

Induced Neuroprotective A2 Astrocytes to Treat Traumatic CNS Injury



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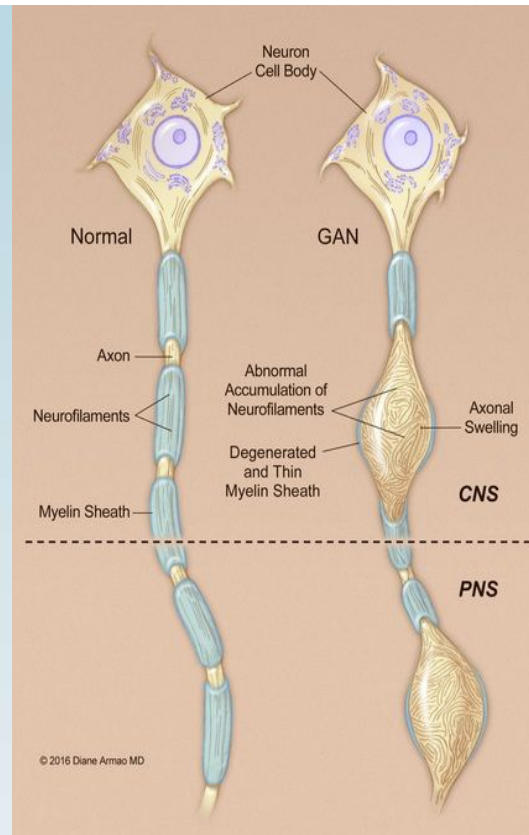
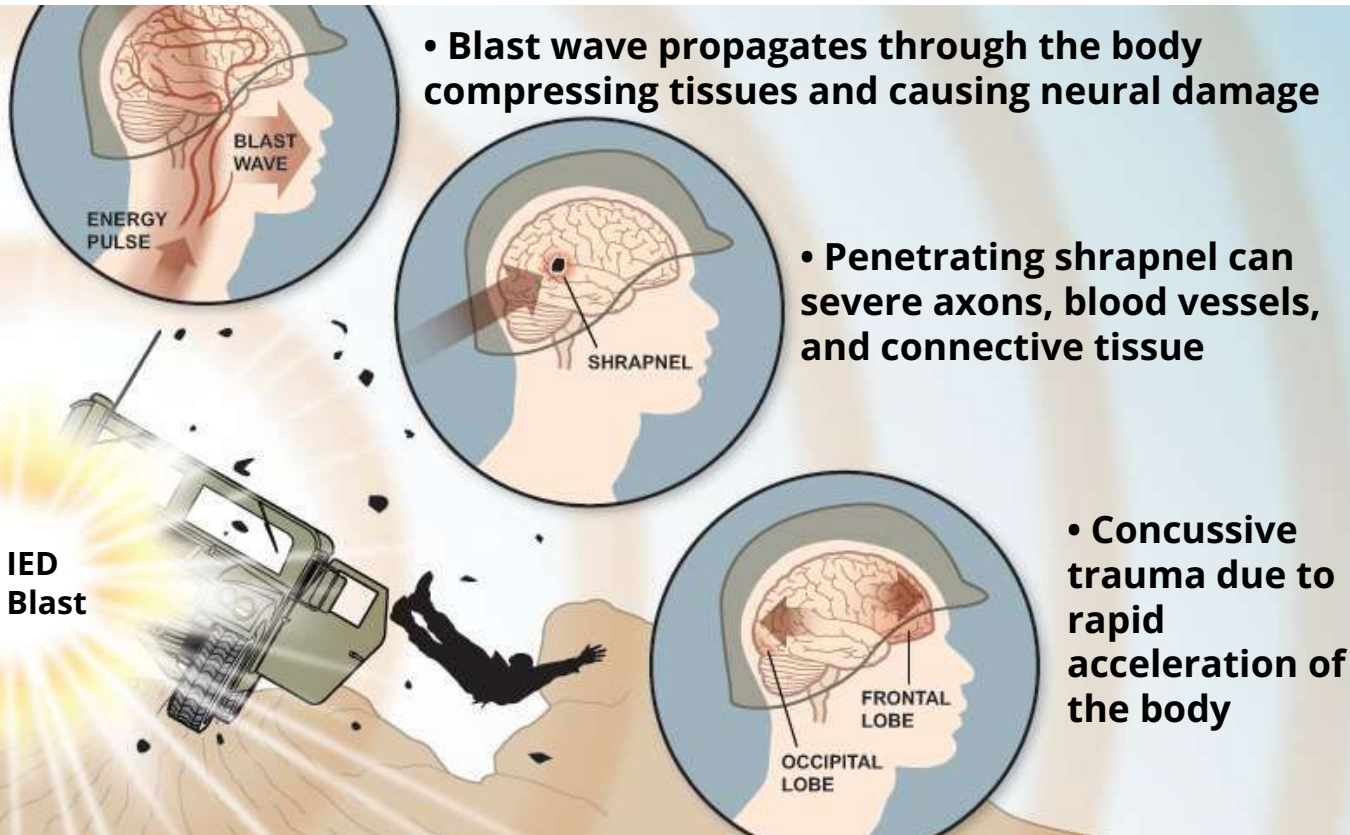
BACKGROUND - Thrust D

Improving treatment for veterans suffering from traumatic brain injury or post-traumatic stress disorder (DARPA)

