Drew University

College of Liberal Arts

The Roles of Neurexin in *Caenorhabditis elegans* Behavior

A Thesis in Biochemistry and Molecular Biology

by

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Submitted in Partial Fulfillment

Of the Requirements for the Degree of

Bachelor of Science

With Specialized Honors in Biochemistry and Molecular Biology

May 2022

Abstract

Neurexin (*nrx-1*) is the presynaptic terminal protein responsible for forming and maintaining the synapse. As opposed to the three neurexin genes that mammals have, *Caenorhabditis elegans* (*C. elegans*) have one copy, which allows for easier characterization and makes these nematodes a great model organism. The *nrx*-1 gene of *C. elegans* also has different promoters, which lead to an alpha (α) and a gamma (γ) isoform. This study focuses on how *nrx*-1 isoforms affect the behavior of worm strains that have mutations in this gene by using chemotaxis as the assay. Two attractants and one repellent were tested on the wildtype N2 strain, TV13570 mutant strain (non-functional α and γ regions of *nrx-1*), and the SG1 mutant strain (non-functional α region of *nrx-1*). Based on the number of worms and where they moved during the assay, the chemotaxis index was calculated to determine to what degree of attraction or repulsion was experienced by the worms. For the diacetyl chemotaxis assays, there was statistically significant difference between N2 strain and both TV13570 and SG1, but there was no statistically significant difference between TV13570 strain and SG1 mutant strain. Therefore, it can be concluded that the α region of *nrx-1* influences the behavioral change between the tested *C. elegans* strains.

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Introduction

Neurexin (nrx-1)

Neurexins are a class of proteins that are "neuronal cell-surface receptors" (Dean et al. 2003). Their role is primarily for "synaptic formation and maintenance"; it is also known to be a part of signaling pathways for synaptic formation, regulating and maintaining steady conditions inside the body, and allowing for synaptic growth (Calahorro and Ruiz-Rubio 2013; Yamagata et al. 2003). These proteins are typically presynaptic terminals to their partnering proteins, known as neuroligins, that act as the postsynaptic terminal. In mammals, there are three types of neurexin genes, *NRXN1*, *NRXN2*, and *NRXN3* (Craig and Kang 2007; Rowen et al. 2002). In mammals, *NRXN1* codes alpha (α), beta (β), and gamma (γ) isoforms; meanwhile, *NRXN2* and *NRXN3* have α and β isoforms (Sterky et al. 2017). Despite these genes having a limited number of isoforms, it is known that neurexins have many more isoforms from alternative splicing than those mentioned (Missler et al. 1998). The α-neurexin resides in the extracellular region and is long, in comparison to β-neurexin, which is also in the extracellular region but is short (Reissner et al. 2013). These genes are commonly associated with several neurodevelopmental and psychiatric disorders, such as autism spectrum disorders (ASD) (Gomez et al. 2021; Kim et al. 2008).

From the thousands of neurexin isoforms, there is much known about the major ones (Missler et al. 1998). "Cloning revealed that mammals contain at least three neurexin genes, each of which has two promoters. The upstream promoters in the neurexin genes generate long transcripts that encode α-neurexins, and the downstream promoters generate shorter transcripts encoding β -neurexins" (Missler et al. 1998; Ushkaryov et al. 1994). The γ isoform of neurexin is a recent discovery; it has a different start site than the α and β isoforms of neurexin (Kurshan et

al. 2018). Overall, it was found that the group of neurexin genes formed a variety of "transcript isoforms" with several genes; each of these would have different promoters (α, β, γ), as mentioned above (Gomez et al. 2021).

C. elegans and their Nervous System

Caenorhabditis elegans (*C. elegans*) are nematodes that have a life cycle of 3.5 days and can grow to be 1.3 millimeters in length (Brenner 1974; Kaletta et al. 2006; White et al. 1986). Most of these nematodes are hermaphrodites, which mean that they can self-fertilize to reproduce. In fact, only one in 10^3 offspring are males who can sexually mate with hermaphrodites. In addition, each of these hermaphrodites will lay eggs when there is plenty of food and are able to have around three hundred offspring. In a laboratory setting, their food supply would be a lawn of *Escherichia coli* on an agar plate (Kaletta et al. 2006).

The normal lifecycle of a *C. elegans* nematode proceeds through four larval stages before reaching adulthood (Byerly et al. 1976). These stages are known as L1, L2, L3, and L4. However, when food is scarce or unavailable, the nematode will go into a stage called "Dauer", which is a stage that happens between L2 and L3 stages (Figure 1). Being in the "Dauer" stage redirects the growth of the worm; it goes into this stage after L1 and comes out of it as an L4.

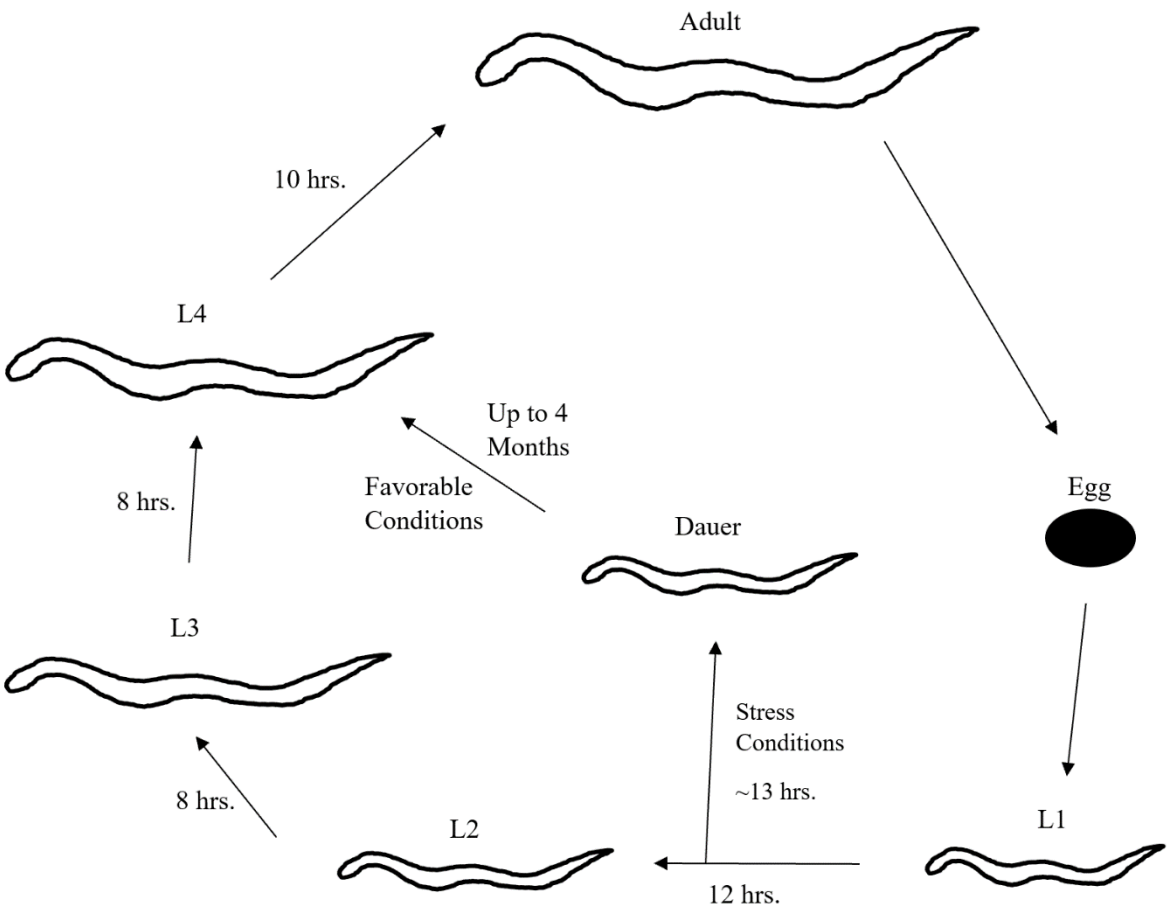


Figure 1. The Life Cycle of *C. elegans* adapted from Byerly et al. 1976. This is each of the developmental stages between the time a hermaphrodite lays eggs to when the nematode grows to be an adult.

C. elegans make good models for complex experiments due to its small, well-defined nervous system. Each hermaphrodite has 132 muscles, 26 end organs (not including muscles), and 302 neurons, which all have distinct functions (Cook et al. 2019; White et al. 1986). Most of these neurons can be located on the head of the nematode, and 126 of these neurons are classified as sensory (Cook et al. 2019). A variety of these neurons (a large portion of the nervous system and "more than 5% of its genes") connect in ways that allow the organism to move and sense its environment (Bargmann 2006). Each connection functions differently and influences the organism. For instance, for both hermaphrodites and male nematodes, the neuron responsible for taste (ASEL) has a strong connection with the class of neurons for smell (AWC) (Cook et al. 2019); both senses can be responsible for the nematode's response to substances, which can include food (Bargmann et al. 1993). In addition, *C. elegans* are the first and only organism to have its connectome completed (Cook et al. 2019). In fact, the connectome for both sexes have been analyzed to understand the basics of all the connections present that can influence its behavior (Cook et al. 2019).

