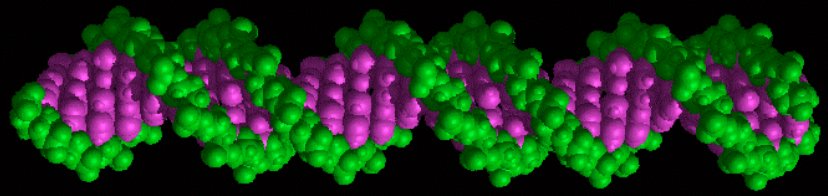
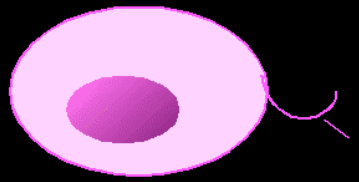
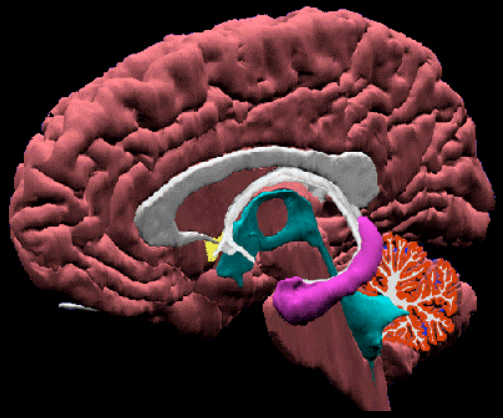
Gene Therapy





