An Optogenetics Approach: The Formation of Chemosensory Habituation in *C. elegans* Affected by the γ-Aminobutyric Acid and Acetylcholine Neurotransmitters

Verona Yue | Forest Ridge School of the Sacred Heart | Bellevue, WA

It had been found that γ -aminobutyric acid (GABA), an important inhibitory neurotransmitter, can influence the process of memory storage. Acetylcholine (ACh), an excitatory neurotransmitter may enhance memory by increasing the strength of afferent input relative to feedback. The aim of this study is to investigate how stimulated secretion of the neurotransmitters ACh and GABA would affect C. elegans' memory formation by using optogenetics techniques. The results indicate that GABA neurotransmitter might have a positive effect on memory storage; ACh neurotransmitter may accelerate the memory losing process or interrupt the memories formation process. These results may have implications for the studies of Parkinson's disease and Alzheimer's disease.

Introduction

Optogenetics is a biological technique that uses light stimulation and genetics to control and monitor the activities of individual neurons in living tissue. Control of neuronal activity is achieved using optogenetic actuators like channelrhodopsin (ChR2), while optical recording of neuronal activities can be made with the help of optogenetic sensors for calcium (GCaMP), vesicular release, neurotransmitter, or membrane voltage. Control (or recording) of activity is restricted to genetically defined neurons and performed in a spatiotemporal-specific manner by light (Deisseroth et al., 2006; Mancuso et al., 2010). However, the photosensitive channel, like ChR2, can be inactivated by excessive exposure to light as well. (Honjo, Hwang, Daniel, & Jr, 2012).

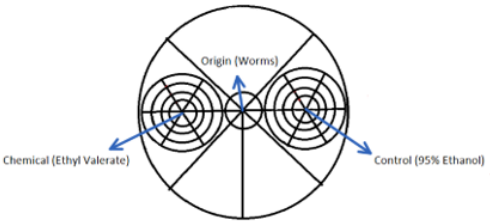
GABA (γ -aminobutyric acid) is a ubiquitous inhibitory neurotransmitter which participates in a wide number of physiological responses. In previous work using mammalian cells, it has been found that the GABAergic neurons can critically modulate the electrical activity of the brain area, like the cortex, amygdala, during the "multiple consolidation" process of memory storage, which means that the process of memory storage takes place in different brain circuits and at different times after the learning experience. *unc-47* encodes a vesicular GABA transporter protein which is involved in the uptake of GABA into the synaptic vesicles. Excess amount of GABA neurotransmitter is transported and released in *unc-47* optogenetic strains, which show a body elongation of up to 5% depending on the intensity of the simulation (Schultheis, Brauner, Liewald, & Gottschalk, 2011; McIntire, Reimer, Schuske, Edwards, & Jorgensen, 1997).

Acetylcholine (ACh) is a neurotransmitter which gives muscle signals—to start or stop a movement. This neurotransmitter may enhance memory formation by increasing the strength of afferent input relative to feedback, by contributing to theta rhythm oscillation, by activating intrinsic mechanisms for persisted spiking, and by increasing the modification of synapses (Rand, 2007). *unc-17* encodes a vesicular acetylcholine transporter protein which is involved in acetylcholine transport into synaptic vesicles. *unc-17* optogenetics strains show a strong body contraction when the excess ACh being produced.

Chemotaxis is the behavior in which C. *elegans* is attracted or repelled to numerous soluble, volatile or odorants chemicals. (Swoboda, Adler, & Thomas, 2000). Chemosensory habituation was discovered by studying C. *elegans*' chemotaxis performance. Colbert and Bargmann (1995) found that by continuous exposing to an attractive odorant, C. *elegans* will lose the chemotactic response to that odorant but recover if they leave the environment with the chemicals. This may work through C. *elegans* odorant receptors expressed in its olfactory neurons (Ardiel & Rankin, 2010).

The purpose of this study is to investigate how stimulated secretion of neurotransmitters, ACh and GABA, affect chemosensory habituation and chemotaxis behavior. By observing and interpreting C. *elegans* and its chemotaxis behavior, I can have a better understanding of the effect of each neurotransmitter on C. *elegans*' memory formation, which may impact the memory formation and the treatment in pathological states like Parkinson's disease and Alzheimer's disease. I hypothesized that by stimulating neurotransmitter release, both Ach and GABA (GABAergic system) will enhance the chemosensory adaption of worms which will decrease interest to the odorant.