Neurexin in C. elegans

In *C. elegans*, there is only one gene, *nrx*-1, for neurexin expressed throughout almost every stage of life and expressed in almost all its neurons; this gene also has the same isoforms mentioned earlier (Calahorro and Ruiz-Rubio 2013; Craig and Kang 2007). Therefore, creating mutant strains with non-functional *nrx-1* and determining its effects are easier to do with this organism as a model. Therefore, to see what impacts the α and γ isoforms of neurexin had, mutant strains were created. The wildtype (N2 strain) has both a functional α and γ isoforms

(Figure 2). However, the TV13570 strain has a non-functional α and γ isoforms, and the SG1 mutant strain has a non-functional α isoform (Figure 2).

More specifically, the TV13570 mutant strain, phenotypically known as *nrx-1 (wy778)*, is made by deleting a large portion of *nrx-1* that most of the NRX-1 isoforms have in common and by deleting all the short isoform (Maro et al. 2015; Offenburger et al. 2018). Therefore, this strain can be used to demonstrate how a non-functional neurexin gene affects the organism and its behavior, especially when detecting food. Meanwhile, SG1 mutant strain, phenotypically known as *nrx-1(ds1)*, is made with a deletion in the long isoform, which results in a non-functional α isoform as mentioned earlier, and no changes to the short isoform (Philbrook et al. 2018).

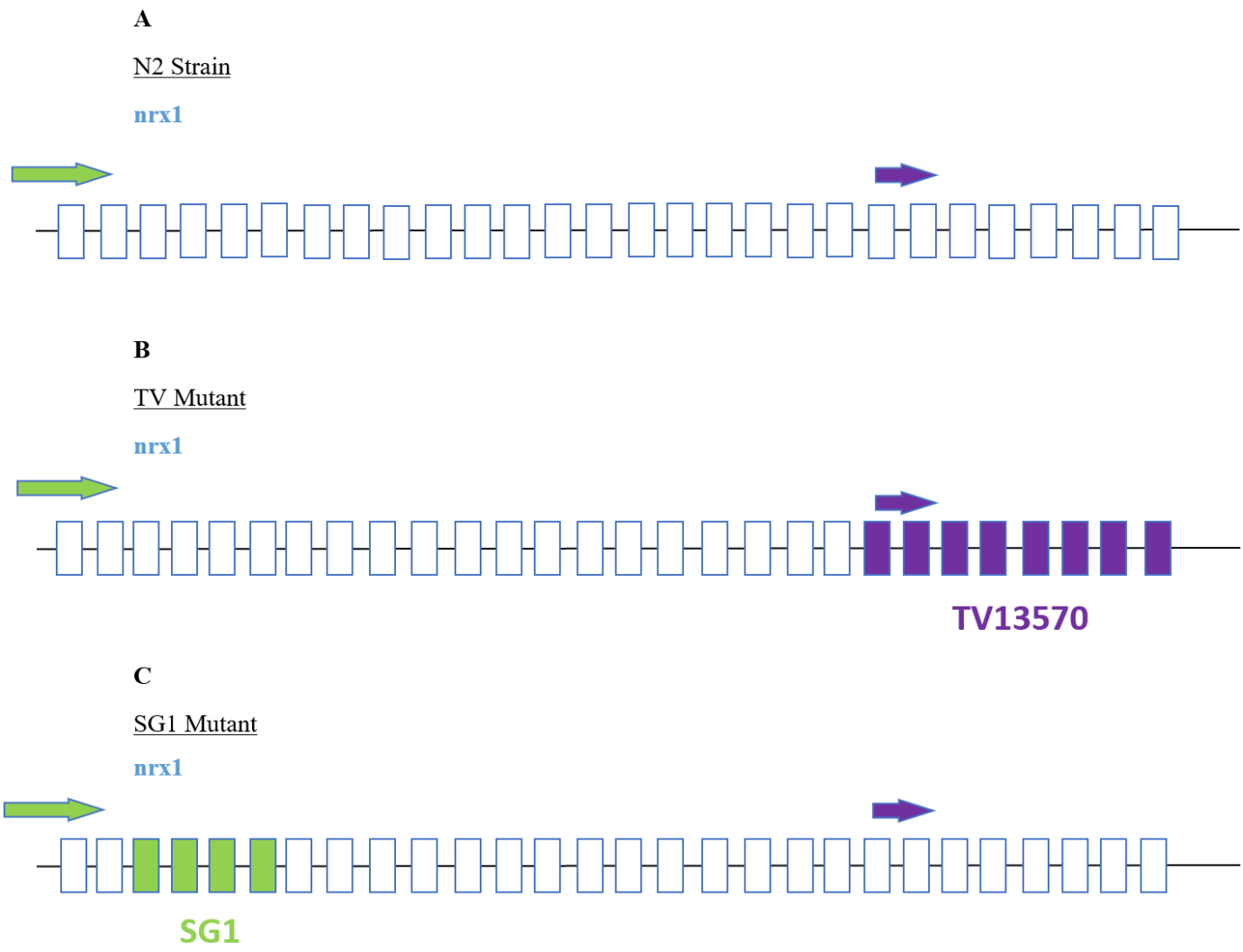


Figure 2. Visual Representation of *nrx1* for each tested strain. A) This represents wildtype, N2 strain. B) This represents mutant strain TV13570. C) This represents mutant strain SG1. Deleted exons are colored in. The green arrow is the start of transcription for the alpha form, while the purple arrow is the start of transcription for the gamma form.

Chemotaxis

Chemotaxis assay is a behavioral assay that determines the strength of an attractant or repellent based on the number of worms that move towards or away from the chemicals from their point of origin (Bargmann et al 1993). To see the strength of these chemicals, ethanol, which is known to be a relatively neutral stimulus for *C. elegans* and is the solvent used to dilute the test chemicals, is included on the agar plates as controls. There is high importance for all the sensory neurons to be functioning for the *C. elegans* to detect the attractants and repellents as the wildtype do. To detect the importance of these neurons, different neurons were killed to see the differences in responses to the same attractants and repellents the wildtype were tested with (Bargmann et al. 1993).

More specifically, *C. elegans* have neurons in their olfactory system to detect food, which are known as AWA and AWC (Zhang et al. 2016). On the other hand, their AWB neuron is used to detect chemicals that could be considered dangerous or pathogenic. The neurons mentioned earlier (AWA, AWB, AWC) play a significant role in attracting and repelling the *C. elegans* in chemotaxis assays. According to Bargmann et al. (1993), the AWA and AWC neurons are needed for chemotaxis assays with attractants. There are some odorants that are detected without having a functional AWA neuron, while others need both functional AWA and AWC neurons to behavior normally. For diacetyl, AWA neuron is responsible for detection of this chemical, while AWC neuron is responsible for the detection of 2,3 – Pentanedione (Bargmann et al. 1993; Wes and Bargmann 2001). Lastly, AWB neuron is used to detect the repellent, 1-Octanol.

The common trend for predicting if a chemical will be an attractant or repellent for wildtype *C. elegans* is based on its size and structure. An alcohol that is composed of 4 to 6 consecutive carbons with a hydroxyl group at the end, would be considered an attractant

(Bargmann et al. 1993). However, if the structure is altered, then the chemical can have a reduced attractive effect. On the other hand, if the alcohol being tested has greater than 5 consecutive carbons, then it will be considered a repellent for the wildtype *C. elegans*. The chemicals utilized in our chemotaxis assays are congruent with this rule since the attractants, although they are not alcohols, are smaller in size with the appropriate number of carbons; meanwhile, the repellent exceeds the number of carbons (Figure 1).

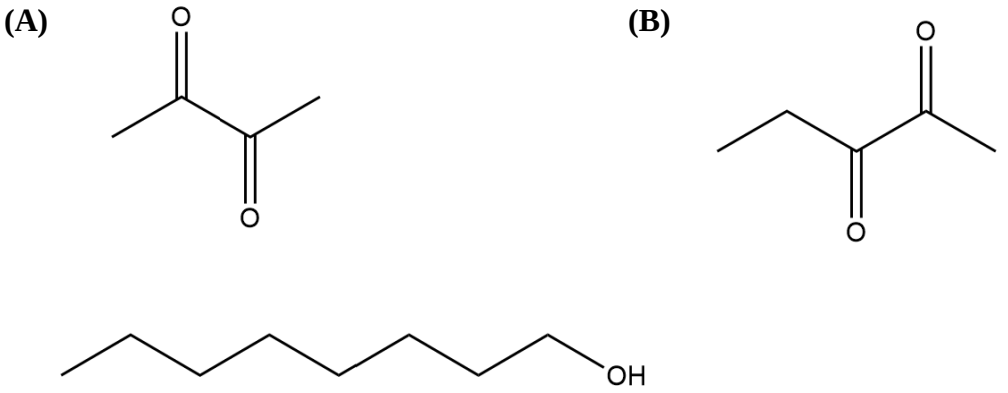


Figure 3. Structures of the Attractants and Repellents Used. (A) This is the structure of diacetyl, the attractants used. (B) 2,3- Pentanedione, the second attractants used. (C) 1-Octanol, the repellent used.