Neural Stem Cells



Neurodegenerative Disorders

Schaffer Lab
Chemical Engineering
U.C. Berkeley

Neuronal Protection and Replacement

- Gene delivery for control of adult stem cells

- Gene therapy for neuroprotection: ALS and spinocerebellar ataxia

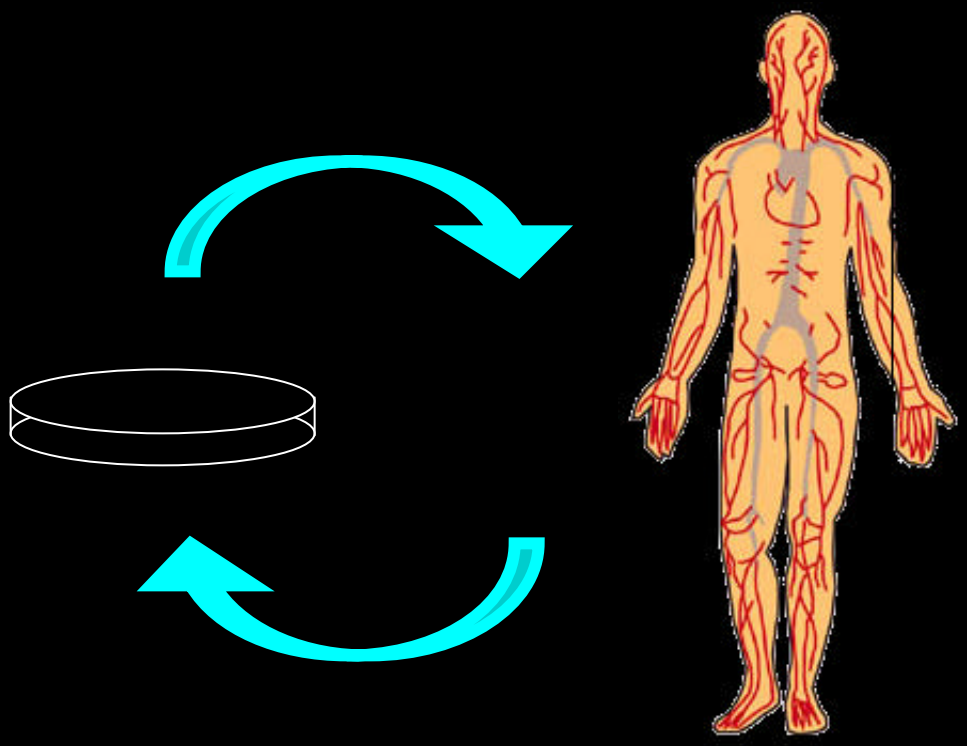
Neuronal Protection and Replacement

- **Gene delivery for control of adult stem cells**

- Gene delivery for neuroprotection: ALS and spinocerebellar ataxia

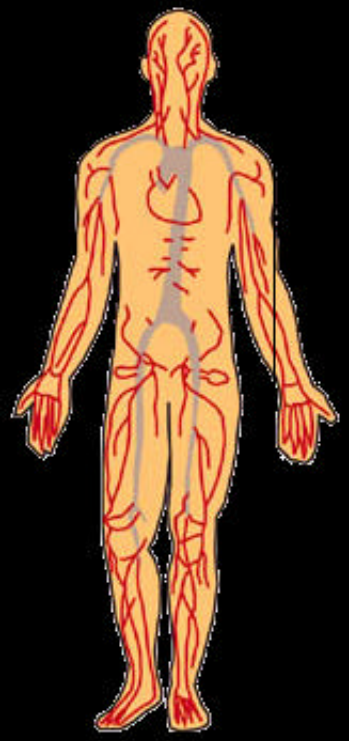
Stem Cell Therapy: In Vitro & In Vivo

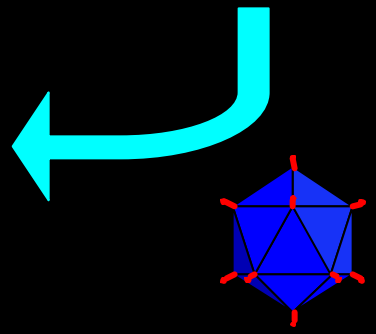
Harvest, Grow, and Reimplant



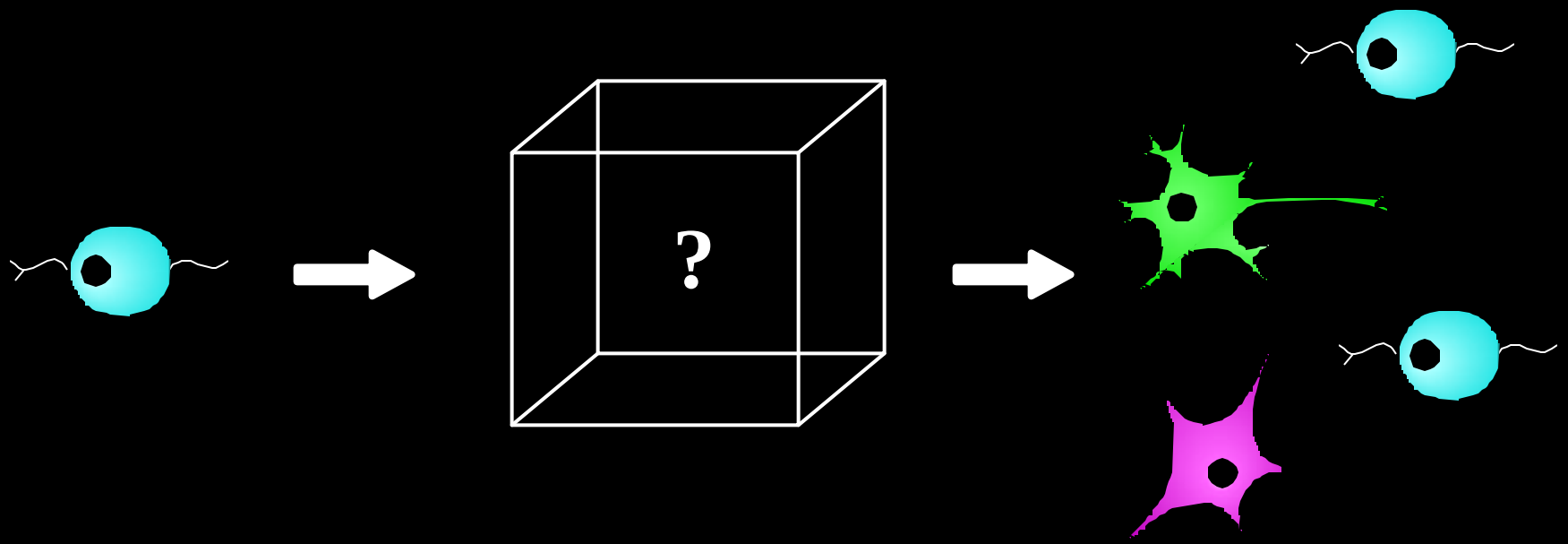
Stem Cell Therapy: In Vitro & In Vivo

Direct Control in the Nervous System by Delivery of Drugs or Genes

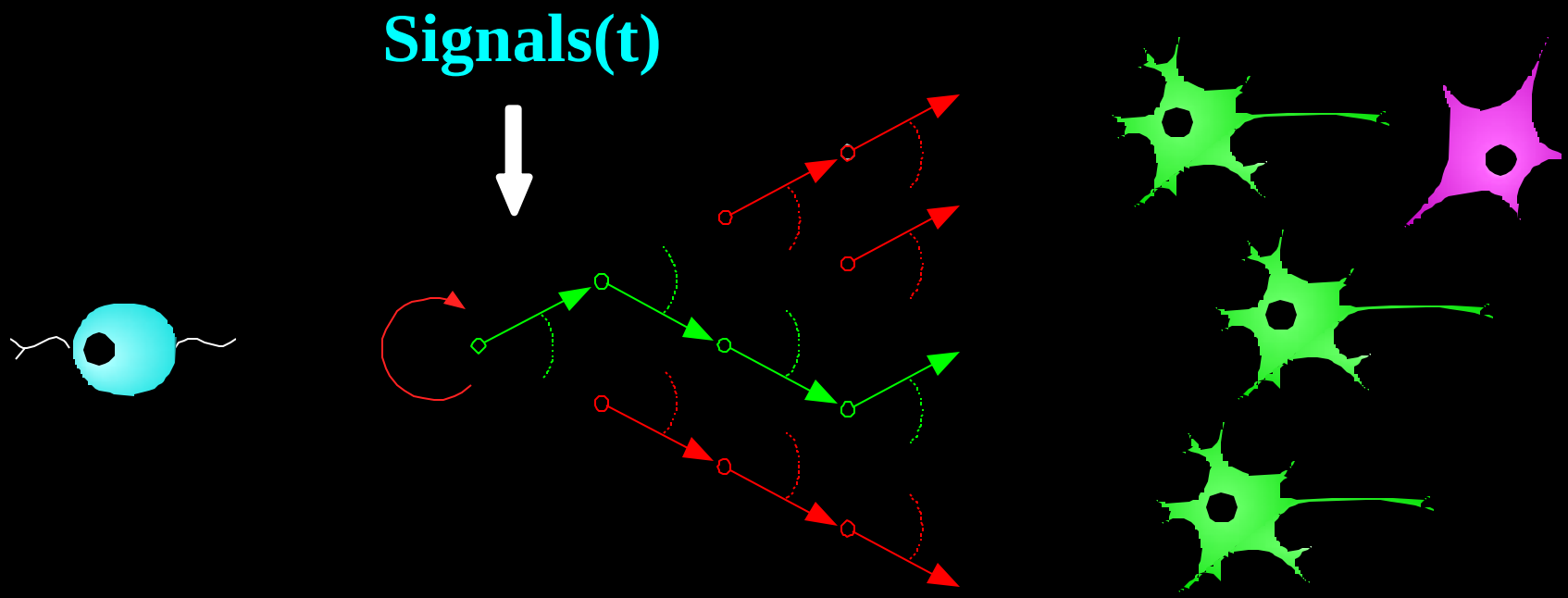




Underlying Challenge: How to Control the Stem Cells



Underlying Challenge: Cellular Control

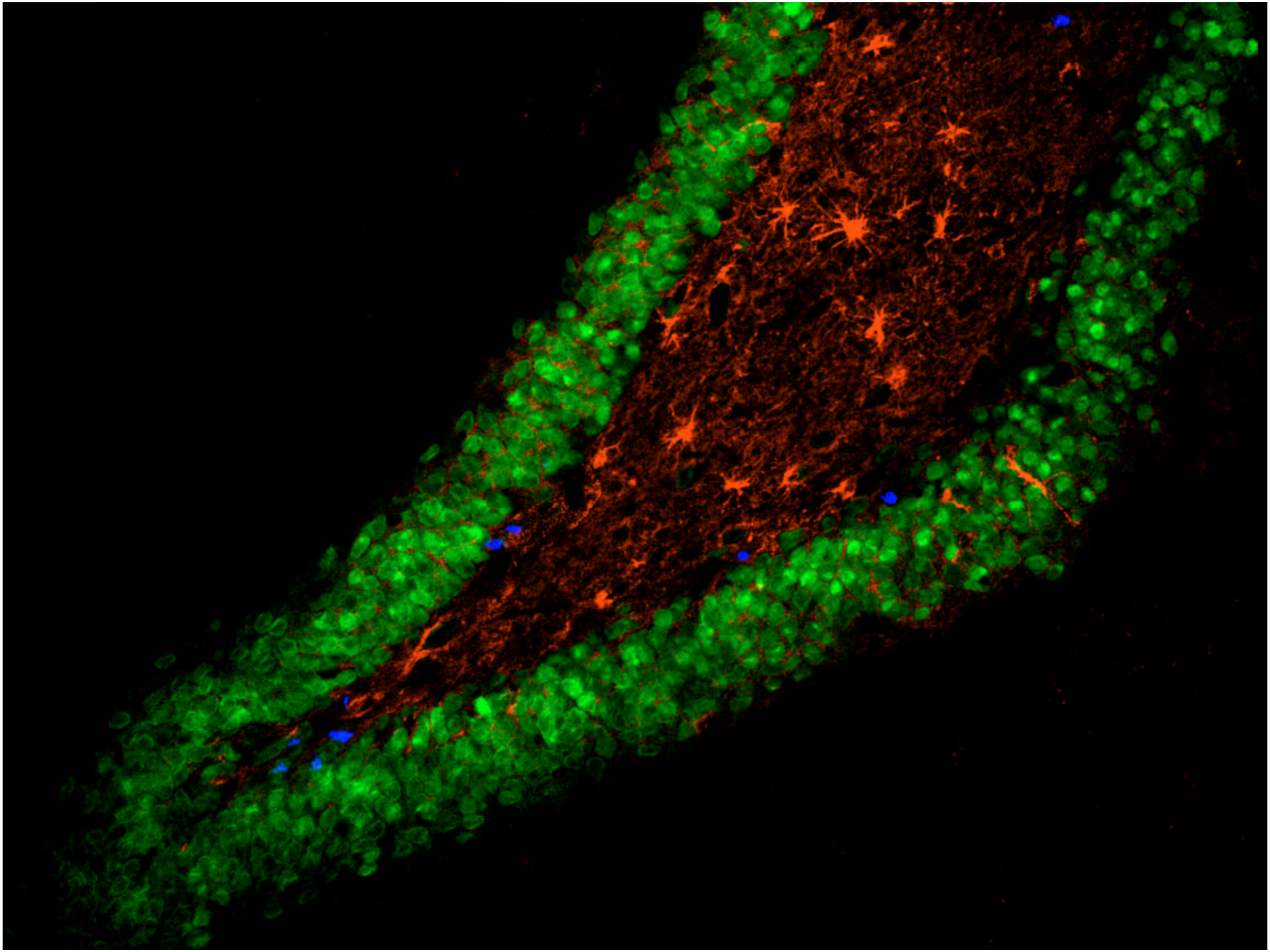


Adult Neural Stem Cells

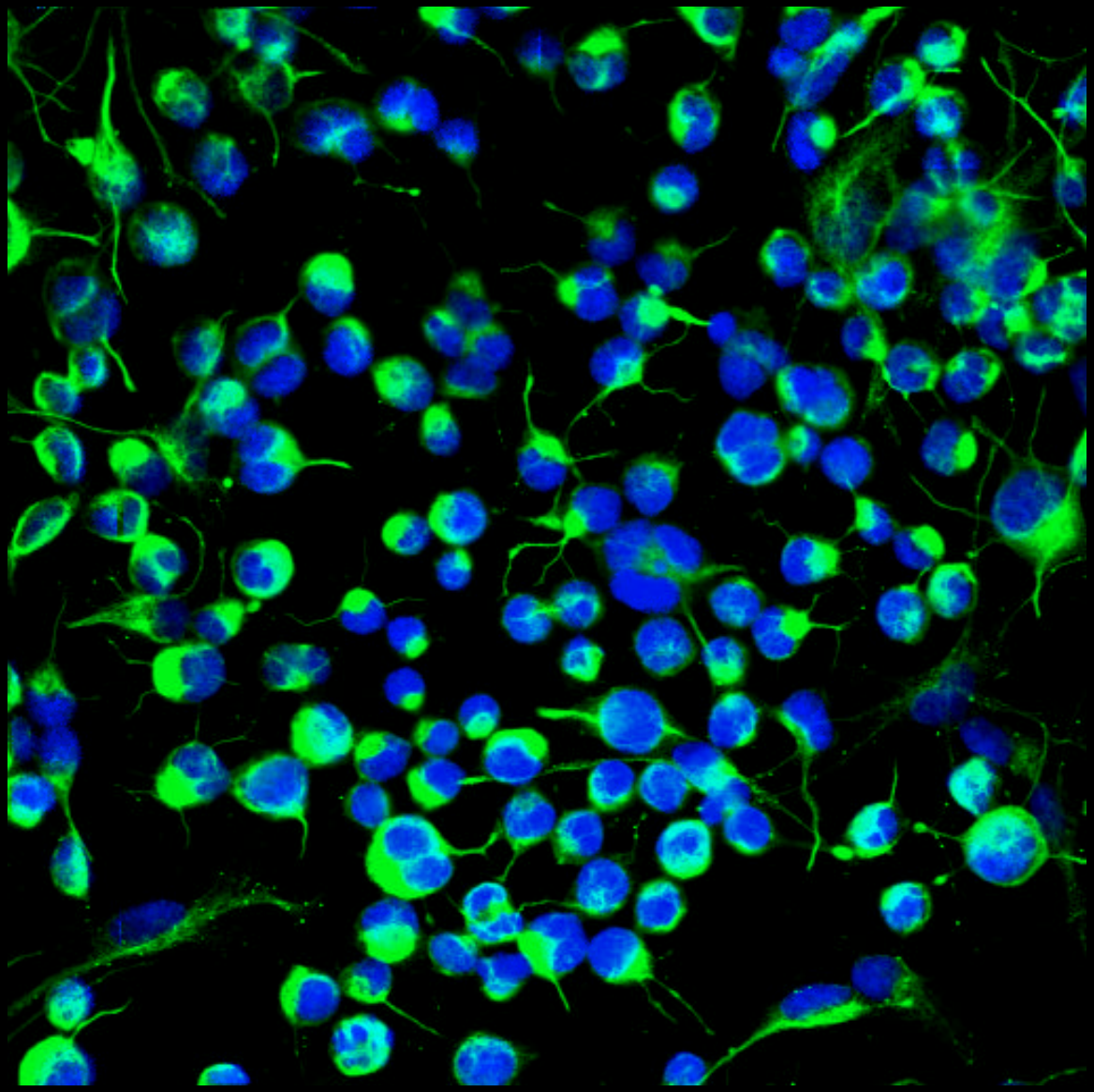
- Shown very recently to exist throughout the nervous system
- Significant potential for neural regeneration
- Requires better understanding of signaling mechanisms that regulate these cells' function

Brain Section: Hippocampus

1) Site of active adult neural stem cells
2) Site affected by Alzheimer's Disease



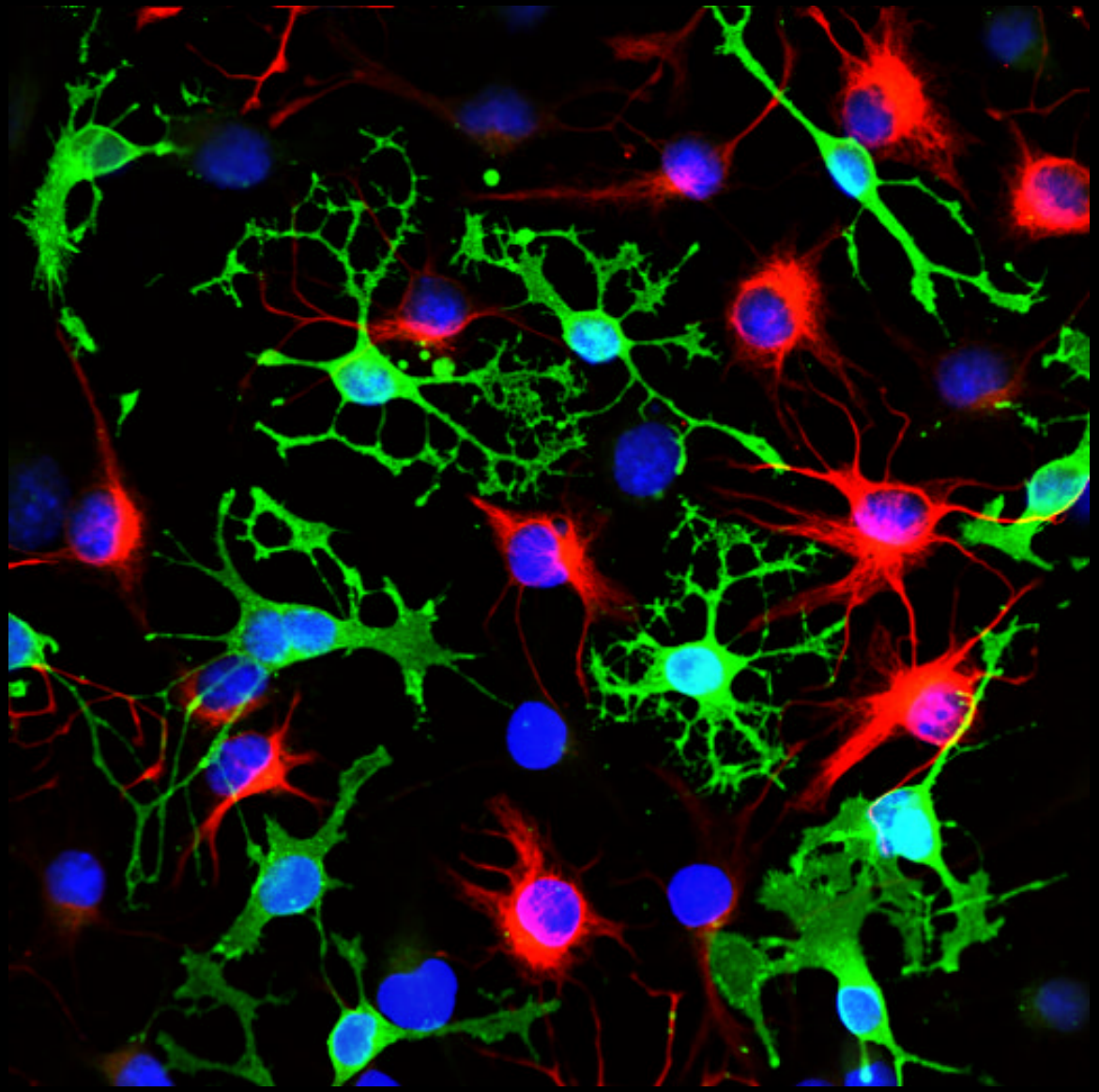
Propagation of Immature Neural Stem Cells



