

**BEHAVIORAL ANALYSIS OF THE NEURAL ESCAPE  
CIRCUIT AND DOPAMINE FOOD SENSING  
CIRCUIT IN *C. ELEGANS***

A Major Qualifying Project Report

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## ABSTRACT

The behavior of *Caenorhabditis elegans* is largely affected by predation and food. In this project, the locomotion of *C. elegans* was artificially modulated by inducing dopamine release and inhibiting backward locomotion. Using optogenetics, worms were observed yielding the following results: halorhodopsin inhibits backward locomotion; dopamine slows the nematode's locomotory rate; despite lacking a dopamine transporter, worms do not paralyze in the presence of excess dopamine expression. Based on these results, the following conclusions were made: the AVA neuron, the backward locomotion command neuron, plays a major role in *C. elegans* ability to move backwards, and when inhibited, backward motion is stunted and nothing assumes the role of the AVA to compensate; an acute expression of dopamine induces a decrease in *C. elegans* locomotory rate; and worms compensate for *dat-1* deficiency by regulating dopamine through other mechanisms.

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## **BACKGROUND**

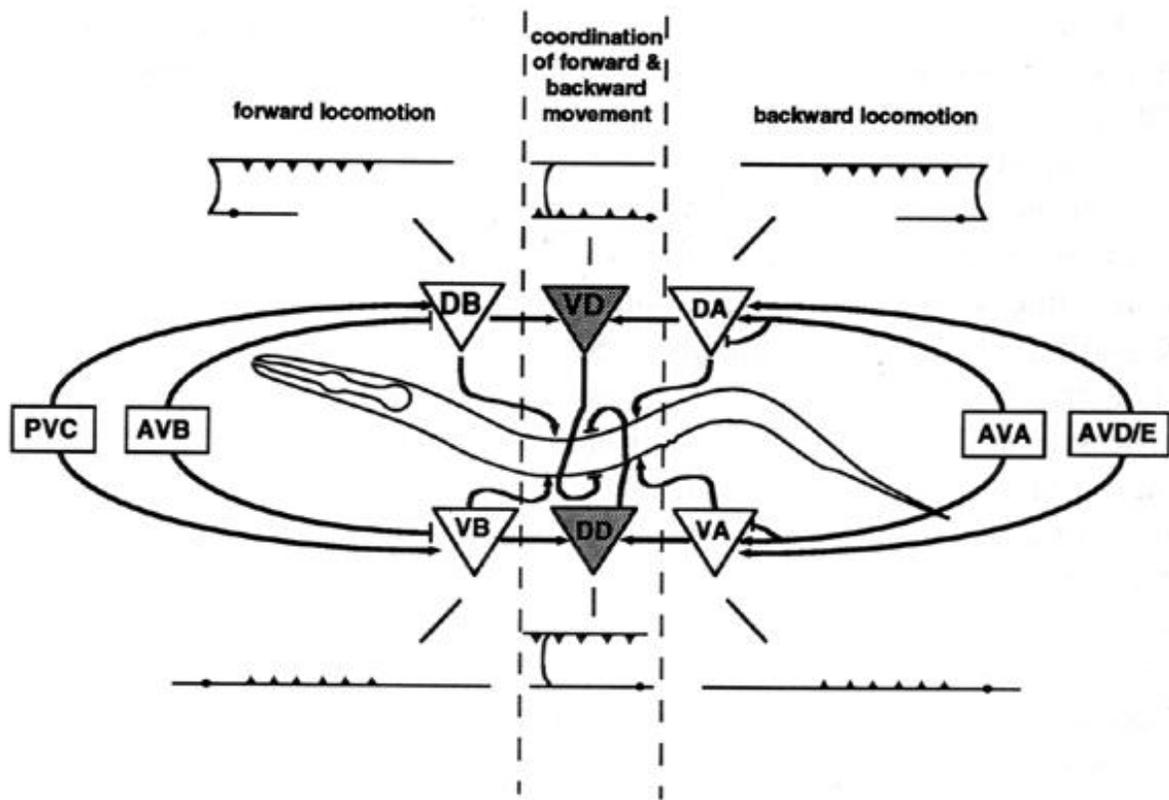
### ***C. elegans* Neurobiology**

*Caenorhabditis elegans* is a popular model organism used for research in neurobiology. It is very useful for investigating neuromodulation at behavioral, cellular and molecular levels due to its transparent and compact nervous system. The nervous system is comprised of 302 neurons, of which 32 are chemosensory and 28 mechanosensory (Bargmann and Mori, 1977; Escurra et al., 2011; Chalfie et al., 1985). It uses a large number of neuromodulators, such as monoamines, and neuropeptides to sense a diverse variety of chemical and mechanical stimuli (Rand and Nonet, 1997). There are several factors that guide behavior. Among these factors include responses to external cues such as predation and food, which contribute to the locomotion of *C. elegans*. Biogenic amines, such as dopamine, have a pivotal role in the modulation of behavior in various organisms. Dopaminergic signaling plays a major role in the locomotory response of the nematode.

### **Locomotion of *C. elegans***

The locomotion of nematode *C. elegans* consists of forward and backward motions. It is also characterized by exploratory head movements in which the *C. elegans* move their heads laterally. Muscle contractions dictate the locomotion of the nematode. The body wall is lined with 95 muscle cells arranged in four quadrants along the body (Altun and Hall, 2008). The muscle cells of the body wall an adult hermaphrodite are innervated by 75 motoneurons comprising eight distinct classes. Four classes innervate

