Do Levels of EPA in *C. elegans* Alter Transcriptional Regulation of NPR-1?

**Introduction**

Alcohol addiction is a widespread disease among humans. An estimated 88,0008 people die from alcohol-related causes annually, making alcohol the third leading preventable cause of death in the United States (Alcoholism). Current drug treatments are inadequate, because the molecular nature of acute response to ethanol is not well known (Bettinger). Lots of research has been conducted on ethanol regulation and the mechanisms involved, but there is still a lot to be discovered. *Caenorhabditis elegans* is a type of worm that is usually used in the study of molecular mechanisms relating to alcohol consumption due to its well-understood and simplistic nervous system. Alcohol consumption alters locomotion (movement) in *C. elegans* in a similar way in mammals (Raabe). Acute functional tolerance (AFT) is thought to be a neuronal response to the depressive effects of ethanol on locomotion, which develops within a single drug exposure. In *C. elegans*, at 30 minutes after exposure to ethanol, AFT shows up as in increase in locomotion, compared to the speed of the animals 10 minutes after exposure (Raabe).

Eicosapentaenoic acid (EPA), a long- chain polyunsaturated fatty acid (LC-PUFA), was discovered to be necessary for the development of AFT in *C. elegans* and is also present in mammals (Bettinger). LC-PUFAs are enriched in the brain and retina in mammals, and mutations that alter fatty acid metabolism in worms have been shown to affect neurotransmitter signaling by decreasing synaptic vesicle recycling and neurotransmitter release in *C. elegans*. The *fat-1* gene encodes an omega3 fatty acyl desaturase, which is the enzyme that is necessary for the conversion of arachidonic acid- another LC-PUFA, to EPA. In Raabe et al, they tested animals carrying a mutation in the *fat-1* gene. The worms were treated with 400 mM ethanol and locomotion was recorded 10 minutes and 30 minutes into ethanol exposure. Below are the results of the experiment. The N2 (Wild Type) animals, which were the normal strain, developed AFT at normal levels, while *fat-1* mutants failed to develop AFT. Figure 1A and 1B shows a comparison of the speeds of the two groups. At 10 minutes into exposure, the speed of the two groups of animals was the same. At the 30-minute time point, the speed of N2 animals was higher, showing that these animals were able to develop AFT. In contrast, the speed of the *fat-1* mutants was lower at the 30-minute time point than at the initial 10-minute time point. This shows that *fat-1* mutants failed to develop AFT, proving that EPA is essential for the development of AFT. (Raabe).

***Figure 1***

***A***

***B***

It was also found that NPR-1, a neuropeptide Y receptor-like protein, plays a role in the regulation of AFT in *C. elegans* during ethanol exposure, and has a conserved function of NPY related pathways in ethanol responses in humans as well as other species (Davies). To test what exactly the role of NPR-1 in the development of AFT was, Davies et al designed an experiment that involved increasing the expression of the *npr-1* gene in N2 worms. They transformed N2 animals with a high copy number of the PM4 genomic DNA fragment containing the *npr-1* gene, in order to increase the level of NPR-1 protein production. PM4 was injected at a concentration of 200 ng/ l into N2 animals. The animals were placed on nematode growth medium plates and copper rings were melted into the surface of the plates. Ethanol was added to reach a concentration of 500 mM. The animals that were to be compared were placed in individual rings on the same plate and their movement was recorded at 10, 30 and 50 minutes. A relative speed for each strain was calculated (Davies).

Figure 2 below shows a decrease in the development of AFT in two out of three animals tested, compared with N2 animals, which had normal levels of NPR-1. In N2;egEx2 and N2;egEx3, the speed of these animals 30 minutes into exposure was significantly slower, compared with the speed of N2 animals. This showed that NPR-1’s role in the regulation of AFT in *C. elegans* is to suppress development of AFT. However, the exact mechanisms involved in this are not yet known (Davies).

 (Davies)

*Figure 2*

Knowing that EPA is required for the development of AFT, and NPR-1 negatively regulates the development of AFT, I would like to find out if NPR-1 and EPA work together. Because these two mechanisms regulate the development of AFT in opposite ways, there is a possibility that EPA might be working through NPR-1. Therefore, the purpose of this experiment is to find out if levels of EPA in *C. elegans* affect transcriptional regulation of NPR-1.

**Experiment**

In this experiment, the N2 strain of *C. elegans* will be used. There will be two groups of worms used and the levels of *npr-1* mRNA in both groups will be quantified. The worms from the first group will be fed dietary supplements of EPA, resulting in the accumulation of greater than wild-type levels of EPA (Raabe). EPA will be added to nematode growth medium solution with Fatty acid salts on plates and then seeded with bacteria. The fatty acid salts (arachidonate and eicosapentaenoate) will be added to 100 mM in ddH20 prior to making plates. Nematode growth solution will be prepared by adding 0.1% tergitol. Lipids will be added slowly to a final concentration of 160 microMolar. Plates will then be seeded with E. coli for 48 hours. (Kahn-Kirby). The worms will be left on the plates for 19 hours (Raabe). The second group of worms will be *fat-1* mutants, which do not have EPA. These mutants will be purchased.