Blue - nucleus

Green - nestin

Differentiation of Stem Cells into Glia

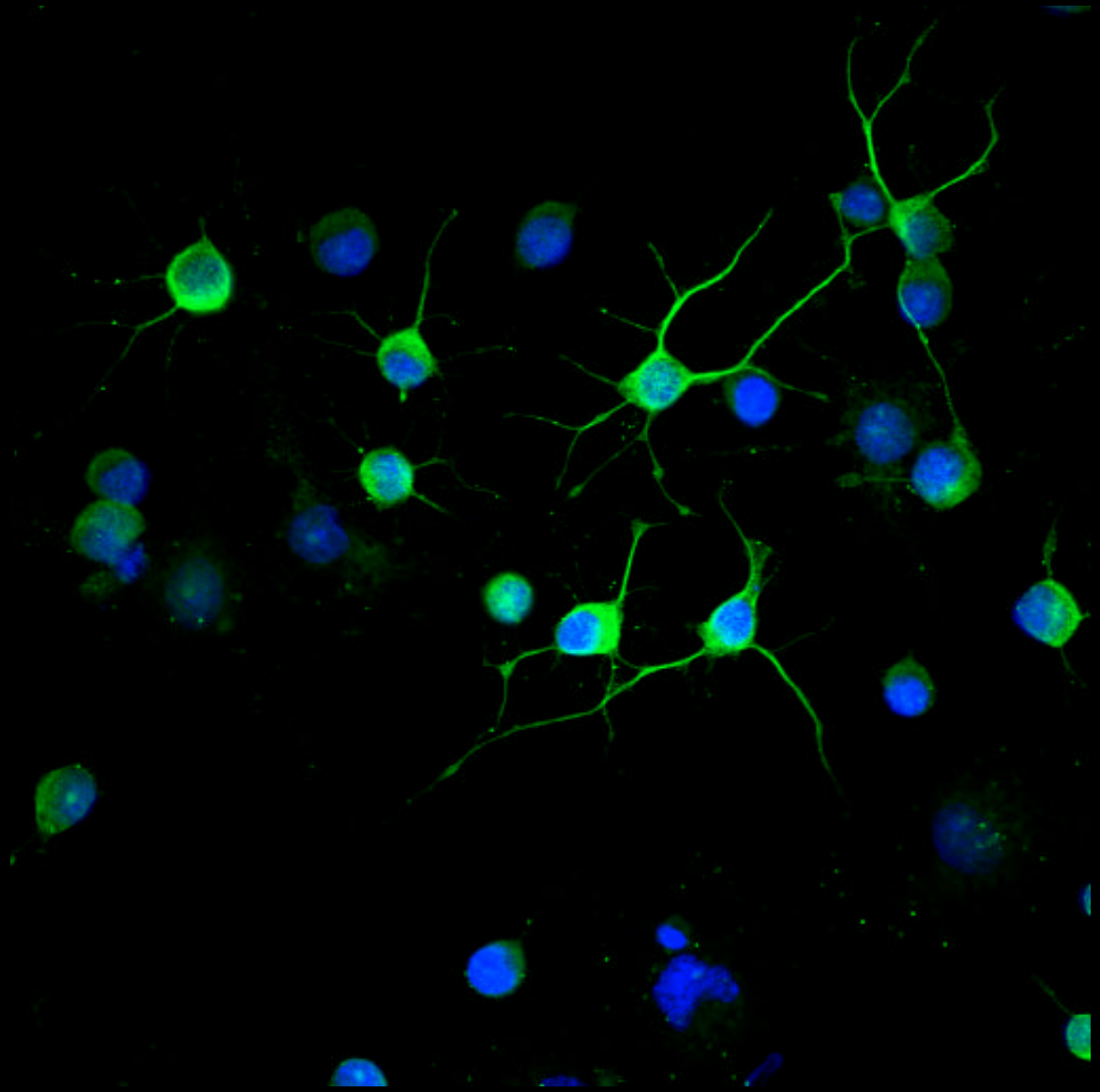


Blue - nucleus

Green - MBP

Red - GFAP

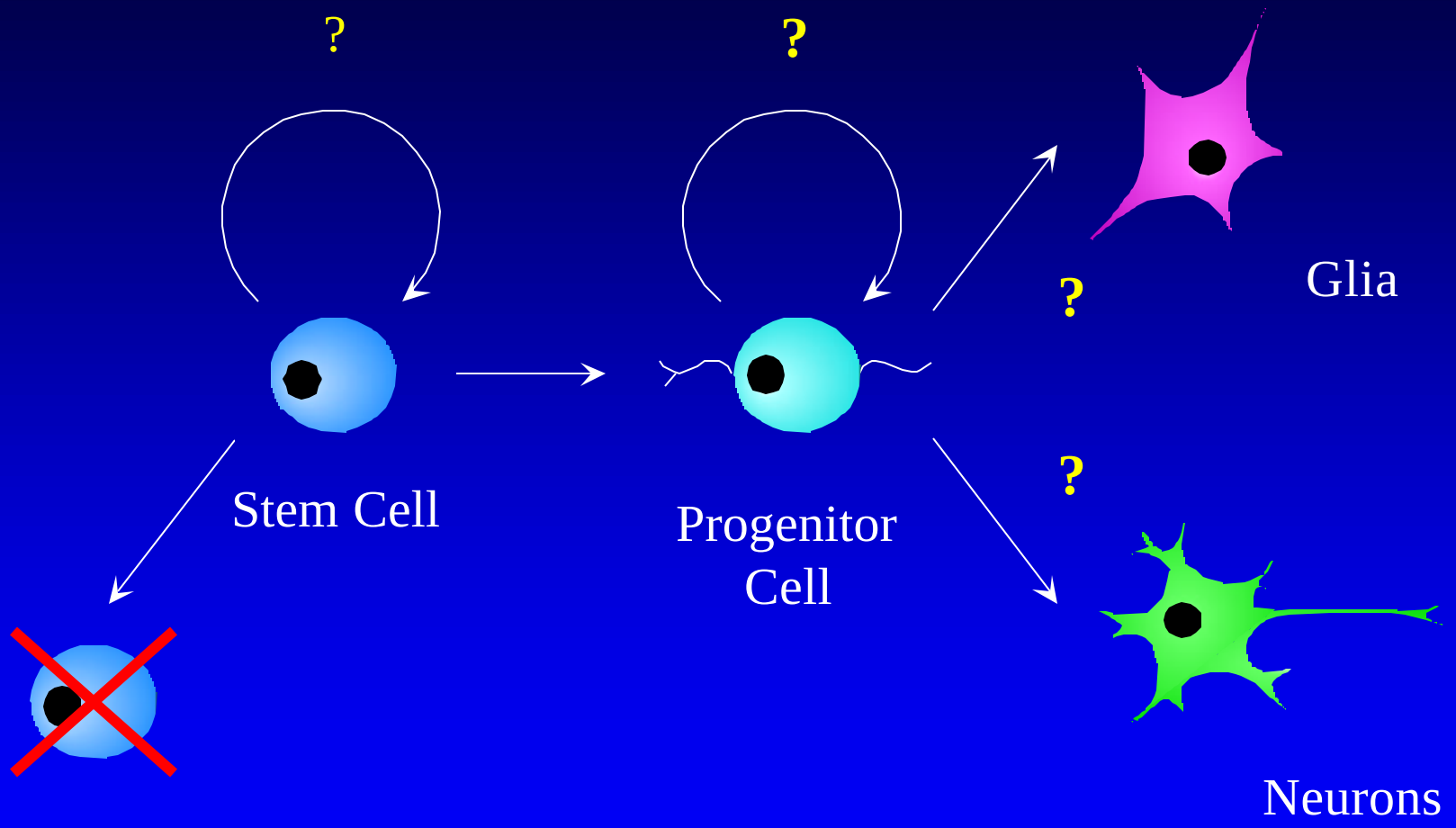
Differentiation into Neurons



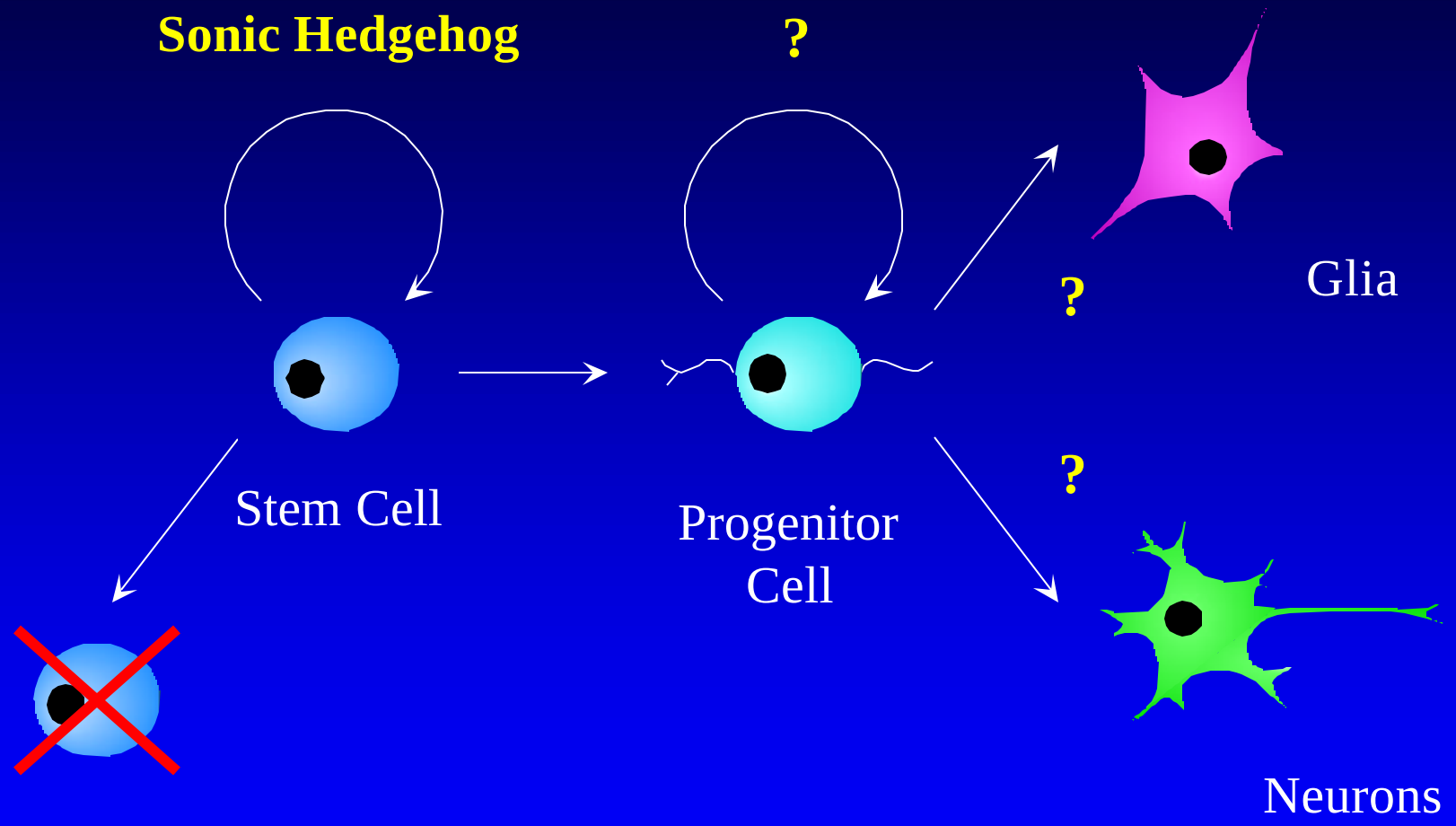
Blue - nucleus

Green - NF200

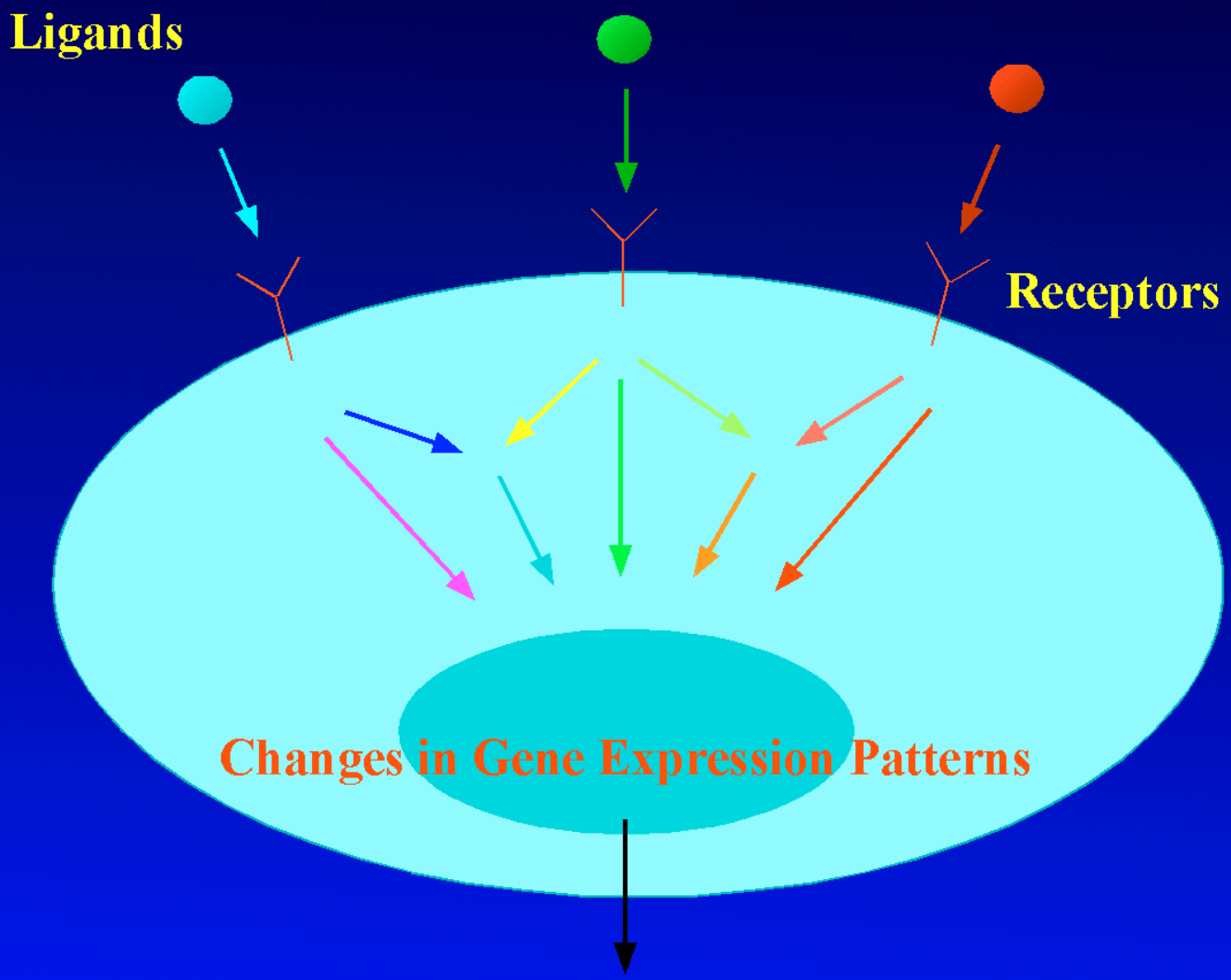
