

Effects on neuronal activity through the deletion of primary cilia

Abstract

Primary cilia are microtubule based cellular organelles that regulate cell signaling and the cell cycle through various ciliary components, and are present in almost all cell types, but are most commonly found in epithelial cells, and play an important role in the WNT and the Sonic Hedgehog (SHH) pathways. [1] The transition fibers of the cilia are the docking site of the intraflagellar transport (IFT) particles, which is responsible for transporting proteins into other ciliary compartments. [3] Deletions found in cilia genes often result in loss of primary cilia expression and cause changes in the structure and function of the cilia gene, resulting in neurological disorders in the brain. [8] I hypothesize that the removal of cilia specifically in the prefrontal cortex of the brain would result in cognitive impairments and uncover the role that cilia may play in this region of the brain.

Background:

Primary cilia (PC) are microtubule organelles that detect extracellular cues, and control physiological processes by regulating cell to cell communication and maintaining energy for homeostasis via the cell cycle. [3] The cilia is enclosed with a membrane that allows the transmembrane receptor located on the cilium membrane to detect changes in the extracellular environment, and send information to nearby cells through cell to cell communication. [4] PC also play an important role in neuronal pathways such as Sonic hedgehog (SHH) and WNT pathways, and utilize a set of motor transporters called intraflagellar transport 88 (IFT88), which is essential for the assembly and function of primary cilia and mediates key signaling pathways. [5] The IFT88 mice possess loxP sites flanking exons 4-6 of the intraflagellar transport 88 gene, which mediates bidirectional movement of proteins along microtubules, and are separated into

large complexes of proteins. [3] The proteins of the IFT particles are transported along axonemal microtubules by kinesin 2 motor proteins in the anterograde (base to tip) direction and are delivered to the tip by cytoplasmic dynein 2 in the reverse direction (tip to base). [6-7]

There have not been many studies on how the brain will respond to the removal of cilia in the prefrontal cortex of the brain. Many previous studies have focused on mutations present in the IFT88 gene and its effect on SHH and WNT neuronal signaling. By studying the effects of the deletion of the IFT88 gene on the Shh and WNT signaling pathways, we can understand the role cilia plays on the prefrontal cortex of the brain and learn how the deletion of cilia in this specific region affects the brain.

Material and Methods

Animal Preparation

I will use 8 week-old *Ift88* flox/flox obtained from the Jackson laboratory. These *Ift88^{fl}* mice possess *loxP* sites flanking exons 4-6 of the intraflagellar transport 88 (*Ift88*) gene. Mice will be split into two groups (n=10), where the cre recombinant virus will be delivered to the prefrontal cortex via stereotactic injection in controls and experimental animals.

Immunocytochemistry

Some mice will be perfused with 4% paraformaldehyde, and we will harvest mice brains and frontally section the brain at 20 μ m. I will stain the sections with anti IFT88 antibody to confirm the deletion of cilia at the injection site and measure the length of the cilia. I will also stain with anti-c-fos in various regions of the brain, which includes the prefrontal cortex, striatum, nucleus accumbens, ventral tegmental area, and hippocampus to see neural activity as a result of removing cilia.

Imaging

After performing immunohistochemistry, I will mount the brain sections on slides and perform fluorescent imaging. I will utilize the microscope Keyence BZ-9000 to take images of the brain sections. The images will be stitched using the BZ-9000 analyzer and the Mouse Brain Atlas to observe the immunofluorescence of the brain and confirm the specific regions of the brain.

My Responsibilities

My responsibility will be to perform stereotaxic injections in specific brain regions of the mice. I will then perfuse the mice, and perform cilia staining on the tissues. I will then image the stained tissues using a fluorescent microscope and analyze the collected brain sections. Lastly, I will regularly report to my mentor and principal investigator on the progress of my project and any struggles or findings.

Timeline

The total amount of time needed to complete the following proposal is about 4 months. Animals will be ready to use at the beginning of Summer quarter. Stereotaxic surgery will require a total of 3 weeks. About one week for the surgery and another week for the animal's recovery. Once recovered the animals will be perfused and the brains will be cut and stained, I allotted about 4 weeks to complete this. Another 4 weeks will be dedicated to imaging and analysis.

Itemized Budget

Rabbit IFT88 Antibody	\$ 450
Cfos Antibody	\$450
Goat anti-rabbit Secondary Antibody	\$ 165
Animal Maintenance	\$ 300
Cre recombinant adenovirus	\$475
Total	\$1,840

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