What The F*ck Do I Do In Lab?

A semi-comprehensive guide to my research



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Introduction

Hi! If you're reading this, you're probably a close friend or family member of mine who's curious about my research. Sometimes, it's hard for me to explain what I do without getting too technical and jargony. So instead, I made this booklet! While there is still a lot that is outside of the scope of this booklet, my hope is that after reading this, my research will be at least a little easier to understand than before.



In this booklet, you can expect to learn about some basic concepts of biology and how they apply to my research, what I actually do in the lab, and why it's all important. You will also see some images taken by me (denoted by a star)!

First, let's break down the title of my research project:

Long Non-Coding RNA Regulation of Neuroimmune Response to Alcohol...

Long Non-Coding RNA (lncRNA)

The Central Dogma of Biology

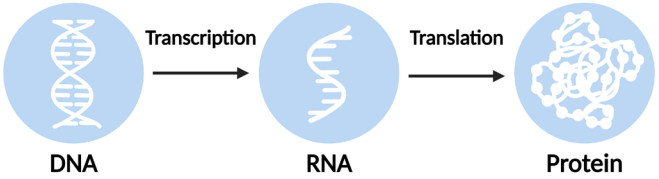
The Central Dogma is the fundamental concept that all other aspects of biology build off of. It states that genetic information flows from DNA to RNA to protein. DNA is **replicated** into more DNA or **transcribed** into RNA, and RNA is **translated** into protein.

What is RNA?

Both DNA and RNA are made up of long chains of small molecules called **nucleotides**. Nucleotides contain the bases adenine (A), cytosine (C), guanine (G), and thymine (T) or uracil (U). The order of these bases is what gives DNA/RNA its specific code. While DNA is a double-stranded helix, RNA is single-stranded. Another difference is that the entire DNA sequence is present at equal levels in all cells. However, different RNA at different levels can be present in different cell types and conditions (this is called **gene expression**).

Gene Expression

the level at which a certain DNA sequence is transcribed into RNA



Long and Non-Coding?

Remember how I said that RNA is translated into protein? It turns out that this is only true for some RNA. RNA that isn't translated is called **non-coding RNA** because it doesn't *code* for a protein. Instead of being translated into a protein, non-coding RNAs are able to perform their function as an RNA molecule. These non-coding RNAs used to be thought of as junk, but it turns out they have many important roles, such as regulation of other genes.

Long non-coding RNA (**lncRNA**) is just a non-coding RNA that is more than 200 nucleotides long. This distinction is important because there are many different kinds of shorter non-coding RNAs with different functions.

For example, ***Neat1*** is the lncRNA that I study. *Neat1* is involved in regulating biochemical activity in the cell, cancer progression, and neuroimmune activation. It is also VERY long (~22,000 nucleotides).

The Neuroimmune System

What is It?

While it used to be thought that the nervous system and immune system were completely separate, recent research shows that they have a complex interaction. This is the **neuroimmune system** – the interface between the brain and the immune system.

Its purpose is to protect **neurons** from whatever is causing immune activation, such as infection or injury. This causes inflammatory signaling molecules to be released into the body, which make their way to the brain to communicate the need to be on high alert.



Microglia

Microglia are **immune cells** that reside in the brain. They mediate the neuroimmune system by monitoring for and releasing signaling molecules. They also act as the garbage trucks of the brain by consuming dead cells and debris within the brain.

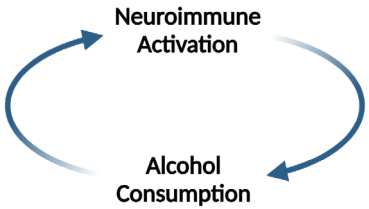
Alcohol Use Disorder

The DSM-5 defines Alcohol Use Disorder (**AUD**) as "a problematic pattern of alcohol use leading to clinically significant impairment or distress". It can be caused by both environmental and genetic factors and can lead to changes in brain function.

The Neuroimmune System & AUD

A lot of data exists that suggests a connection between neuroimmune signaling and AUD. Most notably, alcohol consumption causes an increase in levels of immune signaling molecules in the brain. Microglia also become activated in response to alcohol. It is hypothesized that the neuroimmune system plays a role in the development of AUD.

