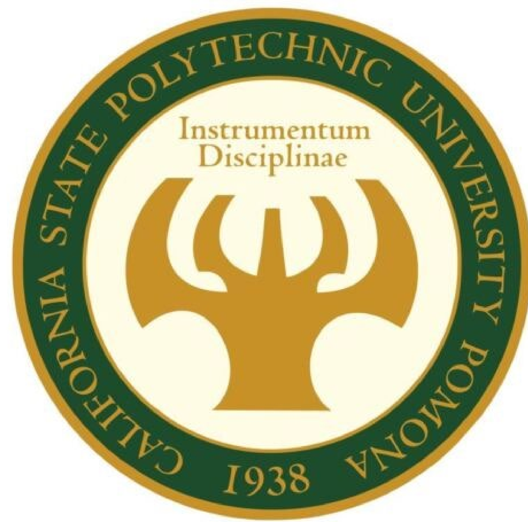


Characterization of an novel adeno-associated virus across varied genetic backgrounds in mice

Irene B. Tran[1]
Faculty Mentor: Damien Wolfe[2], Andrew D. Steele[1]*, Tim Shay[2]

[1]Department of Biological Sciences, California State Polytechnic University Pomona; Pomona, CA; USA, *Corresponding author
[2]Division of Biology and Biological Engineering, California Institute of Technology; Pasadena, CA

Abstract

Adeno associated viruses (AAV) are viruses that are non-pathogenic and therefore can be used as vehicles for genetic cargo delivery, without causing significant damage to the organism. Naturally occuring AAVs are used to engineer variants through a process called directed evolution with the goal to target specific cell types, such as neurons and glial cells, in the brain for gene therapy. To test the efficiency of variant "AAV9-150", an engineered virus that packages the gene for GFP, a series of three genetically different inbred strains of mice (C57BL6/J, NOD, and DBA/2) were injected retro-orbitally with AAV9-150. The mice were incubated for three weeks, and then euthanized, transcardially perfused, and fixed for the collection of the brain and liver. The brains and livers were then sectioned and stained using immunofluorescence to mark neurons, glia, and endothelial cells. GFP was used as a reporter gene to visualize successful viral delivery. A confocal microscope was then used to collect images for visualization and quantification. Through engineering, qualitative, and quantitative analysis we were able to characterize the expression pattern of AAV9-150, which revealed that this variant infected endothelial cells in the brain but was not successful in infecting neuronal or glial cells.

Introduction

Figure 1: Inbred mouse strains of different origin. A) DBA/2J mice (gray). B) C57BL/6J mice (black). C) NOD/ShiLtJ mice (white).

