Verification of Viral Vectors

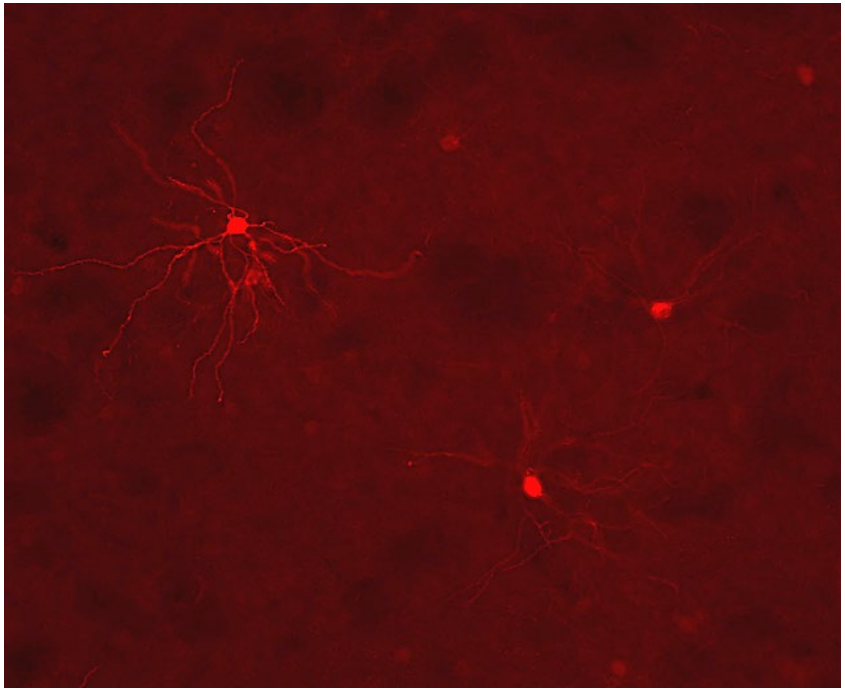
UW Neurological Surgery Summer Student Program
Allen Institute for Brain Science
CJ Andrews & Haleigh Schwartz

ALLEN INSTITUTE for BRAIN SCIENCE

(Doughton, 2015)

"Our brains are what make us human… Yet despite decades of research—and impressive knowledge gathered about other aspects of the human body, including our entire genetic sequence—the brain remains largely unknown…The Allen Institute for Brain Science was established to answer some of the most pressing questions in neuroscience, grounded in an understanding of the brain and inspired by our quest to uncover the essence of what makes us human" (Allen Institute for Brain Science).

Project Objective



Verify viral vectors' effectiveness in tagging medium spiny D1 or D2 neurons in the striatum

The Big Picture

Diseases/Conditions Affected by the Basal Ganglia:

- Huntington's Disease
- Parkinson's Disease
- Obsessive Compulsive Disorder
- Tourette Syndrome
- Addiction
- Schizophrenia
- Depression

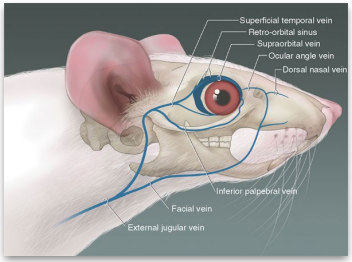
Possible Applications:

- Studying D1 and D2 neurons
- Gene therapy

Method:

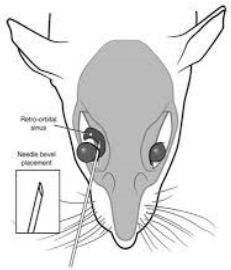
Retro-Orbital Injection → Perfusion → Immunohistochemistry & Electrophysiology

Retro-Orbital Injection



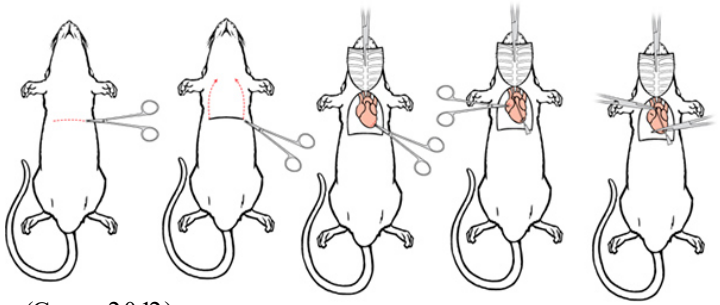
(Yardeni et al., 2011)

3 Weeks



(Yardeni et al., 2011)