Under this hypothesis, alcohol consumption causes neuroimmune activation, which leads to cravings, anxiety, and further alcohol consumption. This cycle is what eventually develops into AUD.



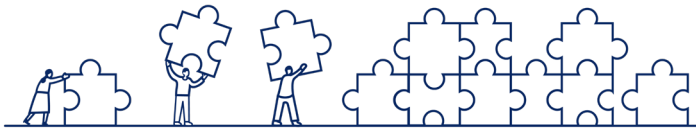
Putting It All Together

While you may be able to connect some puzzle pieces from the last few pages, let's put it all together into one, simple(ish) page.

The idea behind my research project is that *Neat1* is somehow involved in regulating neuroimmune signaling in response to alcohol (or ethanol, **EtOH**). I focus on microglia since they are the immune cells of the brain. To decipher this role, I grow microglia and prevent them from making *Neat1*. This allows me to compare what happens in certain conditions when *Neat1* is present or absent and then infer how these differences occur.

Rationale

I hope for my research project to make a valuable contribution to the field of alcohol research and lead to a better understanding of the molecular mechanisms behind AUD development. While this is just the beginning for me, the ultimate goal for this field is to identify novel targets for medications to treat AUD.



So What Do I Actually Do???

All of this biology is really cool, but to actually study any of it, you need to put on your lab coat and get ready to do some experiments in the lab!

Cell Culture

Cells are extracted from the brains of baby mice and transferred to a container similar to a petri dish. The cells are given "food" (**media**) and allowed to grow in an incubator for two weeks. After two weeks, microglia are extracted from this mix of brain cells using special magnetic beads. These microglia can then be used in all experiments that follow.

Cell Culture FAQ



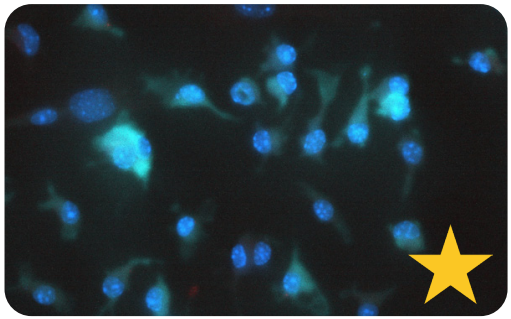
Do you have to kill the mice?
Yes, but in research it is called "sacrificing".

Does it make you sad?
Yes, but the process is very humane and I always remind myself that it is for the greater good.

Why does it need to happen?
Mice are the most comparable model organism to humans. Although it is sad, these mice allow us to better understand diseases and conditions that afflict humans.

Assessment of Microglia Purity

When cells are grown as described on pg. 9, both **microglia** and **astrocytes** (another cell type found in the brain) will be found in the mixture. Even after purification, sometimes astrocytes like to be annoying and stick around. To make sure this hasn't happened, the cells are labeled and imaged. The labels are fluorescent and cell-type specific, meaning that microglia and astrocytes are labeled with a different color. A stain called **DAPI** labels the **nuclei**, which is the compartment in the cell that contains the DNA. Once labeled, a special (and very expensive) fluorescent microscope takes pretty pictures of the cells.



DAPI
Microglia
Astrocytes

Above is what the image *should* look like, with only DAPI and migroglia staining. This is a pure microglia sample.

This is an impure sample where astrocyte contamination (red) has occurred. As frustrating as astrocyte contamination is, this image deserves an entire page because they are still very pretty.