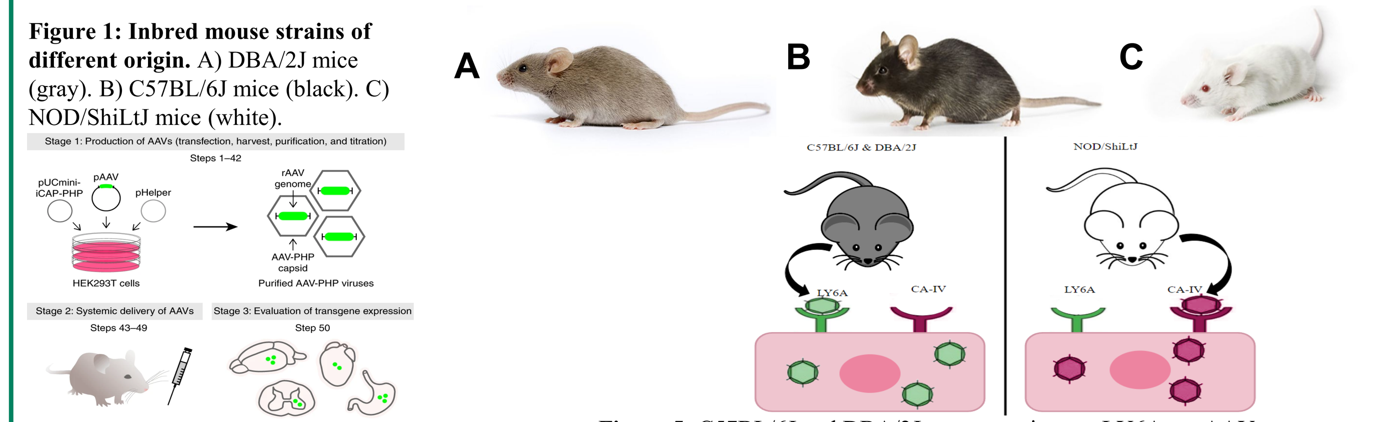


Figure 2: Production of engineered AAV's, a systemic delivery and evaluation of expression in the brain (Challis, R.C., et al. 2019 Systemic AAV vectors for widespread and targeted gene delivery in rodents. *Nat Protoc* 14, 379–414)

Figure 5: C57BL/6J and DBA/2J mouse strains use LY6A, an AAV receptor to which AAV vectors bind, facilitating the crossing of the blood-brain barrier. This mechanism is nonfunctional in the NOD/ShiLtJ strain. Instead, NOD/ShiLtJ utilizes carbonic anhydrase IV (CA-IV) as an AAV receptor to facilitate the crossing of the blood-brain barrier. (Shay, Timothy F., et al. 2023 Primate-conserved carbonic anhydrase IV and murine-restricted LY6C1 enable blood-brain barrier crossing by engineered viral vectors. *Sci. Adv.* **9**.).

Figure 3: Retro-orbital Systemic Injection - noninvasive, simple procedure, systemic spread throughout the body/brain, potential liver toxicity at high doses

AAV9 capsid
Distance from center (Å) 110–140
Interaction between monomers
AA449–468 - VR IV, monomer 1
AA579–594 - VR VIII, monomer 1
AA487–504 - VR V, monomer 2

Figure 4: The AAV9 capsid proteins amino acid structure is engineered to contain random additional amino acids and then selected through directed evolution to obtain AAVs that have better delivery to the brain (Goertsen, D., et al., 2022 AAV capsid variants with brain-wide transgene expression and decreased liver targeting after intravenous delivery in mouse and marmoset. *Nat Neurosci* 25, 106–115).

- A biochemical approach was taken to select novel AAVs based on known receptor binding assays
- The pipeline includes characterizing these viruses and identifying their tropism by using mice as an animal model
- Three genetically different mouse strains were chosen to characterize the expression patterns of our virus: C57BL/6J, DBA/2J and NOD/ShiLtJ mice, representing the different types of receptors.
  - Our objective is to engineer a virus that can be broadly useful for gene therapy, specifically one capable of effectively crossing the blood-brain barrier across diverse species.
- An engineered AAV virus encapsulated the gene for green fluorescent protein (GFP), functioning as a reporter gene to visualize the successful delivery of the virus.

Methods

- We use HEK293 cells in order to package our capsid and cargo together so that we can produce enough virus to inject at a dose of 3.00E+11 vg (viral genomes) per mouse
- The virus was administered using retro-orbital injection and after 3 weeks of incubation the mice are perfused and the organs collected
- We took the brains and sectioned 100 micron sagittal slices and stained them using the cell markers NeuN (neurons) and s100B (astrocytes), and GLUT1 (endothelial cells).
- They were then imaged on a Nikon Confocal microscope and the images were quantified for co-staining of GFP and the cellular markers