To check for a change in NPR-1 function, quantitative reverse transcription polymerase chain reaction (qRT-PCR) will be used to determine the levels of *npr-1* mRNA in the animals that have been fed EPA and those with a *fat-1* mutation. Complementary DNA (cDNA) will be derived from the worms treated with supplemental EPA and *fat-1* mutant worms by isolating *npr-1* mRNA using an RNAqueous- 4PCR Kit (Davies). cDNA synthesis will be performed using a SuperScript cDNA synthesis kit and an oligo(dT) primer, used for priming reactions catalyzed by reverse transcriptase (Link). The cDNA will then be amplified by PCR. *Npr-1* mRNA levels in both groups will be quantified by comparing against a non-variable control gene in *C. elegans* (F22E5.6). CTATGC GTGGCTGAACCCAAGTTTC (forward) and GTGTCGTTGACGCTGA ACTCGATC (reverse) primers will be used for *npr-1* mRNA (Davies). TCCCCATACGAAACAACACA and CTCCTCCCAGCTTTTCCACAA will be used as forward and reverse primers in the control gene (Link). A standard curve will be used to quantify the *npr-1* mRNA levels in both groups of animals. This construes relative expression levels of the gene of interest (Qiagen). The entire procedure will be repeated twice.

**Discussion**

It is expected that in the worms with higher EPA levels, the results will show a decrease in the levels of *npr-1* mRNA. This is because higher EPA relates with higher levels of AFT, which means that levels of *npr-1* mRNA in these worms will have to be lower than WT, as NPR-1 suppresses the development of AFT. Subsequently, in the worms with no EPA, there will be higher levels of *npr-1* mRNA. This prediction is due to the experiment performed in Davies et al. When the animals were given too much *NPR-1*, they had no AFT. If EPA were acting through NPR-1 to affect AFT, it is expected that *fat-1* mutants, who do not have EPA and so have no AFT, should have more NPR-1 as well. If there are changes in *npr-1* mRNA levels as predicted, we can make the observation that EPA does act through NPR-1, and they indeed have some sort of connection. However, if the levels of *npr-1* mRNA remain unchanged, we can make the observation that these two mechanisms of ethanol tolerance are independent of each other relating to the development of tolerance.

Because we know the effect of NPR-1 on AFT it is also expected that these levels of *npr-1* mRNA will go on to affect the regulation of AFT in *C. elegans*. The mechanism for how NPR-1 levels affect AFT is not yet known. We just know that NPR-1 negatively regulates AFT. If there was more information on how exactly this occurs, maybe further experiments would be conducted to figure out how exactly varied EPA levels determine the level of NPR-1 function.

# Works Cited

1. Alcoholism, N. I. (n.d.). *Alcohol Facts and Statistics*. Retrieved from https://www.niaaa.nih.gov: <https://www.niaaa.nih.gov/alcohol-health/overview-alcohol-consumption/alcohol-facts-and-statistics>

2. Bettinger, J. (n.d.). *Lipid Environment Modulates the Development of Acute Tolerance to Ethanol in Caenorhabditis elegans.* Retrieved from http://journals.plos.org: 4 May 2012 <http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0035192>

3. Davies, A. (n.d.). *Natural Variation in the npr-1 Gene Modifies Ethanol Responses of Wild Strains of C. elegans.* Retrieved from http://www.sciencedirect.com: Journal 45, Issue 5, 10 June 2004, Pages 731-743 <http://www.sciencedirect.com/science/article/pii/S0896627304002922>

4. Kahn-Kirby, A. (n.d.). *Specific Polyunsaturated Fatty Acids Drive TRPV-Dependent Sensory Signaling In Vivo.* Retrieved from http://www.sciencedirect.com: Volume 119, Issue 6, 17 December 2004, Pages 889–900 <http://www.sciencedirect.com/science/article/pii/S0092867404010475>

5.Link, C. D. (n.d.). *Gene expression analysis in a transgenic Caenorhabditis elegans Alzheimer’s disease model.* Retrieved from http://www.sciencedirect.com: Volume 24, Issue 3 May–June 2003, Pages 397-413 <http://www.sciencedirect.com/science/article/pii/S0197458002002245>

6. Nolan, T. (n.d.). *Quantification of mRNA using real-time RT-PCR.* Retrieved from [http://www.nature.com: http://www.nature.com/articles/nprot.2006.236](http://www.nature.com:%20http://www.nature.com/articles/nprot.2006.236)

7. Qiagen. (n.d.). *What is the standard curve method for qPCR assay data analysis? How is the standard curve method for qPCR assay data analysis performed?* Retrieved from https://www.qiagen.com: <https://www.qiagen.com/us/resources/faq?id=411524f1-fe07-41bc-bbd2-32e1ce16b497&lang=en>

8. Raabe, R. (n.d.). *The Omega-3 Fatty Acid Eicosapentaenoic Acid Is Required for Normal Alcohol Response Behaviors in C. elegans .* Retrieved from https://www.ncbi.nlm.nih.gov: 27 August 2014 <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4146551/>