Evaluating AAV Tropism for Neonatal Mouse Cerebellum

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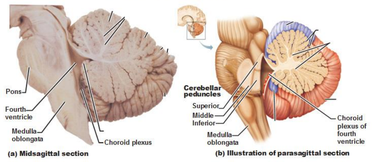
Abstract

The cerebellum is a vital area of the brain that receives sensory information and helps regulate motor movements such as posture, balance, coordination, and speech. More recently the cerebellum was discovered to play a role in emotion as well. Damage to the cerebellum and loss of some of these functions can occur as a result of neurodegenerative disorders, such as Alzheimer's Disease. Problems with coordination that occur in later stages of Alzheimer's have been linked to amyloid plaque build-up in areas of the brain, including the cerebellum. Adeno-associated virus (AAV) has the potential to be a successful gene therapy for Alzheimer's disease as well as many other neurodegenerative disorders. AAV is a non-pathogenic virus that has been used as a tool in gene therapy and in creating isogenic human disease models. Recombinant AAV (rAAV) is used for gene delivery because it can target specific tissue types through rAAV transduction. There are different serotypes of AAV that may have different efficacies in different areas of the brain. The purpose of this study was to compare AAV1, AAV5, AAV6, and AAV8 and evaluate each AAV serotype tropism in the cerebellum.

The Cerebellum



Introduction

Alzheimer's disease is a neurodegenerative disease that causes dementia. Alzheimer's disease causes problems with memory, cognitive skills, and behavior. The level of these symptoms increase over time, and eventually interfere with important daily tasks. Those with Alzheimer's have a buildup of beta-amyloid plaques, which are protein fragments that end up damaging and killing nerve cells when they are built up.. The cause of Alzheimer's disease is unknown and, at the moment, there is no cure either. Research that focuses on the areas of the brain that are affected by these beta amyloid plaques may help shed light on the cause and a cure for Alzheimer's disease. The cerebellum is an important part of the brain that plays a role in motor control and cognitive functions. As previously mentioned, decrease in cognitive function is a result of Alzheimer's disease. Therefore, learning what can be used to treat the beta amyloid plaques in this area of the brain can improve treatment for those with Alzheimer's disease.

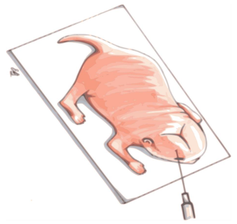
Aim

The aim of this study was to evaluate the level of expression of multiple Adeno-associated viruses (AAV) on the cerebellum in neonatal mice. The results of this study would provide insight into targeting cerebellum that have been adversely affected by Alzheimer's disease.

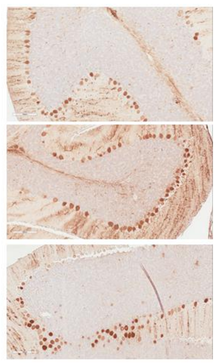
Methodology

- Capsid serotypes 1, 5, 6, and 8 were evaluated through a microscope at 4X, 10X, and 20X.
- rAAV vectors were utilized which encoded an EGFP protein driven by a CBA promoter
- Vectors were then sent towards the cerebellum of the mouse
- Analysis of the pictures were based on the brown color; a stronger and more apparent brown color means a stronger expression of the AAV in the cerebellum

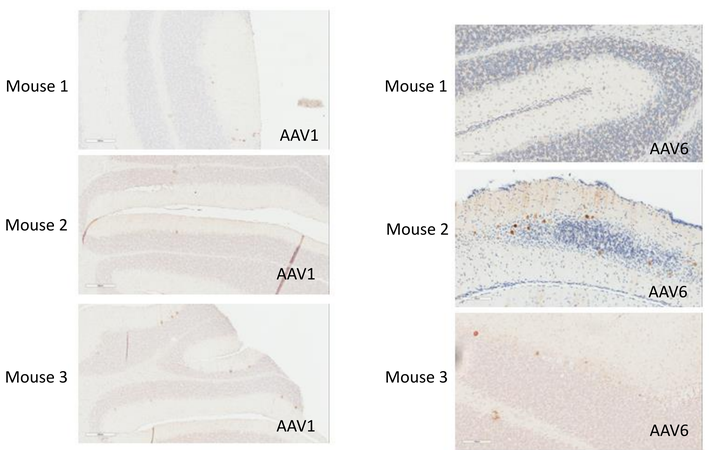
Surgical Procedure

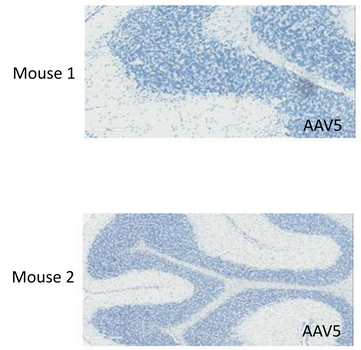


Strongest AAV Expression



Other AAV Expression





Conclusion

- AAV8 demonstrated the strongest expression through viral cells because there was a large amount of "brown" in all three of the pictures taken and shown in the previous section.
- AAV1 and AAV5 provided the weakest form of expression because there was almost no "brown" in any of the mice cerebellum.
- AAV6 had a few areas of "brown", especially in mouse 2, however, it was still a significantly weaker expression than that of AAV8.
- Overall the most optimal vector for successfully reaching the cerebellum was AAV8, therefore, it is the most effective method of protein transduction out of the 4 AAVs used in this study.

Future Work

Using AAV8 to successfully transduce proteins into the cerebellum may help in repairing cells in this area for those with Alzheimer's and other neurodegenerative disorders with symptoms in the cerebellum. The ability to regenerate and repair of the nerve cells that have been damaged in the cerebellum could possibly serve as a form of treatment for those with Alzheimer's disease.

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