CRISPR

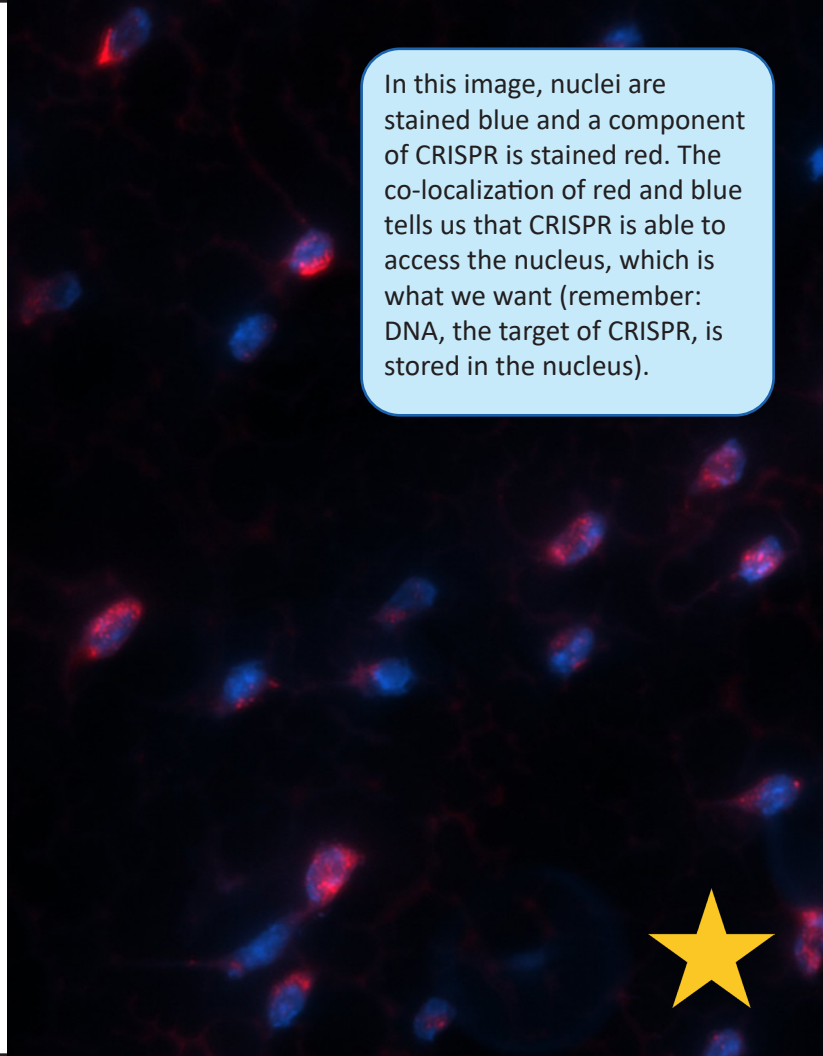
You may have heard of **CRISPR** and that it can be used to edit the genome (i.e. your DNA/genes). CRISPR technology is most well known for its ability to be "programmed" to induce mutations in very specific regions of DNA. Any gene can be "**knocked out (KO)**" (yes, that is really what it's called) by making the gene and its product (a protein or non-coding RNA) nonfunctional. We can then look at what else happens to the cell once the gene is knocked out. For example, in my research, I look at how the loss of the *Neat1* gene affects how other genes are working.

PCR and Sanger Sequencing

Polymerase chain reaction, or **PCR**, is a technique that amplifies specific regions of DNA from a small amount of DNA in a sample.

Sanger sequencing is a popular method of sequencing DNA. My lab sends DNA that has been *amplified by PCR* to a specialized lab that will do it for us, and they send us the nucleotide sequence of that DNA.

These two methods together allow us to look at the actual DNA sequence and confirm that CRISPR has successfully induced mutations where we want.

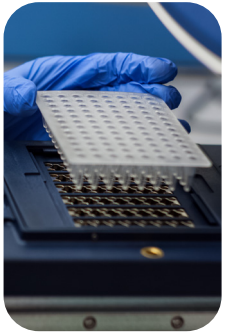


RT-qPCR

There is a lot going on with RT-qPCR, but the most important thing to remember is that the goal is to quantify RNA levels in a given sample.

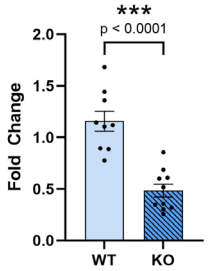
You may remember that transcription is the process where DNA is made into RNA. **Reverse transcription**, or "RT", is the opposite process where RNA is turned back into DNA (this is called **cDNA**).

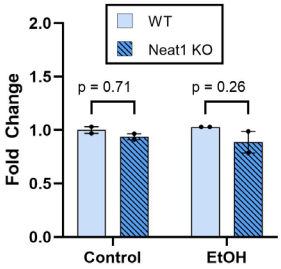
The "q" in qPCR stands for **quantitative**. Normal PCR can only be used for qualitative assessment or as a preliminary step before another experiment (such as DNA sequencing). qPCR can actually quantify how much amplification is occurring during the PCR process (this allows for comparison between samples).