Results

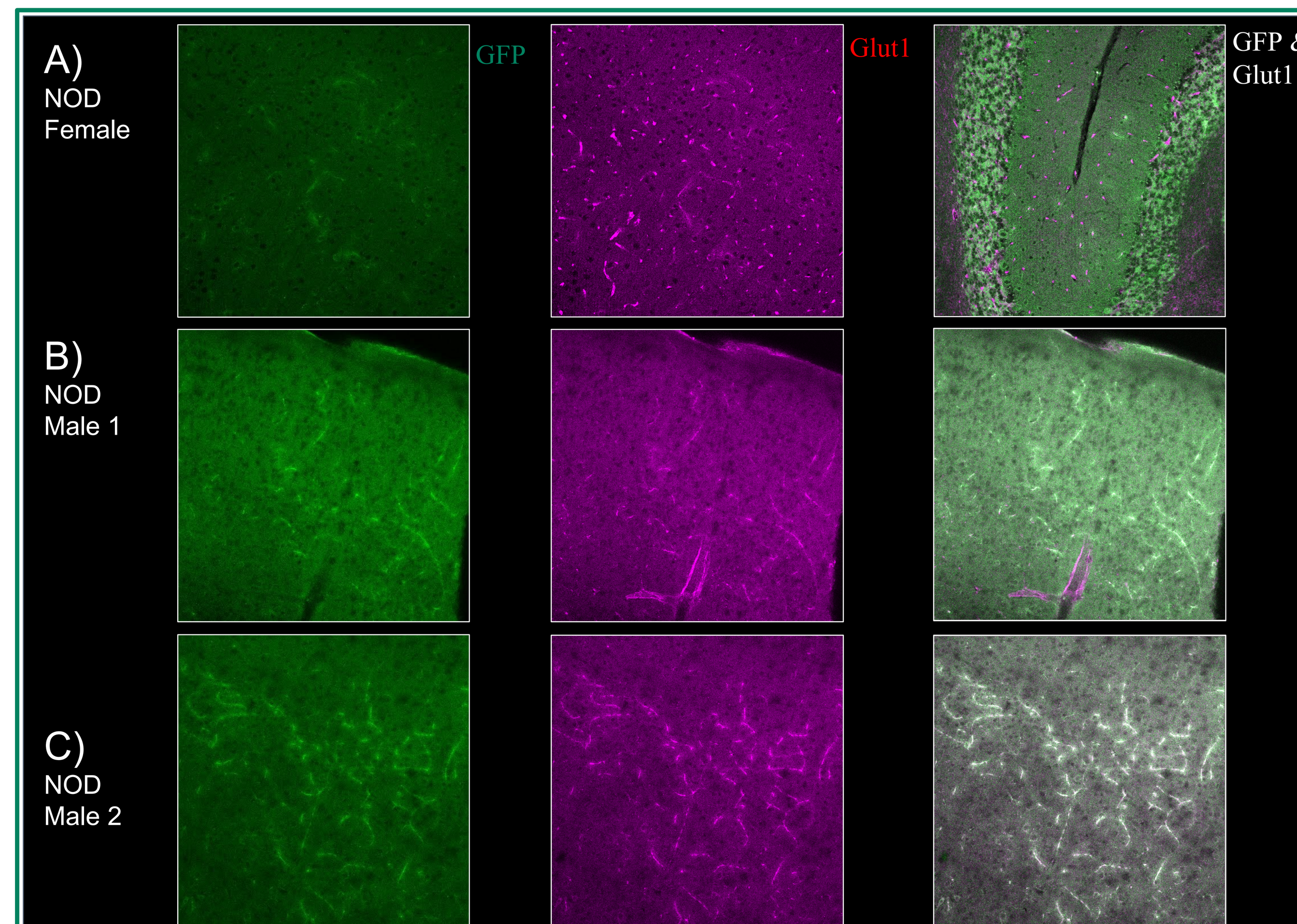


Figure 6: Staining for neuronal marker in the cortex to characterize the expression pattern of AAV9-150 in NOD: Confocal images were taken after using IF to stain for endothelial cells using Glut1 (red) to analyze the expression pattern of the AAV9-150 capsid reporter gene GFP (green).
A) NOD Female
B) NOD Male 1
C) NOD Male 2