ventral muscles (VA, VB, VD, and VC) and four innervate dorsal muscles (DA, DB, DD, and AS). Motoneurons that are active during forward locomotion are the B-motoneurons, and those that are active during backward locomotion are the A-motoneurons. A-motoneurons are active during forward locomotion and B-motoneurons are active during backward locomotion. DB, BV, and VA classes of motoneurons are active during one direction of locomotion but not the other. Therefore, in order to create a sinusoidal pattern of movement it is necessary for some motoneurons to contract in one direction while the remaining motoneurons relax in the opposing direction. This pattern is a result of the interaction between excitatory and inhibitory motor neurons. Locomotor muscles, on the other hand, are multifunctional and are active during both directions of locomotion due to the presence of four to eight motoneurons of different classes in each muscle cell (Haspel et al., 2010). In the adult worm, three sets of ventral cord motor neurons seem to be responsible for the muscle cell activity: (a) the A motor neurons (12 VA cells and 9 DA cells), (b) the B motor neurons (11 VBs and 7 DBs), and (c) the D motor neurons (13 VDs, and 6 DDs). The D cells differ from A and B in that they don't have interneurons synapses. They receive synapses from the other classes of motor neurons on the opposite side of the animal to that on which they form neuromuscular junctions. There are four pairs of interneurons (AVA, AVB, PVC, and AVD) which span the length of the ventral cord and synapse onto the motor neurons (**Figure 1**). These interneurons synapse onto each other and onto the A and B motor neurons of the ventral cord (Chalfie et al., 1985).

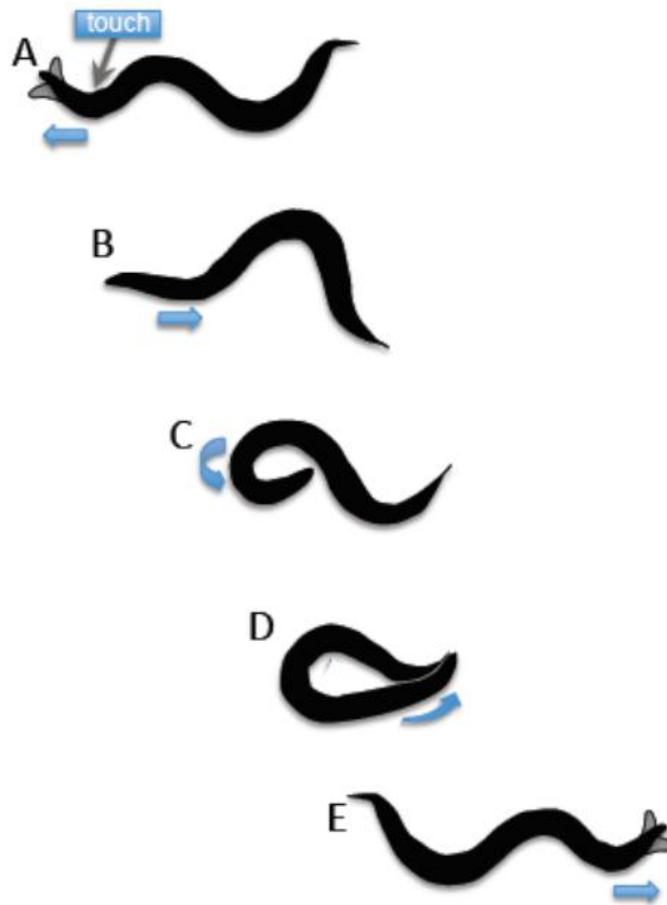


**Figure 1: Locomotory Circuit.** The inverted triangles represent six major motor neuron class (VB, DD, VA, DB, VD, DA), and the squares depict the interneurons (AVA, AVD/E, AVB and PVC). The circuit consisting of AVB and PVC interneurons and the B-motoneurons direct forward locomotion; the circuit including AVA, AVD/E, and the A-motoneurons drive backward locomotion (Driscoll and Kaplan, 1997).

### Locomotion in Response to Predation

A survival instinct of an animal is its response to predation by organisms with higher trophic levels in the food chain. Sensory perception plays a crucial role in dictating an animal's response to predation. Nematode *C. elegans* demonstrate this relationship when undergoing tactile stimulation. When undisturbed, *C. elegans* move backward and forward in a sinusoidal manner. When acted upon by an external stimulus i.e. a touch, the animals exhibit an escape response. Namely, observation has shown that touching the animal on its anterior half, or near the head, incites a backward locomotory

escape response and touching the animal on its posterior half, or near the tail, evokes a forward locomotory escape response (Chalfie et al., 1985). Thus, the animal responds to the external stimulus with a motion characterized by moving away from the point of contact. Figure 2 depicts the sequence of movements assumed by *C. elegans* after experiencing a gentle touch upon the anterior half of the animal's body.



**Figure 2: Movement of *C. elegans* During an Escape Response.** (A) The initial locomotion. (B) Reversal in direction. (C) Sharp turn following reversal. (D) Omega turn. (E) Direction of motion opposite of the initial direction (Alkema et al., 2005).

The animal's initial response is shown in **Fig 2A**, as rapid movement of the animal's head from side to side. This response is followed by a reversal in the worm's locomotory direction in which lateral head movements are suppressed (**Fig. 2B**). Following this

reversal the animal makes a sharp turn (**Fig 2C**) sliding its head down the ventral side of its body (**Fig. 2D**). When the Omega [sharp] turn is completed the worm's locomotory direction has shifted nearly 180 degrees concluding the evasive response initiated by the gentle touch (**Fig, 2E**). The explained escape response of *C. elegans* shows the role of the nervous system, which receives sensory information from the external environment and issues to the worm an appropriate behavioral response. It is achieved by the coordination of motor programs activated by command neurons within the organisms converting sensory information into a distinct motor response, such as the locomotory sequence explained above (Alkema et al., 2005).