Since *C. elegans* use their olfactory neurons (AWA, AWB, AWC) for the detection of food, this research was designed to investigate how different mutations in different strains vary their behavior in the presence of attractants and repellents. Also, since there are three of these olfactory neurons, an attractant or repellent was tested for all: diacetyl for AWA neuron, 2,3-Pentadione for AWC, and 1-Octanol for AWB neuron. It was hypothesized that since *C. elegans* have only *nrx-1* gene that plays a role in the formation and maintenance of the synapse, a mutation that results in a non-functional *nrx-1* would result in the nematodes to behave differently than their wildtype counterparts.

The goal of this study is to see what aspects of *nrx-1* influence the potential behavioral changes of *C. elegans* in the detection of food. There are several possibilities that can occur. Having a fully intact and functional *nrx-1*, the N2 wildtype strain is expected to be attracted or repelled with the appropriate chemicals used. If the TV13570 strain behaves differently and is defective in comparison to the N2 strain, then it can be concluded that having a non-functional α and/or γ isoforms of *nrx-1* affects natural behaviors. If the behavior of the TV13570 strain is more defective than the SG1 strain, then it can be concluded that the α and γ isoforms are responsible for the behavioral changes, but if the SG1 strain and TV13570 behaviors are defective to a similar degree, it can be concluded that the α isoform is responsible.

Methods

General worm maintenance

The *C. elegans* stock for each strain is maintained on NGM agar plates that are seeded with a lawn of *E. coli* OP50. Once the plate is overcrowded, a chunk of the old plate is transferred to a new seeded NGM plate. The technique to synchronize the worms is batch

bleaching. This involves growing worms at room temperature for a few days. Once these plates are populated, 5 mL of M9 buffer (3.0 g KH_2PO_4, 6.0 g Na_2HPO_4, 0.5 g NaCl, 1.0 g NH_4Cl, and 1.0 L H_2O) and a cell scraper are used to wash off all the worms and eggs. This material is then transferred using a glass pipette into a 15 mL conical tube. The tube is then centrifuged at 4000 rpm for 1 minute to pellet all the worms and eggs in the M9 buffer. The supernatant is removed without disturbing this pellet, and 20% alkaline hypochlorite solution (3.0 mL non-germicidal bleach, 3.75 mL 1 M NaOH, and 8.25 mL distilled H_2O) is added to the pellet. The tubes are then inverted for approximately 5 minutes or until the worms have dissolved, leaving just the eggs behind (Figure 1). Once this occurs, the tubes are returned to the centrifuge to spin for 1 minute at 4000 rpm. The supernatant is removed again without disturbing the pellet. New M9 buffer (5 mL) is added, and this process is repeated for a total of two times before the final 7 mL of M9 is added. The tubes are then left rocking at room temperature overnight. Under these conditions, the eggs will hatch, and the larvae will remain in the L1 stage without food (Figure 4B). After 24 hours, the worms can be stored at 15°C until needed or be plated at 15°C or 25°C for use in assays.

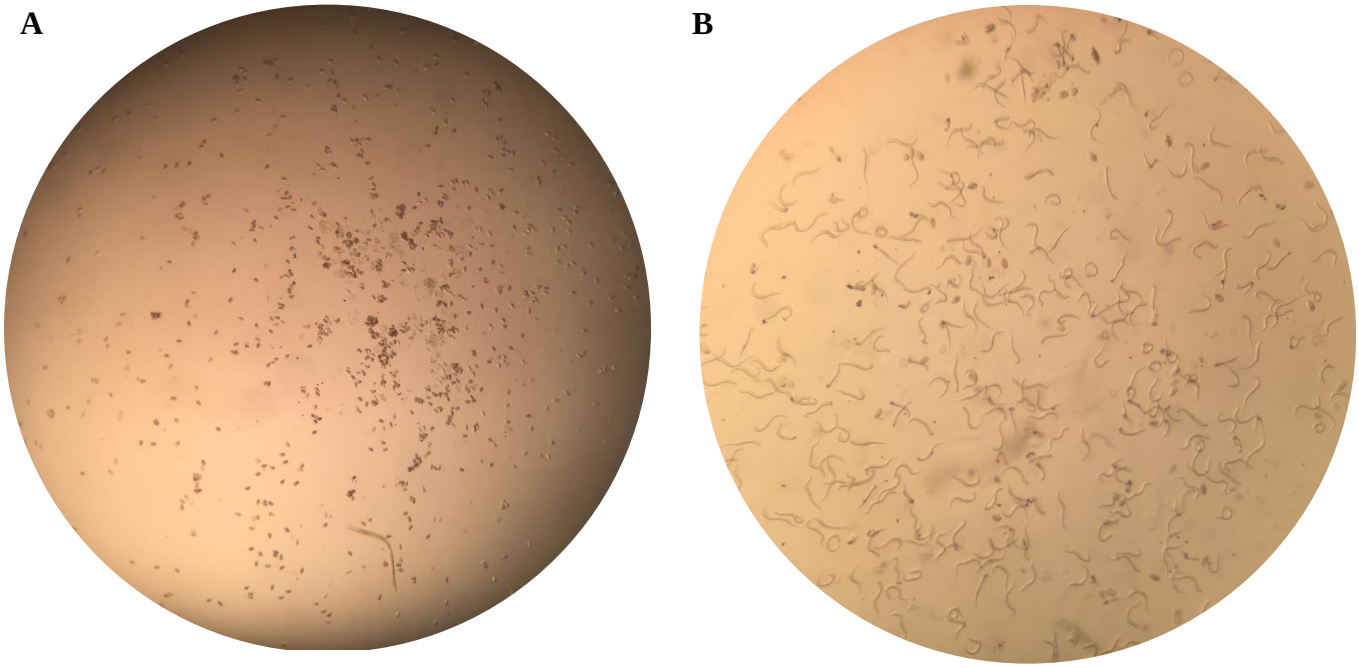


Figure 4. (A) Image of N2 eggs. This is what should be left behind after dissolving the worms with 20% alkaline hypochlorite solution. **(B)** Image of N2 strain L1s after batch bleaching. After 24 hours, the eggs will hatch and all of those will be age synchronized as L1s.

Tested C. elegans strains

All strains used were obtained from the *C. elegans* Genetic Stock Center (CGC) at the University of Minnesota in Minneapolis, MN. For the negative controls in the diacetyl and the 2,3 – Pentanedione assays, the IK105 strain (pkc-1(nj1)) was used, while the JN1715 strain (peIs1715) is used for the 1-Octanol assays. For all of the assays, N2 (C. elegans wild isolate), TV13570 mutant (unc-119(ed3) III; nrx-1(wy778[unc-119(+)])), and SG1 mutant (nrx-1(ds1)) were tested.

Genomic DNA Preparation

Single TV mutant worms were used to prepare the DNA for the PCR, agarose gel electrophoresis, and DNA sequencing. The worms are lysed following the protocols from the InVivo Biosystems Worm Lysis Kit. A single worm is added to 7 µl of water in a PCR tube, then 2 µl of 5X Buffer A and 1 µl of 10X Buffer B are added. The tubes are then placed into the Thermocycler and incubated at 75°C for five minutes and then at 95°C for ten minutes. Once incubation times are completed, 90 µL of water is added to each tube, and the tubes are centrifuged at maximum speed for 30 seconds. The supernatant containing the DNA is stored at -20°C until needed.

PCR and Agarose Gel Electrophoresis

A fragment from the 3' end of the *C. elegans* neurexin gene from strain TV13570 was amplified using the long amp kit from New England Biolabs:

25 µL 2x Solution
4 µL Forward Primer (TV2F) (5µM)
4 µL Reverse Primer (TV2R) (5µM)
5 µL Genomic DNA
12 µL Nuclease Free H_2O

TV2F Forward Primer: 5' CATTCAGCCATTCATGTACG 3'

TV2R Reverse Primer: 5' GTACCCGTTTTGAAGTTGTG 3'

PCR primers were designed using Primer-BLAST tool by NCBI and the appropriate Tm was calculated by NEB Tm Calculator (v 1.12.0).

All these reactions were set for annealing at 58°C and elongation for 5 minutes for a total of 40 cycles. This begins with being at 94°C for two minutes, then 40 cycles of the following:

94°C for 30 seconds, 58°C for 30 seconds, and 65°C for three minutes. Finally, the PCR reactions end with being at 65°C for 10 minutes.

