

Invertebrate Learning: What Can't a Worm Learn?

Dispatch

Catharine H. Rankin

The nematode worm *Caenorhabditis elegans* can learn and remember the stimuli it encounters, the environment it is in, and its own physiological state. Analyses of mutations in *C. elegans* that affect different aspects of experience are beginning to address the nature of learning.

Sydney Brenner [1] introduced *Caenorhabditis elegans* over 35 years ago as a model system for geneticists and developmental biologists; more recently it has become an important model system for neurobiologists. In early studies on *C. elegans* John Sulston and colleagues [2,3] followed all of the cell divisions and determined the lineage of each of the 959 cells in the adult hermaphrodite. One of the important take-home messages of this work was that *C. elegans* development is determined — precisely the same cell lineage pattern can be traced in every member of the species. This led to the discovery by Robert Horvitz and colleagues of genes that regulate programmed cell death [4].

The unintended, and often unspoken, outcome of this Nobel Prize winning science was that researchers began to view *C. elegans* as a 'programmed, hard-wired organism'. For example, John White [5] published an enormous study using electron microscopic serial section reconstruction of the nervous system to generate a wiring diagram including all of the putative chemical and electrical synapses. In many parts of the worm, only a single animal was examined and parts of a second worm used to confirm his findings. The underlying assumption for this was that the wiring diagram would be the same for all worms. Taken together the cell lineage data and the nervous system reconstruction gave the impression that the 302 neuron nervous system of *C. elegans* is 'hard-wired'.

Until 1990, no one investigated the possibility that *C. elegans* might show behavioral plasticity and be able to learn from experience. This has changed dramatically over the last 14 years! Now, instead of asking "what can a worm learn?" it might be better to ask "what cannot a worm learn?" Since the first paper focusing on learning and memory in *C. elegans* [6], there have been a large number of papers that have shown that, rather than being strictly hard-wired, *C. elegans* is exquisitely designed to benefit from its experience, and to learn and remember a variety of different types of information about its environment (see Box for a glossary of learning terms).

These studies have shown that *C. elegans* can learn about mechanosensory input [7], chemosensory input [8,9] and thermosensory input [10,11]. The worm can learn to approach or avoid tastes, odors or temperatures that predict the presence or absence of food. They show non-associative forms of learning, such as habituation and dishabituation, as well as associative forms of learning, such as classical conditioning and differential classical conditioning [7–11]. And they show both short-term and long-term forms of memory [7].

Recent studies have shown that *C. elegans* can also undergo more sophisticated forms of learning. For example, I [12] have reported that *C. elegans* exhibits context conditioning to a taste cue during habituation to mechanosensory stimulation, where the worms demonstrated both latent inhibition and extinction. These results suggested that *C. elegans* is capable of integrating and remembering experiences across different sensory modalities. Bettinger and McIntyre [13] found that *C. elegans* can show state-dependent learning. In this case, worms adapt to an odor in the presence of high levels of ethanol, equal to those that intoxicate

Box 1

Glossary of psychological terms.

Classical conditioning: a type of associative learning first described by Pavlov. Pavlov found that when a neutral stimulus (the conditioned stimulus, CS, the sound of a bell) was presented just before the delivery of a rewarding stimulus (the unconditioned stimulus, US, food) the CS would come to elicit the same response as the US (salivation). This transfer of the response (salivation) to the CS (the bell) after sufficient pairings is the conditioned response (CR).

Differential classical conditioning: a paradigm to study classical conditioning in which there are two CSs, one of which is paired with the US (CS⁺), the other is not (CS⁻). Organisms should develop a conditioned response (CR) to the CS⁺ and not to the CS⁻.

Latent inhibition (also called CS preexposure effect): when the CS is presented to the organism before any training (without exposure to the US) it can retard or eliminate the subsequent ability of that stimulus to serve as a CS in classical conditioning.

Extinction: after an organism has been classically conditioned repeated presentations of the CS without the US will lead to the decrease and eventual elimination of the CR.

Context conditioning: cues from the environment, or the context, where training occurred can also serve as CSs for classical conditioning.

Habituation: a non-associative form of learning in which a single stimulus is presented repeatedly (or, in the case of an odor or taste, continuously). Repeated presentations of a stimulus result in decreased responding to that stimulus. This decrease cannot be attributed to sensory adaptation or fatigue (see dishabituation).

Dishabituation: if a novel or noxious stimulus is delivered to a habituated animal immediately after habituation training responding to the original habituated stimulus is re-instated. Dishabituation tests are used to demonstrate that the decrease in responding is not due to fatigue or sensory adaptation (which only recover with the passage of time).

mammals. When tested for retention of the olfactory adaptation an hour later, the worms only show adaptation in the presence of ethanol at high levels (and not at sub-intoxication levels). This supports the view that worms can associate a physiological state with a specific experience.

In a recent issue of *Current Biology*, Law *et al.* [14] have now reported another sophisticated form of classical conditioning: occasion setting. In this case, the worm is hypothesized to learn a conditional relationship between stimuli: in the presence of the cues from normal growth medium, benzaldehyde predicts that no food will be present and the worm does not approach the normal growth medium. When the normal growth medium cues are not present, the worm will approach the benzaldehyde. The data that support the view that this is occasion setting comes from an analysis of mutants [14]: *gpc-1* worms show no classical conditioning to taste, but do still show occasion setting, while *lrn-2* worms do not show occasion setting but still show classical conditioning to taste. The authors argue that this genetic dissociation indicates that the normal growth medium must be serving as an 'occasion setter' and not as a typical classically conditioned 'context'.

