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# Using $\mu$ -Endorphin as an Opioid Addiction Model in *C. elegans*

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# Using $\beta$ -Endorphin as an Opioid Addiction Model in *C. elegans*

A Major Qualifying Project  
Submitted to the Faculty of  
Worcester Polytechnic Institute  
in partial fulfillment of the requirements for the  
Degree in Bachelor of Science  
in  
Biology and Biotechnology  
By

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Michael Savoie

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Bailey Sweet

**Date:**

Project Advisor:

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## Using $\beta$ -Endorphin as an Opioid Addiction Model in *C. elegans*

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## **Abstract**

One of the most prominent addictions of the modern age is to opioids. While opioids originated as a pain relief treatment, their use has increased in the United States steadily since 1990. To study the impact of opioids on a compact neurological system, the model roundworm *Caenorhabditis elegans* was used.  $\beta$ -Endorphin, an endogenous human opioid, was administered to *C. elegans* and observed for changes in locomotive behavior. Our results revealed a reduction of speed of the worms treated with  $\beta$ -Endorphin versus a water control. These findings suggest that  $\beta$ -Endorphin has an effect on *C. elegans* and can be useful model in studying addiction in these systems

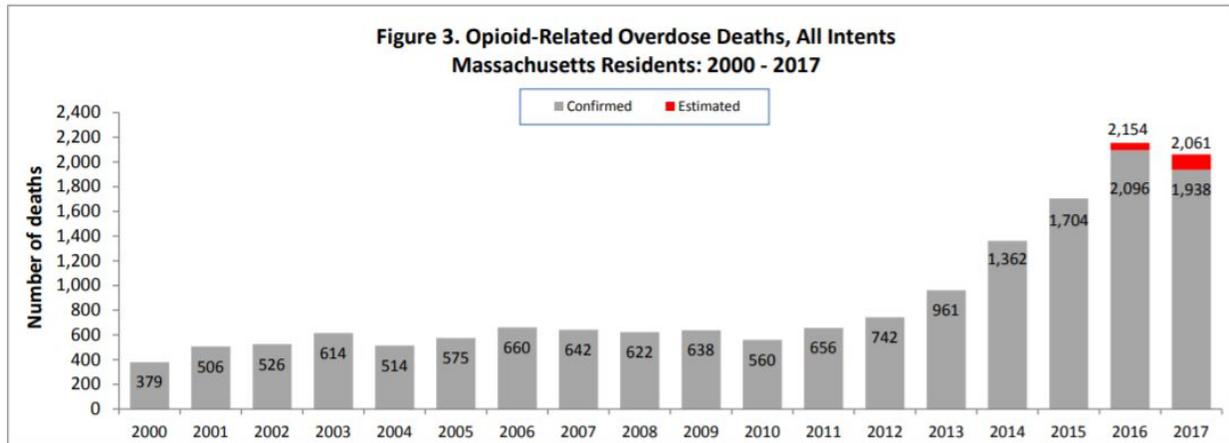
## **Introduction**

Addiction is defined as using a substance or acting in a behavior that results in a rewarding effect (Addiction, n.d.). Addiction substances can include, alcohol, opioids, nicotine and behaviors like gambling. These substances and behaviors activate the reward pathway due to the neurological release of dopamine. This pathway reinforces behaviors that become difficult to control. Addiction can disrupt relationships and other obligations, as well as degrade the body. One of the most destructive addictions of the modern era is to opioids.

Opioids are a class of medication used for pain relief treatment. Commonly, they are used for short term treatment, like after surgery. Their main purpose is to attach to receptors in the brain to block pain signaling. Opioids include prescription medications like Morphine, Codeine, Percocet, Oxycodone, and illicit drugs like Heroin (The National, 2014). Medical use of opioids has increased their use within the United States. The peak of opioid prescriptions occurred in 2012, to a total of 81.3 prescriptions per 100 patients in the United States. This number has declined to 58.5 in 2017 (Opioid, 2018). However, even though a decline in prescriptions has occurred, overdose deaths have continued to increase. Both prescription and illicit opioids contribute to overdose deaths.

Opioid addiction in Massachusetts has shown a similar trend. In 2015, Massachusetts prescribed 59.9 opiates per 100 patients (National, 2018). Figure 1 shows the opioid related overdose deaths of Massachusetts residents from 2000 through 2017. The number of opioid

related overdose deaths peaked in 2016, where it was estimated that 2,154 people died due to opioid overdose. In 2000, the number of overdose deaths was 379, less than 5 times as many deaths than when compared to 2016. There is a correlation between the increase of prescriptions and the increase of opioid related overdose deaths.



**Figure 1:** Opioid Overdose Deaths in Massachusetts (Data, 2018).

Addiction to opioids in humans is a common side effect due to prescriptions post-hospitalization. Between 21 and 29 percent of patients with prescriptions for opioids misuse them. Opioids are common pain medications due to their ability to relax the body and ease pain. Common prescription opioids include Vicodin, Oxycontin, Percocets, Morphine, and Codeine (NIDI, 2018). Physical side effects from opioids include drowsiness, confusion, nausea, constipation, euphoria, and slowed breathing. Slowed breathing can result in a more intense condition called hypoxia, where the brain does not receive enough oxygen. Hypoxia can cause coma, brain damage, and even death. Opioid addiction has become such a prevalent issue across the United States, that it has been deemed an epidemic. This is supported by the fact that about 80%, of heroin users began with misuse of prescription opioids (National, 2018).