Methodology



$$Chemotaxis\ Index = \frac{\#Chemical - \#Ethanol}{\#Chemical + \#Ethanol}$$

Figure 1. Chemotaxis Assays. Each region was precisely counted under microscpe one by one once after the worms had run for more than 4 hours. The regions were also color-coded for easier identification.

Organism: The organisms used for this experiment were *Caenorhabditis elegans* transgenic strains, including the genotypes of zxIs3 [unc-47p::ChR2(H134R)::YFP + lin-15(+)] (hereafter referred to as *unc-47*)., and zxIs6 [unc-17p::ChR2(H134R)::YFP + lin-15(+)] V (hereafter referred to as *unc-17*).

Chemicals: The chemical used for this experiment was Ethyl Valerate. Chemicals were diluted with the 95% Ethanol solvent to 10^{-2}.

Incubation Assays: Worms were bleached to isolate stage-synchronized embryos; about 400 eggs were put on the 6 cm diameter agar plates with bacteria-retinal on the surface of agar and seed the bacteria-retinal solution.

Chemosensory Assays: To establish a chemical gradient, the following solutions were applied to the chemotaxis plates: 5μL drop of the chemical (the dilution of 10^{-2}) to the left chemical spot, 5 μL 95% ethanol to the right control spot. I pipetted a small drop of worms evenly. I timed the assay run for 4 hours, then put the plates in a refrigerator at 4 degrees Celsius to stop the worms from moving. After that, I counted the worms in each circle (Figure 1) and calculated the chemotaxis index. The numbers of the worms under the same condition but from different trials were added together.

Table 1. Experimental Group Setting

Experimental group	Worms contained in the assays	Chemicals on lids	Under the blue light for 30 mins
1	*unc-47*	YES	YES
2		NO	YES
3		YES	NO
4		NO	NO
5	*unc-17*	YES	YES
6		NO	YES
7		YES	NO
8		NO	NO

Result

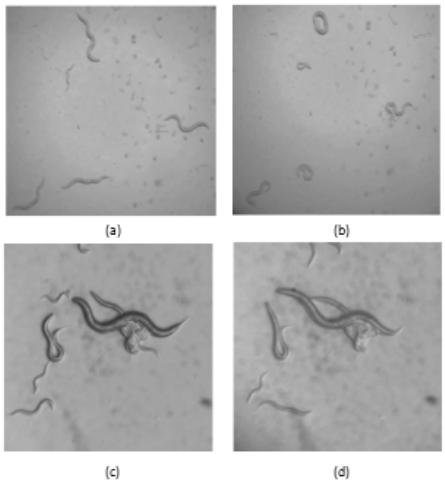


Figure 2. Optogenetic simulation of the *unc-47* and *unc-17* strains. (a) *unc-17* strains before the blue light simulation. Animals bend normally. (b) *unc-17* strains after the blue light simulation. Animals are intensively bended. (c) *unc-47* strains before the blue light simulation. Animals move smoothly. (d) *uns-47* strains after the blue light simulation. Animals would suddenly stop and extend their bodies.

The performance of the transgenic strains under blue light is consistent with the performance of the same species under the same situation in previous studies. As the Figure 2 shows, When the blue light applies, *unc-47* strains would suddenly stop moving; unc-17 strains would contract and bend their bodies.

As the table 2 and figure 3 show, worms have different responses to the chemical under the situations. Both *unc-47* and *unc-17* C. *elegans* are attracted by the Ethyl Valerate with no previous exposure under the blue light and chemical (groups 4 and 8) and repelled the chemical after previous exposure under the chemical without exposure to the chemical or light simulation (groups 3 and 7). This is consistent with the previous study that C. *elegans* is attracted by esters on first exposure and are repelled by the ester after exposing them to the chemical for half an hour, due to the chemotaxis adaptation. It's interesting that both *unc-47* and *unc-17* worms were repelled from the ester when pre-exposed to the blue light but no chemical (groups 2 and 6).

Comparing experimental group 1 (*unc-47* with exposure to the chemical and with light simulation) and group 3 (*unc-47* with exposure to the chemical and without light simulation), where exposing worms to blue light means opening the GABA neurotransmitter channel, C. *elegans* had a stronger aversive reaction from the chemical because the chemotaxis index of group 1 is smaller than group 3. By comparing the group 1(*unc-47* with exposure to the chemical and with light simulation), 2 (unc-47 without exposure to the chemical and with light simulation) and 3 (unc-47 with exposure to the chemical and without light simulation), the combination of having previous exposure under both the blue light and the chemical caused the worms' strongest aversion for the chemical, which showed a synergistic increase in avoidance behavior.