- Explosives in the battlefield:
 - **3,500+** Americans killed by IEDs
 - **30,000+** Americans wounded by IED's
 - *Traumatic brain injury is correlated with **higher incidence of Alzheimer's** amongst veterans*

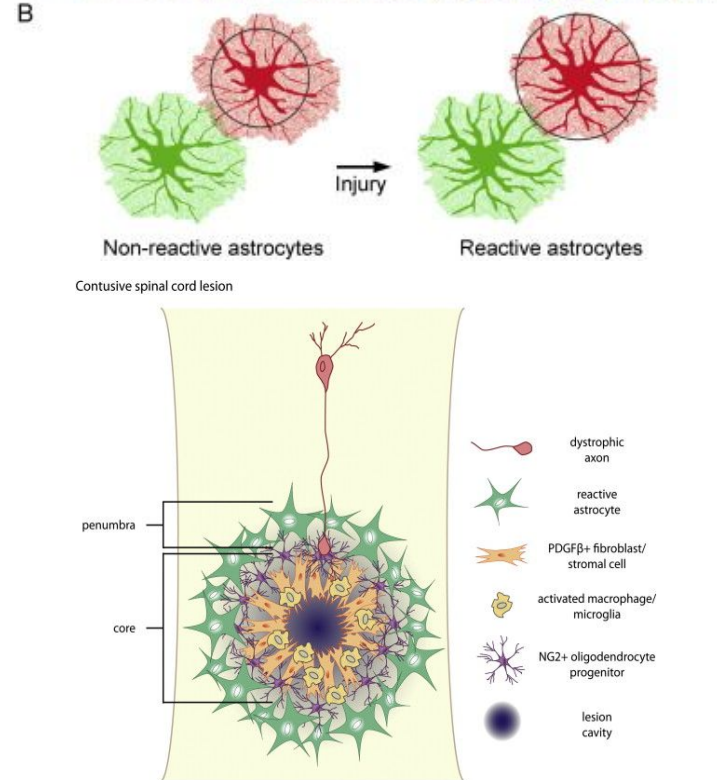


BACKGROUND - Thrust D



Gliosis is a damage response in the CNS that depends on reactive astrocyte composition

- Astrocytes become reactive after CNS trauma
 - **Functionally harmful or helpful?**
- Reactive astrocyte population is **heterogeneous**
- Scar prohibits healing long-term
 - BUT if reactive astrocytes are removed than the damage is **worse**
- How can scarring be both beneficial and harmful?



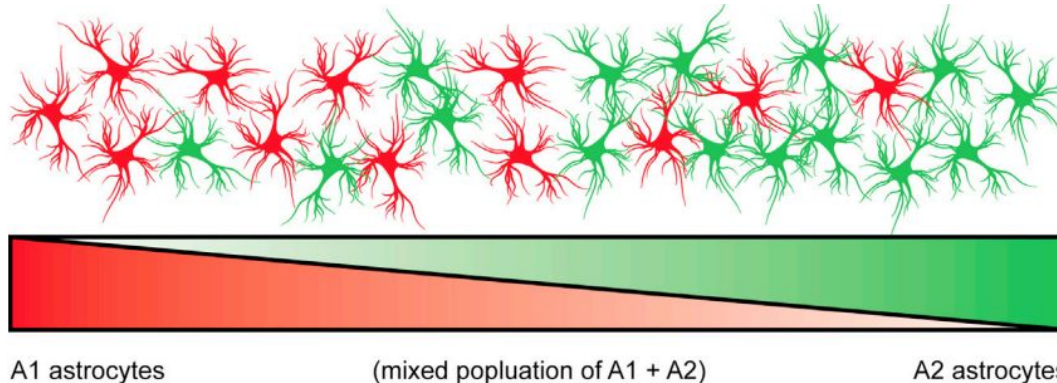
A1 astrocytes are neuro-inhibitory and **A2 astrocytes** are neuroprotective

A1 astrocytes: induced by neuroinflammation

- Prohibits axonal regeneration
- Promotes neuronal and oligodendrocyte death

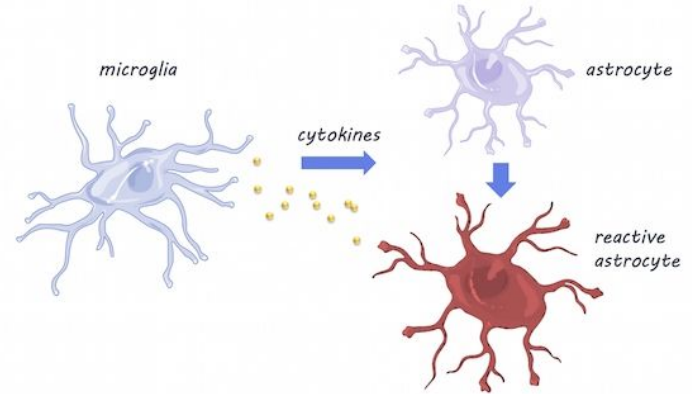
A2 astrocytes: induced by ischemia

- Promotes axonal repair and neurogenesis



Literature Review: Liddelow et al. 2017

- **M1/M2 macrophages:**
 - M1 microglia are pro-inflammatory
 - M2 microglia are neuroprotective
- **M1 → A1 Paradigm:**
 - Pro-inflammatory microglia induce neurotoxic astrocytes
- **Current understanding of A2 induction is limited**
 - Potential theory: **M2 → A2**



OVERVIEW

Aim 1: Quantify cytokines that induce A2 astrocyte development following ischemic injury ***to understand how to induce neuroprotective A2***

