THE MICHAEL J. FOX FOUNDATION
FOR PARKINSON'S RESEARCH

Update Regarding MJFF & UNC-generated AAV Viral Vectors -- April, 2013

Based upon more extensive, long-term analyses using these viral vectors, Drs. Ron Mandel and Deniz Kirik determined that the extent of neurodegeneration elicited varies between the AAV2 vs. AAV5 viral vectors encoding alpha-synuclein. Additionally, transduction of cells with AAV5-eGFP induced a greater loss of TH expression than did AAV2-eGFP. The reason for this is not clear though transduced neurons in both cases retained eGFP expression at both 4 and 12 week time points.

The purpose of sharing this data is so that investigators requesting these viral vectors can appropriately design their own studies and utilize the vectors in paradigms that are most suitable (in vitro vs. in vivo). The following pages outline the experimental design employed and the results of this characterization effort.

Stereological assessment of dopamine neuron loss in response to rAAV-α-synuclein/rAAV-GFP injections in the SNc.

Animal Groups and AAV Vectors:

rAAV Vector Production:

- rAAV vectors were constructed and titered at UNC.
- Vectors were used in stock, undiluted form.

Surgical Procedure:

2 µl of vector solution was injected unilaterally into the right SNc of female Sprague-Dawley rats at the following coordinates (coordinates should be confirmed in PI's own laboratory):

AP (Bregma)	ML (Bregma)	DV (Dura)	Tooth Bar
- 4.8	- 2.0	- 7.2	- 2.3

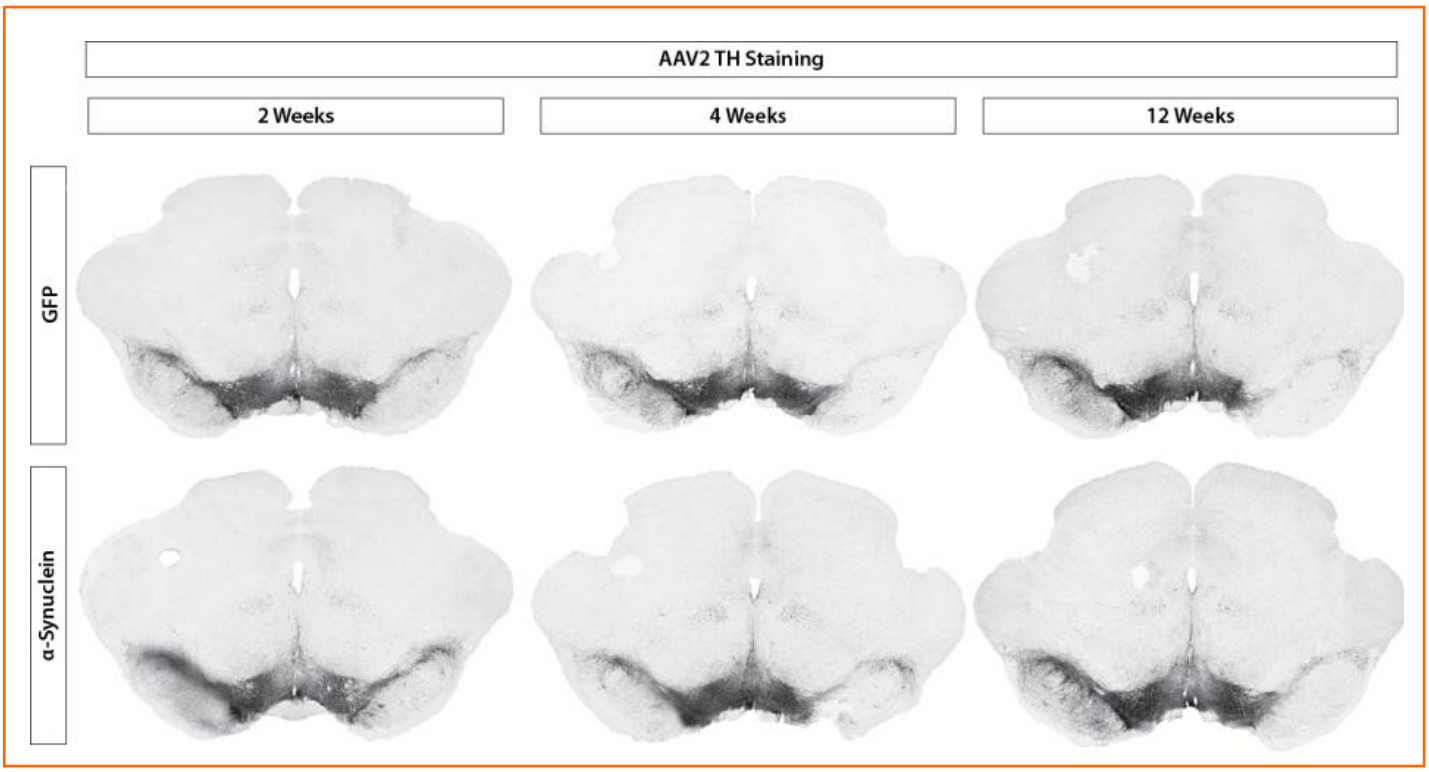
- Vectors were injected at a rate of 0.4 µl/min (5 minute total injection) using a 5 µl Hamilton syringe fitted with a 60-80 µm-diameter glass capillary.