### **Locomotion in Response to Food**

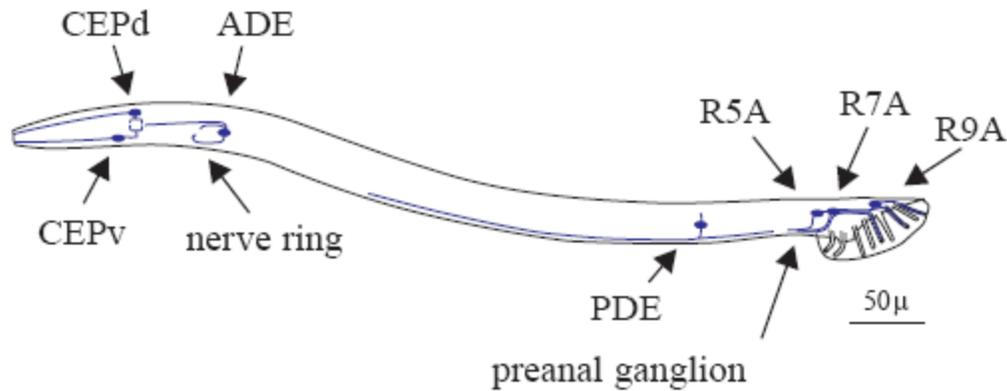
Food is integral to the survival of all organisms. Animals rely on sensory perception for survival, detecting food in their environments through smell, taste, and/or touch. As a result, animal behavior can be significantly altered by the presence or absence of food. The nematode *C. elegans* is no exception, demonstrating a pronounced relationship between food and behavior. Study of the nematode show that the presence of food stimulates feeding (Avery and Horvitz, 1990), high turning frequency and slows the rate of locomotion (Sawinet et al., 2000), while the absence of food elicits a low turning frequency increasing the rate of locomotion. To obtain food for survival, animals apply different foraging strategies. Many species, including the nematode, apply the area restricted search (ARS) strategy in order to obtain food. As the name implies, through this strategy, animals restrict the area from where they search for food to the proximity where food was last encountered. The area restriction results from a behavioral response

to finding food where the animal turns more frequently. Thus, the frequency of the animal's motion increases when food is present and is reduced when food is absent. In addition, since lack of food reduces locomotion, the rate of turns following the detection of food decreases over time as food is no longer available. As the time without food from the last food encounter increases the animal's turn rate drops ultimately resulting in a progressively more linear path of motion. Through ARS, the time spent in proximity to where food is first located is increased keeping the animal in an area where food likely will be found. Over time, ARS allows the animal's food search to be expanded to new extents outside the restricted area when food supply is depleted (Hills et al., 2004; Ezcurra et al., 2011).

### **Dopaminergic signaling in *C. elegans***

In nature, *C. elegans* exhibit the heightened turning frequency observed in the ARS foraging strategy. Though, a slowing response to food exposure, called the basal slowing response, has been observed when the worms are well feed. This response requires functional dopaminergic signaling. When the *C. elegans*'s appetite is satiated dopaminergic neurons release dopamine to modulate the increased turning frequency induced by the presence of food. These neurons signal the presence of food and modify sensory perception in response to the feeding state. (Hills et al., 2004; Ezcurra et al., 2011). As in the reduction of the turning frequency of *C. elegans* around food, dopamine signaling regulates a variety of complex behaviors. Dopamine signaling is facilitated by sensory receptors and downstream signaling mechanisms similar to those that operate in the mammalian brain. Defects in dopamine neuron function can lead to many severe

behavioral disorders in humans such as attention deficit hyper disorder, schizophrenia, and Parkinson's disease (Hills et al., 2004; Chase and Koelle, 2007). Dopamine can act before or following the synapse to modulate signaling (Hills et al., 2004). Dopamine can be found in four different pairs of neurons in a hermaphrodite worm and in six additional neurons in a male worm (**Figure 3**). In a hermaphrodite, the eight dopamine neurons supporting mechanosensory functions are ADE(R and L), PDE(R and L), CEPD(R and L) and CEPV(R and L). The four CEPs can be found in the nose, two ADEs in the head and two PDEs in the body of a hermaphrodite worm. All the dopaminergic neurons are ciliated and consist of mechanosensory dendrites (Ezcurra et al., 2004; Sanotshi et al., 2009; Carvelli et al., 2004; Chase and Koelle, 2007). "The ADE and PDE neurons, also known as the anterior and posterior deiridsensilla, are involved in mechanical texture sensation. ADE neurons are located posteriorly and ventrally to the terminal bulb. The dorsal ADE process sends off a short branch on the side, which extends to the lateral wall and terminates as a cilium (Altun and Hall, 2008). PDE sensory neurons are located next to the dorsal body wall muscle along the lateral side of the posterior body. Neurons of the cephalic sensillia, the CEP neurons, have cell bodies and dendrites that extend to the tip of the nose. Like ADE and PDE neurons, CEP neurons are involved in mechanosensory functions (Altun and Hall, 2008)."



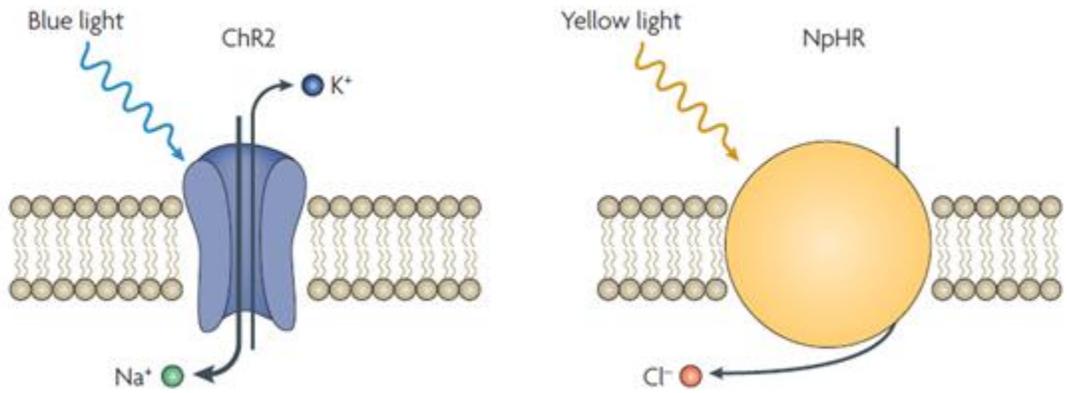
**Figure 3: Dopaminergic Neurons in a Male *C. elegans*.** CEPd (R and L), CEPv (R and L), ADE (R and L) and PDE (R and L) dopaminergic neurons are found in both male and hermaphrodite worms. R5A (R and L), R7A (R and L) and R9A (R and L) dopaminergic neurons are only present in male *C. elegans*. Note, each dopaminergic neuron depicted consists of a right and left neurons (Lints and Emmons, 1999).