Addiction to opioids is challenging to overcome. Physical dependence is the main reason why it is difficult for patients to overcome addiction. Opioids are best prescribed for short term pain regulation, due to tolerance development. When patients become tolerant to opioids, they can experience withdrawal when their prescription is complete. Symptoms of withdrawal include runny nose and eyes, muscle aches, anxiety, agitation, nausea, and more. Dependence on opioids

can occur even when taking the medication as prescribed by a licensed physician. Opioid abuse is characterized by “misusing opioids with the intention to get high or avoid withdrawal symptoms” (Merchant, 2017) .

Studies about opioid addiction can not be studied in human subjects due to ethical concerns. Therefore, the purpose of this study is to observe the effect that exposure to opioid-like analogs have on normal *C. elegans* behaviors. The analog being tested is  $\beta$ -Endorphin. This is an endogenous opioid produced in humans. Since  $\beta$ -Endorphin is not produced in *C. elegans*, it could be considered to be a synthetic treatment. This is a parallel to how prescription opiates are synthetic to humans.

## **Background**

### **Opioids**

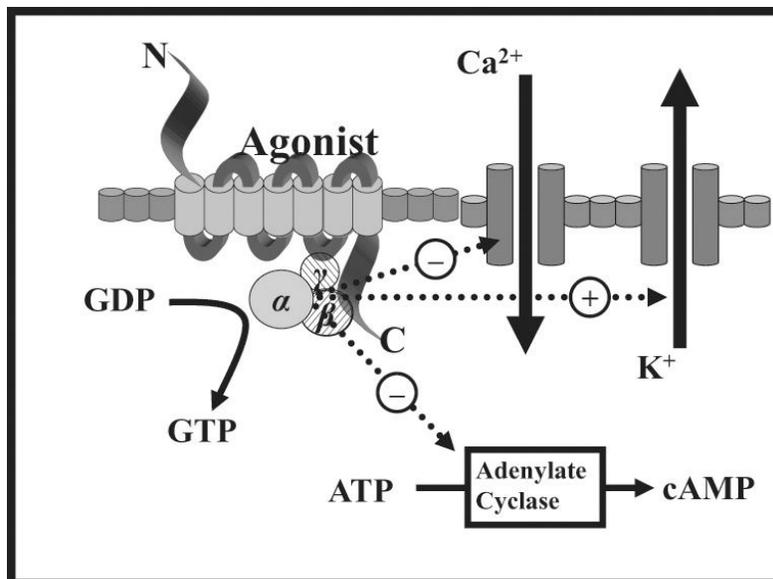
The first discovery of opioids occurred in 1804, when morphine was first extracted from opium poppy (Mitchell, 2017). It was used for medicinal pain relief, but side effects made scientists hope to find an opiate alternative that did not cause tolerance to the drug. In 1896, heroin was developed and claimed to be less addictive than previous medicinal opioids. Oxycodone was developed in 1916, which was beneficial for patients with short term injuries. Experimental trials on the effects of these opiates did not begin until the 1930's.

However, Purdue Pharma is currently being sued by the state of Colorado, and 5 other states due to wrongful marketing and misrepresentation of the side effects of OxyContin and other opioid pain relievers (Moritz, 2018). Some of the claims include; failing to disclose addiction risk of opiates, not claiming a maximum dosage that was dangerous to patients, claiming that addiction symptoms mean that patients need to increase their dosage, and claiming that their formula reduces risk of addiction in general.

### **Mechanism of Action of Opioids**

Opioids bind to opioid receptors within the body. While there are a multitude of receptors, all have similar activation responses. When an opioid binds, the bound guanosine diphosphate (GDP) is replaced by a guanosine triphosphate (GTP) on the  $\alpha$  subunit (Pathan,

2012). The new  $\alpha$ -GTP is removed from the structure. Adenylyl cyclase is inhibited and cyclic adenosine monophosphate is reduced. For neuronal cells, this reduction caused a reduction in released neurotransmitters. This pathway is shown in Figure 2.



**Figure 2:** Opioid binding activation pathway (Pathan, 2012).

## Opioid Receptors

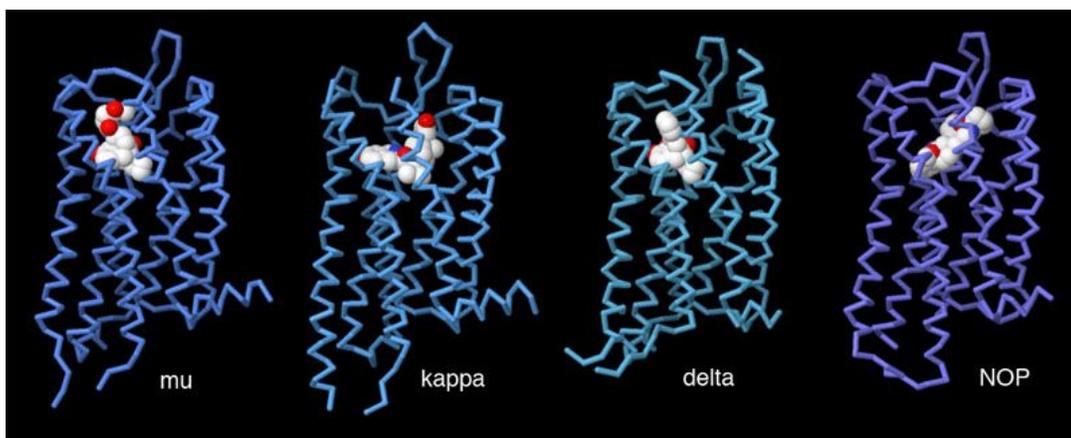
Opioid receptors belong to a class of 7 transmembrane spanning (7TM) G-protein coupled receptors (Feng, 2012). This class of receptors help to mediate both neurotransmitter and hormonal action within humans. These receptors can be activated by two types of opioids, exogenous and endogenous. Exogenous opioids are administered outside of the body, while endogenous opioids originate naturally within the body.

