Studying Caenorhabditis elegans: Using RNAi to Knock Down UNC-3 and LPR-5 Genes to Determine Gene Necessity for Chemoperception

**Katelyn Cheslick**
**University of California Santa Barbara**

**ABSTRACT:**

In this study, RNAi technology was used on the model organism Caenorhabditis elegans to knock down the UNC-3 and LPR-5 genes to determine whether they are necessary for chemoperception. Four RNAi plates were set up (one positive control, one negative control, UNC-3 and LPR-5), and bacteria and larval stage L4 C. elegans were added to each plate. After a seven day incubation period, a chemotaxis assay was conducted, and results were analyzed using a Z test, which was utilized in order to determine whether differences between the experimental and control treatments were due to random error or the knocking out of chemosensory genes. Data sets from both the class section, and the whole winter 2023 cohort were utilized. Only one Z test rendered statistically significant results, which was the positive versus negative comparison for the winter 2023 data set. No other RNAi treatments rendered statistically significant results, even for the UNC-3 and LPR-5 genes within the winter 2023 data set, which had a larger replication number. Therefore, it is unlikely that either UNC-3 or LPR-5 are involved in chemosensory perception. Because C. elegans is a model organism, understanding the functions of genes within the nematode may aid in furthering understanding about human neuronal pathways, due to evolutionary conservation of neuronal activity. Though this study supports the conclusion that UNC-3 and LPR-5 genes in C. elegans are not involved in chemoperception, it would be logical to conduct further studies with larger replicate numbers, as well as to conduct further studies using other genes in order to broaden the understanding of C. elegans', as well as humans', neuronal pathways.

**INTRODUCTION:**

Within molecular biology, model organisms are often studied in order to conveniently ascertain information that is applicable to other species, as a result of genomic conservation throughout evolution [1]. Caenorhabditis elegans is one such organism, which has been studied extensively. It is known that sensory neurons within the nervous system of C. elegans are responsible for chemoperception, and that attractive and repulsive neurons act together in a network to enact locomotion [2]. It is also known that C. elegans have large gene families, with over 1,000 chemoreceptor genes [3]. This study aims to answer the question of whether the genes UNC-3 and LPR-5 function in chemosensory perception in the model organism Caenorhabditis elegans.

This study is being conducted because the information obtained by studying model organisms such as C. elegans can often be generalized to other organisms, including humans, due to

genomic similarities. Though C. elegans have much simpler neuronal pathways than humans, understanding the basic mechanisms involved in C. elegans chemosensory pathways can reveal information about human neuronal networks, as the fundamental molecular and chemical pathways are similar [2 ]. Due to these similarities, C. elegans studies have the potential to aid in furthering our understanding of mechanisms of drug action and diseases [4], and are therefore very valuable.

In this experiment, RNAi will be used to knock out certain genes, and a chemotaxis index will be used to determine whether the genes UNC-3 and LPR-5 were involved in C. elegans chemoperception. It is hypothesized that if the genes UNC-3 and LPR-5 are knocked out using RNAi, then the C. elegans will still be capable of normal chemoperception, since the genes UNC-3 and LPR-5 are not expected to function in chemoperception [5,6].

## METHODOLOGY

In this experiment, the organism C. elegans was studied, and RNAi was used to knock out the function of the UNC-3 and LPR-5 genes in order to determine whether they were necessary for chemoperception. First, four plates were set up by adding bacteria containing various RNAi treatments to each plate (one positive control with ODR-10, one negative control with L440, an experimental with UNC-3, and an experimental with LPR-5). Next, 3-5 larval stage 4 (L4) worms were transferred to each plate. The plates were then incubated at 15 °C for seven days such that the worms could reproduce and progeny could grow in the presence of the RNAi.

