Characterization of an Olfactory Subsystem in Mice

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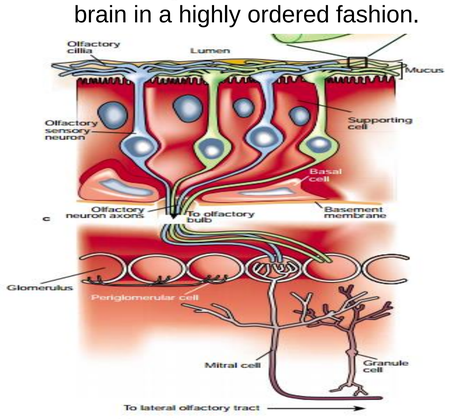
Introduction

I did my most recent coop in the Datta Laboratory at Harvard Medical School. The lab focuses its study on olfaction, and how learning about the sense of smell can translate into learning about behavior.

Olfaction is one of the most primal senses, and is vitally important in a variety of animal species. Typical olfactory information is encoded through the main olfactory system. Odorants bind to olfactory sensory neurons, each of which express only one type of olfactory receptor. The neurons that express the same receptors converge onto a single mitral cell. The synapses of the receptor neurons and the mitral cell form a glomerulus. Information is then carried from the glomerulus to the rest of the brain in a highly ordered fashion.



Main olfactory system overview. Adapted from Firestein 2001

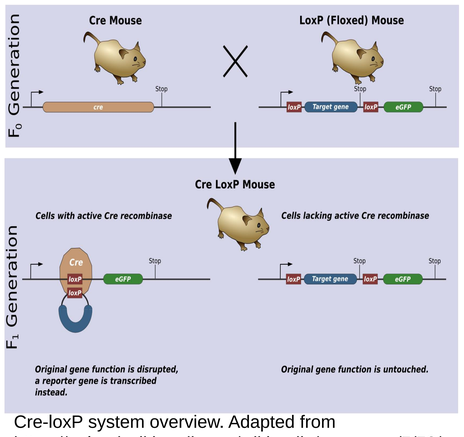
Mice have several different olfactory subsystems that are distinct from this main olfactory system. One of these is the GC-D necklace system, so named because it looks like beads strung together. The neurons in the GC-D necklace use a different signaling pathway than typical olfactory receptor neurons, and mediate specific behaviors such as carbon dioxide detection and acquisition of social food preferences. Almost nothing is known about the system's downstream targets in the brain.

The project I worked on during my coop aimed to elucidate where this necklace projects to in the brain by using both anterograde and retrograde tracing methods.

Activity

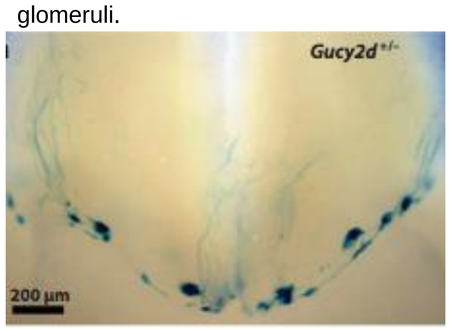
My main duty during coop was performing stereotactic injections on mice. A stereotax is a useful tool for pinpointing exact brain regions, so that reagents, like virus or dye, can be directly implanted into the region of interest.

We traced projections back to the olfactory bulb by injecting cre-dependent virus into candidate brain regions. We used mice that were cre-positive for protocadherin, a marker for mitral cells. The virus contained a cassette that would allow infected protocadherin positive cells to express green fluorescent protein.