Opioids interact with opioid receptors found on neuronal cell membranes. This coupling action induces a signaling cascade via a G-coupled receptor that, in humans, induces a variety of effects. Opioids, such as morphine, can produce extreme mood changes, addictive symptoms, and general physical dependence as they induce a ‘reward’ sense. In humans these receptors come in three different types  $\delta$ ,  $\mu$ ,  $\kappa$ , and NOR receptor variants. These receptors can be found in both the peripheral and central nervous systems. In humans, opioids act in a twofold manner on the nervous system, at the presynaptic terminal as well as the postsynaptic neuron. In the postsynaptic case, opioids have inhibitory actions, hastening the release of neurotransmitters, but

in the presynaptic condition, opioids amplify their corresponding neuronal response, including that of the post neuronal case (Chahl, 1996).

There are three types of opioid receptors that have been cloned; MOR, DOR, and KOR. These receptors are present in the body for many reasons. These include pain modulation, regulation of membrane ionic homeostasis, cell proliferation, emotional response, immune function, feeding, cardiovascular control and more (Feng). Not much is known about all types of opioid receptors, but DOR has been shown to mediate neuro and cardio protection. Upregulation of the DOR increases neural tolerance to hypoxic stress.

*C. elegans* lack particular opioid structures that are evident in vertebrates, with no comparable ortholog genes nor antagonists (Cheong, 2015). However, they have many peptides and structures similar to vertebrate opioid receptors. *C. elegans* have FMRFamide-related peptides (FaRPs) or Neuropeptide Receptor 17's (NPR-17s), which are structurally and functionally similar to vertebrate opioid receptor (Cheong). Several studies have been conducted to illustrate the effects that opiates can have on worm behavior and physiological function. These studies pertain mostly to noireception (pain modulation) or feeding behavior, two common research areas of opioid addiction. In the case of feeding behavior opioids were shown to affect the pharyngeal pumping speed, illustrating that they have an effect on a worm physiologically and behavior, similar to vertebrate cases (Chahl, 1996).

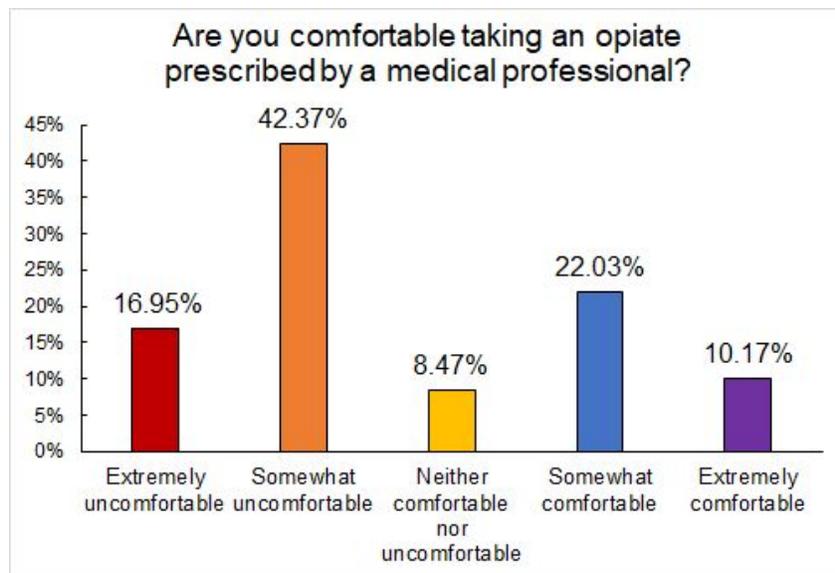


**Figure 3:** Reward pathway in the human brain (pdb101.rcsb.org)

## Opioid Use Within The WPI Community

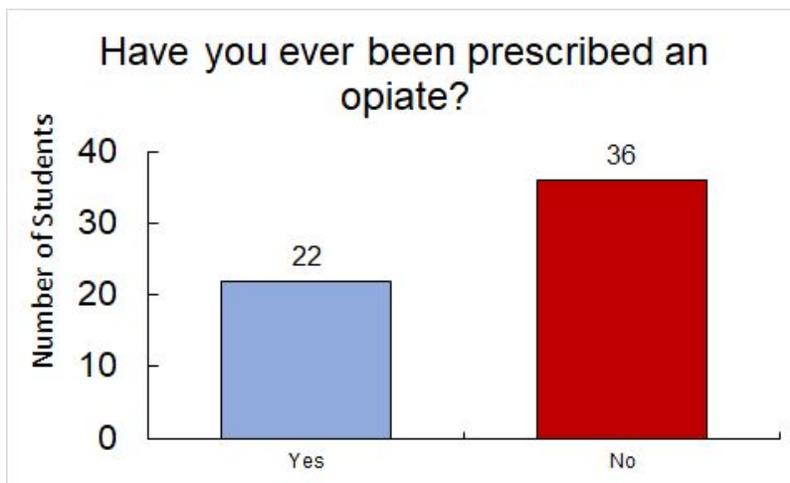
A study was conducted to gain insight about misconceptions of opioids at WPI. The goal of the study was to determine how comfortable WPI students are with opioids. Another goal was to determine how students may be able to access opioids while at college, whether it be medically or recreationally.