Two genes that are expressed in the dopamine neurons, and are vital in regulating dopamine levels are *cat-2* and *dat-1* (Flames and Hobert, 2009). Dopamine uptake in *C. elegans* is mediated primarily by the dopamine transporter gene *dat-1*. *Dat-1* encodes a dopamine transporter (DAT), found in the axons and dendrites of dopamine neurons, responsible for the re-uptake of dopamine into presynaptic terminals (Jayanthi et al., 1998). DATs belong to a neurotransmitter transporter gene family that has been observed to be electrogenic. They translocate neurotransmitters by coupling transport to ion gradients (Carvelli et al., 2004). Loss-of-function mutations in *dat-1* results in increased synaptic dopamine concentrations. When swimming in water *dat-1* mutants paralyze while wild type worms continue to thrash. The paralysis exhibited by *dat-1* worms results from dopamine spillover into extrasynaptic sites, which inhibits locomotion (McDonald et al., 2007).

## Optogenetics in Neurobiology

The use of optogenetics has advanced the manner in which organisms are studied in the field of Neurobiology. It enables researchers to investigate an animal without the need for the animal's dissection. This way neural dynamics responsible for certain behaviors can be studied in live, freely behaving animals. The nematode *C. elegans* is amenable to this technique due to its transparency, and compact nervous system. Optogenetics relies on light-activated proteins. These proteins are single membrane conductance regulators encompassing light sensitivity and fast membrane potential control within a single open reading frame, which can be used to achieve fast bidirectional control of specific cell types even in freely moving animals (Gradinaru et al., 2007).

Channelrhodopsin-2 (ChR2) and halorhodopsin (Halo) are two light-activated proteins that are used to stimulate or inhibit neurons. Each protein is activated upon exposure to a specific wavelength of light. ChR2 is a monovalent cation channel that allows Na<sup>+</sup> ions to enter the cells following exposure to blue light (470 nm), whereas halorhodopsin is a chloride pump that activates upon illumination by yellow light (580 nm). **Figure 4** illustrates the activation of these proteins upon illumination. ChR2 is used *in vivo* to excite action potentials in neurons, and halorhodopsin is used for suppressing action potentials, inhibiting the action of the neurons (Zhang et al., 2007).



**Figure 4: Optogenetic activation neurons.** Illumination with blue light activates ChR2 which then allows the entry of cations, specifically  $\text{Na}^+$  and  $\text{Ca}^{2+}$  inside the cell. Halorhodopsin allows the entry for  $\text{Cl}^-$  anions when activated by yellow light illumination (Zheng et al., 2007).

Genes coding for these light-sensitive proteins can be delivered to the target cells by transfection, viral transduction or the creation of transgenic animal lines. Expression can be restricted to cells of interest using specific promoters (Leifer et al., 2011). For instance, *rig-3* promoter was used for expression of halorhodopsin in the AVA command interneuron, and *dat-1* promoter was used for expression of channel rhodopsin in the AVB command (Donnelly, personal communication).

## PROJECT PURPOSE

The purpose of this project was to artificially modulate *Caenorhabditis elegans* locomotion by inducing dopamine release or inhibiting the backward command neuron. A dopaminergic-specific promoter and AVA specific promoter were used to express light sensitive proteins in target neurons in order to use optogenetics to study behavior. Using confocal microscopy, the expression patterns of *rig-3::Halorhodopsin::mCherry* and *dat-1::ChR2::mCherry* were analyzed to confirm expression in neurons. The goal of these experiments was to execute behavioral analysis of the neural escape circuit and dopamine food sensing circuit using transgenic worms that express channel rhodopsin (ChR2) and halorhodopsin, and identifying genes that are required for locomotory behavior by analyzing the effect of biogenic amines, such as dopamine, on behavior.

## METHODS

### *C. elegans* Strains

All strains were obtained from the *C. elegans* Genetics Center (CGC), with the exception of *dat-1::ChR2* and *rig-3::halo* strains which were generated in the Alkema lab. Worms were cultured at room temperature (22°-24°C) on nematode growth media (NGM) agar plates. Plates were seeded with OP50 *E. coli* as a food source (Brenner, 1974). The wild-type strain used was Bristol N2. strains used in this study were *dat-1::ChR2::mCherry; lite-1(ce314)*, *rig-3::Halorhodopsin::mCherry*, *lite-1(ce314)*, as well as *dat-1(ok157)*, a mutant strain.

*rig-3::halo::mCherry* and *dat-1::ChR2::mCherry* worms were backcrossed with N2 worms prior to experiments to eliminate background mutations. *dat-1::ChR2::mCherry; lite-1(ce314)* was made by crossing this integrated *dat-1::ChR2::mCherry* with *lite-1(ce314)* mutant. The *dat-1::ChR2::mCherry* cross was initiated by plating 8 *dat-1::ChR2::mCherry* males with 2 *lite-1(ce314)* mutant hermaphrodites. In F1 offspring, 8 males were picked and again plated with 2 *lite-1(ce314)* hermaphrodites. In F2 offspring, approximately 12 L4 animals were isolated to individual plates and allowed to reproduce. Homozygosity for mcherry strains was completed visually, and the homozygosity for *lite-1(ce314)* strain was confirmed using PCR

### Worm Lysis and PCR

Approximately 3-5 worms were picked and placed in 5 µL of 1X Proteinase K lysis buffer. The worms were lysed at 65°C for 1 hour and 95°C for 15 minutes in an

Eppendorf Thermocycler. For each PCR reaction, a 25 $\mu$ L mix was used containing 2.5 $\mu$ L 10X PCR Buffer, 2.5 $\mu$ L of 2 mM dNTPs, 0.1 $\mu$ L Taq polymerase, 1.5 $\mu$ L worm lysate, 0.25  $\mu$ L of each primer, and 17.9 $\mu$ L dH<sub>2</sub>O. Primers used for *lite-1(ce314)* mutants were TTCGGGAATGAGGATGAGAC fwd primer, TAAATGTGCCCCTTGGCTAC rev primer, CTCGGCAGGTACCATTTCAT sequencing primer. The samples were heated at 94°C for 2 minutes in an Eppendorf Thermocycler, followed by 35 cycles consisting of 94°C for 15 seconds, 60°C for 45 seconds, and 72°C for 1 minute. Finally, the samples were heated at 72°C for 5 minutes. Agarose gel electrophoresis was used to visualize PCR products.