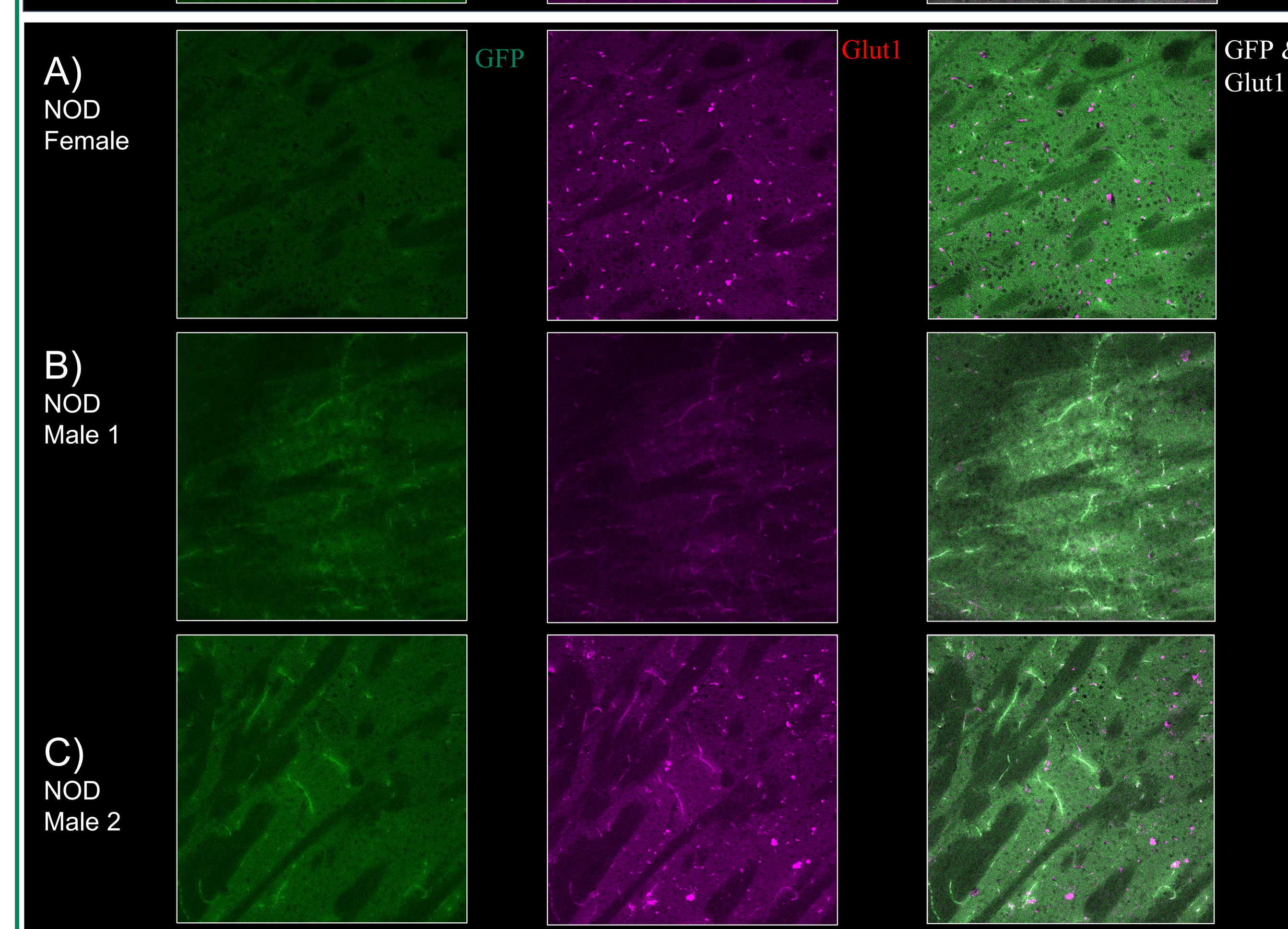