The mutational analyses and conclusions drawn by Law *et al.* [14] speak directly to both the nature of the mutant strains tested and the nature of learning. The categories of learning described by learning theorists are determined by paradigms. We distinguish classical conditioning from instrumental conditioning by the paradigms we use to produce them. But it is important to note that there are often instrumental elements present in classical conditioning, and there are always classical conditioning components present in instrumental learning. For many years, researchers studying the mechanisms of learning have believed that, once we understand the mechanisms of all forms of learning and memory, we will understand how the different types of learning are related to one another.

The results reported by Law *et al.* [14], with earlier work from the van der Hooy lab, offer a new wrinkle to this — their mutants are showing a very strange pattern of results that do not easily fit into categories of learning that we recognize. For instance, the *lrn-2* mutant was isolated in a screen for taste associative learning mutants [8]. In this paradigm, worms were exposed to two salts: one was paired with food, one with no food. In a choice test, wild-type worms would go to the salt that predicted food and avoid the salt that predicted no food; the *lrn-2* mutant worms cannot learn this. In a second study [9], wild-type worms learned to associate the olfactory cue diacetyl with exposure to aversive acetic acid, and no longer tracked diacetyl; again, *lrn-2* worms do not show this olfactory association. In the new work [14] the *lrn-2* worms appear to be able to learn about salts and starvation, and to learn about benzaldehyde and starvation, but they do not learn the occasion setting. What do these results say about the nature of the *lrn-2* mutation? What do they say about these different learning paradigms? It will be important to determine the differences in the training paradigms, and in the testing protocols so that we can understand this strange pattern of results.

Despite the rather odd results from the analyses of mutants, the results from the new paper [14], and other recent papers showing context conditioning and state learning, indicate that *C. elegans* has a remarkable ability to learn about its environment and to alter its behavior as a result of its experience. In every area where people have looked for plasticity they have found it. To address the question "What can't a worm learn?" we can be confident of the obvious answers: for example, without eyes a worm cannot learn visual discriminations, cannot do observational learning, and cannot learn to read. The less obvious answers to the more challenging questions about the depth of a worm's cognitive abilities can only be addressed by researchers who will need to invent creative and clever ways to ask the worm directly.

References

1. Brenner, S. (1974). The genetics of *Caenorhabditis elegans*. *Genetics* 77, 71-94.
2. Sulston, J.E., Schierenberg, E., White, J.G., and Thomson, N. (1983). The embryonic cell lineage of the nematode *Caenorhabditis elegans*. *Dev. Biol.* 84, 64-119.
3. Sulston, J.E., and Horvitz H.R. (1977). Post-embryonic cell lineages of the nematode *Caenorhabditis elegans*. *Dev. Biol.* 56, 110-156.
4. Horvitz H.R., and Sulston, J.E. (1980). Isolation and characterization of cell-lineage mutants in the nematode *Caenorhabditis elegans*. *Genetics* 96, 435-445.
5. White, J.E., Southgate, E., Thomson J.N., and Brenner, S. (1986). The structure of the nervous system of the nematode *Caenorhabditis elegans*. *Phil. Trans. R. Soc. Lond. B* 314, 1-340.
6. Rankin, C.H., Beck, C.D.O., and Chiba, C.M. (1990). *Caenorhabditis elegans*: A new model system for the study of learning and memory. *Behav. Brain Res.* 37, 89-92.
7. Rose, J. K., and Rankin, C.H. (2001). Analyses of habituation in *C. elegans*. *Learn. Mem.* 8, 63-69.
8. Wen, J. Y. M., Kumar, N. N., Morrison, G., Rambaldini, G., Runciman, S., Rousseau, J., and van der Kooy, D. (1997). Mutations that prevent associative learning in *C. elegans*. *Behav. Neurosci.* 111, 354-368.
9. Morrison, G.E., Wen, J.Y.M., Runciman, S., van der Kooy, D. (1999). Olfactory associative learning in *Caenorhabditis elegans* is impaired in *lrn-1* and *lrn-2* mutants. *Behav. Neurosci.* 113, 358-367.
10. Hedgecock, E.M., and Russell, R.L. (1975). Normal and mutant thermotaxis in the nematode *C. elegans*. *Proc. Natl. Acad. Sci. USA* 72, 4061-4065.
11. Mori, I., (1999) Genetics of chemotaxis and thermotaxis in the nematode *Caenorhabditis elegans*. *Annu. Rev. Genet.* 33, 399-422.
12. Rankin, C.H. (2000). Context conditioning in habituation in the nematode *C. elegans*. *Behav. Neurosci.* 114, 496-505.
13. Bettinger, J. C., and McIntire J.C. (2004) State-dependency in *C. elegans*. *Genes, Brain Behav.* Online publication date: 25 May 2004 doi: 10.1111/j.1601-183X.2004.00080.x
14. Law, E.K.-C., Nuttley, W.M., and van der Kooy, D. (2004). A mutational double dissociation of taste adaptation and taste modulation of olfactory learning in *C. elegans*. *Curr. Biol.* July 27 issue.