The combination of these two techniques is called **RT-qPCR**. First, you turn your RNA sample into cDNA. Then, you run a qPCR with the cDNA in a special (very fancy/ expensive) machine. The machine spits out a lot of data, and the result is a quantification of cDNA, and thus RNA, levels. You can then compare quantifications between samples.

For example, I did an RT-qPCR on samples with and without *Neat1* KO (with CRISPR) to confirm that my CRISPR technique was successfully knocking out *Neat1* levels. The results (right) show sufficient *Neat1* KO. Note that WT stands for **wild-type**, which is just another term for "normal" (*Neat1* is not knocked out).





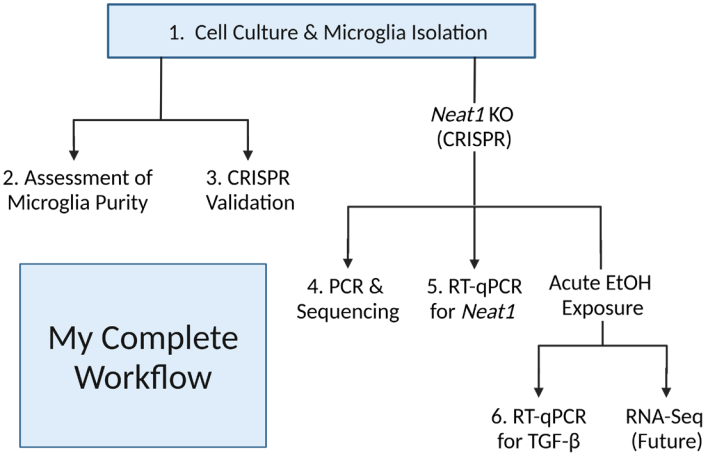
I also ran an RT-qPCR looking at levels of TGF-β RNA in different combinations of EtOH or no EtOH and *Neat1* KO or WT *Neat1*. TGF-β is a gene that is involved in neuroimmune regulation. The results (left) show that TGF-β levels do not vary significantly regardless of EtOH exposure or *Neat1* KO.

RNA Sequencing (RNA-Seq)

RNA-Seq is like RT-qPCR on steroids. After sending an RNA sample to a specialized lab, you will receive data on ALL of the genes that are present in the sample and compare their expression in different samples.

Conclusions

Most experiments described in this booklet produced the expected results. I found that I had pure microglia samples and efficient *Neat1* KO, with the latter being determined by multiple levels of validation (steps 3-5 in the flowchart below). The only step that produced unexpected results was my RT-qPCR looking at TGF-β RNA levels. I had expected to see changes in TGF-β expression following *Neat1* KO and EtOH exposure, however it turned out that there were no changes. This tells us that *Neat1* does not regulate TGF-β specifically in response to EtOH in microglia.



Future Directions (in this research and life)

Research

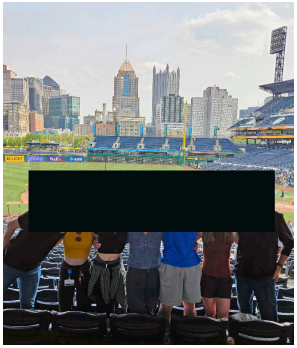
RNA-Seq is the only method in this booklet that I have yet to complete. Currently, I am assisting members of my lab in collecting RNA samples with *Neat1* KO and EtOH exposure - these will be sent for RNA-seq to determine how these two conditions change the expression of all neuroimmune-related genes (instead of just looking at one, like my RT-qPCR with TGF-β).

Life

A lot of people ask me what I will do with my college degree and research experience. Eventually, I hope to attend graduate school and earn a PhD related to molecular biology, disease research, and bridging the gap between research and medicine. This will allow me to continue to be involved in biological research and make valuable contributions to the field. However, I currently plan to take a gap year after graduating to narrow down my research interests.

Lab Members





I would like to thank Drs. [redacted] and [redacted], [redacted], [redacted], [redacted], [redacted], and [redacted] for their continued support throughout my time in the [redacted] laboratories. Without them, this research project would not have been possible.

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