Goal: Control Neural Stem Cell Behavior



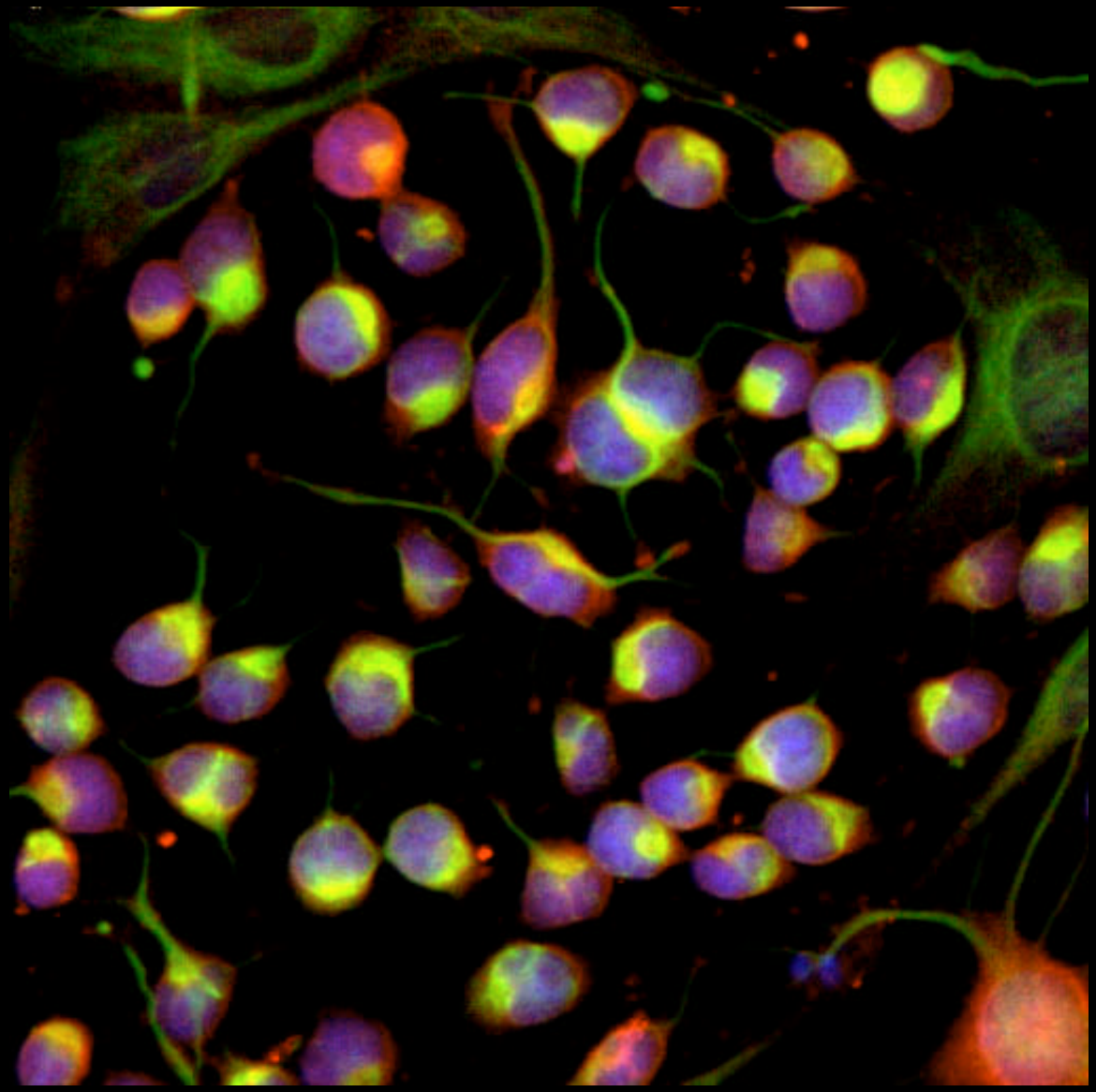
Goal: Control Neural Stem Cell Behavior



Cell Behavior is Regulated by External Environmental Signals



Adult Neural Stem Cells Express Ptc

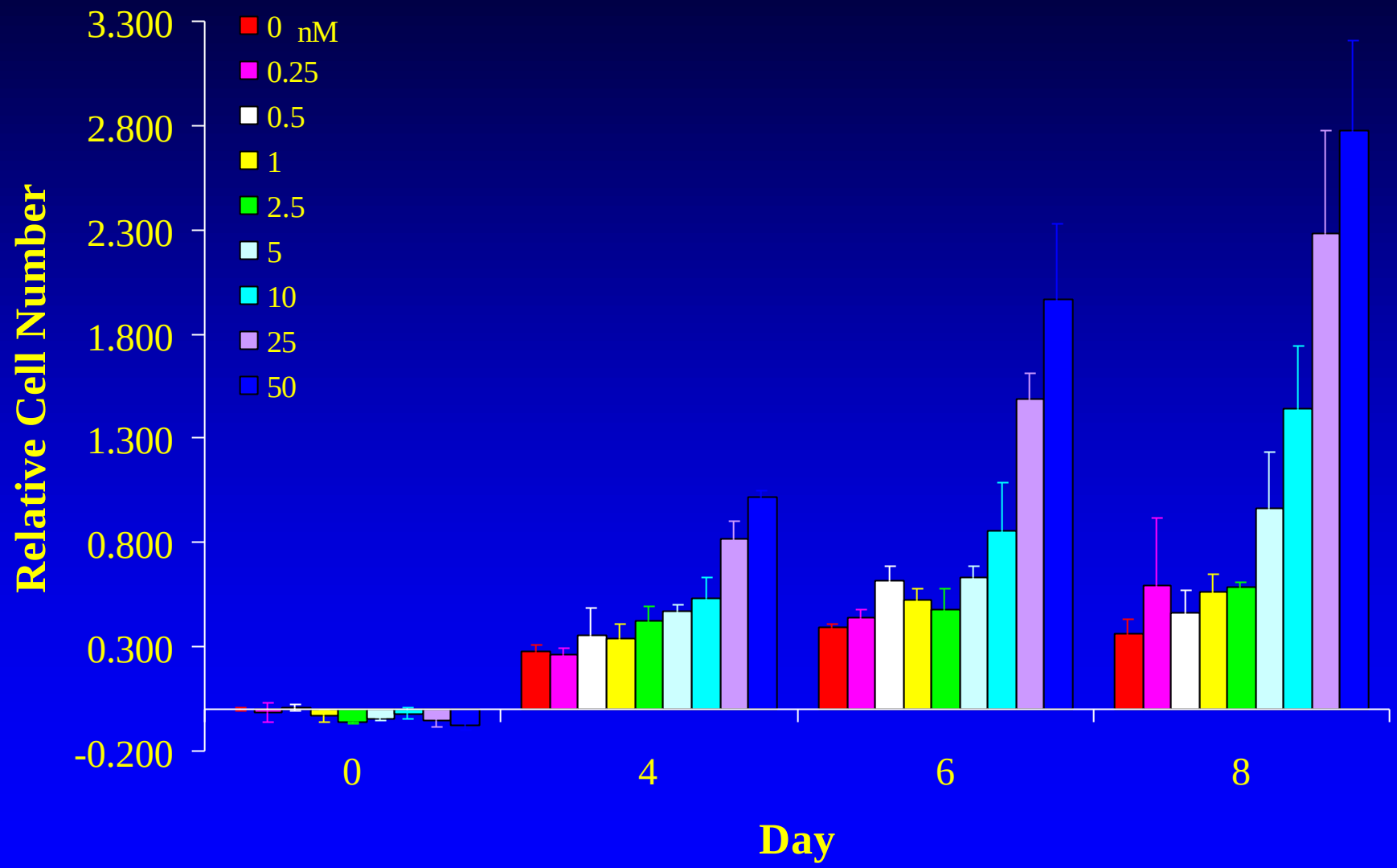


Blue - nucleus

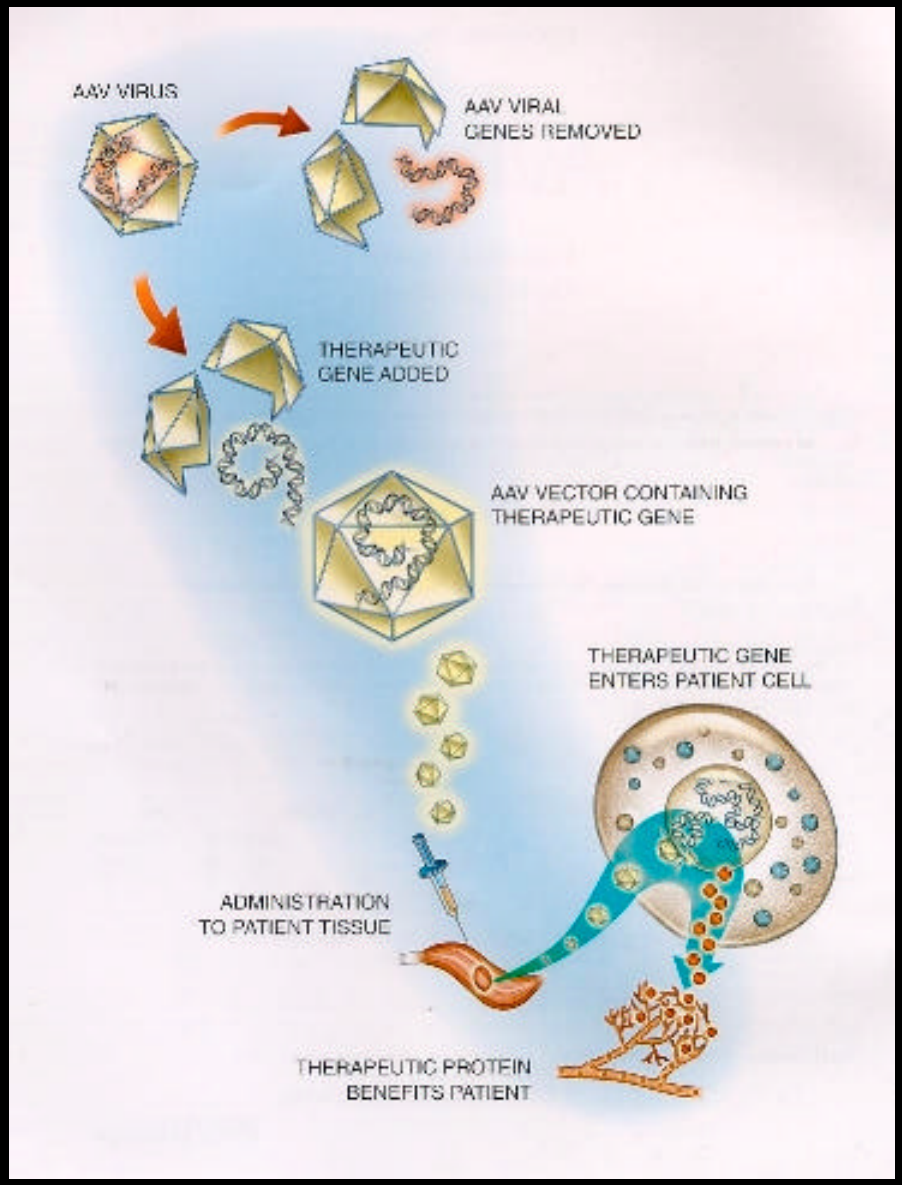
Green - nestin

Red - Shh receptor (Ptc)

