation, repeated experiences cause changes in sensitivity of neural circuits to stimuli, which result in changes in behavior. Prolonged stimulation causing a repeated experience results in enduring homeostatic changes that allows an animal to reset its sensitivity to long-lasting alterations such as day-to-night changes in light intensity (7).

C. elegans also modulates its behavior as a function of experience. The worm will begin to ignore even attractive odors after being exposed to them for a prolonged period of time (8). Chemotaxis can be decreased by a 30 min pre-exposure to an attractive odor while exposures of more than 1 hour will decrease odor-seeking for hours (9). It has been thought that odor sensation occurs within the sensory cilia of the AWC neuron (10). But how these sensory neurons alter their responsiveness as a function of prolonged or repeated experience?

Scientists at the University of California Davis sought to tackle this key question. They previously found that the protein egl-4 is necessary for adaptation

in the C. elegans AWC sensory neurons (11). They found the mRNA of egl-4 to have some interesting properties. The scientists found that mutations in the 3'UTR (untranslated region) of the mRNA for egl-4 interfered with adaptation (11). A region within the 3'UTR of egl-4 was identified to be an NR/FBE region which binds to a protein called FBF-1. Mutations specifically in the NR/FBE region were responsible for loss of adaptation.

In order to further understand why mutations in the NR/FBE region resulted in loss of adaptation, cell-biological techniques coupled with genetics were applied (12). It was hypothesized that FBF-1 directs where egl-4 is translated and changes its level of expression in order for adaptation to take place. A special photoconvertible protein called Kaede was used as a reporter for new protein synthesis. Kaede fluorescence is irreversibly changed from green to red by exposure to UV light (13). Once Kaede is converted to red, only newly synthesized protein will fluoresce green.

The scientists placed Kaede coding sequences upstream of either the wild-type (normal) or ky95-mutated (defective) egl-4 3'UTR. After comparing the subcellular distribution of newly synthesized Kaede under the control of the wild-type 3'UTR to that of the ky95 3'UTR, it was found that accumulation in the cilia and in the cell body was lower with ky95 mutant 3'UTR while accumulation in either the dendrites or axons was not significantly different (12). This demonstrated that the binding of FBF-1 to the 3'UTR region leads to localized expression of egl-4 in the cilia and cell body. It was also observed that NR/FBE mutations decreased overall reporter or green Kaede expression after UV exposure (12). Overall, these studies found that FBF-1's binding to the NR/FBE region increases expression of egl-4 in a localized manner near the cilia and cell body. This increase in egl-4 concentration in localized areas within the cell is then necessary for olfactory adaptation.

This is an example of scientists using molecular techniques to elucidate mechanisms of adaptation in *C. elegans*. This finding demonstrates that alteration of gene expression which alters the neuron results in adaptation. Adaptation is an example of a simple behavior that is exhibited by many organisms including *C. elegans* which makes it a great place for scientists to start unraveling the mysteries of behavior. In this manner scientists can study other organisms and other behaviors using similar molecular techniques. Ultimately these techniques can perhaps even be used to understand human mental function.

"These studies in C. elegans give scientists confidence that complex behaviors in humans can also be understood in terms of neural circuits and cell biological mechanisms."

What these studies indicate

These studies behind the molecular, cellular, and genetic underpinnings of behavior in *C. elegans* show that something as complex as behavior can begin to be understood. Success in understanding a simple behavior like olfactory behavior in a simple organism like *C. elegans* is a start towards elucidating complex behaviors in complex organisms like humans. These studies in *C. elegans* give scientists confidence that complex behaviors in humans can also be understood in terms of neural circuits and cell biological mechanisms.

Yet there is still much mystery remaining in understanding simple behaviors in simple organisms. It may be even more complicated than we presently think. There are also numerous questions that are still left unanswered. For instance, in the neural circuitry behind olfactory behavior in *C. elegans*, it is still unknown what circuitry is downstream of AIB and AIY. How exactly is AIB and AIY able to regulate turning? Another fascinating question regarding adaptation is how binding of FBF-1 to the NR/FBE region increases translation of egl-4? These further questions need to be investigated. However, it looks promising that the road to fully understanding the biological root of behavior may be in the not so distant horizon. H

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