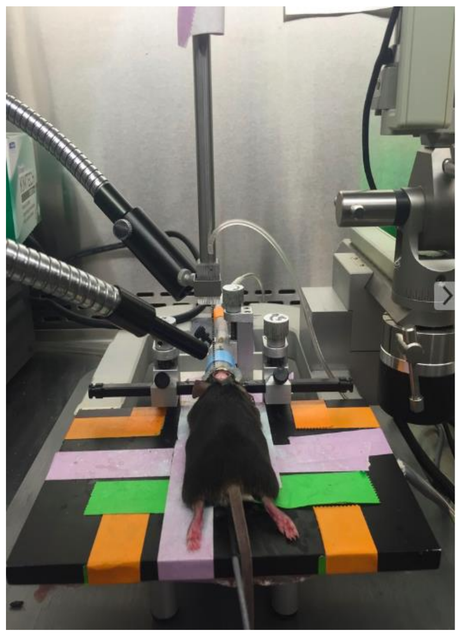


Cre-loxP system overview. Adapted from https://upload.wikimedia.org/wikipedia/commons/5/58/CreLoxP_experiment.png

After allowing the virus to infect the brain tissue for a few weeks, the mice were perfused and their brains were fixed and sectioned. After sectioning, we utilized immunohistochemistry to fluorescently stain the brain tissue, particularly the GC-D necklace glomeruli and the GFP expressing cells that were infected by the virus. The tissue was then be examined and scanned to determine if the injection worked, and if the mitral cells labeled by the virus are innervating GC-D glomeruli.



Visualization of the GC-D necklace system using X-gal histochemistry. Adapted from Leinders-Zufall et. al. 2007.



Stereotax setup used for injection of virus into candidate brain regions. Image taken by Kristen Drummey.

Reflection

I learned a lot of different techniques during this coop that I will be able to apply in a wide variety of future settings.

In addition to doing stereotactic injections, I also perfused, dissected, sectioned, and stained mouse brain tissue. I was also able to learn how to genotype a mouse colony using PCR and gel electrophoresis. Additionally, handling mice now seems like second nature.

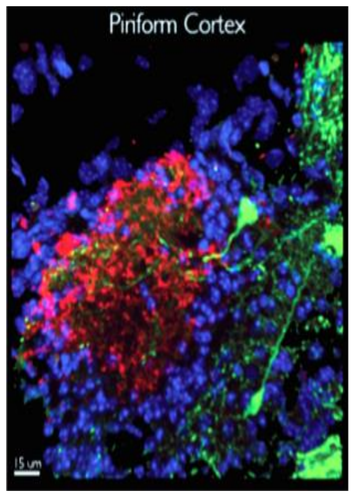
A lot of the techniques that I learned on coop are skills that I would not have gained during class. Additionally, those techniques that I had heard of in class made much more sense and seemed more relevant once they were put into the context of a real lab. Everything I learned is directly relevant to any future research in neuroscience that I would want pursue.

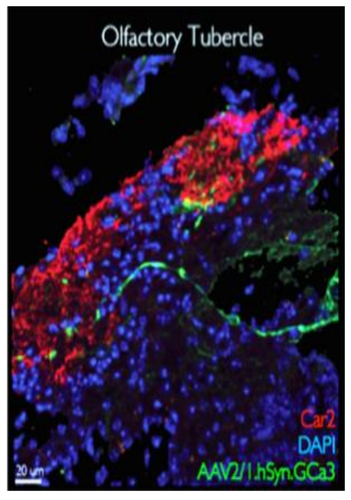
I was also fortunate enough to have a mentor who not only cared that I learned the techniques, but also that I learned the science behind what we were doing. I was able to read and discuss journal articles that contributed to my understanding of the project. Overall, I learned quite a lot on my coop that will no doubt help me no matter what I pursue in the future.

Image from https://upload.wikimedia.org/wikipedia/commons/f/f2/Lab_mouse_mg_3263.jpg

Outcomes

The project is ongoing, but we have so far been able to collect data on a few brain regions of interest, including the piriform cortex and the olfactory tubercle.





High resolution confocal images of GC-D glomeruli (red) and innervating mitral cell fibers (green). Images taken by Taralyn Tan.

Literature Cited

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Munger, S. D., Leinders-Zufall, T., & Zufall, F. (2009). Subsystem organization of the mammalian sense of smell. *Annual Reviews of Physiology, 71*, 115-140.

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Acknowledgments

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