Shh Stimulates Neural Progenitor Proliferation



Adeno-associated Viral Gene Delivery Vehicles



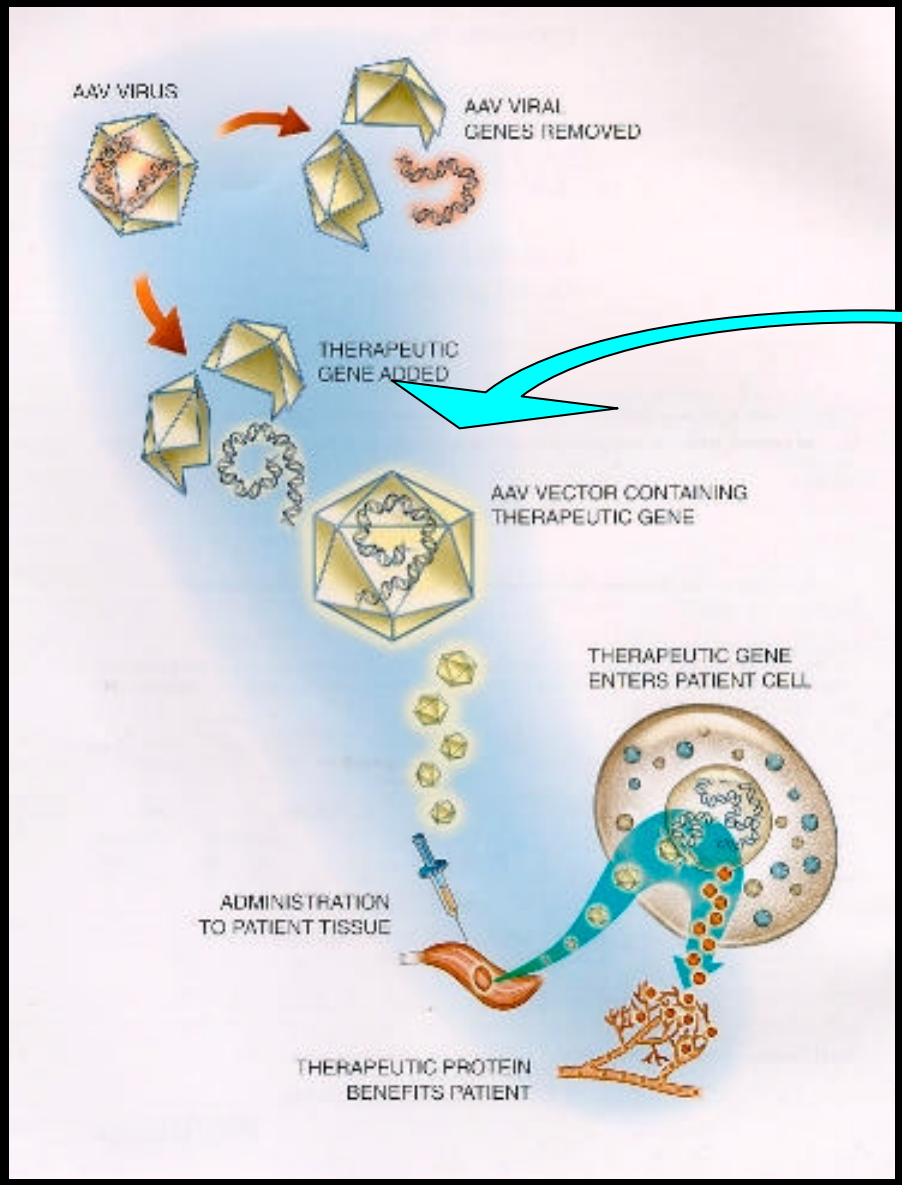
Advantages:

+ Extremely safe

+ Highly efficient

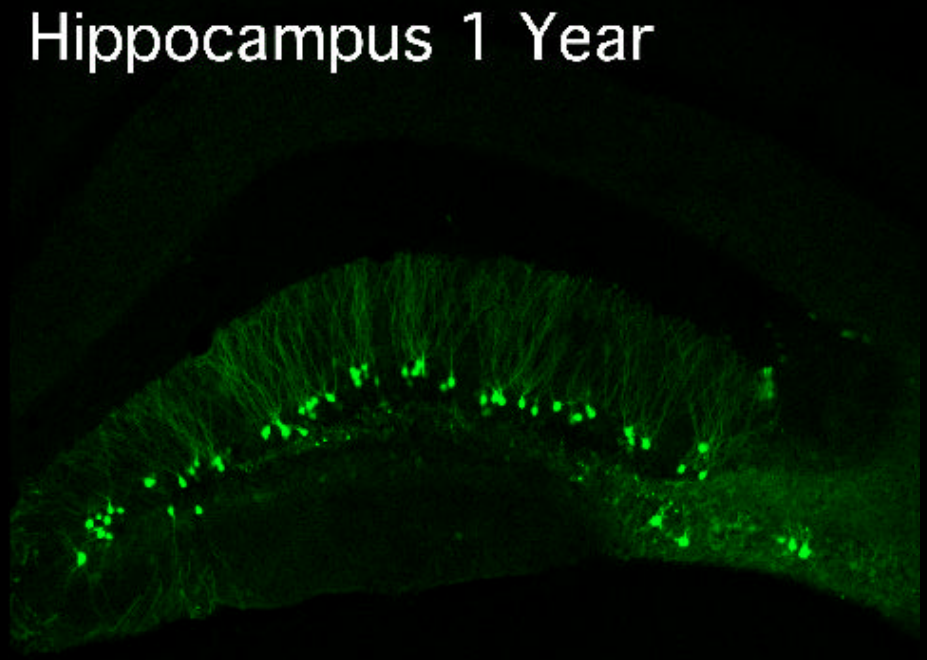
+ Very stable expression

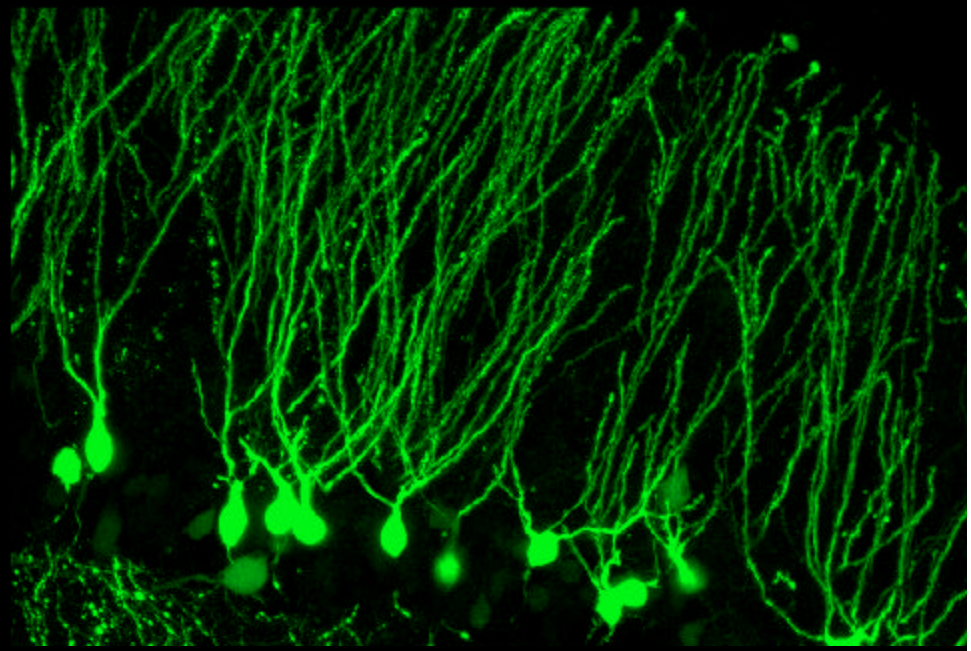
Adeno-associated Viral Gene Delivery Vehicles