The survey began with simple questions asking if students had received any substance abuse trainings during high school or while at college. Most of the respondents had participated in a training within the past 5 years. The topics covered by these trainings was varied, including drinking, recreational drug use, peer pressure, risk awareness, and more. The survey then transitioned into asking specifically about opioids. Opioids were first defined, and then respondents were asked how comfortable would they feel taking an opioid if prescribed by a medical provider. The spread of responses can be seen down below in Figure 4. More than 60% of survey respondents would be uncomfortable to extremely uncomfortable with taking opiates, even when prescribed by a medical professional. About 32% of respondents would be comfortable to extremely comfortable when taking opiates prescribed by a medical professional. Overall, students at WPI are likely to be uncomfortable than comfortable when taking prescribed opioids.



**Figure 4:** Comfort level of WPI students with medically prescribed opiates.

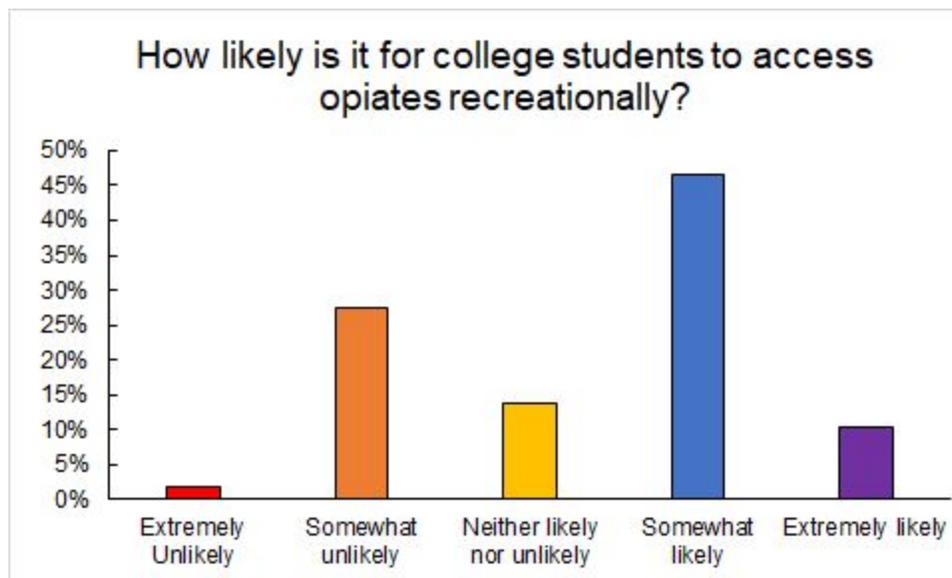
The survey then asked if respondents had ever been prescribed an opiate by a medical professional. Responses can be seen below in Figure 5. 64% of survey respondents had not been prescribed an opiate. However, 35% of respondents had been prescribed an opiate. 22 of the 60 survey respondents were able to have access to opiates through prescriptions. An interesting correlation between the comfort of WPI students with opiates and those who have been prescribed them. An assumption would be that most of the students that have had opiate prescriptions would be comfortable with them. However, of the 22 who have been prescribed opiates, 8 are somewhat comfortable. 7 of the students who have been prescribed opiates are extremely comfortable. The only other response that was extremely comfortable had not been prescribed an opiate. However, the 7 other students who have been prescribed opiates would be somewhat uncomfortable taking them. This was an unexpected result, but shows that even after having a legal experience with opiates, they can still cause some discomfort.



**Figure 5:** Opioid prescriptions of WPI students.

The survey then asked respondents if they knew of anybody taking opiates for both their prescribed use, as well as if they knew anybody who had abused them. At least 38% of respondents knew of somebody who has abused opiates as a way to get high or avoid withdrawal symptoms. However, 91% of respondents knew that using opiates outside of their prescribed purpose was very unsafe. While knowledge about the danger of opioids seems to be clear to WPI

students, there is still dangerous opioid abuse happening to acquaintances of WPI students. The survey then asked respondents how likely it was for college students to have access to opioids recreationally. Responses can be seen in Figure 6. Almost 60% of respondents believe that it is somewhat or extremely likely for college students to be offered recreational opioids.



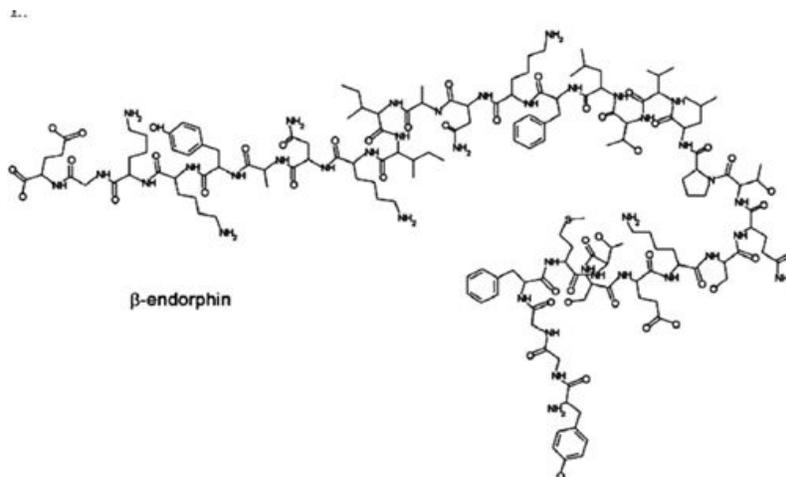
**Figure 6:** Likelihood of college student to recreationally access opioids.

Overall, WPI students tend to be cautious about taking opioids, even when prescribed by a medical professional. However, WPI students are still able to access opioids. Students have access to opioids through prescriptions, as shown in Figure 5. However, based upon Figure 6, it is possible that students also have illicit access to opiates through recreational use. This makes college students able to access opioids through two different routes. This makes college students twice as likely to be susceptible to opioid addiction.