Table 2. The numbers of worms were collected and the unweighted chemotaxis index

Experimental group	Worms contained in the assays	Chemicals on lids	Under the blue light for 30 mins	Side of the testing plates	Worm numbers	Chemotaxis Index
1	*unc-47*	YES	YES	Drug	608	-0.268
				Control	1053	
2		NO	YES	Drug	109	-0.068
				Control	125	
3		YES	NO	Drug	61	-0.032
				Control	65	
4		NO	NO	Drug	86	0.110
				Control	69	
5	*unc-17*	YES	YES	Drug	524	0.039
				Control	485	
6		NO	YES	Drug	1057	-0.130
				Control	1372	
7		YES	NO	Drug	80	-0.126
				Control	103	
8		NO	NO	Drug	195	0.345
				Control	95	

For group 5 (*unc-17* with exposure to the chemical and with light simulation), the worms exhibited neutral or attracted behaviors when both light and chemical conditions were pre-applied. By comparing group 5 (*unc-17* with exposure to the chemical and with light simulation), 6 (*unc-17* without exposure to the chemical and with light simulation), and 7 (*unc-17* with exposure to the chemical and without light simulation), it indicated that C. *elegans* lost interest in Ethyl Valerate under the individual condition, but somehow restored attraction when both conditions applied.

Discussion

The results show that the combination of blue light stimulation of GABA and previous exposure to the chemical caused the strongest aversive response to the chemical, more than when each individual condition was applied. The simulation of ACh neurotransmitter and exposure to the chemical caused the animals to be attracted to the chemical, which is opposite from the results when I test the individual conditions. By comparing the results to my hypothesis, I found that excess GABA neurotransmitter may enhance the chemotaxis adaptation; however, excess ACh neurotransmitter may accelerate the process of recovery from the chemotaxis adaptation or prevent formation of those memories.

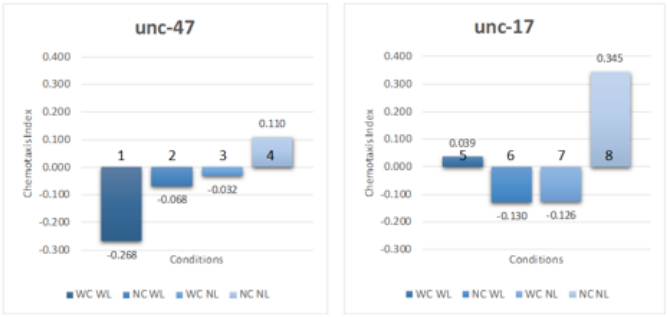


Figure 3. Unweighted chemotaxis index of responses of mutant worms *unc-47* and *unc-17* to the Chemicals with the dilution of 10^{-1}. This graph compares the chemotaxis indexes by different genes with using the same test chemicals. "WC" means the worms had pre-exposed "with chemical". "NC" means the worms had "not pre-exposed with chemical". "WL" means the worms had pre-exposed "with blue light". "NL" means the worms had "not pre-exposed with blue light".

After opening the GABA neurotransmitter channel, C. *elegans* moved away from the chemicals (see data from group 1). Comparing the group 1, 2 and 3, with the light simulation and pre-exposure to the chemical, the worms in group 1 had the strongest tendency to move away for the attractant. This can be interpreted in two ways: one is that this might suggest that excessed GABA neurotransmitter secretion can enhance the C. elegans' chemotaxis adaptation to the chemical; or the aversion to the chemical from group 1, where applying both light simulation and chemical exposure conditions, may because it's the combined effect of the individual conditions

For the worms with over secretion of ACh, abnormal responses to the chemical, one possibility is that the ACh neurotransmitter may take a role of accelerating the process of recovery of chemotaxis adaptation, because the chemotaxis index was negative in group 7 but positive in group 5. However, when the light was applied, the worms did not show any stronger adaptation to the attractant, which means it may not enhance the worm's chemotaxis memory. In another way, with both conditions applied, the habituation memories may not be formed when light-sensitive neurons interact with the chemosensory neurons. Also, I expect that in group 6 when only the light stimulation is applied, the animals should be attracted by the chemical as they did in the control group; in group 5 when both conditions apply, I expect that the animals will have aversive response. However, they performed opposite to the expectation; this indicates that the ACh neurotransmitter may reverse C. *elegans*' normal behavior.