Aim 2: Quantify the effect of an exclusively A2 astrocyte environment on synaptic formation and function *in vitro* ***to understand what A2 does to the uninjured brain***

Aim 3: Investigate therapeutic effect of A2 upregulation following traumatic CNS injury ***to understand how to use A2 to heal injury***

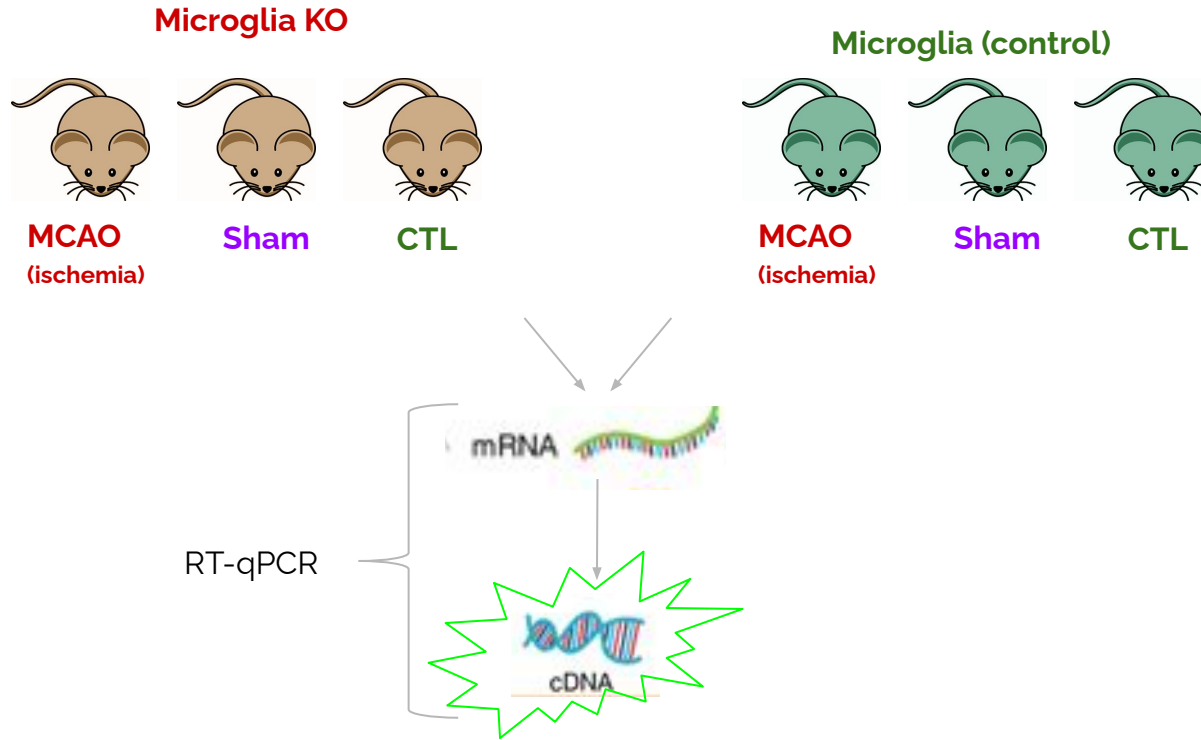
Aim 1:

Quantify cytokines that induce A2 astrocyte development following ischemic injury

...to understand how to induce neuroprotective A2

1.1: Are microglia necessary for A2 astrocyte induction?

In Vivo:
Microglia
KO models

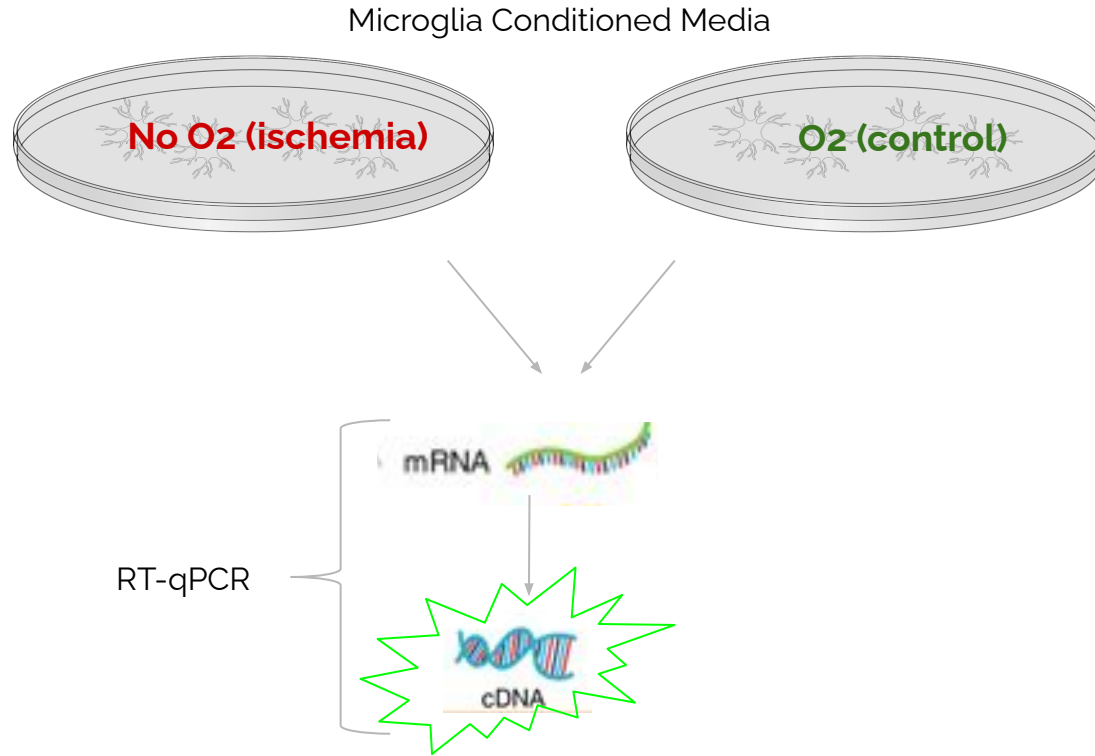


Quantify A2 Gene Expression via Fluorescence ($-2\Delta\Delta Ct$)

