Identification of a Potential A8 Dopamine Projection for Functional Control in Cre Mice

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Introduction

My research seeks to understand the function of the A8 region of the brain in mice. Currently, we believe it plays a role in functional control and motivational behaviors like hunger. Motivational behavior controls how driven an animal will be for certain tasks, such as food, water, or and things necessary for survival. In mice, the Ventral Tegmental Area (VTA) and Nucelus Accumbens (NAc) are two structures in the brain already known to regulate motivational behavior already. The VTA is known for regulating reward consumption and the NAc is a mediator of motivational and emotional processes. Dopaminergic neurons synthesize the neurotransmitter dopamine which modulates motivational behavior – these dopaminergic neurons make up more than 65% of the VTA's neurons (Bouarab et al., 2019). Past research of dopaminergic neurons found they play an important role in behavioral processes such as reward, addiction, and stress (Chinta & Andersen, 2005). In this study, we will be stimulating the A8 region of the brain in mice to study its effect. A8 is in the midbrain reticular formation and is dorsolateral to the substantial nigra. To visualize these dopaminergic cells and their connections, we will be using Tyrosine Hydroxylase (TH), an enzyme involved in the synthesis of dopamine and norepinephrine.

Discussion

The AAV anterograde virus is a non–enveloped, single stranded virus that delivers DNA to target cells, allowing visualization of the neurons axons, which are the nerve cells that carry the electrical impulses away from the cell body. Axons are very long and transport essential molecules and signals. Since this AAV anterograde virus only shows the projections from the brain area, it's only a tool for mapping or tracing projections. The sucrose solution helps prevent ice crystals forming in tissues when the brain is frozen in the cryostat, which is an essential step before the brain can be sliced into 30 micrometer sections to analyze. The sucrose solution disrupts the interactions between the polar water molecules – without it, water within the tissue would form ice crystals and shred the tissue, causing holes in the brain slices.

Methods

Surgery

An anterograde virus is used to map the neural projections of the dopaminergic neurons in the A8 region of the brain of TH-Cre mice. A Hamilton Syringe was used to measure and inject precisely 50 nanoliters of the virus. The two injections were done at the following coordinates – y=-4/05 and –4.1, x= 0.98 and –1.03, and z= 4.1 and 4.3. The mouse was given 100mg/kg ketamine for anesthesia and 5 mg/kg meloxicam to reduce pain and discomfort. After injection, we waited 10 minutes to allow for viral diffusion and then sutured the mouse and monitored it post-surgery to make sure it recovered properly.

Perfusion

The mouse was given a triple dose of ketamine to euthanize it, then perfused it with saline first, then 4% Paraformaldehyde. The perfusion began in the heart, flowing through the rest of the organs following the natural channels. This allows the blood to be cleared from the body and preserves the brain for immunostaining, which allows us to study the neural projections in regions of the brain. PFA can cross-link proteins and DNA molecules to preserve the tissue and cell structure of the brain.

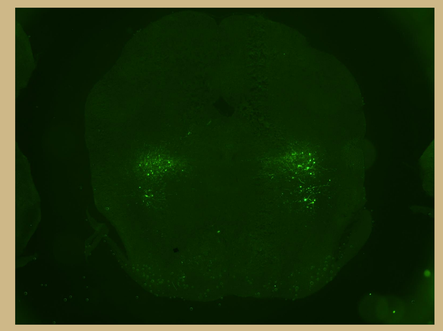
Mounting

After the body is perfused with PFA and the blood is cleared from the body, the mice is decapitated, and the brain carefully removed. It's placed in a small jar of PFA overnight to post-fix, which will allow the brain to shrink, harden, and darken. After 24 hours, the solution is changed to sucrose, which is a cryoprotectant. The brain is then placed into a cryostat, which is microtome machine for cutting tissue at low temperatures. The brain is sliced into 30 micrometer sections and collected in different sections based on brain regions.

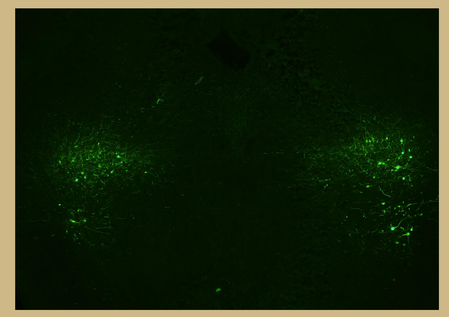
Staining

The brain slices are studied under an epifluorescent microscope to see where the virus and its connections are present. Anterograde viruses show up as green under epifluorescent microscopes and thus we would use an atlas of the brain to study what regions the fluorescent, and therefore virus, are in.

Results



A8-TH-EGFP injections



Future Research

Future studies can utilize special techniques like optogenetics – a technique that modulates the activity of excitable cells using light – to test the food motivation in operant chambers. Additionally, other viruses such as AAV-ChR2 – which can activate neurons with a laser – can be used to study the effects of neuron activation on behavior specifically. In this study, the injections from are too posterior and dorsal to the A8 regions, which contribute to the lack of fluorescent, and can be redone and corrected in future trials.

References & Acknowledgements

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