To determine the sizes of these TV13570 mutant PCR products, electrophoresis was set up with a 0.8% agarose gel and 1x TAE. Ten µL of the PCR reaction product for each and 2 µL of 6X gel loading dye were loaded on the gel. As a size marker, 4 µL of 1kb Plus DNA Ladder from New England Biolabs was added to the agarose gel as well. The gel ran at 2.0 amperes and 80 volts for 2 hours. The remaining PCR products were stored in a -20°C freezer and used for DNA sequencing.

Sequencing TV Mutants

Monarch PCR and DNA Clean-Up kit by New England Biolabs and the manufacturer's included protocol was used to purify the remaining PCR samples that were analyzed previously and stored in a -20°C freezer. All these samples were combined into a 1.5 mL microcentrifuge tube after purification; the combined sample's concentration was then checked on the Nanodrop (59.7 ng/µL).

Initially, there were four 10 µL reactions (2 reactions with TVF2 and 2 reactions with TVR2). Two of these reactions (one with each primer) also included double the concentration of DNA. The single concentration of DNA PCR reactions included the following reagents:

1.0 µL BigDye

1.5 µL BigDye Terminator v1.1/3.1 Sequencing Buffer (5X)

1 µL DNA of 59.7 ng/µL

1 µL Primer TVF2 or TVR2 (5µM)

5.5 µL Nuclease-Free H_2O

For the 10 µL reactions that include double the concentration of DNA, the reagents include:

1.0 µL BigDye

1.5 µL BigDye Terminator v1.1/3.1 Sequencing Buffer (5X)

2 µL DNA of 59.7 ng/µL

1 µL Primer TVF2 or TVR2 (5µM)

4.5 µL Nuclease-Free H_2O

Making sure to cover all tubes that contain BigDye in it with aluminum foil because it is very light sensitive. Eight µL of each synthesis reaction is included in the properly labeled 1.5 mL Eppendorf tubes. Each tube also included 1.0 µL of 20 µg/mL glycogen, 2.25 µL 125mM EDTA (pH 8.0), and 27 µL of 95% ethanol. Each tube is vortexed to mix all these reagents. They are then left covered at room temperature from 15 to 20 minutes and centrifuged at 18°C at full speed for 20 minutes. This forms a small pellet in each tube, so without disturbing the pellet, the supernatant needs to be aspirated. Two hundred and fifty microliters of 70% ethanol is added to each tube and vortexed for 10 to 20 seconds before putting it back in the centrifuge for another 10 minutes. The supernatant is again removed; this is repeated one more time by adding another 250 µL 70% ethanol, centrifuging for 10 minutes, and removing the supernatant. Once all the supernatant is removed without disturbing the pellet in each tube, they are left open to dry in the Speed Vac, which is covered in aluminum foil, for 10 to 15 minutes. Afterwards, 20 µL formamide is added to each and vortexed for around 30 seconds to thoroughly mix the mixtures before being transferred into the sequencing tubes. These reactions were placed in Applied BioSystems SeqStudio Sequencing machine in the following order:

A1 – TV1F
B1 – TV1R
C1 – TV2F (2x DNA)

D1 – TV2R (2x DNA)

Based on the results of the first sequencing reactions, 6 additional sequencing primers were designed based on the sequence of the *C. briggsae* unc-119 gene (Wormbase), TVS1, TVS2, TVS3, TVS4, TVS5, and TVS6, with the use of the sequencing primer design tool from Eurofinsgenomics.

The sequencing was completed again with the new primers and redoing the others. Sequencing was conducted again with the following reagents:

1.0 µL BigDye

1.5 µL BigDye Terminator v1.1/3.1 Sequencing Buffer (5X)

2 µL DNA of 59.7 ng/µL

1 µL Primer TVS1, TVS2, TVS3, TVS4, TVS5, TVS6, TVR2, TVR2, or TVF3 (5µM)

4.5 µL Nuclease-Free H_2O

Ethanol precipitation, which is mentioned previously, was conducted again with these samples, and they were finally loaded onto the Applied BioSystems SeqStudio Sequencing machine (A1, B1, C1, D1, E1, F1, G1, and H1).

Chemotaxis Assay

Synchronized larvae from the 15°C incubator is plated onto new seeded NGM agar plates. They are grown at 15°C for about 4 days or at 25°C for 2 days, which allow all the worms to develop into L4s before performing an assay. The buffer that is used during the duration of these chemotaxis assays (washing worms off plates, washing the worms themselves, the buffer used to transfer the worms, etc.) is S Buffer. It is composed of 129 mL 0.05 M K_2HPO_4, 871 ml 0.05 M KH_2PO_4, and 5.85 g NaCl.

Before the L4 worms are prepared, the treatment and control chemicals are diluted. The diacetyl solution used in the chemotaxis assays is diluted to a 1% solution. Once this solution was mixed in the 1.5 mL Eppendorf tube, 20 µL of it was transferred into a new Eppendorf tube along with 20 µL of 50µM Sodium Azide.

The 2,3- Pentanedione solution for these assays is diluted to a 5% solution in a 1.5 mL Eppendorf tube. In a new Eppendorf tube, 20 µL of this solution and 20 µL of Sodium Azide are mixed, which will be the final treatment solution for the 2,3-Pentanedione chemotaxis assays.

Finally, for the 1-Octanol chemotaxis assays, the treatment solution is diluted to 0.03%. Twenty microliters of this solution and 20 µL of Sodium Azide are combined in a new Eppendorf tube, which is now the final treatment solution for these assays.

Regardless of what chemotaxis is being conducted, the control treatment stays consistent. It is a solution that includes 20 µL of 95% ethanol and 20 µL of Sodium Azide. This is mixed, and 2 µL of this solution is pipetted on each of the control quadrants when the assays are being prepared. Depending on the type of chemotaxis assay, 2 µL of the corresponding treatment solution is added to the treatment quadrants as well.

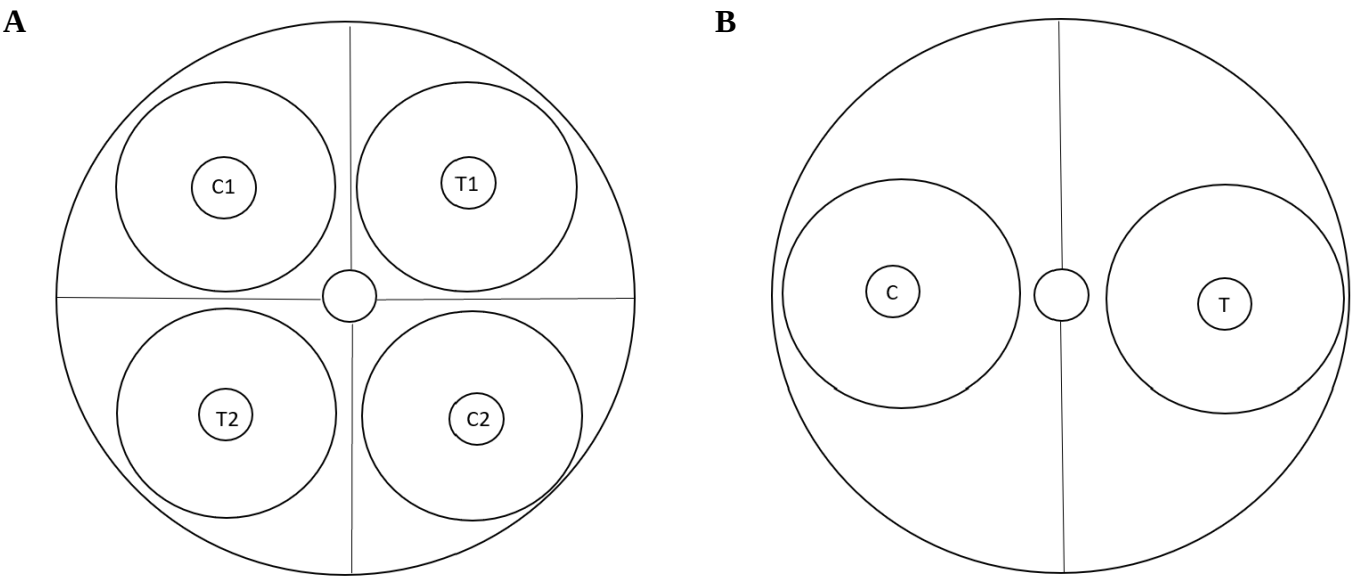


Figure 5. (A) Chemotaxis Assay Set-Up for diacetyl and 2,3 – Pentanedione. The four quadrants set up requires 2 µL of the corresponding chemical for each quadrant. Also, the number of worms in each large circle in each quadrant are counted separately, while the worms outside these large circles are excluded from analysis. **(B)** Chemotaxis Assay Set-Up for 1-Octanol. The two halves set up requires 2 µL of the corresponding chemical for each half. Also, the number of worms in each large circle in each half are counted separately.