C. elegans which had grown in the presence of the various RNAi treatments were then harvested. After the worms were removed from the RNAi plates, they were transferred to a filter screen and washed with distilled water in order to remove all bacteria. Chemotaxis plates were then set up (with a diacetyl chemical attractant mixture on the DA side and an anesthetic mixture on the O side) and labeled, such that a chemotaxis assay could be conducted. Thirty adult worms (at minimum) of each treatment group were then transferred to their respectively labeled chemotaxis plates. After 60 minutes, the number of worms on both sides of the plate were counted for each treatment, and results were recorded.

$$Chemotaxis\ Index\ = \frac{\#\ worms\ on\ DA\ side}{\#\ worms\ total\ for\ both\ sides}$$

$$Z\ = \frac{(P1 - P2)}{\sqrt{\frac{P1\ (1-P1)}{n1} + \frac{P2\ (1-P2)}{n2}}}$$

$P1\ and\ P2$ = *the mean values for the chemotaxis index of the experimental and control groups, respectively*
$n1\ and\ n2$ = *the sample sizes for the experimental and negative control groups, respectively*

In order to interpret the outcome of the experiment and to determine whether differences between the experimental and control treatments were due to random error or to the knocking out of chemosensory genes, the results of the chemotaxis assay were analyzed using a Z test. This particular form of statistical analysis was utilized because it allowed the null hypothesis to be tested.

**RESULTS AND FIGURES**

For all four RNAi treatments, the average chemotaxis index was found for the group data, section data, and winter 2023 data. For the group data, the chemotaxis indexes for the positive (ODR-10), negative (L440), experimental one (UNC-3) and experimental two (LPR-5) treatments were found to be 0.49, 0.83, 0.30, and 0.88, respectively. Our positive control value of 0.49 is predicted, as it is expected that the chemotaxis index will be close to 0.5. Our negative control value of 0.83 is also predicted, as it is expected that the chemotaxis index will be close to 1. For the section data, the average chemotaxis indexes for the positive, negative, experimental one, and experimental two treatments were found to be 0.54, 0.62, 0.60, and 0.52, respectively. For the winter 2023 data, the chemotaxis indexes for the positive, negative, experimental one, and experimental two treatments were found to be 0.53, 0.64, 0.60, and 0.60, respectively.

| Treatment | Experimental Value | Average CI (Section Data) | Number of Replicates | Average CI (Winter 2023 Data) | Number of Replicates |
|---|---|---|---|---|---|
| Positive control | 0.49 | 0.54 | 84 | 0.53 | 256 |
| Negative control | 0.83 | 0.62 | 84 | 0.64 | 256 |
| unc-3 | 0.30 | 0.60 | 40 | 0.60 | 40 |
| lpr-5 | 0.88 | 0.52 | 15 | 0.60 | 44 |

Table 1: The data table describes the various RNAi treatments and the associated chemotaxis indexes for group data (experimental value), section data, and winter 2023 data.

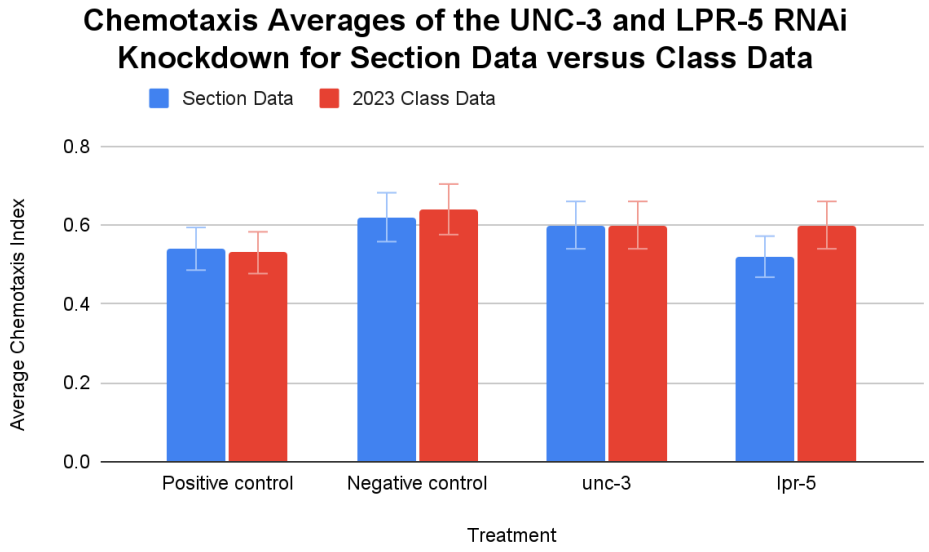


Figure 1: The figure illustrates the various RNAi treatments and the associated average chemotaxis indexes. Error bars show the standard deviation for each treatment.