1.1: Are microglia necessary for A2 astrocyte induction?

In Vitro:

*Oxygen-Glucose
Deprivation of
Microglia*



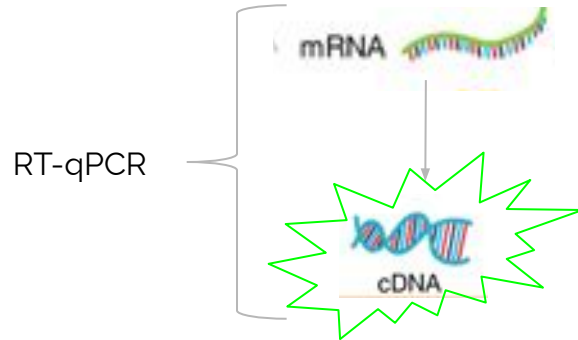
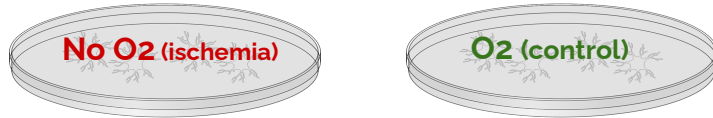
Quantify A2 Gene Expression via Fluorescence ($-2\Delta\Delta Ct$)

1.1: Are microglia necessary for A2 astrocyte induction?

YES

1.2: **Quantify** microglia-released cytokines following ischemia

Microglia Conditioned Media



Quantify Cytokines via Fluorescence ($-2\Delta\Delta C_t$)

NO

1.3: **Identify** upregulated cytokines following ischemia

Middle Cerebral Artery Occlusion



- Euthanise after 3 days
- Cortical Extraction



Identify Cytokines via RNA-seq

Aim 1: Challenges and *Resolutions*

Challenges	<i>Resolutions</i>
Microglia KO Mouse Model Design	<i>Csf1r</i> ^{-/-} (Liddelow)
RNA extraction purity	<i>Purification Kits</i>

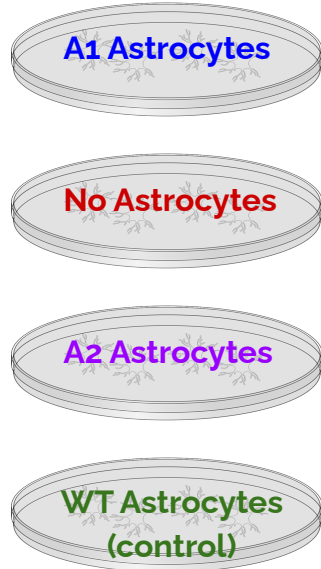
Aim 2:

Quantify the effect of an exclusively A2 astrocyte environment on synaptic formation and function *in vitro*

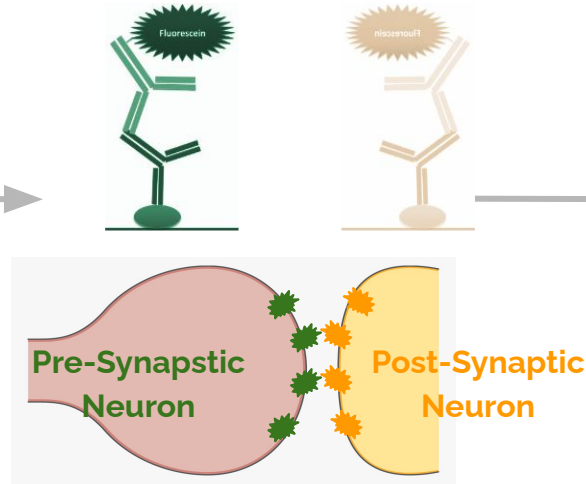
...to understand what A2 does to the uninjured brain

2.1: Quantify A2 effect on **synaptic formation** *in vitro*

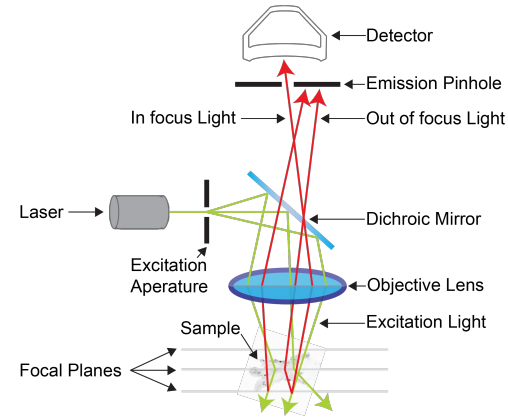
Neurons cultured with varying astrocyte populations