### **$\beta$ -Endorphin**

$\beta$ -Endorphins are a class of hormones secreted in human nervous systems that function as natural pain suppression. They are primarily synthesized and stored in the anterior pituitary gland. Some immune cells, lymphocytes and macrophages have been found to contain  $\beta$ -Endorphins (Sprouse-Blum, 2010).  $\beta$ -Endorphins act post and pre synaptically to mediate pain responses on opioid receptors located in the peripheral nervous system (PNS).

$\beta$ -Endorphins are secreted from the anterior pituitary gland. Figure 7 illustrates the molecular structure of  $\beta$ -Endorphin. Similar to prescription opioids, they bind primarily to MOR receptors located in the olfactory bulb, spinal cord, and the cerebral cortex. It is anticipated since it binds to the same receptor that it will act similarly to other opioids, especially ones used for medical purposes.  $\beta$ -Endorphins are naturally occurring, and increase during traumatic experiences like surgery. Opioids are then prescribed to continue pain treatment, copying the action of natural  $\beta$ -Endorphins (Sprouse-Blum).



**Figure 7:** Molecular Structure of  $\beta$ -Endorphin (Sprouse-Blum)

### *Caenorhabditis elegans*

*Caenorhabditis elegans* (*C. elegans*) are a model organism used to test neurological functions on a smaller, less complex scale than other model systems. *C. elegans* are a small, mostly hermaphroditic (with a small percentage of males), nematodes that are globally abundant in soils and are easily cultivable in a laboratory environment (Riddle, 1997). They have a 3 day life cycle, which involves four larval stages, a young adult stage, and a mature adult stage in which mature adults (who can self fertilize or be fertilized by males) can lay eggs that yield viable progeny. The worms are small in size, measuring about 1.5 mm as an adult, and thus can be held on small petri dishes containing *E. coli* and nutrient growth media (Riddle). It is possible to maintain large population sizes in laboratory settings when using *E. coli* (OP 50 strain) as a primary food source. The “worm” model can be used for various applications, from neuronal,

pathological, and genetic research. *C. elegans* have a small genome, which is only 20 times larger than its food source, *E. coli* (Riddle). *C. elegans* maintain a very small anatomical structure and a relatively small neurological layout, which in hermaphrodites is only 959 somatic cells and 302 neuronal cells (Hermaphrodite, n.d). This neuronal simplicity makes the *C. elegans* model ideal for neurological and behavioral testing. *C. elegans* complete simple functions with their nervous system, such as locomotion, foraging, feeding, and interacting in response to stimuli in their environment with pheromonal cilia. This relatively simple neurological layout still can yield complex behavioral actions.

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

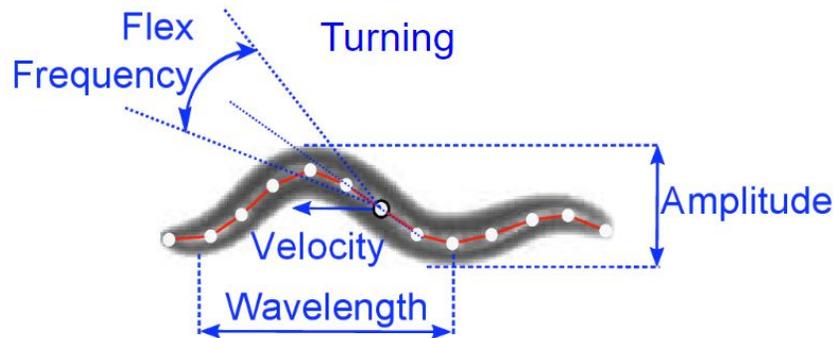
*C. elegans* have 75 innervated motoneurons that enable them to move forward and backward. In order to create thrust, *C. elegans* bend in the direction they wish to move. When swimming, the wavelength of undulation is greater than their body length, but on a surface such as agar the wavelength of undulation is much less than their body length (Gjorgjieva, 2014). The type of motion of *C. elegans* can be defined into four categories: forward locomotion, backward locomotion, dwelling, and quiescence.

Steering is controlled by muscle cells in the head and neck that can freely move compared to the rest of their muscular structure. *C. elegans* have both chemical neuromuscular junctions and gap junctions (Gjorgjieva). These muscles are all anchored throughout the body of *C. elegans*.

There are many different types of motoneurons within *C. elegans*. Each have different functions and structure in order to cause movement within the muscles of *C. elegans* (Gjorgjieva). It is probable that all excitatory motor neurons show activity at the side they innervate, during dorsoventral bending.

The exact nature of *C. elegans* locomotion pattern is still unknown. There are three main hypothesis about how locomotion patterns begin. One theory is that neck muscles create rhythmic bends and other mechanisms propagate this activity throughout the rest of the body. The second idea is that there are coupled oscillators throughout the body that relay the same pattern at the same time. The third hypothesis is that there is sensory feedback throughout the body that creates bends dependent on the received sensory information (Gjorgjieva).

## Behaviors



**Figure 8:** Behaviors exhibited by *C. elegans* during locomotion (Yu *et al.*, 2013)

During locomotion, *C. elegans* exhibit several characterized behaviors that can be observed. For the purpose of this study, three main behaviors were observed and analyzed to understand the impact of  $\beta$ -Endorphins on locomotion. One observed behavior is the speed of the worm. The speed on the worm is defined as the number of micrometer per second moved over the distance of the experimental plate. Another observed behavior of locomotion was the amplitude of the worm. Amplitude is classically defined as the maximum displacement of points in a wave (Zesiger). In terms of *C. elegans*, it is the displacement between the arcs created by the worm as it bends its body to propel itself forward. The last behavior being examined is the turning of the worms or their change of direction on the plate. These behaviors can be useful in determining if there are changes in locomotive ability.