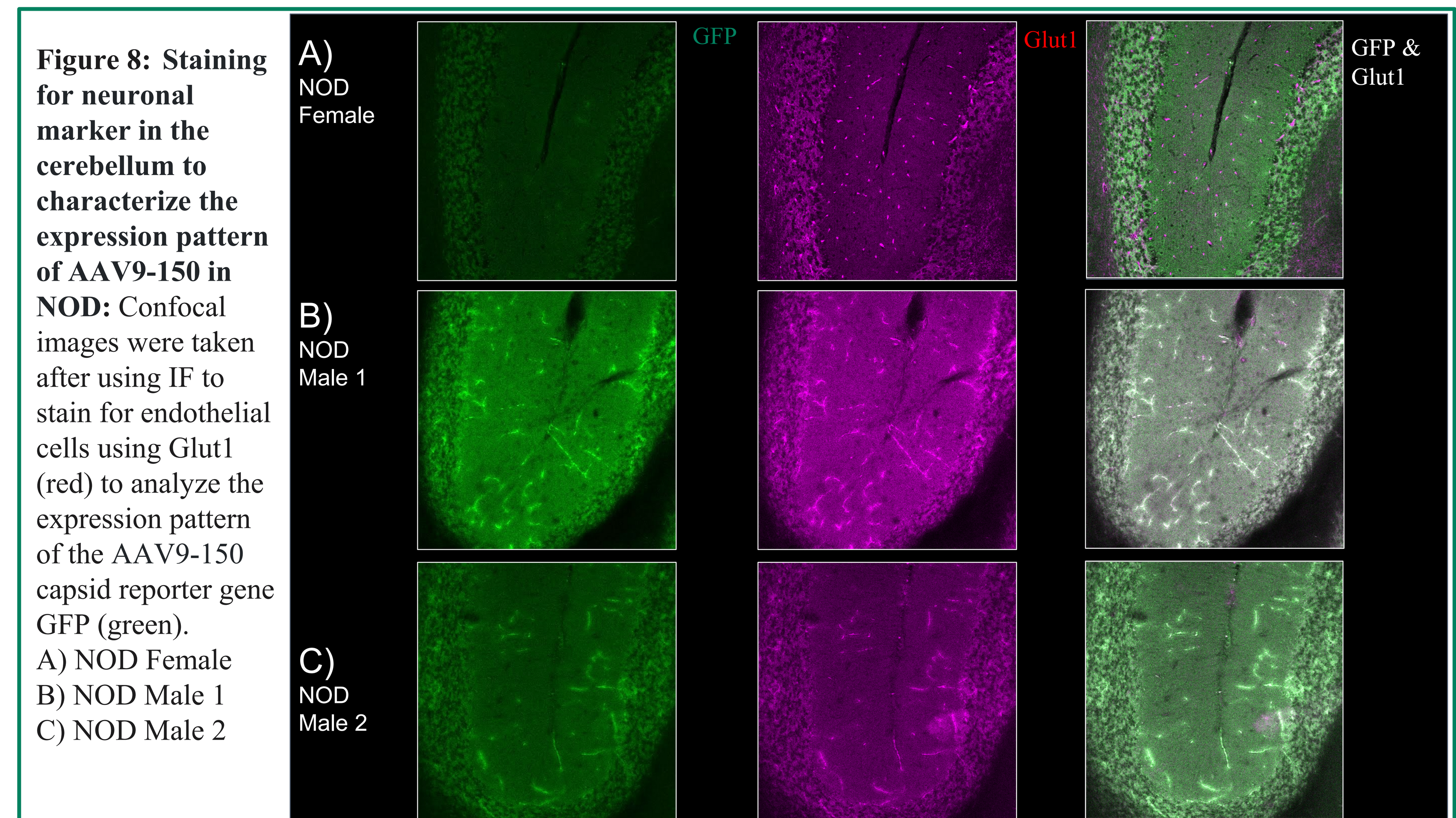