For the diacetyl and the 2,3- Pentanedione assays, the 10 cm Petri dishes are divided into four quadrants with circled spots for the treatment chemical and the control (each had two quadrants) and the worms in the middle (Figure 5A). On the other hand, 1-Octanol assays are divided into halves (Figure 5B). Regardless of the assay and set up of the plate, the L4 worms are washed with S Buffer off the seeded plates they were allowed to grow on for approximately four

days at 15 °C or about two days at 25 °C. The S Buffer from the seeded Petri plates are pipetted using a glass Pasteur pipet into a 1.5 mL Eppendorf tube. The worms are given time to settle and pellet at the bottom of the tube. The supernatant is removed and more S Buffer is added; this is repeated for a total of three times. The final supernatant is removed before the worms can be pipetted on the assay plate.

After the worms in the Eppendorf tube are washed three times, 2 µL of the treatment and control chemicals are added to their corresponding quadrants or half (Figure 5). Afterwards, the worms are pipetted using a 1.0 mL syringe and 50 µL disposable glass micropipettes from the Eppendorf tube where they pelleted. A small amount is pipetted onto the middle circle on the assay plate, and with a microscope, the number of worms is checked. Around 50 to 100 worms are ideal; however, the numbers do fluctuate. Nonetheless, a picture of the worms is taken to count the total number of worms plated. Once the preparation of all the assays is completed, they are set to run for 60 to 90 minutes. The completed assays are then placed in the 4°C refrigerator for at least five minutes to prevent any more movement as the worms are being counted in each quadrant or half.

Chemotaxis Index

After the worms in each quadrant or half is counted and recorded, the chemotaxis index is calculated for each assay plate to see how attracted or repulsed that strain of worms were with the tested chemical (Equation 1). This index ranged from -1 (100% or complete repulsion) to 1 (100% complete attraction).

$$CI = \frac{(Test - Control)}{(Test + Control)}$$

Equation 1. The Chemotaxis Index Equation. This is the equation that was used to calculate the degree of attraction or degree of repulsion.

The statistical analysis done with this data was a Kruskal-Wallis test for an overall comparison. Then, a Whitney-U test was conducted to find differences between each group.

Results

Molecular Composition of Mutant Strains

The N2 strain is used as a control to determine how wildtype *C. elegans* would naturally behave. The TV13570 mutant has non-functional α and γ neurexin gene, while the SG1 mutant has a non-functional alpha neurexin gene, which are being tested to see if they impact their behavior with the chosen chemicals. The negative controls are specific for each assay. For instance, the JN1715 strain (for the repellent assays) has a caspase gene connected to the *str-1* gene (G-protein coupled olfactory receptor), which specifically destroys the AWB neurons. On the other hand, the IK105 strain (for the attractant assays) is used for the diacetyl and 2,3 –

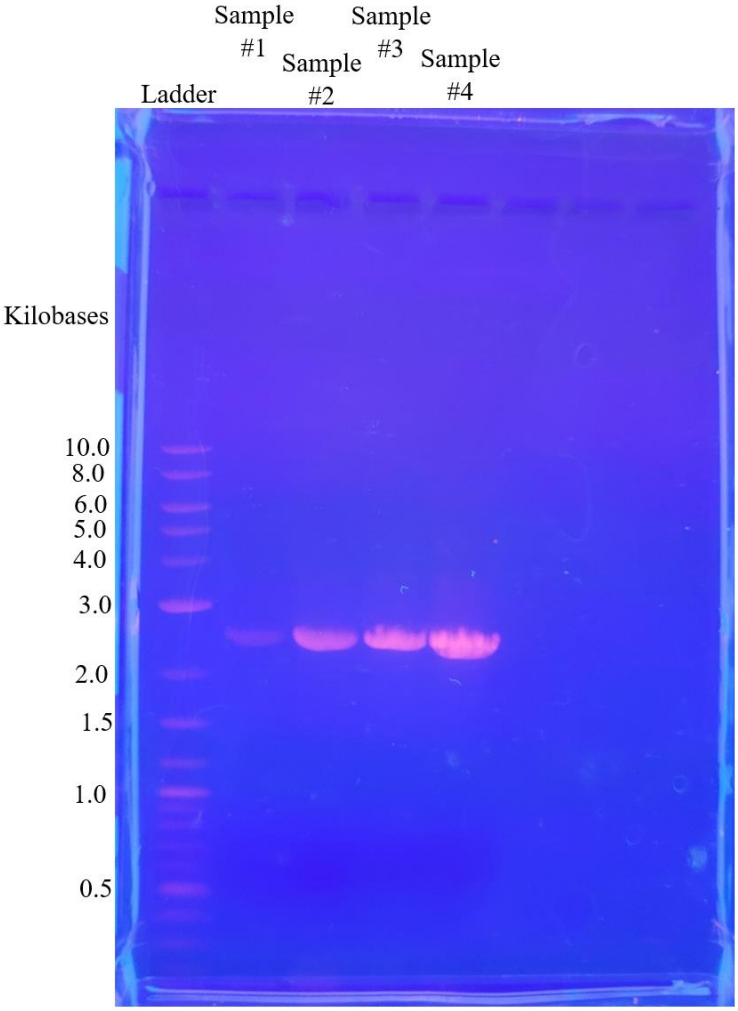
Pentanedione assays because there is a mutation in the *pkc-1* gene that takes part in a signal transduction pathway, which regulates a variety of behaviors including thermosensation and chemosensation.

During chemotaxis, the behavior of the mutant strains was compared with the behavior of the N2 wildtype strain. The SG1 mutant strain was sequenced in the past in the lab, and the deletion that is illustrated on Figure 2 was confirmed (DeFronzo 2020). To confirm the deletion in strain TV13570, genomic DNA was isolated from single mutant worms and a region of the 3' end of the *nrx-1* gene was amplified using PCR primers flanking the deletion site based on the sequence published in Wormbase (Figure 6A). DNA from four individual worms were amplified and an aliquot of the reaction run on an agarose gel. Based on the Wormbase entry, a product of 407 bp was expected. All four PCR samples showed a band running at ~ 2.5 kb (Figure 6B). Analysis of the original description of TV13570 in Maro et al. (2015) indicated that the 12 kb region of the *nrx-1* gene was replaced by a unc-119 rescue construct. DNA sequencing of the amplified PCR products show a 2.1 kb insert of the *C. briggae* unc-119 gene (Figure 6C). The location of the insert is consistent with the location of the ttTi26330 transposon insertion site described in Maro et al. (2015) (Figure 6D).

(A) Sequence of NRX-1 Wy778 allele (TV13570) from Wormbase

CATTCAGCCATTCATGTACGCCCTTTCGTTTCTTAATTTTGAAAACTTACAT
GTATAGTGACCATCTTCAAATAAAACTCTACACAATAGCTGCTGAAACTTTCC
AAAAGCGCAAAAGCCTCGACCTATTTTTAAAACCTCGACCTATTTTTCTAAAT
TTTTCATCAGACTTCTCTTGGTCTGTAGAAATTTTGAATTTTTGATTTTTTTTTT
TGACAAAAATTTCGAGAAAAAGTTGAATTTCATTTCCGTAAGTTAATGATATT
TTGTGTCAATTTTCAGTTTTTTTTCCTCTCATTTTTCTTCTTTCTCTATCTCAGT
C-
-12KB DELETION-
TCCATTTCTTCAATCAAAACTCAATACAATGATGATTAAAAAATTCACTTTTG
TCTGCAAATTGC**CACAAC TTCAAAACGGGTAC**

(B) Gel of TV13570 PCR product



(C) Sequence of 2.5 kb TV13570 PCR product

NNNNNNNNNNNNNNNNTACGCCCTTTCGTTTCTTAATTTTGAAAACTTACATGTA
TAGTGACCATCTTCAAATAAAACTCTACACAATAGCTGCTGAAACTTTCCAAAAG
CGCAAAAGCCTCGACCTATTTTTAAAACCTCGACCTATTTTTCTAAATTTTTCATC
AGACTTCTCTTGGTCTGTAGAAATTTTGAATTTTTGATTTTTTTTTTTTGACAAAAA
TTTCGAGAAAAAGTTGAATTTCATTTCCGTAAGTTAATGATATTTTGTGTCAATTT
TCAGTTTTTTTTCCTCTCATTTTTCTTCTTTCTCTATCTCAGTCTCCTTACTAACTTA
TTTCCCACCCACTAC- **2.1KB INSERTION OF *C. BRIGGAE* UNC-119..**TAA
TCCATTTCTTCAATCAAAACTCAATACAATGATGATTAAAAAATTCACTTTTGTCT
GCAAATTGCCACAACTTCAAAANNNNNNN