Green Fluorescent Protein

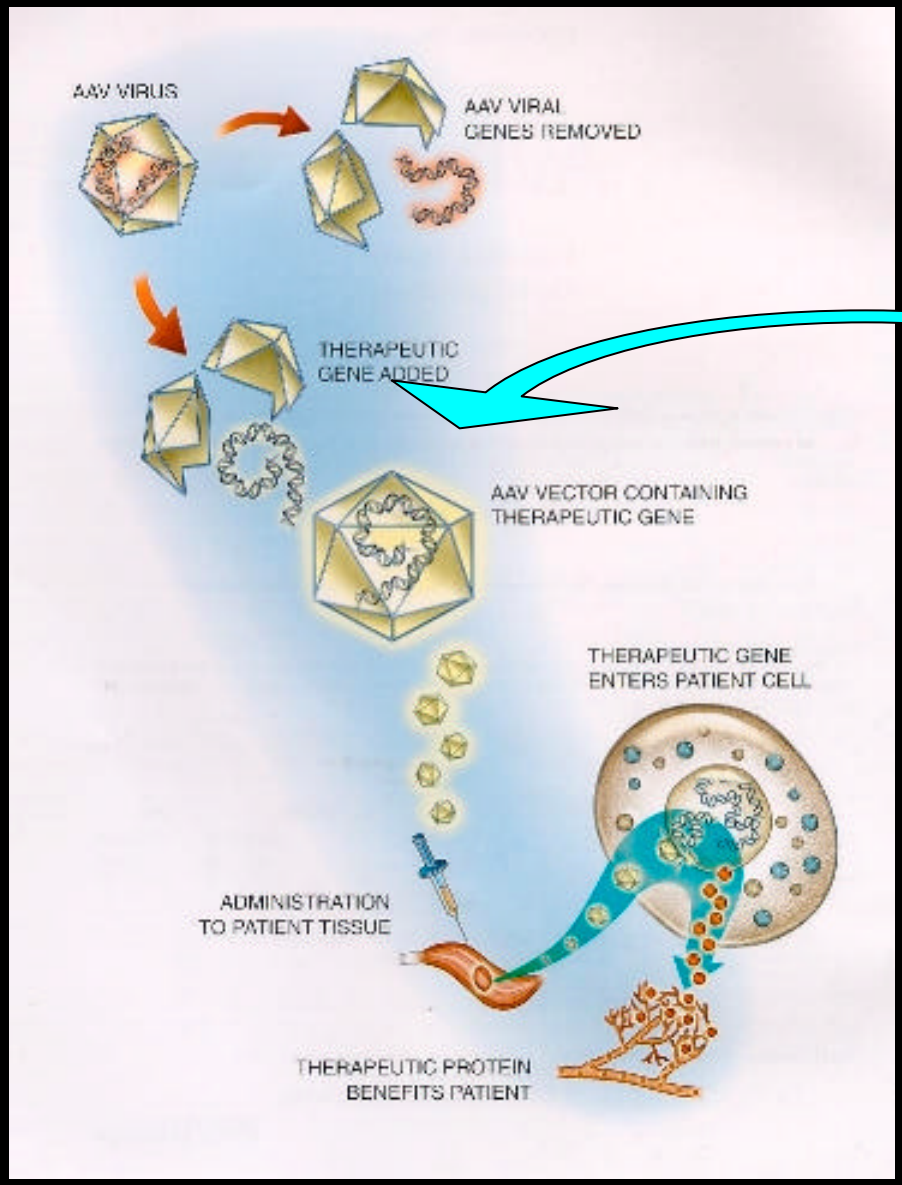
AAV-GFP Delivery to the Brain





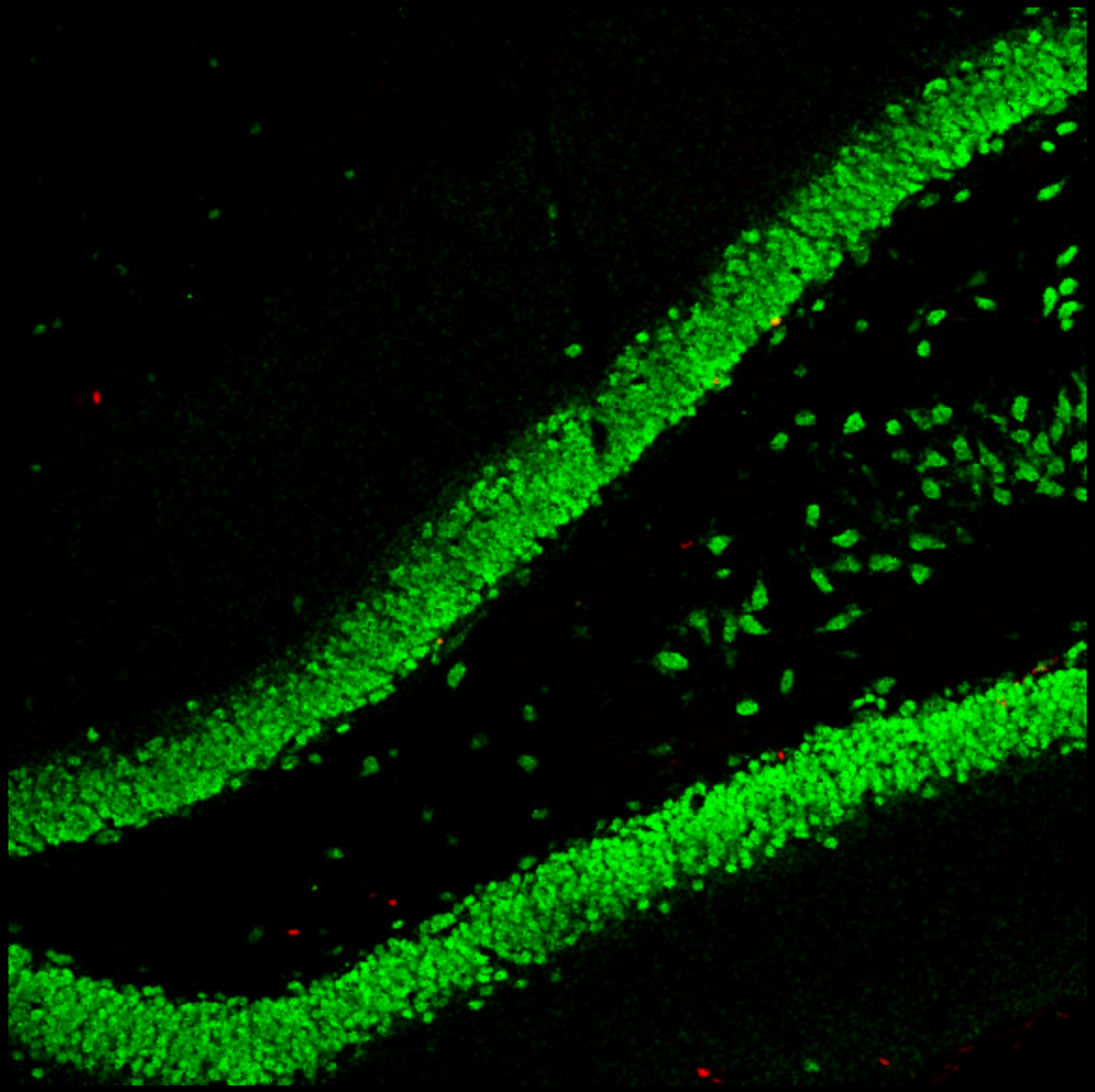
after 1 year

Adeno-associated Viral Gene Delivery Vehicles



Sonic Hedgehog

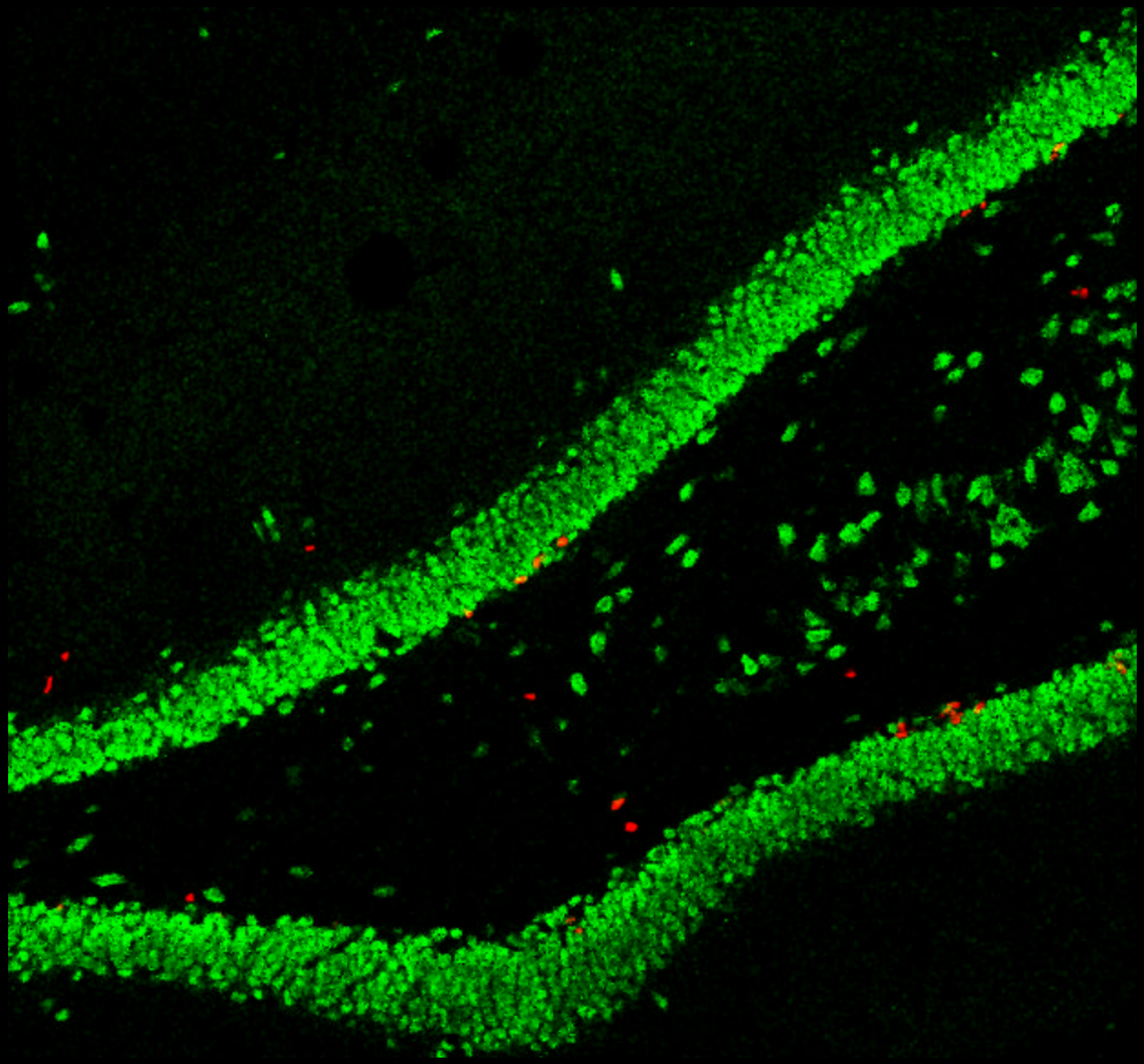
Adult Neural Stem Cell Proliferation: Control



Green - neurons
(NeuN)

Red - mitotic cells
(BrdU)

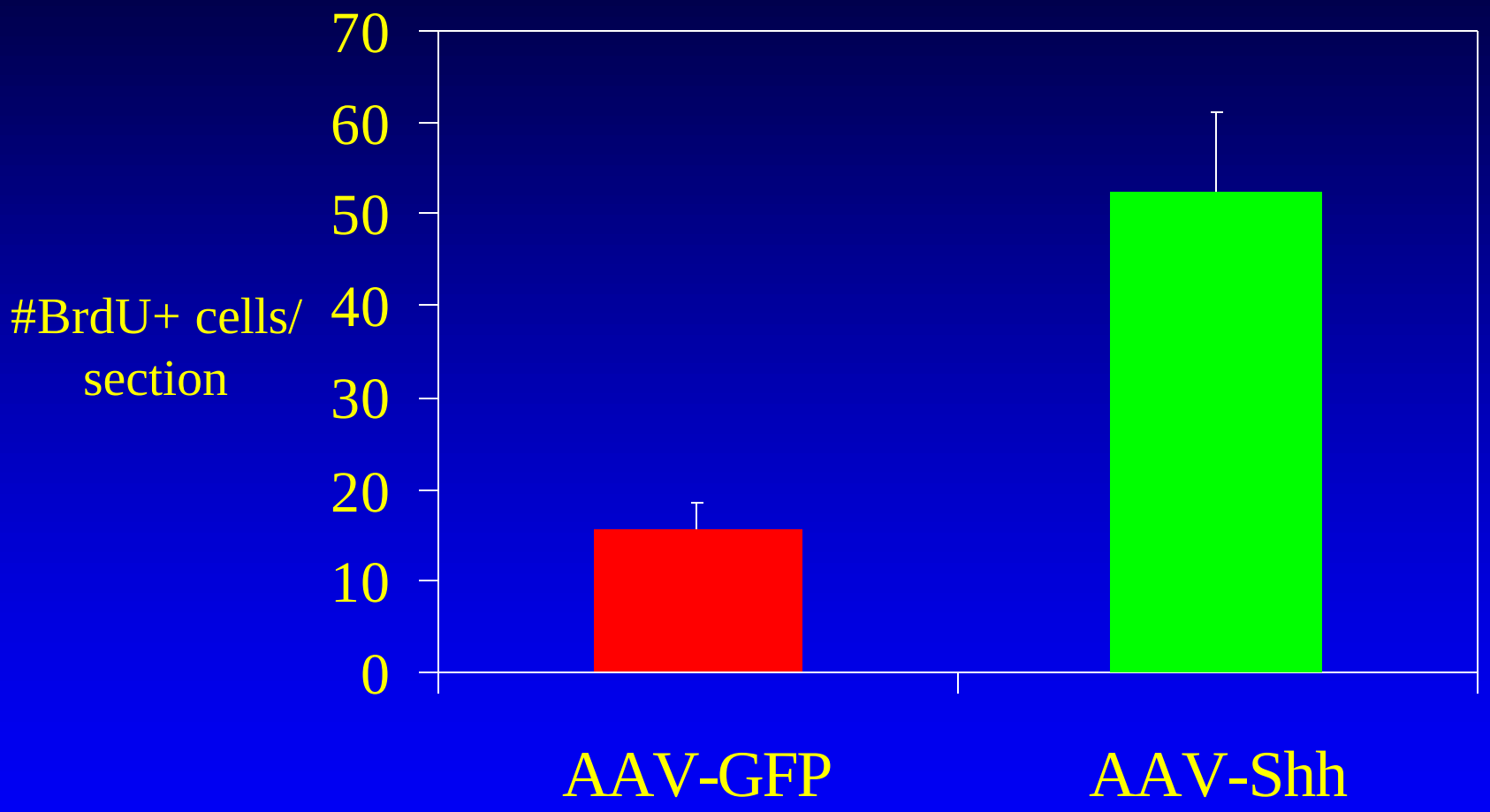
Adult Neural Stem Cell Proliferation: AAV-Shh



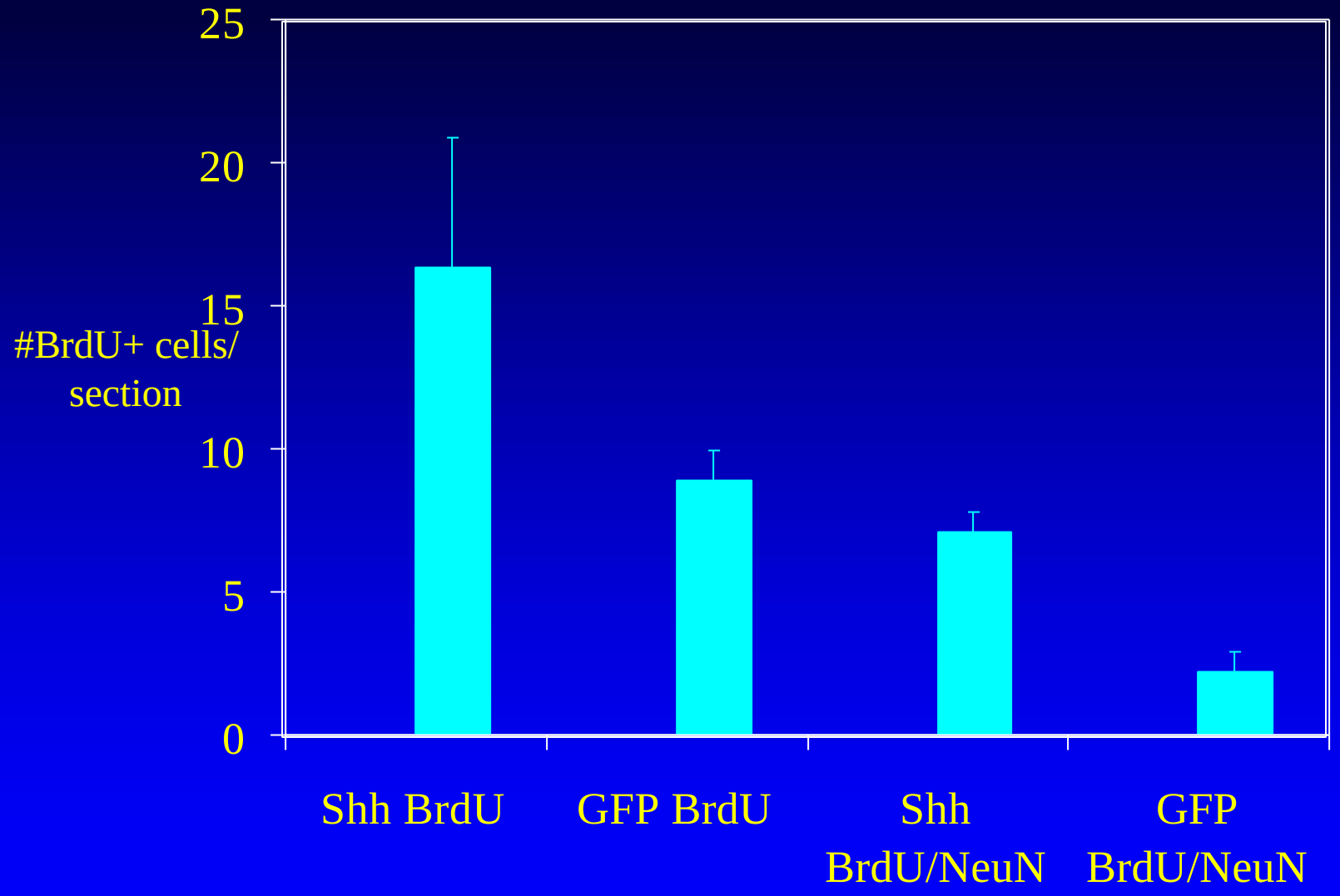
Green - neurons (NeuN)

