Do Higher Levels of EPA in *C. elegans* Affect NPR-1 Regulation of AFT?

**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 in *C. elegans* in a similar way as in mammals (Raabe). Acute functional tolerance (AFT) is thought to be a neuronal response to the depressive effects of ethanol on locomotion that 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).

It was found that NPR-1, a neuropeptide Y receptor-like protein, plays a role in the suppression 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). Davies et al tested the role of NPR-1 in the development of AFT by increasing the expression of the *npr-1* gene in wild type N2 animals. 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 1 below shows a decrease in the development of AFT in two out of three animals tested, compared with N2 animals. In N2;egEx2 and N2;egEx3, the speed of the animals after 30 minutes of exposure was significantly slower, compared with the speed of N2 animals. This shows that NPR-1 suppresses the development of AFT in *C. elegans* (Davies).



*Figure 1*

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). The *fat-1* gene encodes an omega3 fatty acyl desaturase that is necessary for the conversion of arachidonic acid (AA)- another LC-PUFA to EPA. In Raabe et al, they tested animals carrying a mutation in the *fat-1* gene. These mutants lacked EPA and were unable to develop AFT, proving that EPA is essential for the development of AFT (Raabe).

Knowing that NPR-1 negatively regulates the development of AFT and EPA is required for the development of AFT, I would like to find out if NPR-1 and EPA work together. Therefore my research question is “Do higher levels of EPA in *C. elegans* affect NPR-1 regulation of AFT?”

**Experiment**

In this experiment, the N2 strain of *C. elegans* will be used. The worms will be fed dietary supplements of EPA, resulting in the accumulation of greater than wild-type levels of EPA. This will increase the levels of EPA in the animals, thereby increasing the development of AFT (Raabe). EPA will be added to nematode growth medium solution and Fatty acid salts on plates and then seeded with bacteria (Kahn-Kirby). The worms will be left on the plates for 19 hours (Raabe).

To check whether there is an increase in NPR-1 function, reverse transcription polymerase chain reaction (RT-PCR) will be used to determine the levels of *npr-1* mRNA in animals that have been fed EPA. Complementary DNA (cDNA) will be derived from the worms treated with EPA by isolating *npr-1* mRNA and using a cDNA synthesis kit with primers and reverse transcriptase (Davies). The cDNA will then be amplified using PCR. This will also require a set of primers, PCR buffer and DNA polymerase (Nolan). *Npr-1* mRNA levels in worms supplemented with EPA will be compared with *npr-1* mRNA levels of worms that were not fed supplemental EPA.

**Discussion**

It is expected that the results will show an increase in the levels of *npr-1* mRNA, thereby increasing the production of the NPR-1 protein to compensate for an increase in levels of AFT due to higher EPA levels. As the development of AFT increases, so will the function of NPR-1. The mechanism for how NPR-1 levels affect AFT is not 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 higher levels of EPA increase 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. 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)

6. 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/>