(D) Location of ttTi26330 Transposon Insertion Site from Wormbase

CATTCAGCCATTCATGTACGCCCTTTCGTTTCTTAATTTTGAAAACTTACATGTAT
AGTGACCATCTTCAAATAAAACTCTACACAATAGCTGCTGAAACTTTCCAAAAGC
GCAAAAGCCTCGACCTATTTTTAAAACCTCGACCTATTTTTCTAAATTTTTCATCA
GACTTCTCTTGGTCTGTAGAAATTTTGAATTTTTGATTTTTTTTTTTTGACAAAAAT
TTCGAGAAAAAGTTGAATTTCATTTCCGTAAGTTAATGATATTTTGTGTCAATTTT
CAGTTTTTTTTCCTCTCATTTTTCTTCTTTCTCTATCTCAGTCTCCTTACTAACTTAT
TTCCCACCCACTACT-

ACTTTTCAAAAGCTTTTTTCAACACATAACGTGGCCGTACCTCAGTTTTTCGAAA
ATAGTATGCAATCAGGTAGAGAATTCGAGAAAAGAGACTCTCCTTCTTGGTTGCT
TCGTGTCGGTCTTCTTCTATATATCTTCCTTT

Figure 6. DNA Sequencing of TV13570 Mutant. (A) Sequence is from Wormbase for the TV13570 mutant; the bold underlined sequences are the primers TVF2 and TVR2. (B) Agarose gel electrophoresis of genomic DNA PCR from four individual worms. (C) Sequence of the 2.5 kb PCR product. (D) Sequence of the ttTi26330 transposon insertion site from Wormbase.

Chemotaxis Data

Figure 7 and Table 2 shows the number of worms in each quadrant/half and was used to calculate the chemotaxis index (Equation 1; Table 2; Figure 7). Based on these, the degree of attraction and repulsion for each strain is determined. For instance, the wildtype N2 strain had a great tendency to have near perfect attraction for diacetyl (Table 2). Meanwhile, the TV13570 mutant strain had a high degree of attraction for the chemotaxis assays with diacetyl, but not to the same extent as the wildtype N2 strains. The SG1 strain also demonstrated a degree of attraction to both the attractants.

For the assays conducted with 2,3 – Pentanedione, the wildtype N2 strain did not have as strong of a degree of attraction as the strain did for the diacetyl chemotaxis assays (Table 2). The same is true for the TV13570 mutant strain since they still had a degree of attraction but not to the extent that N2 strain has for 2,3-Pentanedione and not the extent of TV13570 mutant strain with diacetyl.

For the repulsion chemotaxis assays, wildtype N2 strain show repulsion based on the chemotaxis index calculated; however, the degree of repulsion is not consistent throughout (Table 2). They tend to vary, which can be said for both the TV13570 strain and the SG1 strain as well. Typically, all the strains showed repulsion, but the extent to which the worms were repulsed varied between each strain and between strains.

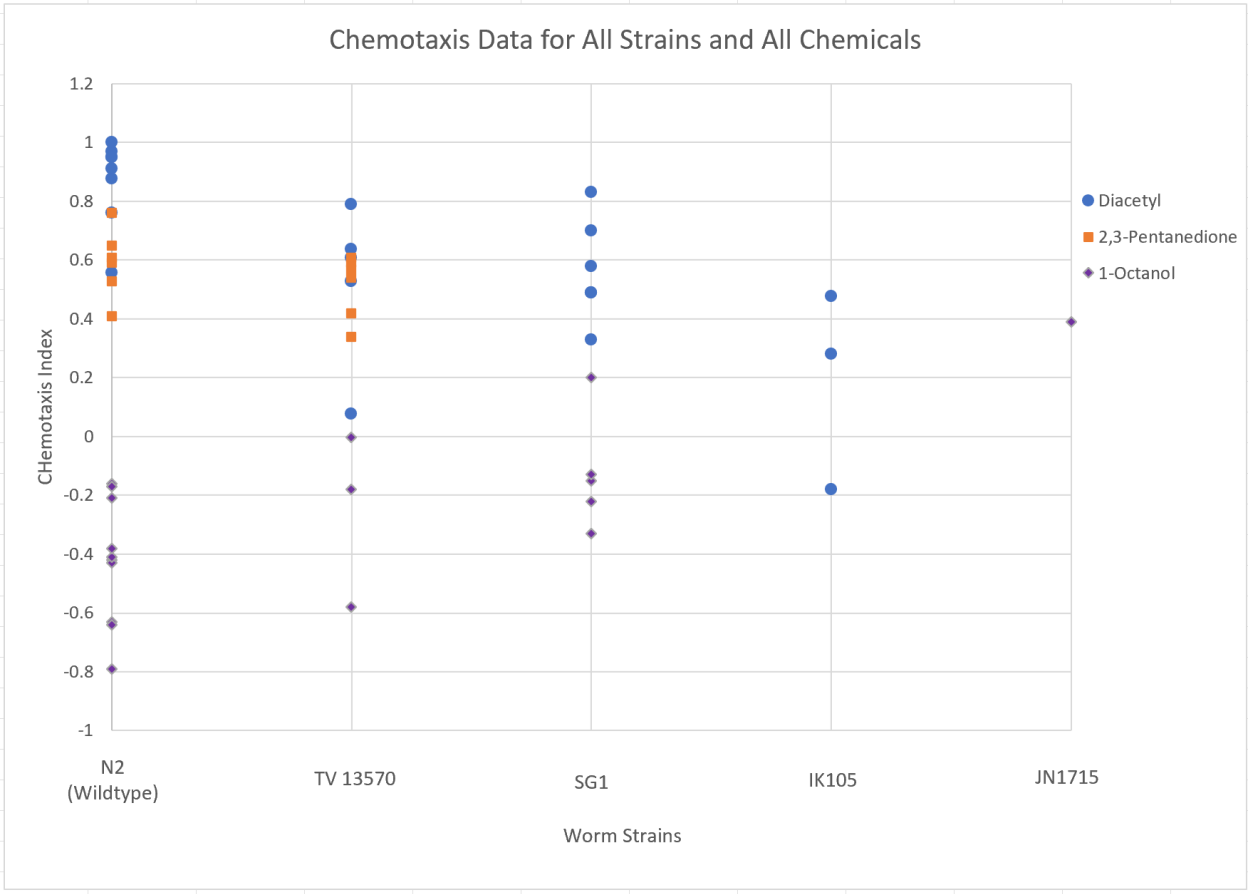


Figure 7. Raw Data for All the Worm Strains and Chemicals. This is a summarizing graph of all the chemotaxis raw data for each strain and each attractant/ repellent used.

Statistical Analysis

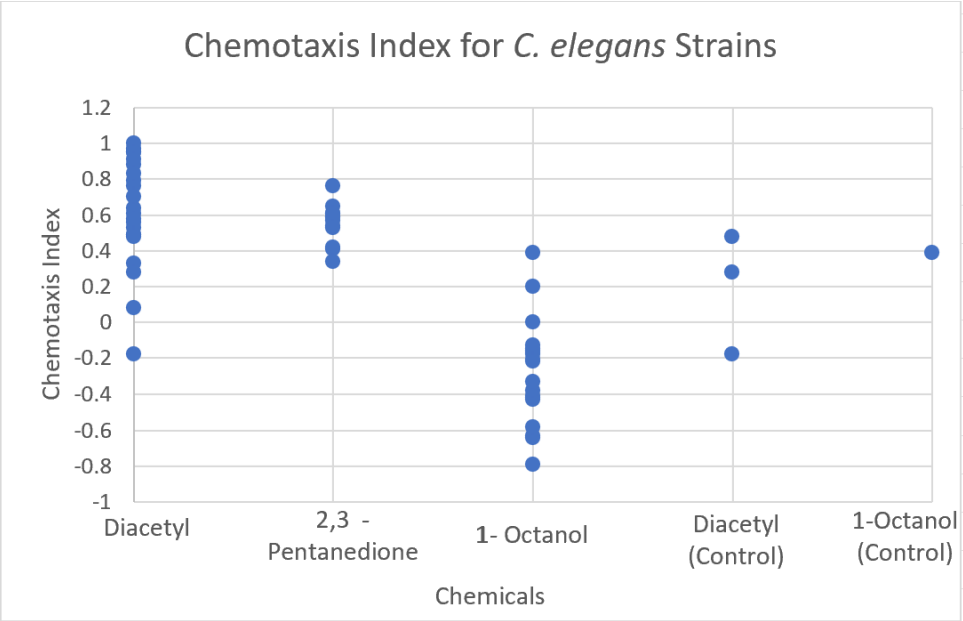


Figure 8. Chemotaxis Indices Graphed Based on Chemical. This is graphing all the chemotaxis indices calculated for each plate based on the attractant/ repellent used.

To get an overall comparison, a Kruskal-Wallis test was conducted, and it was found that when comparing all the plates, the p-value is < 0.01. To compare between each attractant and repellent, the Whitney-U test was conducted. This showed that between diacetyl and 2,3-Pentanedione, the p-value was 0.586. Meanwhile, between diacetyl and 1-Octanol and between 2,3-Pentanedione and 1-Octanol, the p values were < 0.001.