## Methodology

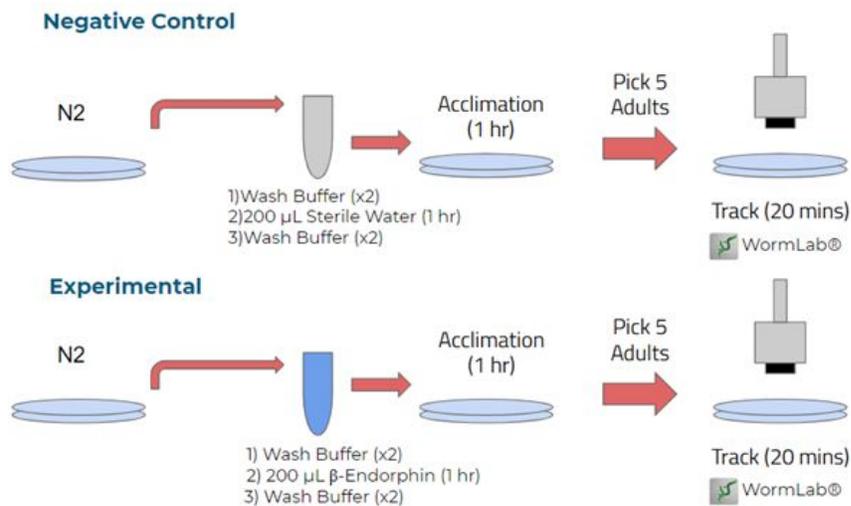
### *C. elegans* maintenance

The *C. elegans* used for this project were of the wild type strain. This strain was originally acquired from the Caenorhabditis Genetics Center (CGC). *C. elegans* were maintained on OP 50 seeded nutrient media plates. All *C. elegans* were incubated at 20° C to allow proper

development. Worms were moved every 3-4 days to new seeded plates to maintain healthy communities.

## Locomotion Assay

Plates with sexually mature adult wild type worms were used for the locomotion assay. The plates were then washed with approximately .5 mL of sterile M9 and transferred to an eppendorf tube. The worms were allowed to settle in the tube for approximately 20 minutes. The supernatant was then removed and the washing procedure was repeated one more time. The worms in the tube were then exposed in the eppendorf tube to 200  $\mu$ L of either sterilized PCR grade water (as a negative control) or the  $\beta$ -Endorphin solution. The worms were left to be exposed for either 10 minutes, 1 hour, or 2 hour intervals. After the exposure, the supernatant was removed and the worms were washed twice more with sterile M9 using the washing procedure outlined previously. The worms were then removed from the tubes and transferred to a 30mm agar plate to acclimate for a 1 hour period. After the acclimation, 5 worms were then transferred from the acclimation plates to a fresh agar plates. The worms were then recorded using Worm Lab for a 20 minute interval.



**Figure 9:** Locomotion assay procedure for N2 (wildtype)

## **Analysis of Locomotion Videos**

Videos of wild type worm locomotion were analyzed using WormLab software. Each video was loaded into the software. The width of each plate was measured for each video to ensure proper tracking. Highlighting on each plate was set to 156. Background smoothing, Gaussian smoothing, and hotspot correction were also selected to help illuminate worms for tracking. A label was placed the illuminated portion of the agar portion of the plate with tracking being limited to this labeled area. This was done to ensure the tracking of only worms in illuminated agarose agar regions of the plate. Each of the five worms on tracked plates were detected using the manual detecting feature. Before each video, the frame rate limiting was set to 35 frames. Each video was saved and then processed using the batch processing feature.

## **Survey Instrument**

A survey about opioid misconceptions at WPI was created with the assistance of a principal investigator, James Doyle and student co-author, Jessica Greenleaf. The survey contained 37 questions, related to beliefs and experiences of WPI students on the topic of opioids. The survey was created using qualtrics software. The SONA systems psychology portal was used to collect responses. 60 responses were collected from January through March of 2019. 55% of responses were from those identifying as women, while 40% of responses were from those identifying as men. 86% of respondents were white. Each undergraduate class was evenly represented throughout survey responses.

## **Results**

This section will discuss how the concentration of  $\beta$ -Endorphin was determined. It will then present data from wild type locomotion trials. Lastly, this section will present findings from the  $\beta$ -Endorphin treatment of *C. elegans*.

### **$\beta$ -Endorphin Concentration**

$\beta$ -Endorphin concentrations were found using a body mass ratio between rats and *C. elegans* exposed to punicagin and  $\beta$ -Endorphins. *C. elegans* have not been tested with

$\beta$ -Endorphins in previous studies and ratios for punicalagin concentrations were used from the MQP completed by Cosedine, Randle, and McNeill. Thus a body mass ratio derived from the concentration of punicalagin in both species was compared to  $\beta$ -Endorphins used in rats to find the  $\beta$ -Endorphin concentration of *C. elegans*. The concentration of  $\beta$ -Endorphin to use was determined to be  $1.6 \times 10^{-11}$  M  $\beta$ -Endorphin.