Perfusion

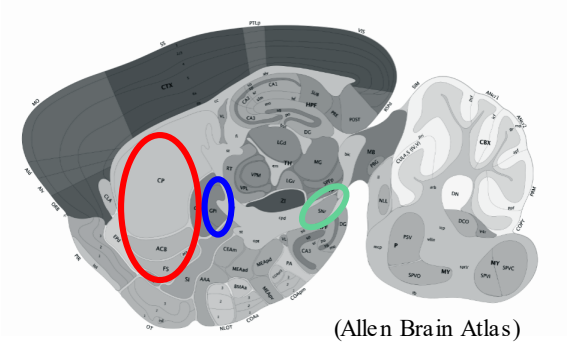


(Gage, 2012)

Method:

Retro-Orbital Injection → Perfusion → Immunohistochemistry & Electrophysiology

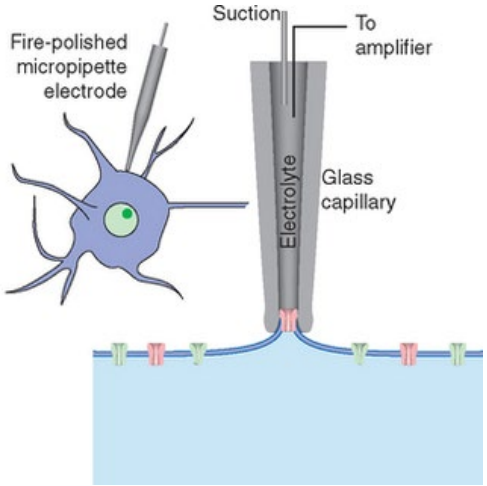
Immunohistochemistry (IHC): using antibodies and fluorescence to detect the location of target antigens in whole tissue slices (Abcam)



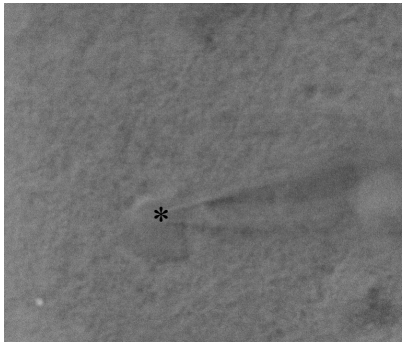
(Allen Brain Atlas)

Striatum (STR)
Substantia Nigra (SNr)
Globus Pallidus (GPe)

Electrophysiology (Patch Clamp Method): "a versatile electrophysiological tool for understanding ion channel" conductance, voltage, and membrane potential (Molecular Devices)



(Neupsy, 2016)