Furthermore, the experiment can be further explored by extending the chemical exposure time into different length and to measure if the time is related to how strong an adaptation does worms have. Also, it should be done over more time to see if the worms lose the ability to detect the chemicals and behave randomly.

Acknowledgements

I am thankful to Dr. Nicole Liachko for providing equipment, protocols and mentoring; Ms. Heather Currey for technical help and mentoring; Dr. Michael Ailion for providing equipment and answering the technical questions; Ms. Corina Rahmig and Mr. Peter Traube for technical help; Dr. Maureen Munn for providing equipment and mentoring; Dr. Michael Heinekey for providing chemicals; Mr. Kenneth Brasel for NWABR mentoring; Caenorhabditis Genetics Center (CGC) for providing other mutant strains; Veteran Affairs Puget Sound in Seattle for providing facilities; Forest Ridge School of the Sacred Heart for providing facilities.

Bibliography

Ardiel, E. L., & Rankin, C. H. (2010). An elegant mind: Learning and memory in Caenorhabditis elegans. Learning & Memory, 17(4), 191–201. https://doi.org/10.1101/lm.960510

Bargmann, C. I., Hartwieg, E., & Horvitz, H. R. (1993). Odorant-selective genes and neurons mediate olfaction in C. elegans. Cell, 74(3), 515–527. https://doi.org/10.1016/0092-8674(93)80053-H

Bargmann, C. (2006). Chemosensation in C. elegans. WormBook, 1–29. https://doi.org/10.1895/wormbook.1.123.1

Brioni, J. D. (1993). Role of GABA during the multiple consolidation of memory. Drug Development Research, 28(1), 3–27. https://doi.org/10.1002/ddr.430280103

Colbert, H. A., & Bargmann, C. I. (1995). Odorant-specific adaptation pathways generate olfactory plasticity in C. elegans. Neuron, 14(4), 803–812. https://doi.org/10.1016/0896-6273(95)90224-4

Deisseroth, K., Feng, G., Majewska, A. K., Miesenbock, G., Ting, A., & Schnitzer, M. J. (2006). Next-Generation Optical Technologies for Illuminating Genetically Targeted Brain Circuits. Journal of Neuroscience, 26(41), 10380–10386. https://doi.org/10.1523/JNEUROSCI.3863-06.2006

Honjo, K., Hwang, R. Y., Daniel, W., & Jr, T. (2012). Optogenetic manipulation of neural circuits and behavior in Drosophila larvae. Nature Protocols, 7(8), 1470–1478. https://doi.org/10.1038/nprot.2012.079

Mancuso, J. J., Kim, J., Lee, S., Tsuda, S., Chow, N. B. H., & Augustine, G. J. (2010). Optogenetic probing of functional brain circuitry. Experimental Physiology, 96(1), 26–33. https://doi.org/10.1113/expphysiol.2010.055731

McIntire, S. L., Reimer, R. J., Schuske, K., Edwards, R. H., & Jorgensen, E. M. (1997). Identification and characterization of the vesicular GABA transporter. Nature, 389(6653), 870–876. https://doi.org/10.1038/39908

Rand, J. (2007). Acetylcholine. WormBook, 1–21. https://doi.org/10.1895/wormbook.1.131.1

Schultheis, C., Brauner, M., Liewald, J. F., & Gottschalk, A. (2011). Optogenetic analysis of GABAB receptor signaling in Caenorhabditis elegans motor neurons. Journal of Neurophysiology, 106(2), 817–827. https://doi.org/10.1152/jn.00578.2010

Swoboda, P., Adler, H. T., & Thomas, J. H. (2000). The RFX-type transcription factor DAF-19 regulates sensory neuron cilium formation in C. Elegans. Molecular Cell, 5(3), 411–421. https://doi.org/10.1016/S1097-2765(00)80436-0

Ward, S. (1973). Chemotaxis by the nematode Caenorhabditis elegans: identification of attractants and analysis of the response by use of mutants. Proceedings of the National Academy of Sciences of the United States of America, 70(3), 817–21. https://doi.org/10.1073/pnas.70.3.817