Immunostain for synapses



Quantify Synapses via Confocal Microscopy



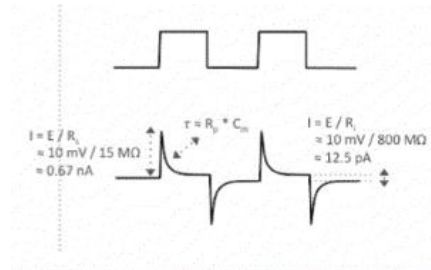
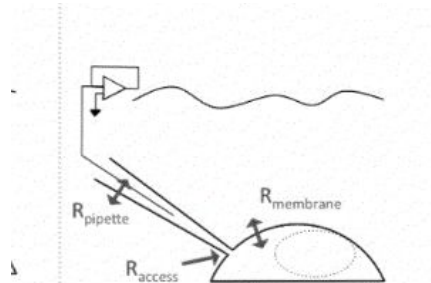
Expect: Neuroprotective A2 → More synapses

2.2: Quantify A2 electrophysiological effect on **synaptic function** *in vitro*

Neurons cultured with varying
astrocyte populations



Whole Cell Patch Clamp
Recording (EPSCs)



Expect:
Neuroprotective A2
↓
Higher Frequency and
Amplitude of EPSCs

Aim 2: Challenges and *Resolutions*

Challenges	<i>Resolutions</i>
Antibody specificity	<i>Multiple ab host species + secondary ab</i>
Whole Cell Patch Clamp Technique Time and Physical Demands	<i>Nanoscale Recording?</i>

Aim 3:

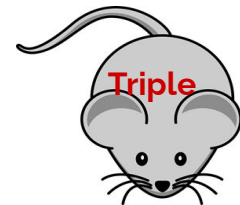
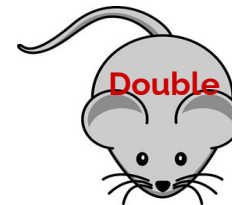
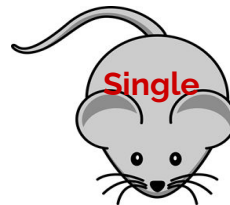
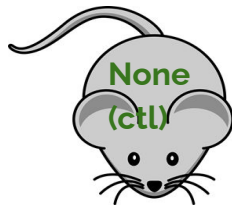
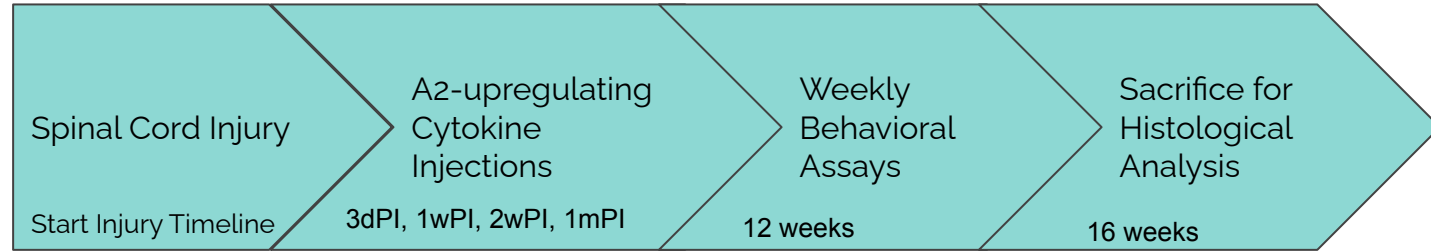
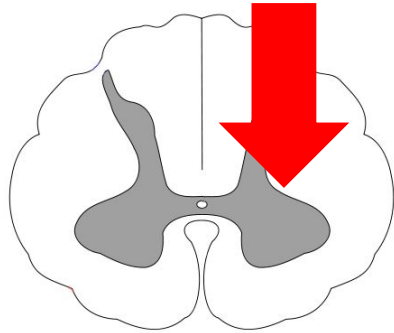
Investigate therapeutic effect of A2 upregulation
following traumatic CNS injury

...to use A2 to heal injury

Aim 3: Investigate therapeutic effect of A2 upregulation following traumatic CNS injury

Traumatic CNS injury model: Contusive spinal cord injury (SCI)

C5 Contusive SCI:
~60 kDynes

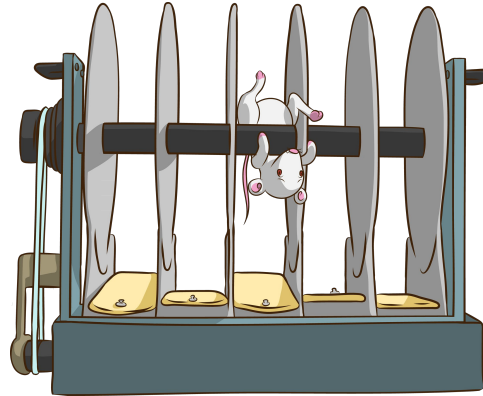


3.1: Does A2 upregulation produce behavioral improvement after traumatic CNS injury?