Red - mitotic cells (BrdU)

Shh Triples Neural Stem Cell Proliferation



Shh Triples New Neurons



Lai et al., *Nature Neuroscience* (2003)

Neuronal Protection and Replacement

- Gene delivery for control of adult stem cells

- **Gene delivery for neuroprotection: ALS** and spinocerebellar ataxia

Gene Therapy: Concept and Current Status

Definition:

the delivery of genetic material to an individual's cells for therapeutic benefit

Recent success:

Hemophilia B using AAV (Avigen)

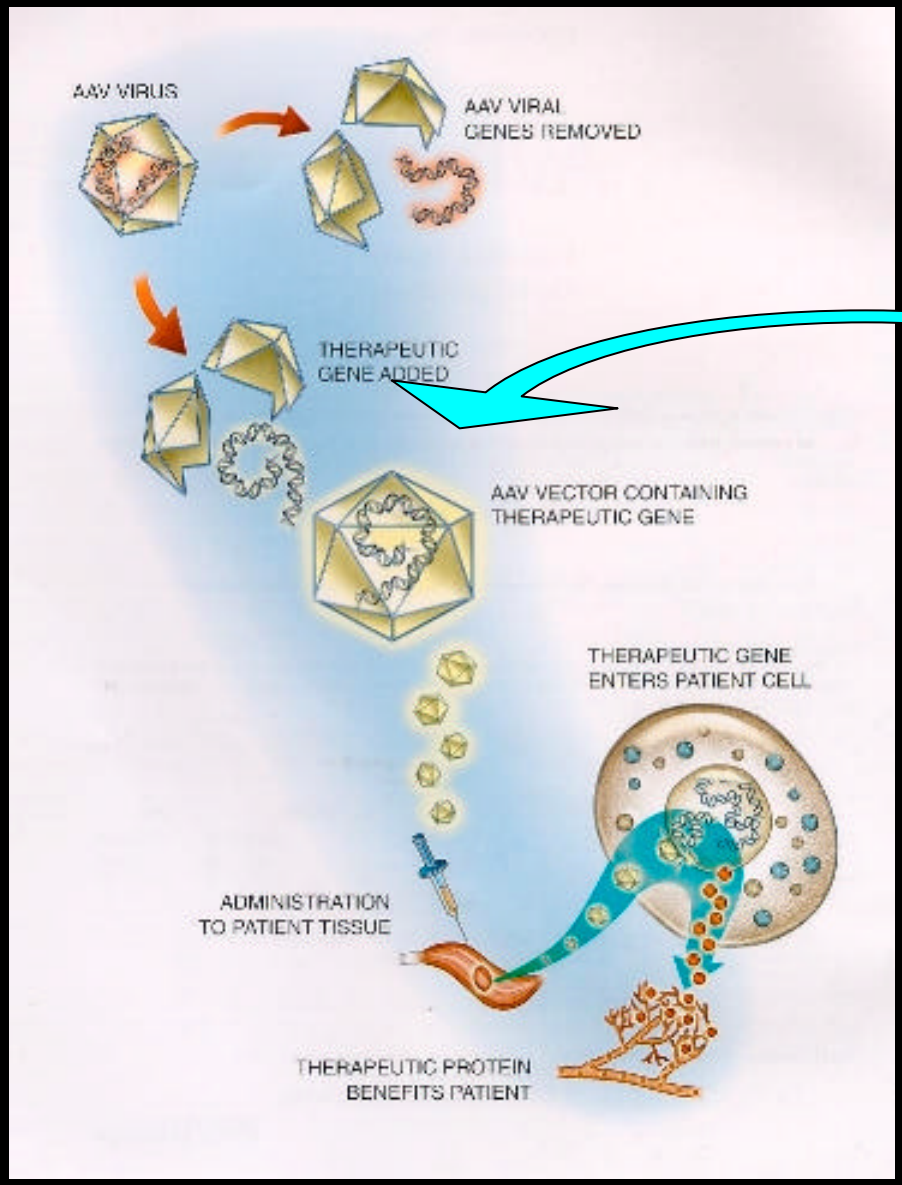
Heart disease using adenovirus (Coll.Thx.)

Cancer using adenovirus (Onyx)

Challenge:

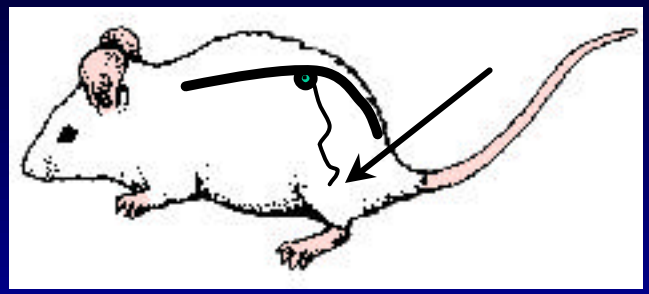
Need better gene delivery technology

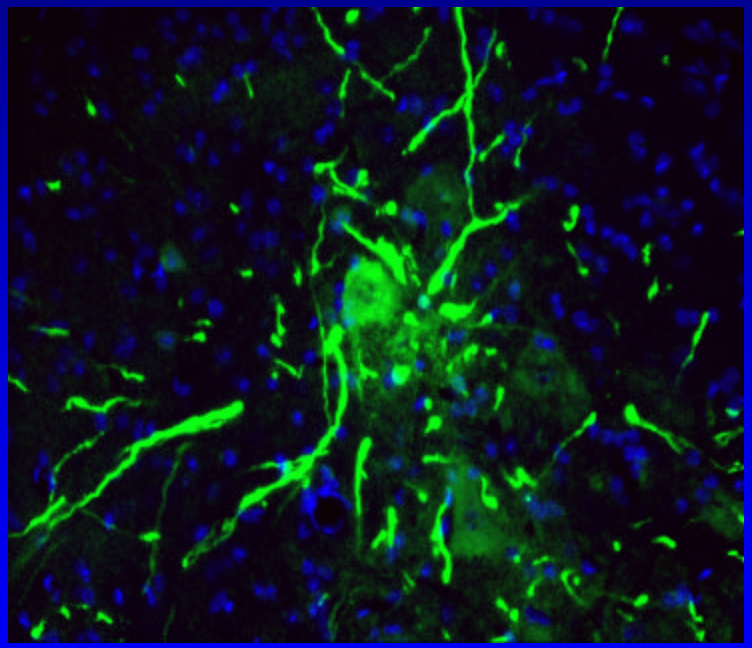
Adeno-associated Viral Gene Delivery Vehicles



Green Fluorescent Protein

AAV Neuroprotection in the Spinal Cord



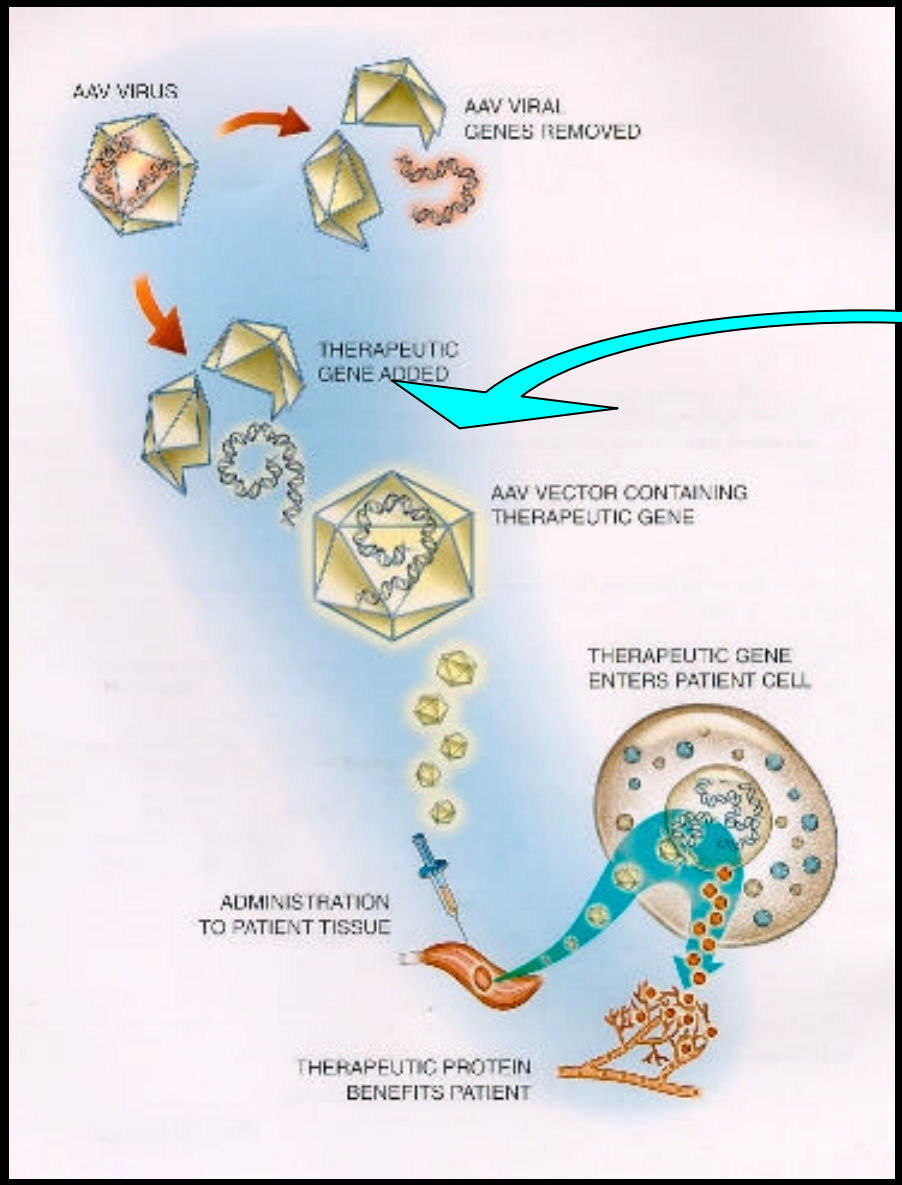


Fred Gage Lab, Salk Institute
Kaspar et al., *Science* (2003)

