



The Effect of Diphenhydramine on Neurotransmitters, Neurons, and Memory in *C. elegans*



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Antihistamines: A Menace to the Mind?

Antihistamines are a commonly used class of drugs that prevent histamine from binding to cell receptors and triggering histamine release and symptoms of allergic reactions (1, 2). Recent studies have correlated the use of antihistamines with an increased risk of dementia (4-6), which is alarming since the US population has an increasing incidence and prevalence of diagnosed food or seasonal allergies (3), and antihistamines are often a first-line medication recommended for initial treatment of these allergies.



C. elegans: A Truly Amazing Organism

Caenorhabditis elegans, or *C. elegans*, are transparent, microscopic soil nematodes. They are ideal organisms to use in several types of experiments because of their rapid life cycle, invariant number of somatic cells, and sequenced genome. Hermaphrodites and males are the two possible genders of a *C. elegans*. Hermaphrodites contain what we consider both the male and female reproductive systems of *C. elegans*. The instance of finding a male *C. elegans* in the wild is extremely low since males are typically only produced in times of stress. All *C. elegans* undergo four larval stages before reaching adulthood; this process is altered slightly if a *C. elegans* in the first larval stage is faced with undesirable conditions such as limited food, overcrowding, and heat, causing the worm to enter a dauer stage where it uses minimal energy and can survive for up to four months without the outside conditions improving before re-entering the life cycle at the L4 stage.

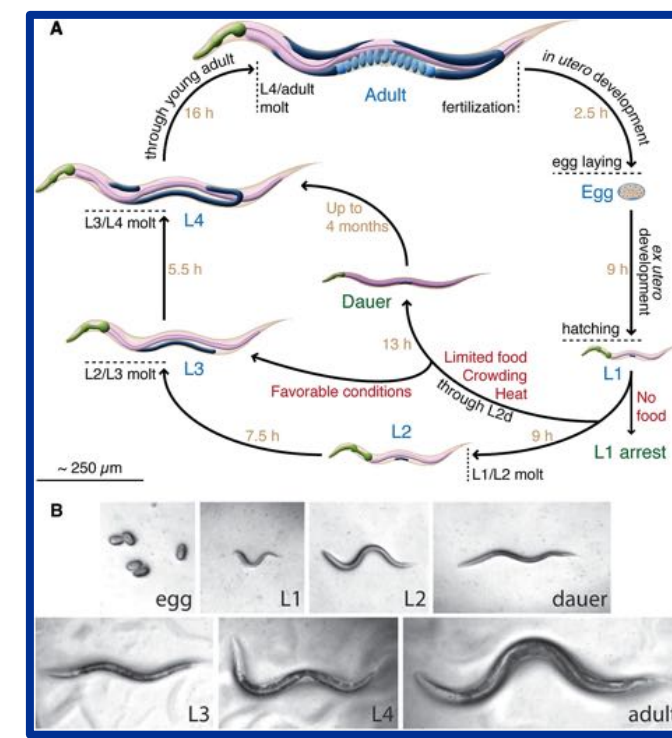


Figure 1. Chen & Kirschlin, *Yeast* (2011). The *C. elegans* life cycle as a model for studying neurodegeneration and disease. *Yeast*, 26, 100-106. doi:10.1002/yea.2112

Materials and Methods

This procedure was performed once. This experiment began with synchronizing *C. elegans* to ensure they were of the same age at the time of the training. The *C. elegans* were trained to stop responding to a series of vibrating taps applied with a Philips Sonicare HealthyWhite HX6711/02 Toothbrush on the first vibration setting to the side of the agar-filled petri dishes the nematodes were on, rotating through training periods according to ten-second timers running on the app "Timers +" on an iPhone. During training, the *C. elegans* were filmed by a Canon EOS Rebel T5i camera (DS 126431) attached to the ocular of an Olympus SZ-40 stereo zoom microscope with 40x magnification. After three hours of training, which took place over two days, the *C. elegans* were exposed to a commonly available and used antihistamine, diphenhydramine (Benadryl), and tapped while the camera recorded their movements. Doses of 1.3 mg of Benadryl/ml of sterile water for were used. These amounts were based on the recommended doses for adult patients taking both medicines. Each medication was crushed using a mortar and pestle and added to 10 mLs of sterile autoclaved water. 10 mLs of sterile water was used as a control. After an additional hour of tapping, images were taken of the nematodes under a fluorescent microscope.