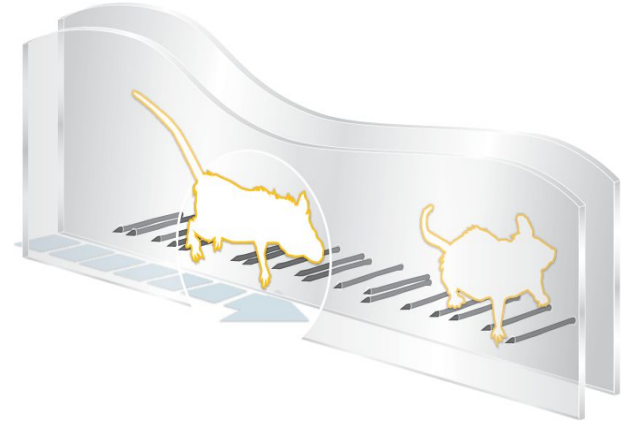
Grooming



Rotarod



Horizontal Ladder



↑ A2-Inducing Injections → ↑ Behavioral Scores

3.2: Does A2 induction cause neural preservation and axonal regeneration *in vivo*?

Immunohistochemical (IHC) Analysis:

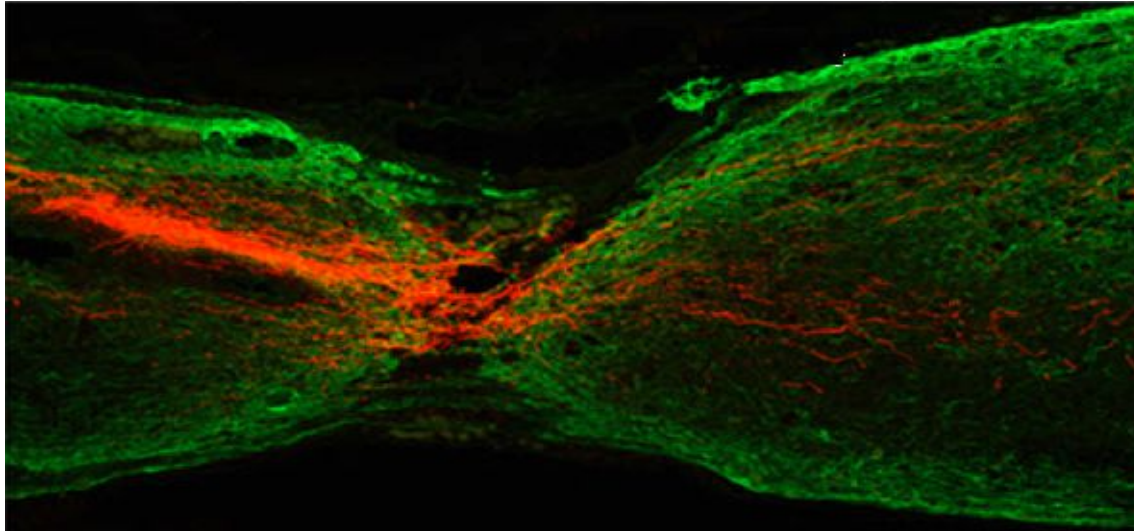
- Axonal regeneration (BDA)
- Functional motor units (ChAT, synapsin)
- Adjacency of functional neurons (NeuN) with neuroprotective A2 (S100A10)

Temporal Analysis:

- Comparison of behavioral/histological data for different injection time points post-injury



Is A2 upregulation best in acute or chronic stages of CNS injury?



Aim 3: Challenges and *Resolutions*

Challenges	<i>Resolutions</i>
Broader inferences of behavioral tests	<i>Correlation with histological data</i>
Experimental consistency for groups with different numbers of injections	<i>Sham injections of saline</i>

Summary

Aim 1: Understand how to induce neuroprotective A2

- Identify necessary cytokines for A2 induction and expression
- Test the M2 → A2 paradigm

Aim 2: Understand what A2 does to CNS *in vitro*

- Quantify the effect of A2 on synaptic formation
- Patch clamping to test electrophysiology of A2-modulated neurons

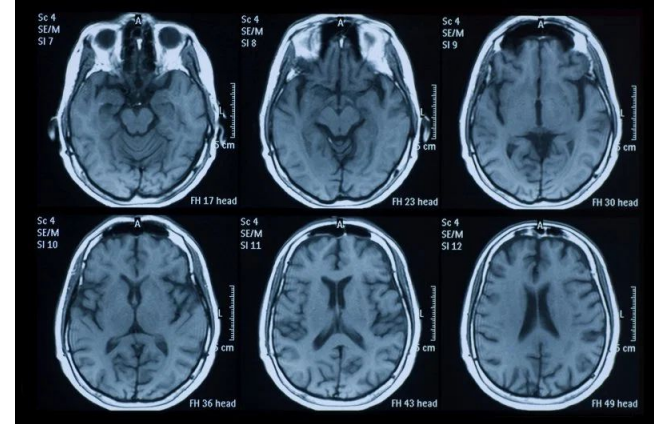
Aim 3: Understand how to use A2 to heal injury *in vivo*

- After induced traumatic SCI, test behavioral recovery with A2 upregulation
- Correlate behavioral results with histology

We predict that A2 induction and upregulation after CNS trauma will serve as promising therapeutic strategy to counteract neurotoxicity and degeneration

IMPACT - Thrust D

- **Thrust D:** Improving treatment for veterans suffering from traumatic brain injury (DARPA)
 - Modulation of A2 astrocyte growth could potentially:
 - Improve recovery time
 - Preserve cognitive function in soldiers affected by BINT