Figure 7: Staining for neuronal marker in the striatum to characterize the expression pattern of AAV9-150 in NOD: Confocal images were taken after using IF to stain for endothelial cells using Glut1 (red) to analyze the expression pattern of the AAV9-150 capsid reporter gene GFP (green).
A) NOD Female
B) NOD Male 1
C) NOD Male 2

Figure 8: Staining for neuronal marker in the cerebellum to characterize the expression pattern of AAV9-150 in NOD: Confocal images were taken after using IF to stain for endothelial cells using Glut1 (red) to analyze the expression pattern of the AAV9-150 capsid reporter gene GFP (green).
A) NOD Female
B) NOD Male 1
C) NOD Male 2



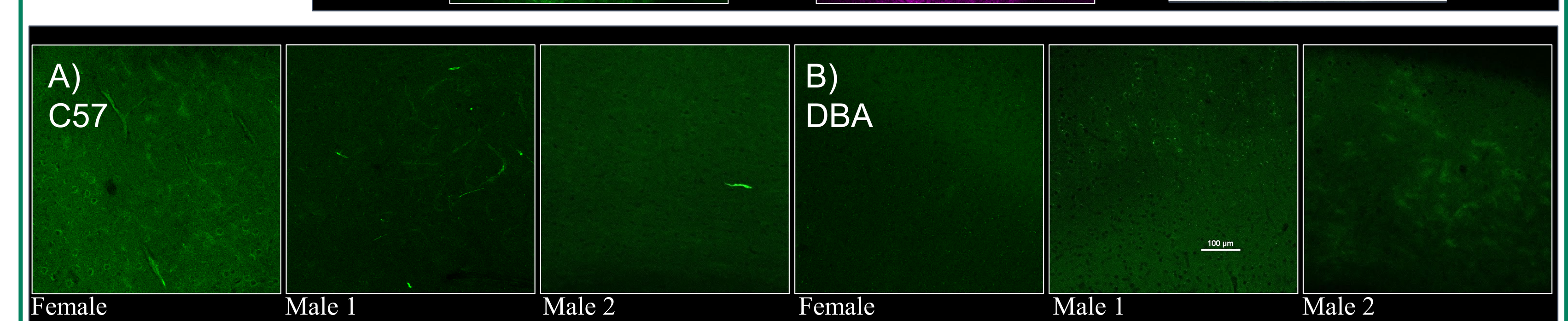


Figure 9: AAV9-150 GFP expression in the cortex across C57 and DBA mouse strains: Confocal images were taken after using IF to to analyze the expression pattern of the AAV9-150 capsid reporter gene GFP (green). A) C57 B) DBA