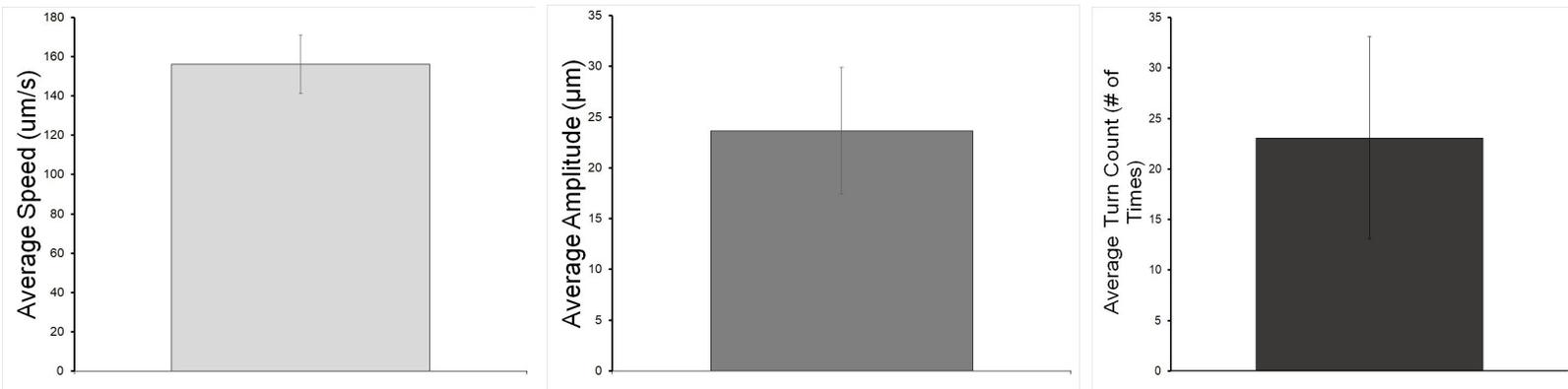
$$\frac{\text{Punicalagin in Rats}}{\text{Beta Endorphin in Rats}} = \frac{\text{Punicalagin in } C.\text{elegans}}{\text{Beta Endorphin in } C.\text{Elegans}}$$

$$\frac{150,000 \text{ ug/mg}}{20 \text{ ug/mg}} = \frac{1.2 \times 10^7 \text{ M}}{x}$$

$$x = 1.6 \times 10^{-11} \text{ M } \beta\text{-Endorphin}$$

### Normalized Wild Type Data

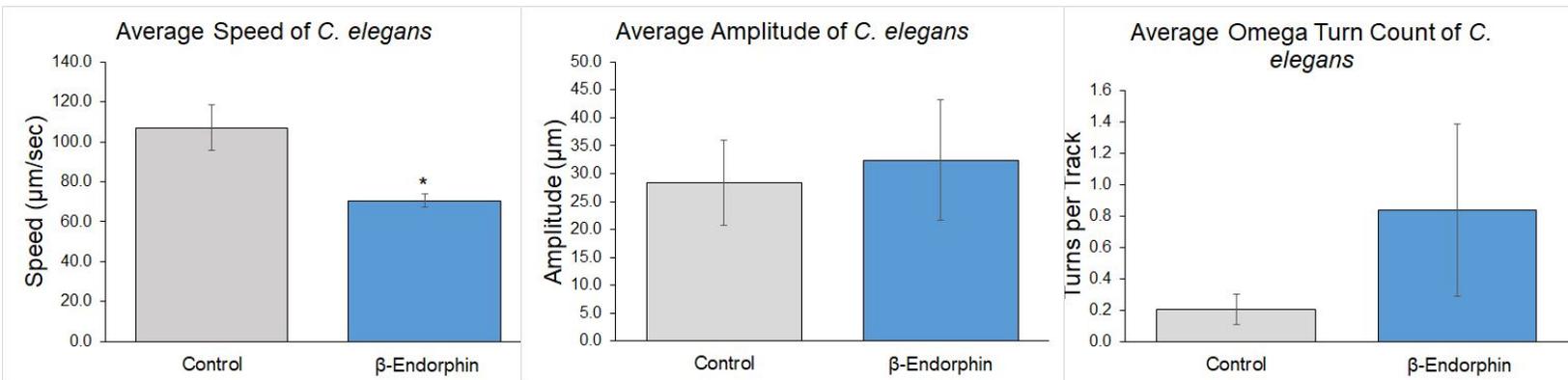
To determine baseline locomotive behavior, 4 trials of 5 worms were tracked according to the Worm Tracker software. Results of the trials are shown in Figure 10 below.



**Figure 10: Wild type *C. elegans* Behavior (n=4).** Wild type *C. elegans* locomotive behavior. The average speed of wild type *C. elegans* (top left), average amplitude (top right), and average turn count (bottom) are shown in this figure. Speed is the average speed over a 20 minute period. An N= 4 was used. Standard Error is graphed.

## $\beta$ -Endorphin Treatment

$\beta$ -Endorphin, at a 16 pM concentration was applied to *C. elegans* for an hour. Comparisons of speed, amplitude, and  $\Omega$  turn count between the water control and  $\beta$ -Endorphin treatment can be seen in Figure 11 below.



**Figure 11: Control vs.  $\beta$ -Endorphin behavior of *C. elegans* (n=5).** Wild type *C. elegans* locomotive behavior after exposure to sterile water versus exposure to 16 pM  $\beta$ -Endorphin treatment tracked over a 20 minute period. The figure shows average speed (left), average amplitude (center), and average  $\Omega$  turn count (right). An N=5 was used. Standard error is plotted on all figures. A student t-test, two-sample assuming equal variances, was performed.

## Discussion

The survey instrument and  $\beta$ -Endorphin treatment of *C. elegans* were used to begin to study opiate addiction at WPI. The survey instrument showed that there is some awareness of the danger of opiates at WPI. However, it also showed that college students have more than one way to access opiates; through both prescriptions and recreationally. This section will discuss the results of the  $\beta$ -Endorphin treatment. It will also touch upon some limitations of the study as well as future directions.