Post-Mortem Tissue Analysis:

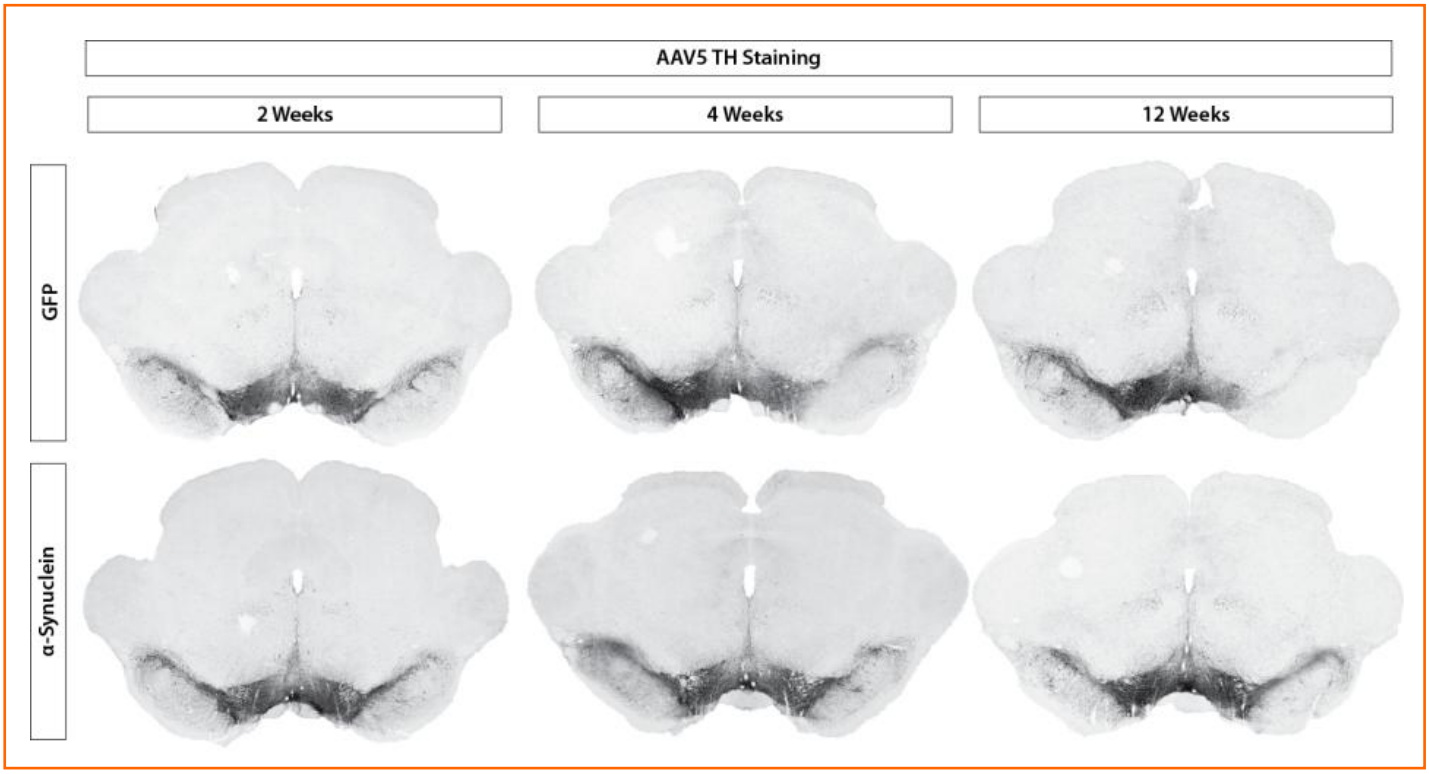
- Perfused brains were sectioned at 35 µm and collected in 8 series.
- Striatal and SN sections were stained for GFP or α-synuclein, and TH.
  - These sections were all sent to Lund
- Stereological assessment of SN dopaminergic neuron loss using TH
- Striatal and SN sections were stained for TH using IR secondary.
  - These sections were all sent to UF
- Densitometric analysis of TH in striatum and SN using LiCor Odyssey
- Striatal DA content analysis of 12 week animals (Lund)
- Western analysis of α-synuclein 4 week animals for over-expression (UF)