### **ChR2 and Halorhodopsin Expression Analysis**

*dat-1::ChR2::mCherry* and *rig-3::halo::mCherry* were visually analyzed using confocal microscopy (Zeiss). The images were formatted using ImageJ software. Cell identification was done by comparing cell morphology to the known *C. elegans* neuronal diagram.

### **Response To Green Light assay**

*rig-3::Halo::mCherry* assays were conducted at room temperature (22°-24°C) on 1.7% agar plates containing no food. Performing the experiment on plates with no food promotes increased locomotion due to the worm's foraging behavior. Since it is difficult to differentiate a 1 day old adult from a 3 day old adult worm, animals used in this experiment were selected at the L4 stage 24 hours prior to assay for consistency. This method ensures that the worms used in the assay are all 1 day old adults producing a

homogenous sample. For the assay, worms were transferred to plates with no food, and observed for reversals. When a reversal was made, green light was shined for approximately 1 second upon the worm to observe its effect. The strains used in this assay were: N2 grown on regular food and two *rig-3::Halo::mCherry* strains; one grown on food supplemented with retinal and the other grown on food without retinal. Plates containing no retinal were used as a control to confirm that *rig-3::Halo::mCherry worms* on retinal were functioning properly, and that the results were not due to some unknown factor. Retinal is required by light activated proteins such as *Halo* and *ChR2* for activation. At least 3 trials, executed on three different days, using an average of 50 worms were conducted on each strain in this study.

### **Swimming Induced Paralysis (SWIP) assay**

As in the green light assay, all worms used for this assay were selected at the L4 stage 24 hours before the execution of the experiment. Single L4 hermaphrodites were placed in 30  $\mu$ l of M9 solution in a single well of Pyrex Spot Plate. Each worm was observed under blue light for a period of 10 minutes. The number of thrashes, or times the worm's body bended from one side to the other with respect to the worm's midsection, was enumerated for five 1 minute intervals over the 10 minute time period. The strains used in this assay were: *dat-1::ChR2::mCherry*; *lite-1(ce314)*, *lite-1(ce314)* grown on retinal and regular food, and *dat-1(ok157)* mutants grown on regular food. *dat-1(ok157)* mutants were observed under blue light and white light to verify that the behavior of the worms is not due to some unknown factor. At least 8 trials, performed on

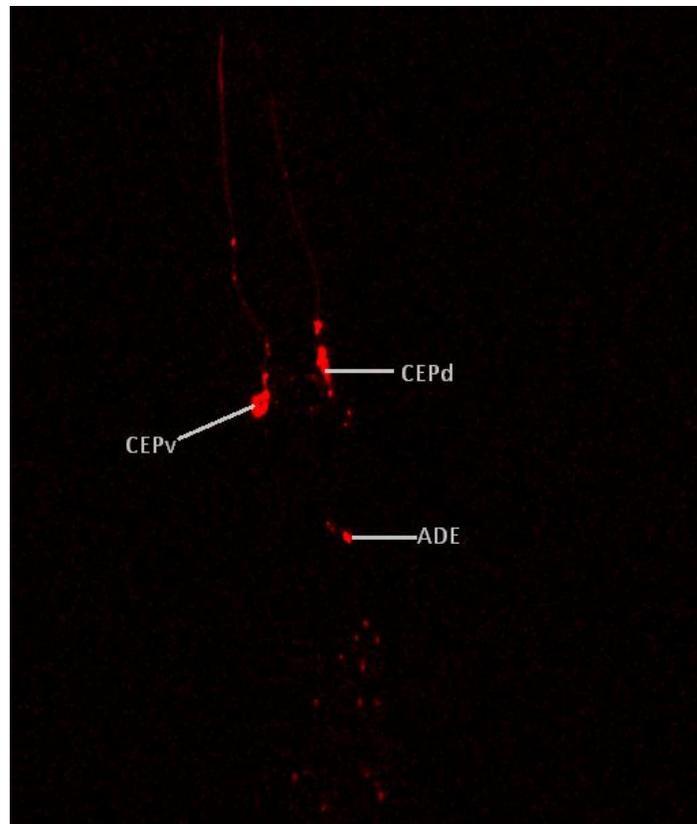
three separate days, were conducted on each strain. The average number of thrashes was determined and graphed using Microsoft Excel.