Z tests were then conducted in order to determine the statistical significance of the data. First, the section data set was used and all RNAi treatments were compared to the negative control. The results were as follows: the positive v. negative comparison yielded a P value of 0.1459, the negative v. negative control yielded a P value of 0.5, the UNC-3 v. negative yielded a P value of 0.4168, and the LPR-5 v. negative yielded a P value of 0.2358. Next, the winter 2023 data set was used, and once again all RNAi treatments were compared to the negative control. As seen in the table, the number of replicates for this data set were significantly larger, and the results were as follows: the positive v. negative comparison yielded a P value of 0.0052, the negative v. negative control yielded a P value of 0.5, the UNC-3 v. negative yielded a P value of 0.3156, and the LPR-5 v. negative yielded a P value of 0.3015. A P value of 0.05 or less is considered statistically significant. Therefore, the only Z test that rendered statistically significant results was the positive versus negative comparison for the winter 2023 data set; no other RNAi treatments rendered statistically significant results.

| Data Set | Comparison | Z score | P value | Number of Replicates |
|---|---|---|---|---|
| Section | pos v. neg | -1.05 | 0.1469 | 84 |
| | neg v. neg | 0 | 0.5000 | 84 |
| | unc-3 v. neg | -0.21 | 0.4168 | 40 |
| | lpr-5 v. neg | -0.72 | 0.2358 | 15 |
| Winter 2023 | pos v. neg | -2.56 | 0.0052 | 256 |

| | | | | |
|---|---|---|---|---|
| | neg v. neg | 0 | 0.5000 | 256 |
| | unc-3 v. neg | -0.48 | 0.3156 | 40 |
| | lpr-5 v. neg | -0.52 | 0.3015 | 44 |

Table 2: The data table describes the Z scores and P values attained by comparing the various RNAi treatments for two data sets.

**DISCUSSION**

By observing the Z scores and associated P values obtained from our experiment, it is apparent that only the positive versus negative control treatment for the winter 2023 data resulted in a statistically significant result, as the P value was less than the statistical significance benchmark of 0.05. This outcome was expected, as the positive control gene was known to be involved in sensory perception, and the negative control treatment was an empty vector.

The P values obtained from the chemotaxis index averages of the UNC-3 and LPR-5 genes were greater than 0.05 for the section data, meaning that the data attained was not statistically significant, and there is a large chance the data resulted from chance alone. However, when the replicate number was increased (i.e. when winter 2023 data was used), the P values obtained from the chemotaxis index averages of the UNC-3 and LPR-5 genes remained greater than 0.05 and statistically insignificant. Because the replicate number was increased and the P value remained statistically insignificant, it is likely that neither UNC-3 or LPR-5 are involved in chemoperception. These genes can be referenced via wormbase and it is found that UNC-3 functions in processes such as DNA binding, protein binding, and and histone binding [5], while LPR-5 is likely to aid in lipid transport [6]. Therefore, neither gene would be expected to be involved in chemosensory perception. As a result, worms with these genes knocked out would still be expected to sense chemoattractants normally and have chemotaxis indexes close to the negative control average, which was experimentally determined to be 0.62. The chemotaxis indexes for UNC-3 and LPR-5 were indeed close to this value. Therefore, the hypothesis that neither UNC-3 or LPR-5 are involved in chemoperception was supported, and the data attained in this study did support the findings of previous studies [5,6] regarding the function of the UNC-3 and LPR-5 genes.

Because *C. elegans* is a model organism, the data has broader implications beyond just understanding chemoperception within one nematode species. By analyzing the function of genes in *C. elegans*, it may be possible to deduce information about molecular and chemical pathways involved in humans, as well, due to the presence of analogous genes [2]. Though the data attained for UNC-3 and LPR-5 in this experiment did support the hypothesis that neither gene is involved in chemoperception, it is still possible that errors occurred. The primary

possible source of error is failing to rinse all of the bacteria off the worms before transferring them to the agar plate, which would result in a lack of movement towards the chemoattractant, and would result in a chemotaxis index closer to 0.5, rather than 1. It would be logical to conduct further experiments using these same genes. By conducting experiments using these same genes and a different experimental approach, additional supporting data for the hypothesis could be attained. One such method could be utilizing a chemorepellent rather than a chemoattractant. Additionally, it would be beneficial to conduct follow up experiments using different genes, other than UNC-3 and LPR-5, such that knowledge of the chemosensory pathway of *C. elegans* can be expanded further.

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