

Jonathan Kindberg

BNF0 491

Molecular Biology Through Discovery

Research Proposal

The effect of a single nucleotide polymorphism in the NPY protein and ethanol consumption in mice

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I. Introduction

Alcohol abuse affects millions of people worldwide. Currently, nearly 14 million Americans (1 in every 13 adults) abuse alcohol or are alcoholic. Current drug treatments are inadequate because the proteins that alter the nervous system when exposed to ethanol are not completely understood. According to Bettinger et al¹, this is partially due to the fact that the molecular nature of acute ethanol response is not well understood. Davies et al² used *Caenorhabditis elegans* (*C. elegans*), a small roundworm to understand the protein interactions in the nervous system of both humans and *C. elegans*. This animal is a good candidate for experimental procedures because it has a simple nervous system and it uses similar neurotransmitters that are used by the human brain.

When *C. elegans* is exposed to a continuous concentration of ethanol for an extended period of time, it undergoes a change in how its neurons respond to the ethanol stimulus, which is known as acute tolerance. This explains why *C. elegans* appears to be less intoxicated over a period of time. The NPR-1 protein was found within *C. elegans* to control how these animals regulate acute tolerance to ethanol². NPR-1 protein is a G protein-coupled neuropeptide receptor. G protein coupled receptors (GPCR) are found only in eukaryotes. The receptors sense molecules and activate cellular responses inside the cell. A neuropeptide is used by neurons for communication. Neuropeptides signal molecules and influence the activity of the brain. The NPR-1 protein is homologous to the neuropeptide Y (NPY) receptor required for regulating anxiety, food consumption, and pain sensation in humans. In *C. elegans*, NPR-1 is involved in differences in social behavior such as social versus solitary feeding.

During one experiment Davies et al² compared the NPR-1 proteins of *C. elegans* that were exposed to ethanol for an extended period of time with *C. elegans* that were not exposed to ethanol. This was achieved through an experimental procedure called "clumping." 20 ethanol-induced animals and non-ethanol-treated animals were placed inside copper rings placed on separate plates. Inside each copper ring was a bacterial lawn or bacterial clump. *C. elegans* are attracted to the bacteria clump because it's a natural food source. The authors then counted the number of animals whose body overlapped the bacterial clump by at least 50%. The results of the experiment are detailed below.

N2 animals (*C. elegans* strain used in the experiment; this strain has a higher functioning NPR-1.) do not clump unless induced by ethanol. The figure 1A below demonstrates how the N2 animals clump and border after they are withdrawn from alcohol. These animals display the same behavior as animals with a lower functioning NPR-1 protein. Figure 1B demonstrates the % of clumping by each strain. The N2 animals show very little clumping when untreated with EtOH, less than 20%. However, when treated with alcohol and then withdrawn, these animals show a significant amount of clumping at almost 60%.

Daf-3 animals, *C. elegans* strain used in the experiment, that does not suppress the clumping behavior of npr-1 loss of function, failed to suppress EtOH withdrawal in this experiment. These animals clump and border when withdrawn from ethanol. In figure 1A, these animals show significant clumping and bordering. In figure 1B, these animals show similar behavior to the N2 animals. The untreated animals show less than 40% clumping but the animals withdrawn from the EtOH show almost 70% clumping.

Osm-9 and tax-4 are *C. elegans* mutation strains. These strains suppress the clumping behavior of NPR-1 loss-of-function. These animals show no visible signs of clumping when exposed to ethanol. In figure 1A, these animals also show no ethanol-induced clumping. In figure 1B, these animals also show little differences in the untreated versus alcohol withdrawal clumping.

Each of the four plates in the diagram above contains no EtOH. The animals were exposed to EtOH for an extended period of time and then withdrawn to determine if the NPR-1 protein function would be affected by the withdrawal of EtOH. The clumping and bordering on the plate indicates a lower functioning NPR-1.

The authors concluded that the strains with a lower NPR-1 function show a higher dependency for ethanol. They also observed that prolonged exposure to ethanol for the animals with a higher NPR-1 function mirrored the lower NPR-1 functioning animals, which indicates that prolonged exposure to alcohol may also increase the chance of dependency to ethanol.

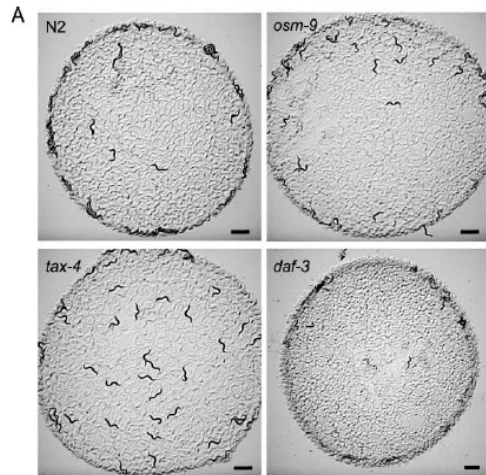


Fig.1 (A) the plates demonstrate the clumping and bordering of each animal strain after withdrawn from EtOH.