Vector	Injected Titer (% of Stock Titer)	Analysis	*N*
rAAV2-CBA-eGFP Stock Titer: $8.1x10^{12}$ vg/ml Production Date: 14/12/2011	100	Histology/Stereology 4 and 12 Weeks Post-Injection	16
rAAV2-CBA-α-Synuclein Stock Titer: $1.5x10^{13}$ vg/ml Production Date: 27/10/2011	100	Histology/Stereology 4 and 12 Weeks Post-Injection	16
rAAV5-CBA-eGFP Stock Titer: $9.5x10^{12}$ vg/ml Production Date: 9/2/2012	100	Histology/Stereology 4 and 12 Weeks Post-Injection	16
rAAV5-CBA-α-Synuclein Stock Titer: $1.0x10^{13}$ vg/ml Production Date: 16/11/2011	100	Histology/Stereology 4 and 12 Weeks Post-Injection	16

In vivo nigral TH+ neuron survival results using AAV2 Viral Vectors

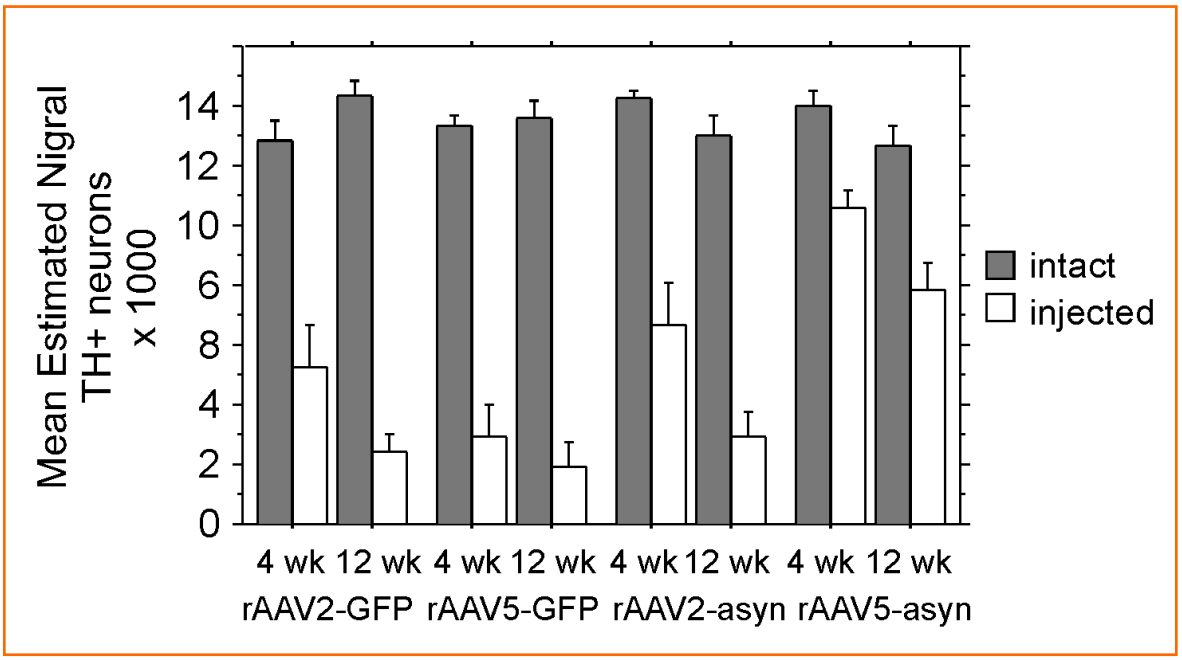


In vivo nigral TH+ neuron survival results using AAV5 Viral Vectors

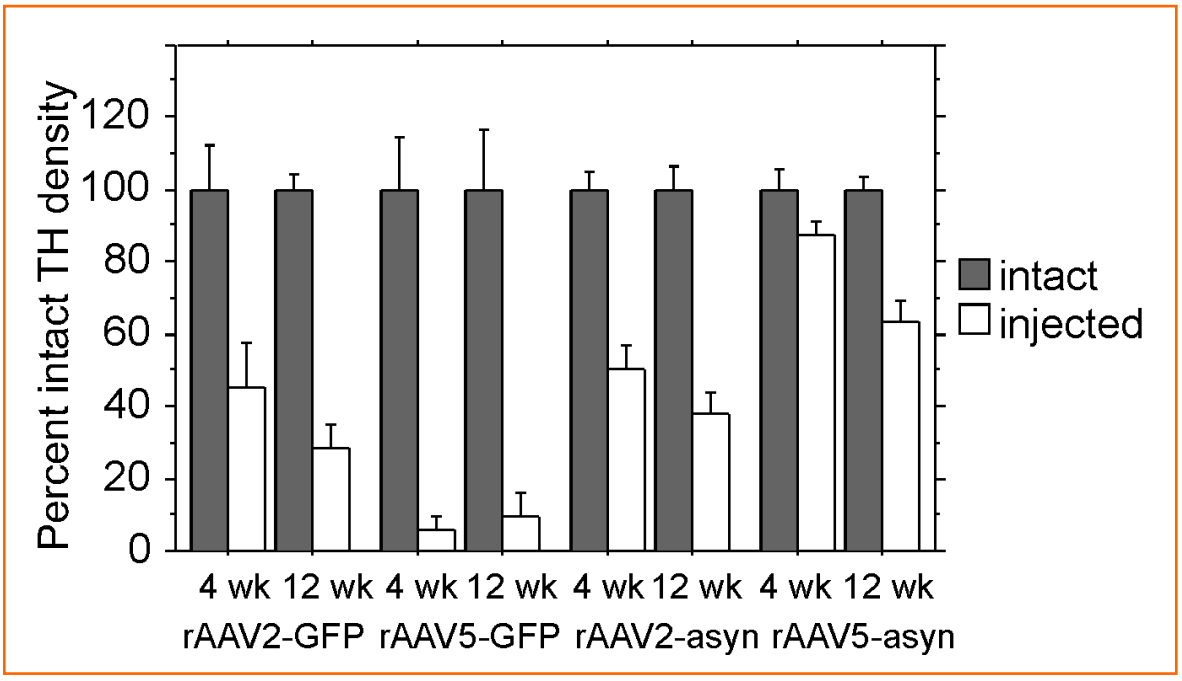


In vivo nigral TH+ neuron survival results using AAV2 or AAV5 Viral Vectors – TH Staining