**dat-1::ChR2::mCherry; lite-1 (ce314) no food assay**

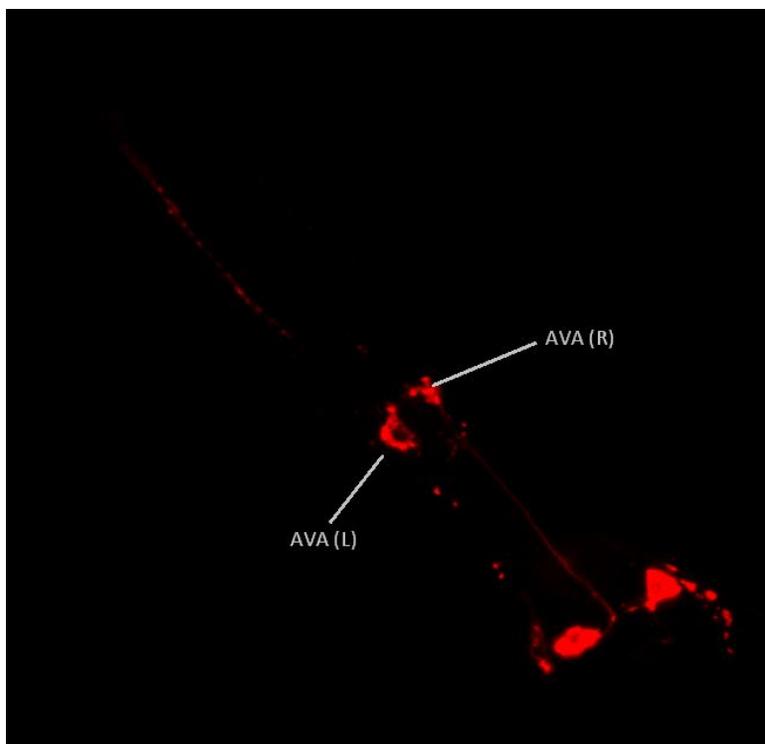
This experiment was conducted on *dat-1ChR2mCherry; lite-1(ce314)* and *lite-1(ce314)* worms both grown on retinal and regular food. Young adult worms, which were chosen at the L4 stage 24 hours before the experiment, were transferred from their original plate to a medium sized plate with no food. The worms were allowed to explore the no food plate and adjust to the new environment for approximately 10 minutes before blue light was shined on them for 10 seconds. A Sony XCD-SX910 camera and Astro IIDC, a video recording program, were used to record the worm's movement for 2 minutes. The resulting movies were analyzed to discern the speed of the worm, determined by the number of body bends completed by the worm over a 10 second interval. In this assay, a body bend is characterized by an undulation of the extrema (head or tail) of the worm's body along a sinusoidal path displayed by the worm during forward or backward locomotion. At least 3 trials, performed on three separate days, using an average of 20 worms were done on each strain.

## RESULTS

Confocal microscopy of the *dat-1::ChR2::mCherry*, and *rig-3::halo::mCherry* strain was used to analyze the expression pattern of *dat-1*, and of *rig-3*, respectively. *dat-1::ChR2::mCherry* was identified in dopaminergic CEP neurons and ADE neurons(**Figure-5**). *rig-3::halo::mCherry* was observed in the AVA command neurons (**Figure-6**).



**Figure-5: ChR2 Expression in Dopaminergic CEP and ADE Neurons.** The *dat-1::ChR2::mCherry* protein fluoresces red.



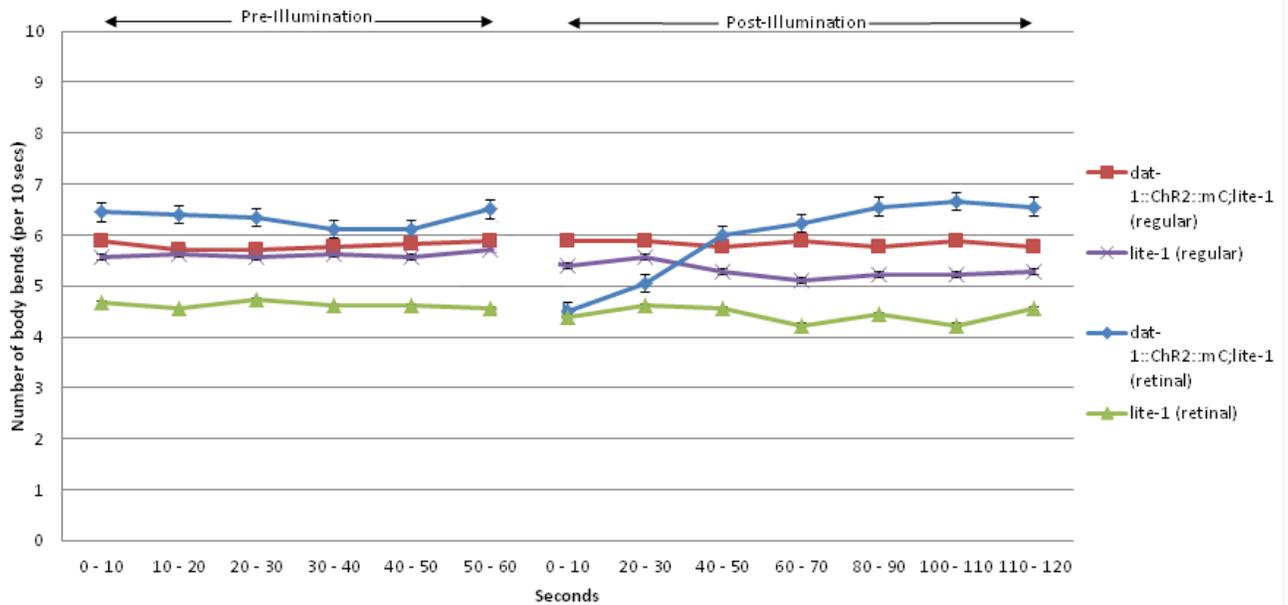
**Figure-6: Halorhodopsin Expression in AVA Command Neurons.** The *rig-3::halo::mCherry* protein fluoresces red.

The protein halorhodopsin inhibits the backward locomotion command interneuron AVA. It was used experimentally to determine the effect of inhibiting backward locomotion on the worm's basal response. Young adult worms from the *rig-3::halo::mCherry* strain grown both on retinal and regular food were tested in this experiment. N2 worms were used as a control. Green light was shined on a worm sample from each strain to observe its effects on the backward locomotion of the worms. Table 1 displays the results from each 50 worm trial for each strain providing percentages for the inhibition of backward locomotion when the green light is shined and the continuation of backward locomotion once the light is removed.