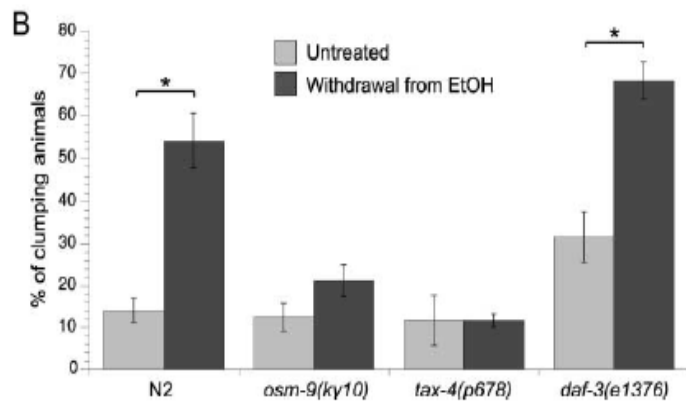


Fig. 1(B) the bars represent the percentage of animals clumping following the withdrawal of ethanol.

NPR-1 gene in *C. elegans* is the homologous to the NPY gene in mammals. The NPY gene encodes a neuropeptide that is widely expressed in the central nervous system and influences many physiological processes, including cortical excitability, stress response, food intake, circadian rhythms, and cardiovascular function. A polymorphism in this gene resulting in a change of leucine 7 to proline in the signal peptide is associated with elevated cholesterol levels, higher alcohol consumption, and may be a risk factor for various metabolic and cardiovascular diseases¹³.

Does a single nucleotide polymorphism in the NPY protein of mice increase the consumption of ethanol? This experiment will correlate the low NPR-1 function found in *C. elegans* and the EtOH dependency with an abnormal functioning NPY in mice. Humans and mice share the same 20-25k genes, which makes the mice genome about 85% similar to humans. The mice can be selectively bred to isolate a single nucleotide polymorphism. As described in the experiment by Martin Davies⁵, he used multiple mice which were bred to be affected by ethanol in order to study a different protein receptor called GABA_A. Indiana University researchers have been able to selectively breed mice that are addicted to alcohol⁴. These mice were then used in other experiments relating to gene functions with alcoholism.

II. Experiment

This experiment will study the NPY function of 20 mice. The experiment will isolate the leucine 7 to proline polymorphism in the signal peptide of the mice. 10 out of the 20 mice will be selectively bred with the polymorphism in the signal peptide and the other 10 mice will be used as control mice. The mice will be exposed to ethanol and the function of the NPY protein will be isolated and compared.

The mice will be exposed to ethanol by adding sweetener to the EtOH. As the mice acquire a taste for the ethanol, the sweetener will be reduced. The mice will be exposed to ethanol for one month and the data will be analyzed at weekly intervals. Each week, the mice will be presented with options of food or ethanol. Data will be captured on whether the mice prefer the ethanol or food. I will then compare the NPY mRNA levels of the mice in each group by extracting tissue samples from the mice after the month long ethanol exposure. The tissue cells will be used to isolate the NPY mRNA in the mice. Reverse transcription polymerase chain reaction (RT-PCR) will be used to compare the NPY mRNA in the animals. This will allow me to quantify the levels of NPY mRNA in each group of mice.

The steps to quantify the NPY mRNA levels will be to add the following together: buffer, DNA primers, Reverse Transcriptase and DNA polymerase. The mRNA from the mice will be added and then incubated at 37 degrees Celsius. This process will isolate the mRNA to quantify the levels of NPY protein in the mice. The standard curve method will be used to compare the NPY mRNA levels of the mice. The expected results would indicate the mice with a lower functioning NPY to have a polymorphism in the signal peptide of NPY. This data would be further analyzed to determine if the mice with the polymorphism in the signal peptide consumed more alcohol over the month long period.

III. Discussion

This experiment correlates closely to the experiment by Badia-Elder et al where they found that mice who were lacking the NPY protein consumed much more ethanol than mice who had a normal functioning NPY protein. They also determined that mice with an over expressed NPY protein would consume less ethanol than both the controls and the NPY deficient mice¹⁴.

In both *C. elegans* and mice, the difference in the G-protein coupled neuropeptide receptor function indicates an increased likelihood of alcohol dependence or consumption. The NPY in humans and mice and the NPR-1 in *C. elegans* control various behavioral processes including alcohol consumption. In mice, the polymorphism in the NPY receptor protein may result in increased consumption by mice over an extended period of time. In *C. elegans*, a lower functioning NPR-1 protein results in higher dependency to ethanol.

In order to explore these results further, more data would need to be gathered on the effect of the single nucleotide polymorphism in the NPY protein. An experiment that analyzes the NPY function of selectively bred mice with additions would provide additional data. The NPY function of these selectively bred mice could be studied and it would be expected for these mice to have an abnormal or lower functioning NPY protein.

The experiments indicate that the NPY neuropeptide receptor may provide a genetic link to alcoholism if further studied.

IV. References

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