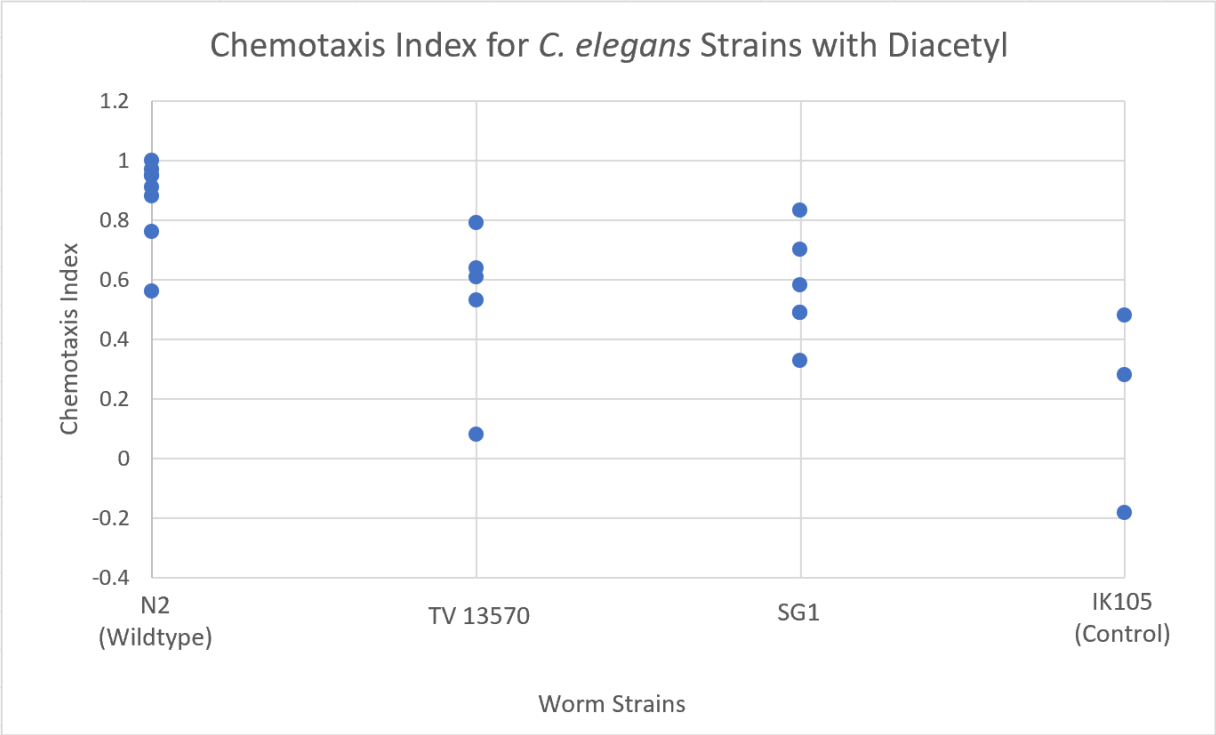


Figure 9. Chemotaxis Indices Between Different Worm Strains for Diacetyl. This is graphing the chemotaxis indices that were calculated for each plate and strain for the assays conducted with diacetyl.

Table 1. Comparison between *C. elegans* Strains with Diacetyl

Sample 1- Sample 2	**Statistical Significance**
IK105 – SG1	0.168
IK105 – TV13570	0.164
IK105 – N2	< 0.001
SG1 – TV13570	0.946
SG1 – N2	0.020
TV13570 – N2	0.033

A Kruskal-Wallis test was conducted to compare the chemotaxis indices between worms strains tested with diacetyl since it appeared to have the largest difference in the raw data (Figure 9 and Table 1). It was found that the difference in behavior between the IK105 strain and N2 wildtype strain was statistically significant ($p < 0.001$), while the IK105 strain was not statistically significant with any of the other worm strains (Table 1). Also, both TV13570 mutant strain and SG1 mutant strain had a statistically significant difference in behavior when compared to N2 wildtype ($p = 0.033$ and $p = 0.020$, respectively). However, there was no statistical significance between the TV13570 mutant strain and the SG1 mutant strain ($p = 0.946$).

Discussion

Sequencing

Chemotaxis was used to compare the behavior of wildtype N2 Strain *C. elegans* with the TV13570 mutant to see if the mutation in *nrx-1* influenced the degree of attraction or repulsion. If this was true, the study went further to investigate if the α or γ region of the gene was the reason for the difference using the mutant strain SG1. Therefore, the sequence for TV13570 mutant strain was confirmed by sequencing genomic DNA (Figure 6). This data is consistent with the description of this mutant strain in Maro et al. (2015), but it did not match the sequence provided on Wormbase. This suggests that there is an error for Wormbase, as the sequence from this study matched the insertion site of the transposable element (ttTi26330) mentioned in the Maro et al. (2015). This confirmed that the mutant strain being used in the chemotaxis assays did have the correct mutations.

Overall Chemotaxis Comparison

The chemotaxis index was calculated after each assay based on the number of worms that moved into each quadrant or half (Kauffan et al. 2011; Equation 1; Table 1; Figure 7). This determined the degree of attraction and repulsion for each of the chemicals; it was a range from -1 (complete repulsion) to +1 (complete attraction). Based on the Kruskal–Wallis test, there was statistical significance when comparing all the chemicals together with all of the strains that were tested (Figure 8). This suggests that there is a notable difference between all the groups and that the behavior did change overall. This was also true when comparing each of the groups with each other with the use of the Whitney-U test. For instance, the difference the *C. elegans* attraction to diacetyl or 2,3 – Pentanedione and repulsion to 1-Octanol is significant, and it indicates that there is a behavioral difference between the attractants and the repellent used. However, there was no statistical significance between diacetyl and 2,3-Pentadione. This conclusion is congruent with what Troemel et al. 1997 mentions; although these attractants are primarily detected by different neurons, both have similar structures, which can cause the worms to respond similarly (Troemel et al. 1997; Figure 3).

Chemotaxis Assays with Attractants

Diacetyl is the first chemical that was tested and confirmed to be an attractant both with the results shown in Troemel et al. 1997 and our results. These show that the wildtype strain has a close to +1 calculated chemotaxis index. Also, based on the results of the chemotaxis assays, it can also be concluded that the diacetyl was a stronger attractant than 2, 3 – Pentanedione for the wildtype N2 strain due to having a higher chemotaxis index (Figure 7; Supplemental Table 2).

Also, the TV13570 strain was found to have a statistically significant difference between itself and the N2 strain when tested with the diacetyl (Figure 9; Table 1).

Due to this change in behavior, the SG1 mutant strain was tested for the attractants to see if its behavior differed from the TV13570 strain to determine what region of *nrx-1* was responsible for the change. From the chemotaxis assays conducted with diacetyl, there was a statistically significant difference between SG1 mutant strain and the N2 strain, which confirms that a mutation is *nrx-1* affects their behavior. However, there was no statistical significance between the behavior exhibited by the SG1 mutant strain and TV13570 mutant strain. Therefore, it can be concluded that the γ region of *nrx-1* does not influence the change the behavior since the only difference between those mutant strains is that TV13570 does not have a functional γ isoform. For 2, 3 – Pentanedione chemotaxis assays, SG1 was not tested; for this reason, no conclusion can be made regarding what region of *nrx-1* is responsible for the behavioral changes for 2,3 – Pentanedione chemotaxis assays.

Chemotaxis Assays with Repellent

Based on the chemotaxis assays for 1-Octanol, it could be concluded that 1-Octanol was an effective repellent for all the tested *C. elegans* strains (Bargmann et al. 1993; Lee et al. 2010; Supplemental Table 2). The wildtype N2 strain appears to have a moderate to strong repulsion that is near -1 for its chemotaxis index (Neto et al. 2016; Figure 7; Supplemental Figure 2). However, the wildtype N2 strain behavior did not appear to be consistent throughout all or majority of the chemotaxis assays. Meanwhile, the TV13570 mutant strain has a repulsion to a lesser degree in comparison to the wildtype N2 strain. Although the data is insufficient, it can be concluded that the repellent has a statistically significant difference overall (Figure 8). However,

due to the lack of consistency, no other definitive conclusions can be drawn other than that the TV13570 mutant strain had a behavioral difference, which can potentially be because of the *nrx-1* mutation. In fact, Hunter et al. demonstrates no response to 1-octanol with changes to *nlg-1* (2010), and with little to no research done to investigate the response to 1-octanol with *nrx-1* mutations, it can potentially be determined that *nrx-1* and *nlg-1* have similar responses.

Due to the changes in behavior, the SG1 mutant strain was tested with 1-Octanol to possible identify what region of *nrx-1* is responsible for the behavioral change. This strain showed a light repulsion to 1-Octanol, which is does not seem to be to the extent of TV13570 mutant strain. Therefore, this data could suggest that the γ region of *nrx-1* could be responsible for the change in behavior of the repellent.