Trial	<i>rig-3::Halo:mCherry</i> (retinal supplemented food)		<i>rig-3::Halo:mCherry</i> (regular food)		N2 (regular food)	
	Backward locomotion inhibited during green light (%)?	Backward locomotion continued after green light (%)?	Backward locomotion inhibited during green light (%)?	Backward locomotion continued after green light (%)?	Backward locomotion inhibited during green light (%)?	Backward locomotion continued after green light (%)?
<b>1</b>	100%	96%	4%	100%	0%	100%
<b>2</b>	98%	96%	2%	100%	0%	100%
<b>3</b>	100%	100%	0%	100%	0%	100%
<b>Average</b>	99.3%	97.3%	2%	100%	0%	100%
<b>Standard Deviation</b>	.011547	.023094	0.02	0	0	0

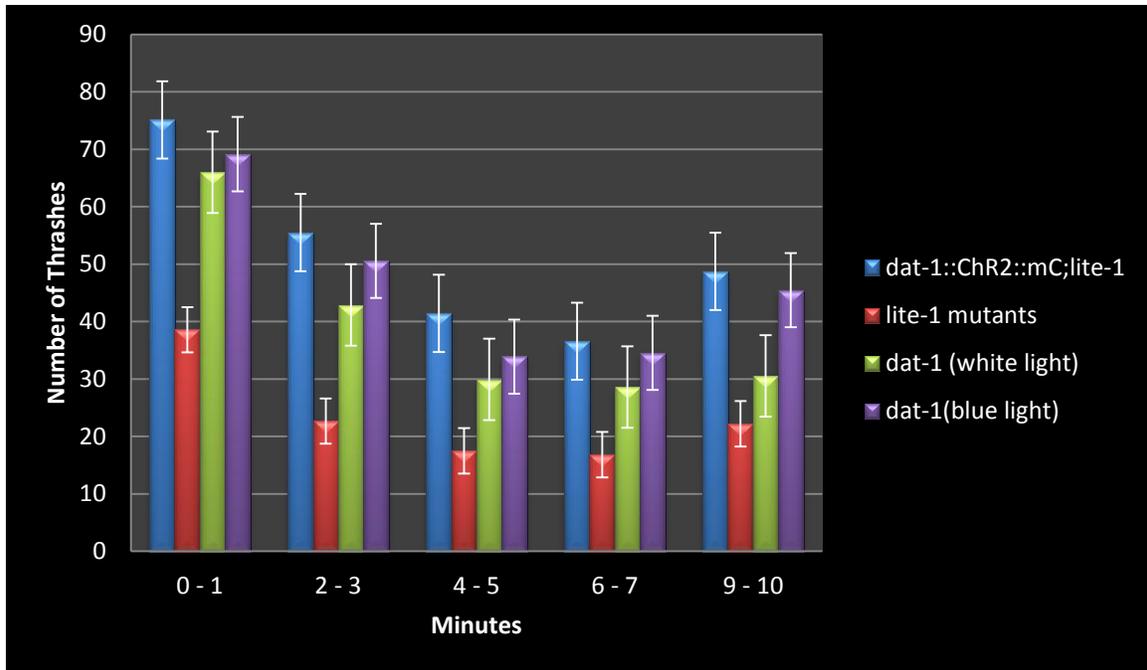
**Table 1: The Effect of Halorhodopsin on Backward Locomotion.** Each trial consisted of 50 worms for each strain. Standard deviation was calculated and is noted in the table.

To further explore the effect of acute dopamine signaling in worms, *dat-1::ChR2::mCherry;lite-1(ce314)*, and *lite-1(ce314)* worms grown on retinal and regular food were observed on plate with no food. Worms were observed under regular light for one minute prior to the 10 second blue light illumination, and for two minutes after the illumination (**Figure 7**). Only *dat-1::ChR2::mCherry;lite-1(ce314)* worms (blue curve) experience a change in locomotion rate after the blue light illumination.



**Figure 7: Optogenetic Activation of the Dopaminergic Neurons Induces Decrease in Rate of Motion.** Each point presents the mean of three trials consisting of 18 worms per trial. Error bars denote the standard error.

A Swimming Induced Paralysis (SWIP) experiment was performed in order to express acute dopamine overload in the synapse. Four different strains of worms were tested: *dat-1::Chr2::mCherry;lite-1(ce314)* grown on retinal food, *lite-1(ce314)* mutants grown on retinal food, *dat-1(ok157)* mutants grown on regular food. Seven worms from each strain were assayed on well plates containing M9 solution, and observed under blue-light to determine the number of thrashes made by worms over a 10 minute time period. *dat-1* mutants were viewed under blue and incandescent white light (**Figure 7**). The rate of thrashing of worms in all the strains decreased from the initial rate, but began to increase over time. The rate of thrashing in *dat-1::Chr2::mCherry;lite-1(ce314)* mutants (blue bar) was observed to be the highest.



**Figure 8: Overexpression of Dopamine in Synapse through Swimming Induced Paralysis.** Each bar represents the mean of three trials which consisted of 7 worms per trial. Error bars denote standard error.

## DISCUSSION

In this study, using optogenetics, the nematode *Caenorhabditis elegans* was observed for behavioral changes induced by artificially modulating the animal's locomotion. This was accomplished by using different strain of worms with varied food sources and mutation. Exploring the backward locomotion command neuron is significant because it plays a crucial role in the worm's escape behavior. The amine, dopamine, was also studied since it is produced in worms in the presence of food and during certain behaviors that induce stress.

To identify the role of the AVA command neuron in *C. elegans* locomotion, *rig-3* promoter, which is unique to the AVA neurons in the ventral cord, was used to express the inhibiting light-activated protein, halorhodopsin. In the assay analyzing the effect of halorhodopsin on backward locomotion it was observed that on an average of 99.3% of *rig-3::Halo::mCherry* worms that were grown on retinal food exhibited an inhibition of backward locomotion. Furthermore, 97.3% of those worms continue to move backwards after the green light was turned off. This indicated that the animal's motion was interrupted by the green light and that the worms were not just reversing directions sporadically. The *rig-3::Halo::mCherry* worms that were grown on regular food served as a control for the same strain of worms grown on retinal. As can be seen in Table 1, they did not show an inhibition in their backward locomotion upon shining green light. Only 2% of the 50 worms tested stopped when the green light was turned on, and a 100% continued the direction of their motion after the light was turned off. This was expected to be the outcome for this strain due to the fact that there was no retinal in the food.