Citations

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Abstract

Caenorhabditis elegans is a transparent, microscopic nematode with an entirely mapped neurological system. This allows for studies on neuronal activity as a simplified model of the human neurological system. Antihistamines have recently been linked to memory loss, specifically dementia, in humans. Last year, we performed an investigation behaviorally training *C. elegans* and exposing "trained" nematodes to antihistamines, hypothesizing behavior change and memory dysfunction would occur when the nematodes were exposed to the antihistamines diphenhydramine (Benadryl) and cetirizine (Zyrtec). The results implied that *C. elegans* became behaviorally trained to cease reacting to vibrations applied to the side of their petri dishes in short taps, then recommenced reacting to vibrations after exposure to antihistamines, indicating a possible loss of the memory of that training. This year, our experiment was based off this prior research. Two tagged strains of *C. elegans*, EAW113 (*dop-1::gfp, dop-3::rfp*) and EAW115 (*unc-17::gfp, mod-1::mCherry*), were synchronized to the L1 larval stage. The *C. elegans* were then conditioned to stop reacting to vibrations applied to the side of the dish with an electric toothbrush. This process was repeated for a total of 180 minutes over two days, resulting in the *C. elegans* showing signs of becoming conditioned to the vibrating "taps", as indicated by a decline in the percentage of *C. elegans* responding to the vibrations. After conditioning, diphenhydramine (Benadryl) or sterile water was added to ensure direct exposure of the *C. elegans*. Groups exposed to diphenhydramine exhibited a loss of conditioned response of the behavioral training, while the control groups retained the conditioned response to the vibration stimulus. The EAW113 and EAW115 strains were utilized because they had specific neurotransmitter receptors tagged with either green fluorescent protein (GFP) or red fluorescent protein (RFP or mCherry). These receptors are for dopamine (*dop-1* and *dop-3*), serotonin (*mod-1*) and acetylcholine (*unc-17*). This enabled us to directly observe specific neural receptor expression. Images taken using fluorescent microscopy indicated that *C. elegans* exposed to antihistamines had lower levels of the neurotransmitters than control *C. elegans*. These findings correlate with previous data gathered from a nearly identical training and antihistamine exposure experiment performed on wildtype *C. elegans*. Results from both experiments suggest a link between antihistamines and behavior change or memory dysfunction in the nematode *C. elegans*.

My Hypothesis

It was hypothesized that *C. elegans* exposed to antihistamines after behavioral training would revert to responding to the stimulus once again ("forgetting" their conditioning), and have dimmer GFP, RFP, and mCherry than *C. elegans* not exposed to antihistamines.

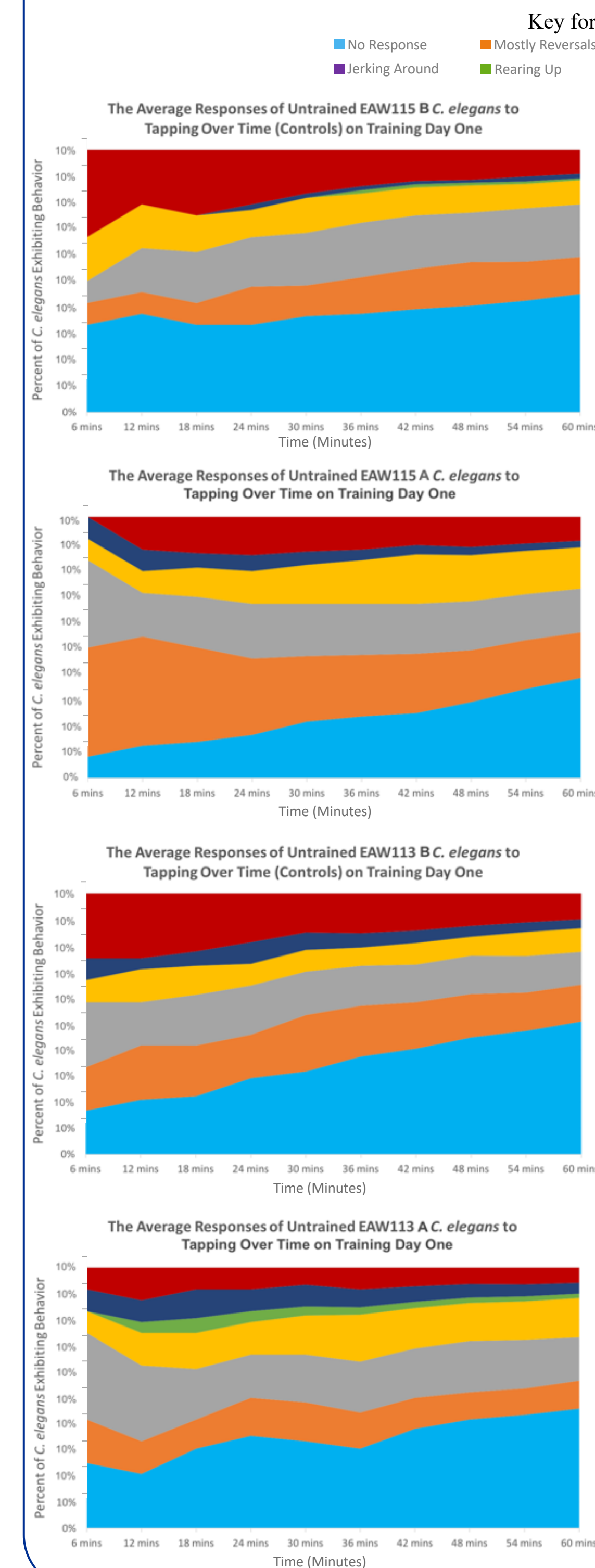
Results and Conclusions

The results for the the most significant tapping portions of the experiment are shown in the eight graphs on the right. The experimental groups' graphs produced similar findings, indicating that *C. elegans* which received the same treatment behaved similarly. The protocol utilized caused the *C. elegans* to become used to the taps and behaviorally trained to not react in a conditioned response. When exposed to the antihistamines, the *C. elegans* seemed to forget their training, as they recommenced responding to the tap stimuli. Interestingly, these new responses varied from the responses observed before the exposure to the antihistamines. This is particularly fascinating because dementia has caused people to behave erratically, sometimes irrationally. These results suggest a possible link between antihistamines and behavior change or memory dysfunction in the nematode *C. elegans*, possibly concerned with dopamine and serotonin. Further studies are needed to confirm and elucidate the mechanism of this response; this project is continuing and different routes of experimentation considered.

Acknowledgements

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Results: The First Training Day



Results: Exposure Day