Temperature and Time for Chemotaxis Assays

Throughout the chemotaxis assays, the temperatures at which the strain was grown in was reported as well (Supplemental Table 2). This was to create a schedule that allowed for L4s to have been generated on days chemotaxis assays were conducted. I also investigated to see if there would be a potential pattern based on the temperature the worms were grown at; however, the chemotaxis indices do not indicate that the temperature was a determining factor in how the strain behaved.

The times that each chemotaxis assay was running for is also recorded (Table 2). This is because of the small number of nematodes moving from the center of the plate where they were initially placed. Increasing the amount of time, the assay ran allowed the worms to have more time to move into the quadrants. Although a few of the assays ran for 90 minutes, most of them ran for 60 minutes to ensure consistency.

Future Directions

In the future, this study should be extended and studied more deeply than I was able to do with the limited time I had. I would start with repeating the chemotaxis assays completed in Table 2, but I would do a consistent number of assays for each *C. elegans* strain, which would include the negative controls since those were not done for all and were not done to the full extent needed. This would give a clearer idea of how *nrx-1* is responsible for the behavioral changes in the TV13570 mutant strain and the SG1 mutant strain. We could also use the new mutant, like CX3260, which expresses green fluorescent protein (GFP) in the AWA neurons. If this strain mates with the TV13570 mutant strain, then this could detect abnormalities to the AWA neurons and provide insight into a change in behavior with the first attractant, diacetyl.

In addition to building on top of this study, I would also suggest seeing if the TV13570 mutant worms moved at a slower pace in comparison to the wildtype N2 worms. This would eliminate a potential confounding variable or shed light on the amount of time the chemotaxis assays should be run. This could also be done with the other strains used in the chemotaxis assays as well to compare if the speed at which the worms are moving for each strain differs significantly. This could also contribute to the conclusions that could be made about the behavioral differences between the *C. elegans* strains. A similar assay is conducted with the lab's current project on Parkinson's Disease to see how the degradation of the neurons affect the worm's ability to move (Fairweather 2022).

The same strains that are used in the chemotaxis assays and the movement assays could also be tested with thermotaxis assays to see if the behavioral changes are also applicable to temperature. The strains would be grown to L1s at different temperatures and would see if they are more inclined to move towards the temperature in which they were grown in. This assay can

also be used to see if the chemicals or temperature is a stronger force in influencing the behavior of *C. elegans* (DeFronzo 2020).

Lastly, a future project could also see how these same assays compare to other *C. elegans* strains, such as those that are *nlg-1* mutants like the VC228 strain or even a double *nlg-1*/ *nrx-1* mutant. With having both sets of data for *nrx-1* and *nlg-1*, it can be analyzed to see which had a greater effect on behavior towards attractants, repellents, and temperature. This would also allow for a larger understanding of what parts of this protein interaction influences *C. elegans* behaviors. More specifically, a new strain can include creating a strain that makes only the α isoform and not the γ isoform with the use of CRISPR/ Cas9 (Patel 2021). This strain could give a more definitive conclusion on what isoform of *nrx-1* is responsible for the behavioral defects in the TV13570 strain, the SG1 strain, and potentially this new strain when searching for food. Gathering more information about neurexin allows us to understand more about the micro perspective of the proteins (neurexin and neuroligin) that are said to be associated with autism spectrum disorder (ASD).

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Supplemental Figures

Table 2. Chemotaxis Data for the Attractants and the Repellent

(A) Diacetyl

Types of Worms	Temperature	Time	Plate	Quadrant	Number of Worms	Chemotaxis Index
N2	15°C	60 minutes	Plate 1	C1	1	0.947368421
				C2	1	
				T1	56	
				T2	18	
			Plate 2	C1	1	0.969230769
				C2	0	
				T1	41	
				T2	23	
			Plate 3	C1	0	1
				C2	0	
				T1	25	
				T2	10	
N2	25°C	60 minutes	Plate 1	C1	1	0.949685535
				C2	3	
				T1	82	
				T2	73	
			Plate 2	C1	3	0.875647668
				C2	9	
				T1	93	
				T2	88	
			Plate 3	C1	2	0.905325444
				C2	6	
				T1	85	
				T2	76	
			Plate 4	C1	2	0.76
				C2	4	
				T1	36	
				T2	8	
			Plate 5	C1	7	0.5625
				C2	0	
				T1	11	
				T2	14	
TV	15°C	90 minutes	Plate 1	C1	10	0.606557377
				C2	2	
				T1	20	
				T2	29	
			Plate 2	C1	3	0.083333333
				C2	8	
				T1	9	
				T2	4	
TV	25°C	60 minutes	Plate 1	C1	1	0.636363636
				C2	7	
				T1	23	

				T2	13	
			Plate 2	C1	4	0.531914894
				C2	7	
				T1	16	
				T2	20	
			Plate 3	C1	2	0.785714286
				C2	1	
				T1	13	
				T2	12	
SG1	25°C	60 minutes	Plate 1	C1	14	0.582608696
				C2	10	
				T1	52	
				T2	39	
			Plate 2	C1	10	0.487804878
				C2	11	
				T1	29	
				T2	32	
			Plate 3	C1	12	0.325
				C2	15	
				T1	21	
				T2	32	
SG1	15°C	60 minutes	Plate 1	C1	2	0.830508475
				C2	3	
				T1	25	
				T2	29	
			Plate 2	C1	7	0.492537313
				C2	10	
				T1	23	
				T2	27	
			Plate 3	C1	2	0.7
				C2	4	
				T1	13	
				T2	21	
IK	25°C	60 minutes	Plate 1	C1	1	0.476190476
				C2	10	
				T1	9	
				T2	22	
			Plate 2	C1	74	-0.178294574
				C2	2	
				T1	30	
				T2	23	
			Plate 3	C1	2	0.275862069
				C2	19	
				T1	23	
				T2	14	

(B) 2,3 – Pentanedione

Types of Worms	Temperature	Time	Plate	Quadrant	Number of Worms	Chemotaxis Index
N2	15°C	60 minutes	Plate 1	C1	9	0.761363636
				C2	12	
				T1	84	
				T2	71	
			Plate 2	C1	4	0.649484536
				C2	13	
				T1	42	
				T2	38	
			Plate 3	C1	23	0.405128205
				C2	35	
				T1	61	
				T2	76	
N2	25°C	60 minutes	Plate 1	C1	13	0.533980583
				C2	11	
				T1	42	
				T2	37	
			Plate 2	C1	8	0.611510791
				C2	19	
				T1	47	
				T2	65	
			Plate 3	C1	12	0.590909091
				C2	6	
				T1	38	
				T2	32	
TV	15°C	60 minutes	Plate 1	C1	9	0.416666667
				C2	5	
				T1	17	
				T2	17	
			Plate 2	C1	3	0.594202899
				C2	11	
				T1	31	
				T2	24	
			Plate 3	C1	5	0.34375
				C2	16	
				T1	33	
				T2	10	
TV	25°C	60 minutes	Plate 1	C1	15	0.565217391
				C2	10	
				T1	48	
				T2	42	
			Plate 2	C1	9	0.542857143
				C2	15	
				T1	60	
				T2	21	
			Plate 3	C1	5	0.608247423
				C2	14	
				T1	41	
				T2	37	

(C) 1- Octanol

Types of Worms	Temperature	Time	Plate	Quadrant	Number of Worms	Chemotaxis Index
N2	15°C	60 minutes	Plate 1	C1	17	-0.416666667
				T1	7	
			Plate 2	C1	31	-0.631578947
				T1	7	
			Plate 3	C1	17	-0.789473684
				T1	2	
			Plate 4	C1	30	-0.428571429
				T1	12	
N2	25°C	90 minutes	Plate 1	C1	79	-0.410714286
				T1	33	
			Plate 2	C1	78	-0.155555556
				T1	57	
			Plate 3	C1	38	-0.206349206
				T1	25	

			Plate 4	C1	23	-0.642857143
				T1	5	
N2	15°C	90 minutes	Plate 1	C1	17	-0.172413793
				T1	12	
			Plate 2	C1	20	-0.379310345
				T1	9	
TV	25°C	90 minutes	Plate 1	C1	11	0
				T1	11	
			Plate 2	C1	45	-0.578947368
				T1	12	
			Plate 3	C1	30	-0.176470588
				T1	21	
SG1	25°C	90 minutes	Plate 1	C1	20	-0.333333333
				T1	10	
			Plate 2	C1	10	0.2

				T1	15	
			Plate 3	C1	35	-0.147540984
				T1	26	
			Plate 4	C1	36	-0.220338983
				T1	23	
			Plate 5	C1	27	-0.125
				T1	21	
JN	15°C	90 minutes	Plate 1	C1	7	0.391304348
				T1	16	