## $\beta$ -Endorphin Treatment versus Water Treatment

The results from Figure X, seem to show that there may be a correlation between the speed of *C. elegans* and the  $\beta$ -Endorphin treatment. This study analyzed three main behaviors;

speed, amplitude, and turn count. The difference between the speed of the control and  $\beta$ -Endorphin treated worm was statistically significant ( $p < 0.05$ ). The control speed was around 110  $\mu\text{m}$  per second, while the  $\beta$ -Endorphin treated worm speed was around 70  $\mu\text{m}$  per second. This is a fold change of about 1.5.

Both the amplitude and  $\Omega$  turn count of the control versus treated worms were not statistically significant. It is possible that this is due to a variability within these behaviors. The wild type locomotive behaviors, from Figure 10, show that the standard error of these 2 behaviors is greater than that of speed. The data of the  $\beta$ -Endorphin treated behaviors had a greater standard error than unexposed wild type worms. This indicates that exposure to  $\beta$ -Endorphin created more variability and less stability in the behaviors of normal *C. elegans*.

$\beta$ -Endorphin does change wild type behavior of *C. elegans* to a degree. The statistically significant reduction of speed shows that the  $\beta$ -Endorphin is somehow interacting with *C. elegans* neural structure. The increased variability of movement could also be a result of interaction of  $\beta$ -Endorphin with *C. elegans* neural structure. Due to these correlations, *C. elegans* could be used with  $\beta$ -Endorphin as a method to test opioid addiction.

## **Limitations**

This study was limited by a variety of factors. These include timing of worm passing, the Worm Tracker software, and issues with maintaining consistencies with controls. The goal of the project was to look at both locomotion assays and egg-laying assays. Egg laying was removed as an experimental observation after difficulty with initial tests on wild type worms. These issues stemmed from the inability to synchronize *C. elegans* populations for appropriate egg laying. They were not passed early enough in age, and therefore did not produce as many eggs as was expected based on other studies.

Originally three  $\beta$ -Endorphin concentrations were tested to understand the range of effects on speed. However, only one  $\beta$ -Endorphin concentration, 16 pM, was included in this final report. The 1.6 and 160 pM concentrations were conducted but encountered difficulties in gathering data and interpreting the information. It is possible that the  $\beta$ -Endorphin could have degraded over time after being dissolved in water and stored in the freezer. However, there is no

literature that discusses if it degrades when dissolved in water. One complication was due to trouble with the Worm Tracker software. The software did not always produce data after analysis was conducted on a video. It was deduced that this was caused by various factors including: size of the worm, darkness of the worm's body, exposure of the plate, or complications in opening multiple project windows in the software. Another persistent issue with the software was the inability to track more than one frame. This often made it difficult to analyze the videos captured from these experiments.

Another major issue was the lack consistency in wild type controls. Controls had to be repeated multiple times due to fluctuations from previously viewed values. This could have been due to changing humidity and temperature in the laboratory. Observed behaviors in both controls and exposed worms fluctuate with temperature and humidity change. This made it difficult to keep observed data trend consistent. Lastly, time was a factor in the inability to complete analysis of the desired concentrations. The analysis process using the Worm Tracker software took extended periods of time. Due to multiple uses of the machine, it was difficult to reasonably time analysis of collected data.

Some of these limitations could be resolved by studying egg-laying of *C. elegans* more in depth, potentially by finding a new procedure to replicate. To resolve some issues with the Worm Tracking software, it is important to ensure that it is up to date. It may also be beneficial to use smaller plates when recording, to improve resolution of the video taken. Lastly, another machine with the Worm Tracker software available would improve efficiency of video analysis.

### **Future Directions**

Future studies with this project should focus on optimizing data analysis using the Worm Tracker software. There were some issues with the software itself that made it difficult to run batch analysis on multiple videos overnight. There were some times when the software would shut down before it was able to finish analysis. Due to this, it would be beneficial for future groups to familiarize themselves with the software at the beginning of the project. It would also be beneficial to contact the Worm Tracker representative, to understand what type of analysis would be best for them, depending on what behaviors their project is focused on.

The future directions of this project specifically include the expansion of concentrations analyzed in this study. In this study, only a 16 pM concentration was fully analyzed. The 16 pM concentration was a good starting concentration because it did show an effect on the speed of *C. elegans*. However, different concentrations could depict a stronger or weaker response to the  $\beta$ -Endorphin. There could be a concentration that is a lethal dose of  $\beta$ -Endorphin. While studies have shown that humans cannot overdose on  $\beta$ -Endorphin, it is possible that it could happen in *C. elegans*. This would be a beneficial way to model opioid overdoses.

Exposure time could be another variable to test. This project only focused on a one hour exposure, but it is possible that a longer exposure could change observed behavior as well. It would be valuable to test exposures less than an hour to see if decreasing exposure time would yield a different behavioral changes. Multiple exposures on the same worms would model opioid addiction. A single individual would be exposed multiple times to the  $\beta$ -Endorphin over the course of a day, a few days, or even a week. This chronic exposure can be compared to an acute exposure to understand differences between the two.

Furthermore, different assays can be conducted to understand  $\beta$ -Endorphins impact on other behaviors. One behavior that was considered but not added to this study was egg laying. It would be of interest to see how opiates impact the ability for worms to lay eggs. A biochemical study could be conducted to determine what is impacting the speed of *C. elegans* when treated with  $\beta$ -Endorphin versus the water control. Other assays, such as avoidance and attraction assays could be performed after  $\beta$ -Endorphin exposure. These are normal behaviors, that influence the success of an organism to feed and reproduce.

There are many future directions to be explored with this project. However, the goal of the project was achieved. This project sought to determine if *C. elegans* and  $\beta$ -Endorphin can be used as a model of opioid addiction. While there are many more directions to explore, this baseline study showed that administering  $\beta$ -Endorphin at a 16 pM concentration reduced the speed of *C. elegans* but also increased variation in normal locomotive behaviors.

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