THANK YOU

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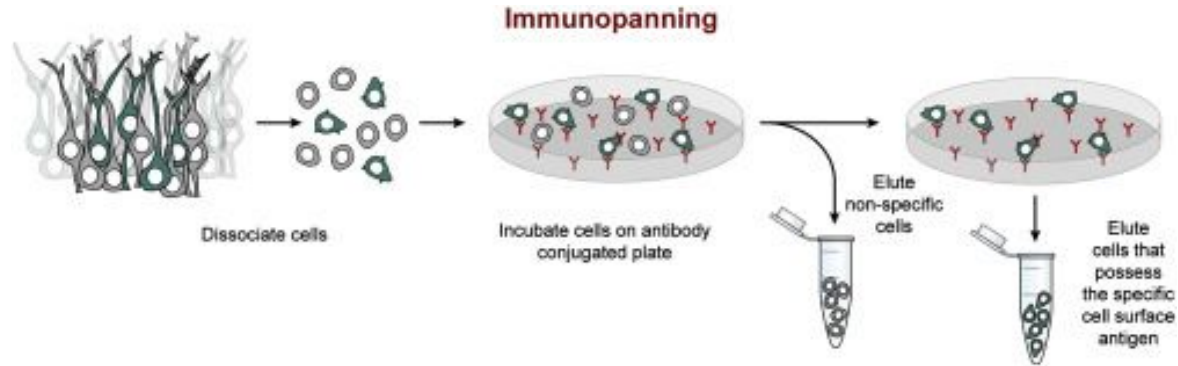
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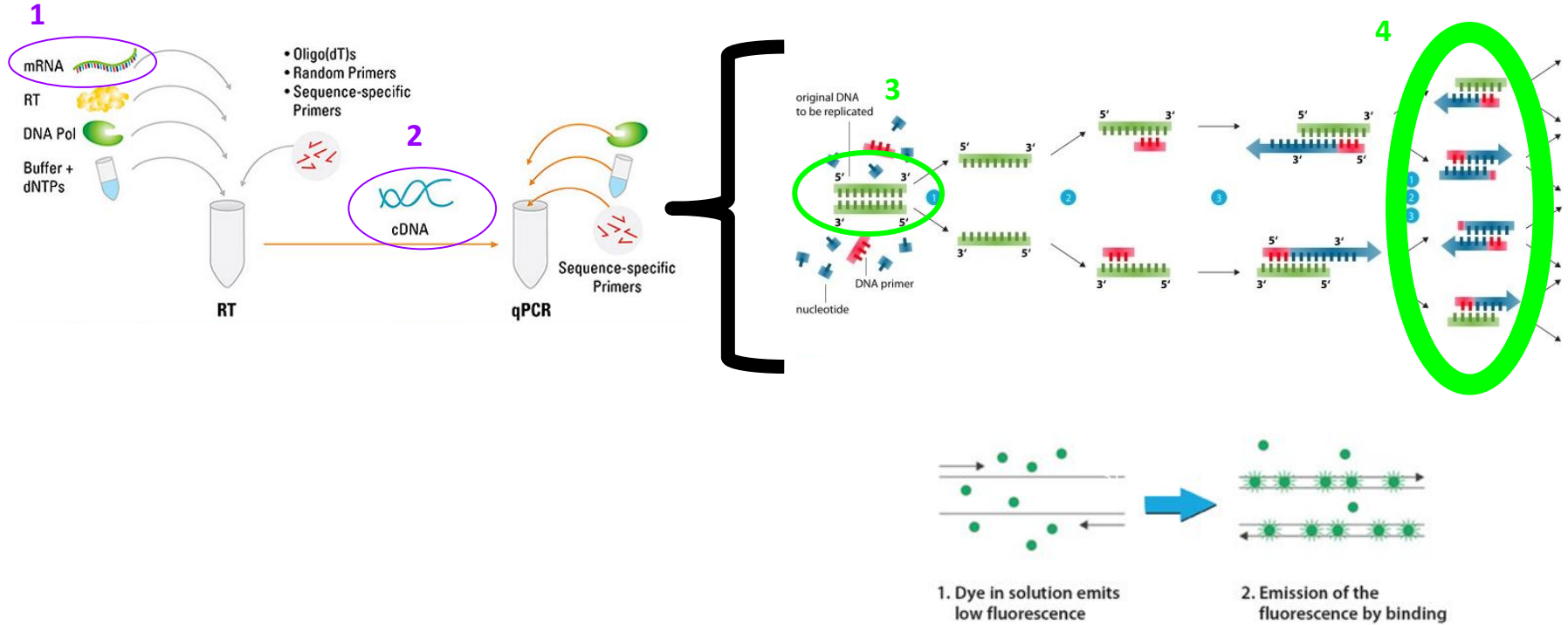
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Supplemental