Substantia Nigra

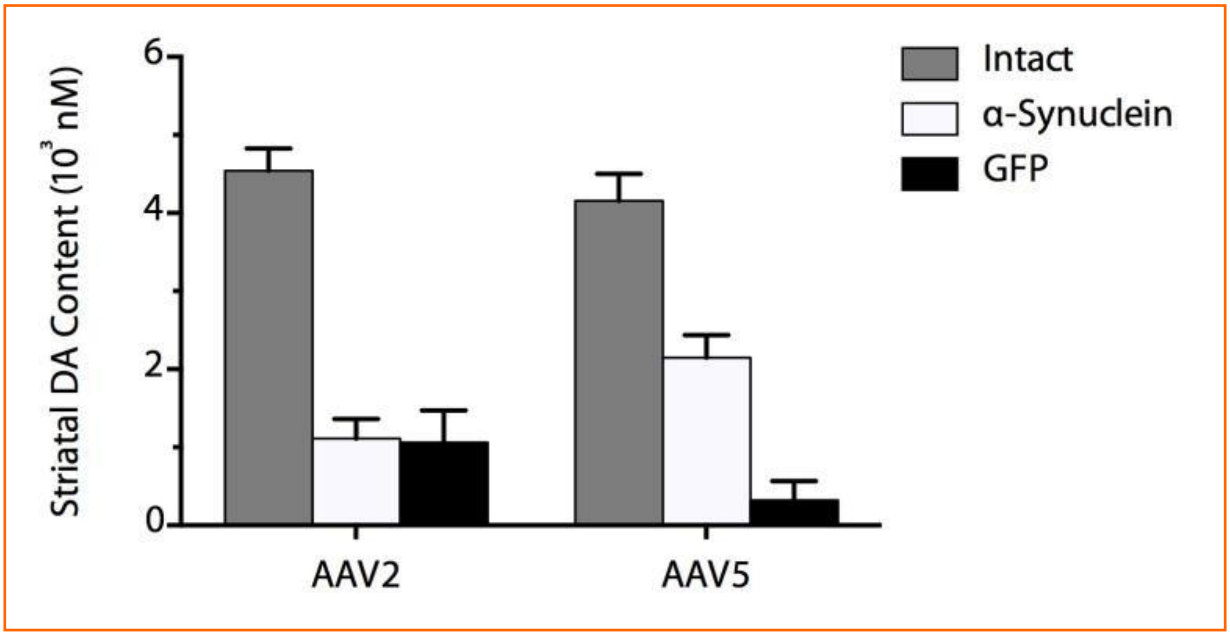


Striatum



In vivo nigral TH+ neuron survival results using AAV2 or AAV5 Viral Vectors – Dopamine Content

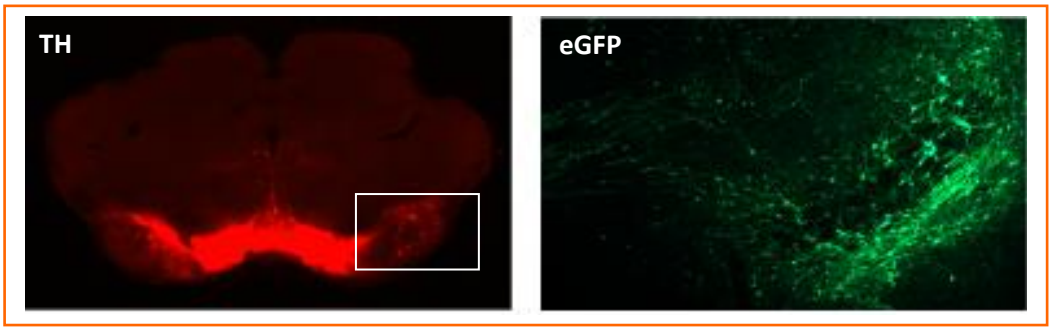
Striatal DA content -- 12 week survival



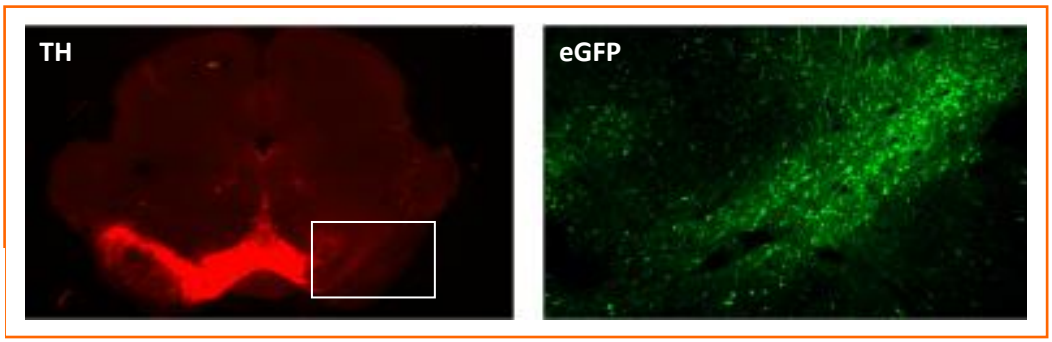
Loss of TH staining in neurons expressing does not correlate with cell death

Inspection of native GFP fluorescence in infrared stained sections reveals cells still expressing GFP*

4 week survival:
AAV2-eGFP



12 week survival:
AAV5-eGFP



**This indicates transduced cells are still alive. TH images taken with a scanner*

CONCLUSIONS

- **rAAV2-α-synuclein recapitulates the model better than rAAV5-α-synuclein**
- **rAAV2-GFP and rAAV5-GFP both cause significant loss of TH expression and DA**
- **Vector titer is not correlated to toxicity of α-synuclein or TH down-regulation by GFP**
- **In the case of α-synuclein, TH densitometry is highly correlated to nigral TH+ cell counts, GFP expression does not follow this pattern**
- **These studies are meant to serve as a guide and Investigators should optimize conditions in their own laboratory**