Amyotrophic Lateral Sclerosis

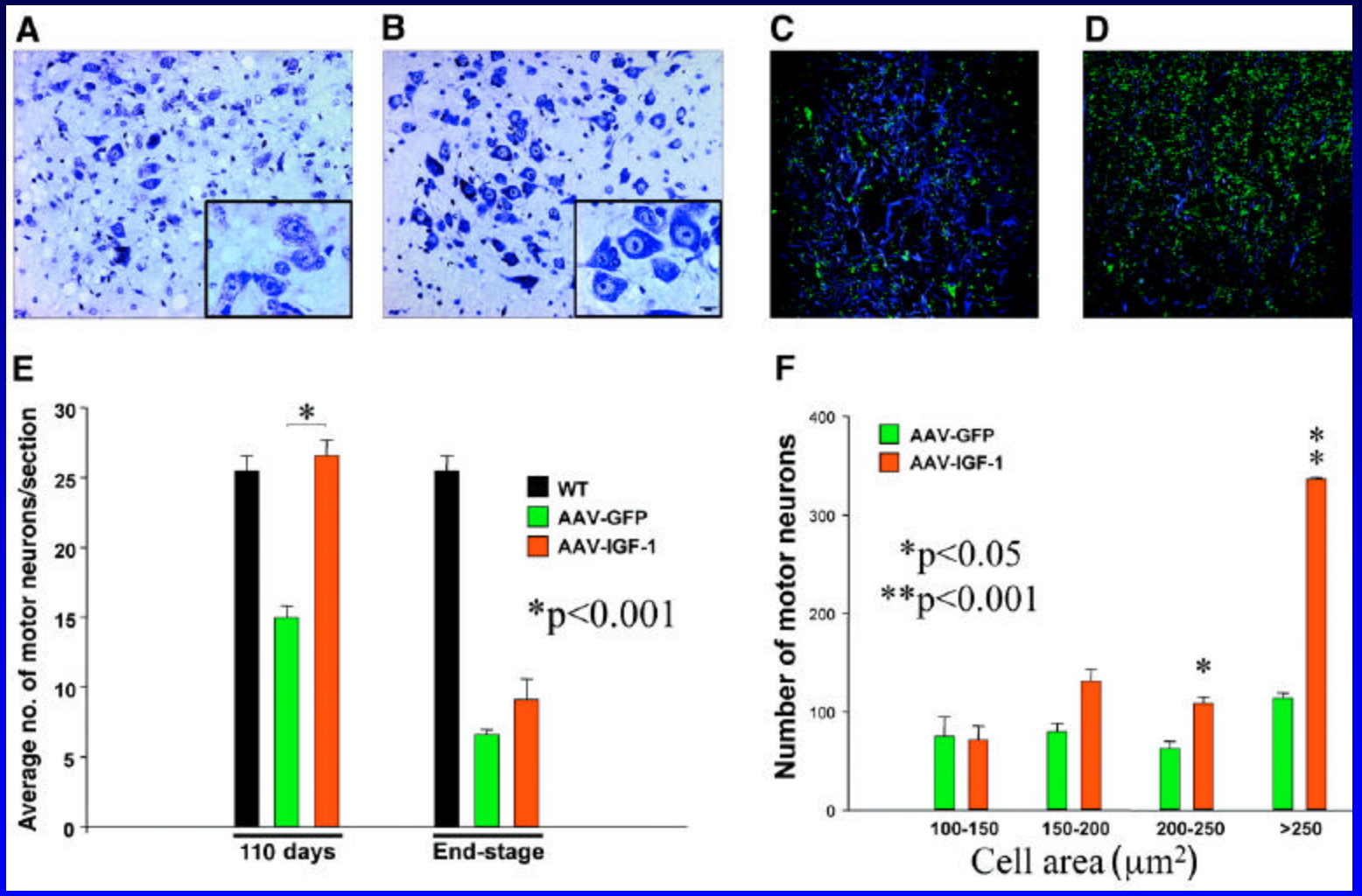
- **Progressive disease that selectively kills spinal cord motor neurons (Lou Gherig's Disease, Stephen Hawkins)**
- **Fatal within 1-5 years of onset**
- **Prevalence of 2-3 per 100,000 people**
- **Causes of disease remain unknown**
- **5-10% of cases, inherited in a dominant manner [SOD-1 mutations (Superoxide dismutase-1) 90 mutations known**

AAV Gene Therapy for ALS



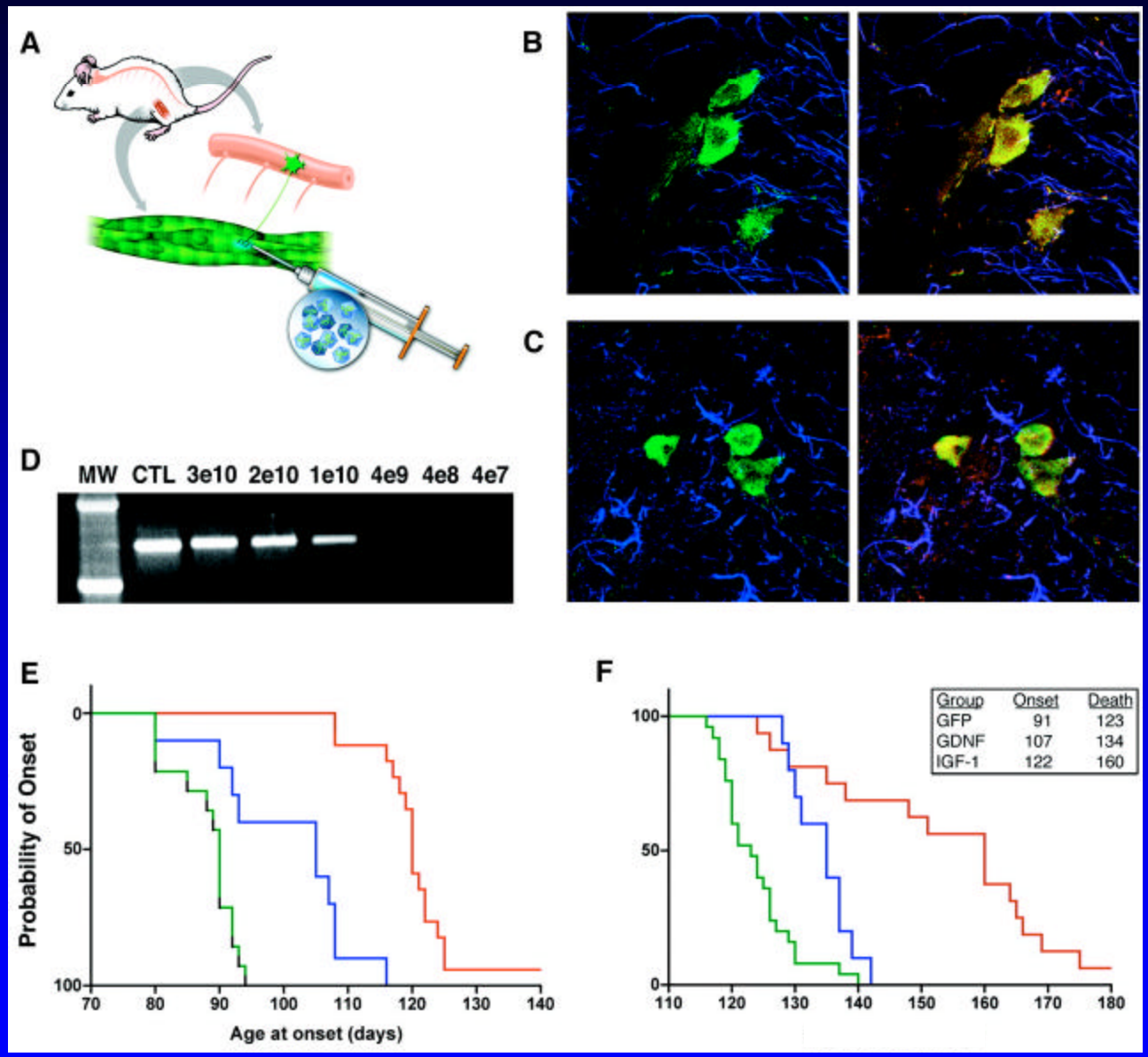
Neuroprotective Factor IGF-I

Gene Delivery Protects Motor Neurons



Kaspar et al., *Science* (2003)

Gene Therapy Significantly Delays Symptoms



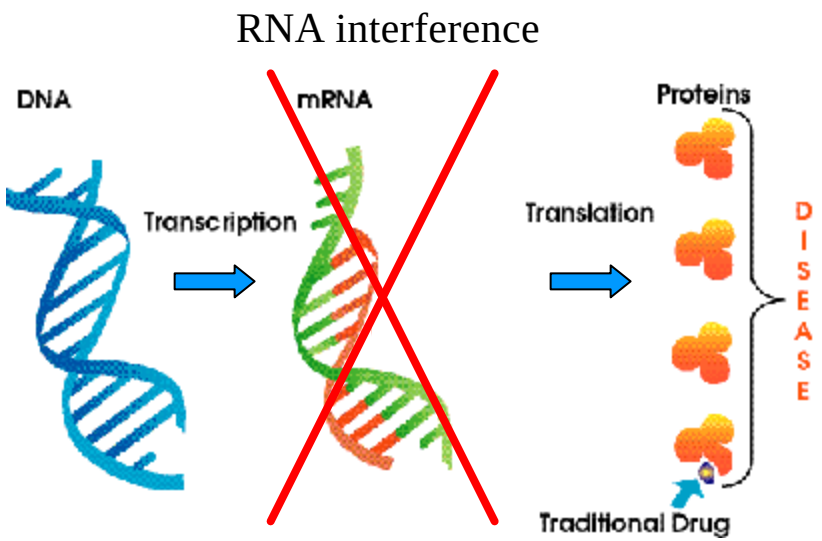
Kaspar et al., *Science* (2003)

Neuronal Protection and Replacement

- Gene delivery for control of adult stem cells

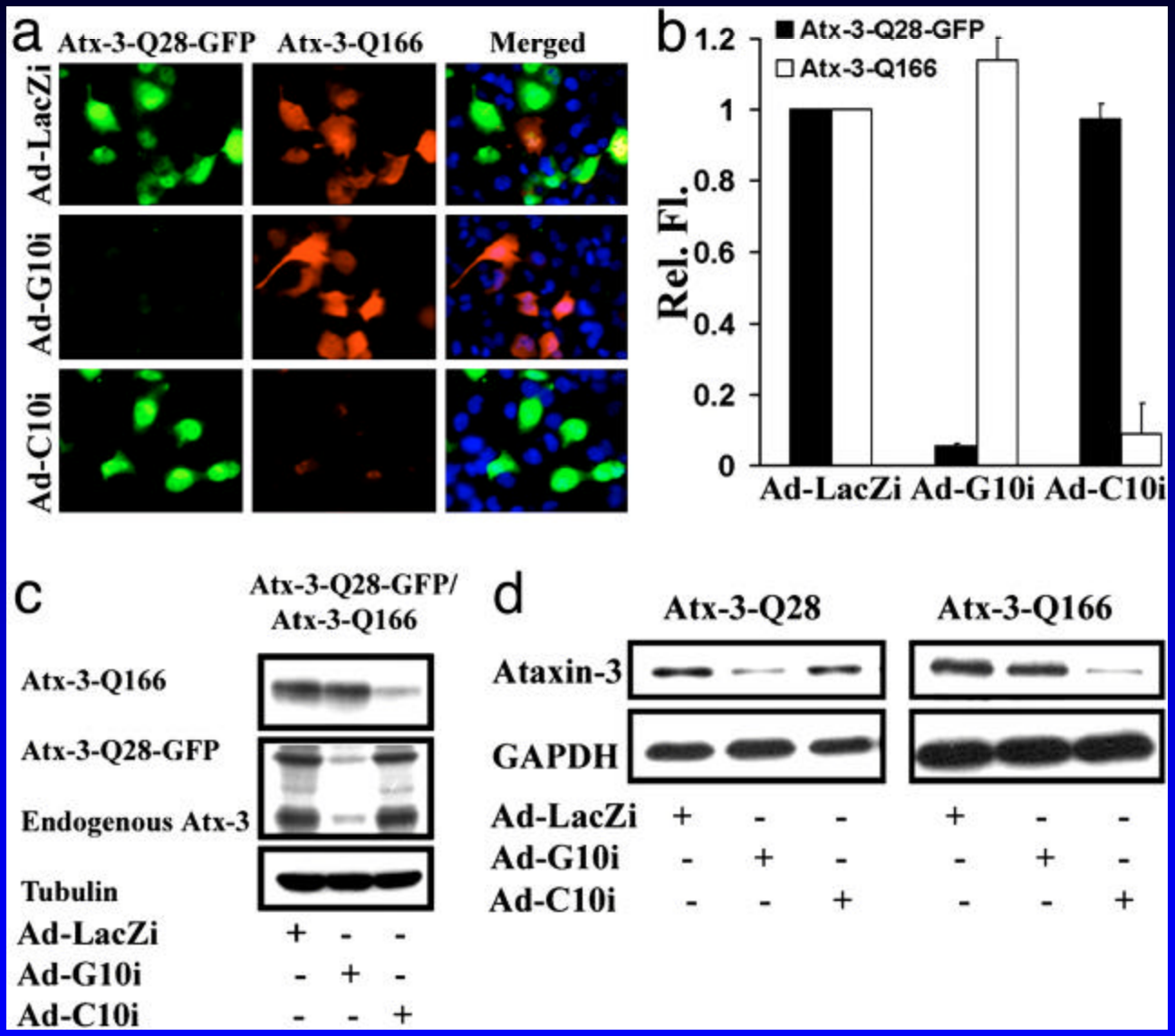
- **Gene delivery for neuroprotection:** ALS and **spinocerebellar ataxia 3**

Challenge for Dominant Disorders: Blocking Defective Genes



RNA interference can degrade mutated RNA sequences

RNA Interference to Knock Down Mutant Ataxin



Gene delivery can reduce mutant Ataxin expression in cell culture

Miller et al., *PNAS* (2003)

Summary

- Gene delivery can be used to control adult neural stem cells in the nervous system for neuron replacement
- Gene therapy can be used to deliver genes for general neuroprotection
- Targeted degradation of defective mRNA
- Animal models of ataxia
- Combining stem cell therapy and gene therapy may prove a powerful approach

Acknowledgments

Adult Neural Stem Cells

Karen Lai
Brian Kaspar
Fred H. Gage

ALS Work/AAV Studies

Brian Kaspar
Jeffrey Rothstein
Fred Gage
Project ALS

Ataxia

Victor Miller et al.
Henry Paulson