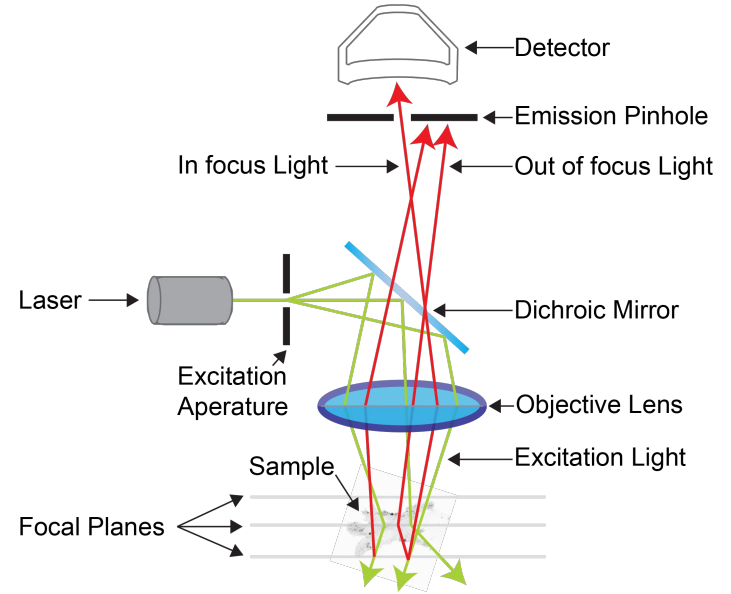
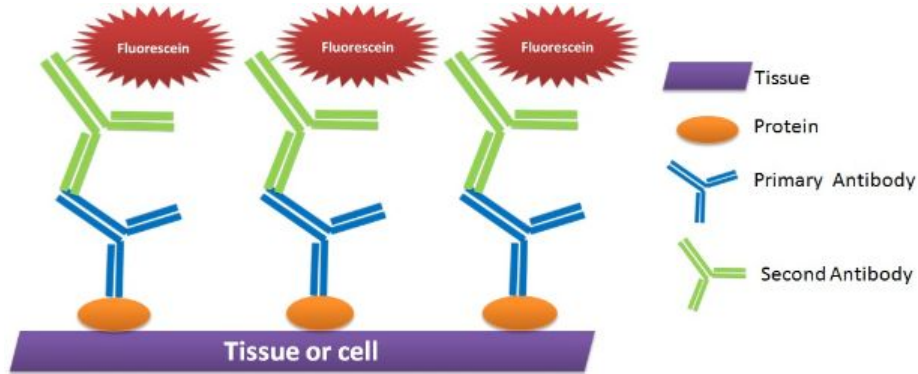
Methods: **immunopanning** selects target cells



Methods: RT-qPCR quantifies gene expression

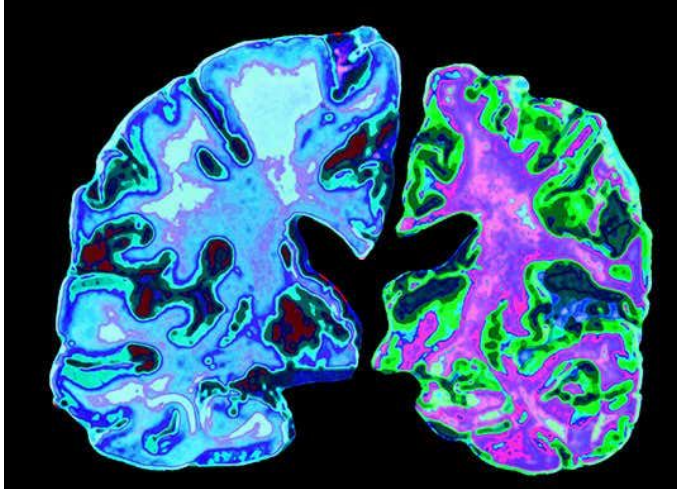


Methods: Immunofluorescence + Confocal Microscopy



BACKGROUND - Thrust B

The diagnosis and/or treatment of neurological diseases (NIH)



Alzheimer's Disease

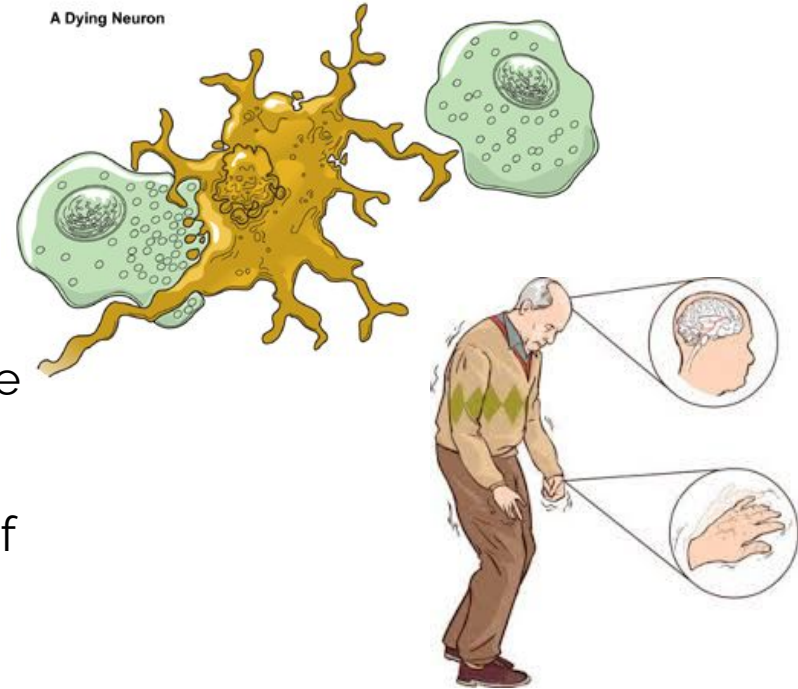
- **>5 MM** Americans affected
- Most common cause of dementia in elderly individuals
- 6th leading cause of death in the US

BACKGROUND - Thrust B

The diagnosis and/or treatment of neurological diseases (NIH)

Parkinson's Disease

- **>1 MM** Americans affected
- Caused by death of dopamine producing neurons
- Can lead to loss of motor function, cognitive decline, depression, and anxiety
- By time motor symptoms appear, 60-80% of dopamine producing neurons are dead



IMPACT - Thrust B

- **Thrust B:** The diagnosis and/or treatment of neurological diseases (NIH)
 - New potential therapy through stimulating the growth and development of A2 astrocytes