Retinal is a co-factor that is required by the light-activated proteins in order for them to function (Guo et al., 2009). Since there was no retinal in the worm's internal system, there was no effect of green light on this strain of worm, and therefore no inhibition in locomotion occurred. N2 worms acted as another control in the experiment for the mutated strains since they are wild-type worms with no mutations. They were also unaffected by the green light. The results from this assay prove that AVA plays a major role in the ability of *C. elegans* to move backwards, and that when inhibited, backward motion is stunted and nothing assumes the role of AVA to compensate.

Expression of dopamine was artificially induced in *C. elegans* to observe its effects on the animal's behavior. One assay performed to this end was the *dat-1::ChR2::mCherry* no food assay. No food allowed for an observation of greatest unbridled locomotion due to their food searching behavior (Hills et al., 2004). *dat-1::ChR2;;mCherry;lite-1(ce214)* double mutants grown both on retinal and regular food, as well as *lite-1(ce314)* mutants also grown on both regular and retinal food were tested in this assay. As expected, *dat-1::ChR2;;mCherry;lite-1(ce214)* grown on retinal worms experienced a noticeable change in their speed of locomotion after blue light illumination, while the other worms did not experience a significant change (Figure 8). *dat-1::ChR2;;mCherry;lite-1(ce214)* grown on retinal strain was the only strain of *C. elegans* in which dopamine was produced artificially due to *ChR2* mutation. The *lite-1(ce214)* mutant worms lack the light-activated protein which stimulates the dopaminergic neurons to produce dopamine. The *dat-1::ChR2;;mCherry;lite-1(ce214)* strain grown on regular food served as a control for the *dat-1::ChR2;;mCherry;lite-1(ce214)* strain grown on retinal since it lacks the *ChR2* co-factor. This assay indicates

that acute expression of dopamine induces a decrease in the locomotory rate of *C. elegans*, but does not induce paralysis. This was expected due to the fact that the *dat-1::ChR2;;mCherry;lite-1(ce214)* did not have a *dat-1* loss of function mutation, and therefore still had the transporter intact responsible for the reuptake of dopamine.

In the SWIP assay, *dat-1::ChR2::mCherry;lite-1(ce314)*, and *lite-1(ce314)* worms grown on retinal were submerged in M9 solution and observed under blue light for the animal's activity for a period of 10 minutes. In addition, *dat-1(ok157)* mutants were grown on regular food and were observed under both blue and white light. In this assay, the *lite-1(ce314)* mutants served as the control for the *dat-1::ChR2::mCherry;lite-1(ce314)* double mutants. This is so since both strains have *lite-1* mutation but the *lite-1(ce314)* mutants do not have the *ChR2* mutation. Thus, with these two strains, the significance of the *ChR2* mutation on dopamine expression can be isolated and observed. Furthermore, the *dat-1::ChR2::mCherry;lite-1(ce314)* served as the control for the *dat-1(ok157)* mutants. This is so since the *dat-1::ChR2::mCherry;lite-1(ce314)* mutants have the transporter necessary to regulate the dopamine expression that *dat-1(ok157)* mutants lack. Consequently, with these strains, the significance of the dopamine transporter *dat-1* on dopamine expression can be isolated and observed. According to a previous study done using the SWIP assay, when swimming in water *dat-1* mutants paralyze while wild type worms were observed to continue to thrash. The paralysis exhibited by *dat-1* worms results from dopamine spillover into extrasynaptic sites, which inhibits locomotion (McDonald et al., 2007). As can be seen in Figure 7, *dat-1* mutants, which were observed under blue and white light, did not paralyze. This was unexpected since the *dat-1* mutants lack the dopamine transporter *dat-1*, which facilitates the re-uptake of

dopamine preventing overexpression and dopamine spillover. Unobserved paralysis in the mutants could have been a result from some compensatory factor which allowed for the regulation of dopamine even in the absence of the dopamine transporter *dat-1*. Namely, the worms may have compensated by secreting less dopamine, by activating another uptake mechanism, by breaking down dopamine through metabolic activity, by regulation at a post-synaptic site (as the experiment was observed pre-synaptically), etc. When compared to the *dat-1::ChR2::mCherry;lite-1(ce314)* strain, and the *lite-1* mutant strain, the same trend of motion is observed. All the worms showed a decrease in rate of thrashes with an eventual increase towards the end of the 10 minute observation period. When placed in water the animals undergo maximum physical activity producing increased amounts of dopamine (McDonald et al, 2007). In the SWIP assay, as dopamine was produced due to the animal's physical exertion the animal's rate of thrashing decreased gradually as dopamine rushed in the synapses of the animal's nervous system. An eventual increase in the worm's thrashing rate resulted from the ultimate re-uptake of dopamine relieving the inundated worm's synapses. In this assay, the *dat-1::ChR2::mCherry;lite-1(ce314)* double mutants exhibit the highest average thrashing rate over the 10 minute observation period out of all strains. All the other strains experienced increased dopamine levels only through the stress of swimming while the *dat-1::ChR2::mCherry;lite-1(ce314)* double mutant produced extra dopamine through the activation of *ChR2* by blue light illumination (under which all strains were observed).

In the future the SWIP assay experiment can be further improved by conducting the experiment on *dat-1::ChR2::mCherry* worms grown on regular food as well as food supplemented with retinal. Worms consisting of a double mutation of *dat-1* and *ChR2*

can be another strain used to observe the effect of excess dopamine on worms. Future experiments can be done to explore how dopamine is being regulated without the dopamine transporter. Dopamine expression levels could be monitored in *C. elegans* with *dat-1* mutation to observe whether worms compensate for the lack of dopamine transporter by producing less dopamine. Furthermore, expression levels of dopamine at the post-synaptic end can be monitored in future experiments to determine whether that mechanism is used by worms lacking *dat-1*.

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