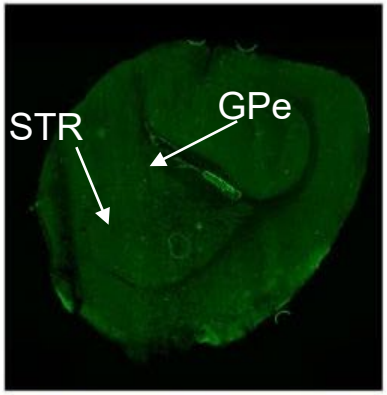
Project Results

CN2011

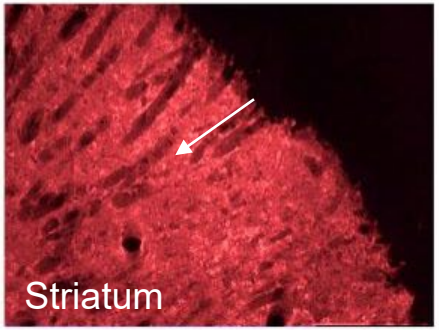
DARPP32

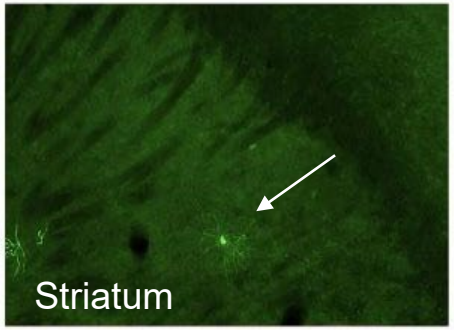
anti GFP

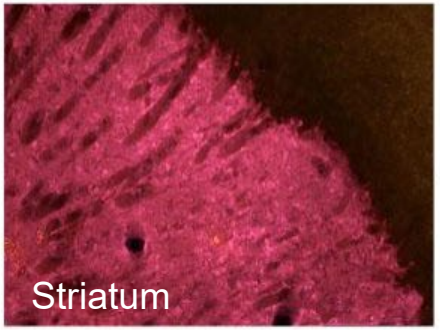
Combined



4x





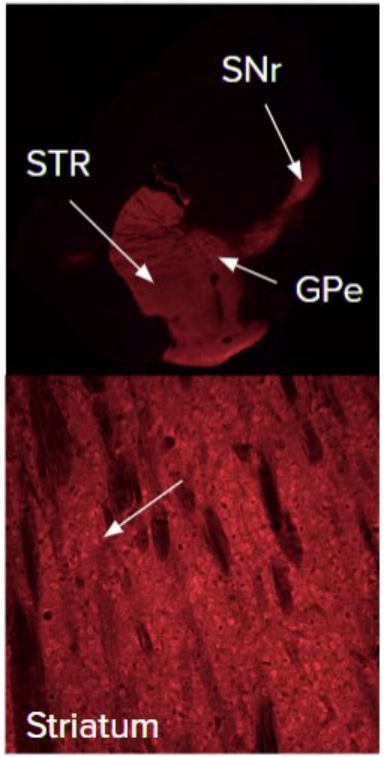


20x

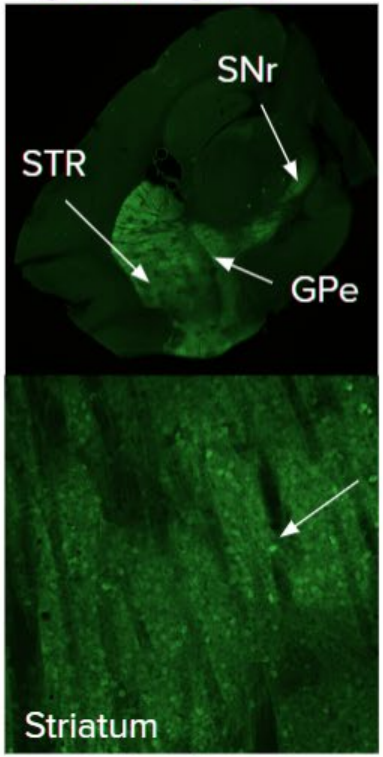
Project Results

CN2025

DARPP32



anti GFP



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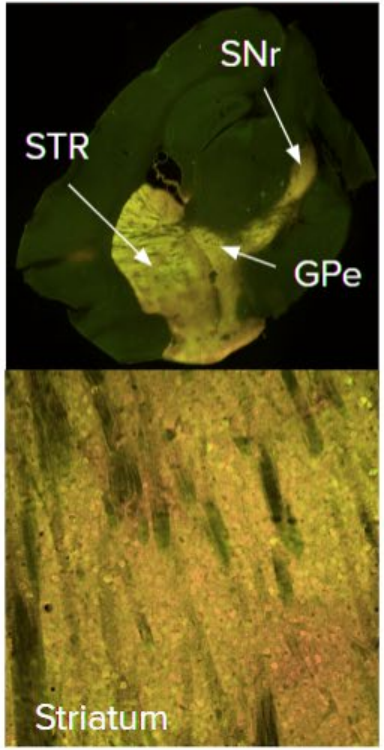


4x

20x

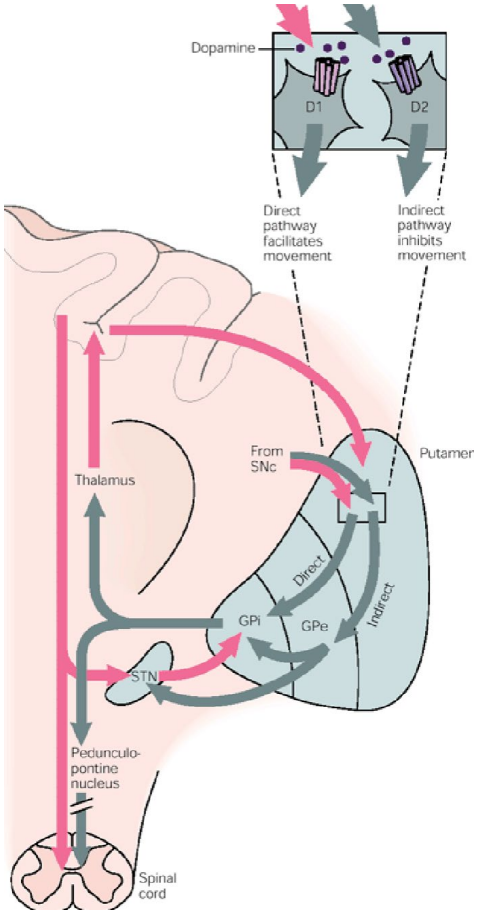
Possible Application

Parkinson's Disease (PD): a degenerative neural disease as a result of dopamine (DA) depletion that disrupts the normal neural circuitry linking thoughts and action

Medium spiny D1 and D2 neurons (D1 and D2 MSNs) are apart of the basal ganglia circuit that help facilitate movement, learning, and cognition but become dysfunctional with DA depletion (Wei, 2017).

The mechanisms behind the development of symptoms of PD are unknown.

Understanding how D1 and D2 MSNs function in healthy tissue is a necessary first step in discovering their role in PD.



(Delong, 2006)

Acknowledgements

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Assistant Investigator: Jonathan Ting, PhD
Senior Scientist: Meanhwan Kim, PhD
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