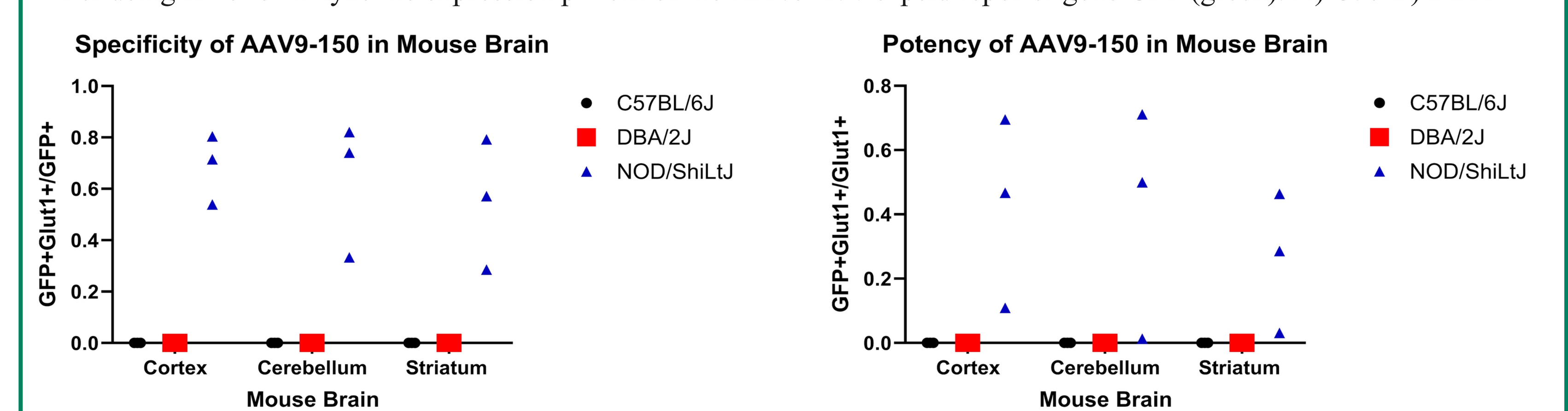
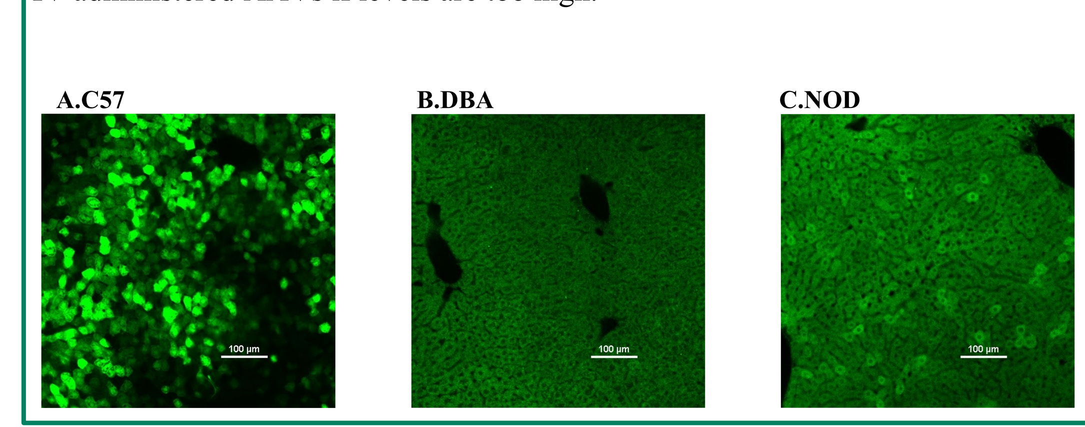


Figure 10: Quantified specificity of AAV9-150 in the cerebellum, cortex, and striatum within the genetically different mice strains

Figure 11: Quantified potency of AAV9-150 in the cerebellum, cortex, and striatum within the genetically different mice strains

Liver Expression

Figure 12: AAV9-150 expression in livers across mouse strains: Magnified images of mouse livers across our 3 strains. Presence of GFP indicates transduction of liver cells which is common and can potentially be cytotoxic in IV administered AAVs if levels are too high.



Conclusion & Future Direction

- AAV9-150 successfully infected endothelial cells in the brain but was unsuccessful in infecting neuronal or glial cells.
- AAV9-150 utilized CA-IV to cross the blood-brain barrier as it only showed expression in NOD mice.
- Currently, we are using a different pool of AAVs selected through **M-CREATE** method (**M**ultiplexed **Cre** **RE**combination-based **A**AV **T**argeted **E**volution) for the next patch of viruses
  - A technique developed to evolve and engineer viruses in order to deliver genetic cargo to cells of a specific cell type
- We are using a cellular infectivity assay as an intermediate step by using HEK cells transiently transfected with receptors to test the viral transduction of an AAV before moving on to in vivo characterization of the virus.

Acknowledgements

We would like to thank the California State Polytechnic University Department of Biological Sciences for their support in our research. In addition, we would like to acknowledge Cindy Tessler and the Animal Care Facility staff for their assistance in the care and maintenance of our research animals. Funding for this research has been provided by the NIH Brain Initiative award 1U24MH131054. Additional thanks to Dr. Viviana Gradinaru, Steele lab, and CalTech

Key Sources

Challis, R.C., Ravindra Kumar, S., Chan, K.Y. et al. Systemic AAV vectors for widespread and targeted gene delivery in rodents. Nat Protoc 14, 379–414 (2019).
Goertsen, D., Flytzanis, N.C., Goeden, N. et al. AAV capsid variants with brain-wide transgene expression and decreased liver targeting after intravenous delivery in mouse and marmoset. Nat Neurosci 25, 106–115 (2022)