Glia-Neuron GPCR Signaling as a Therapeutic Route to Axon Regeneration

cAMP-PKA Activation Elevates Excitability and Regrowth in Neurons

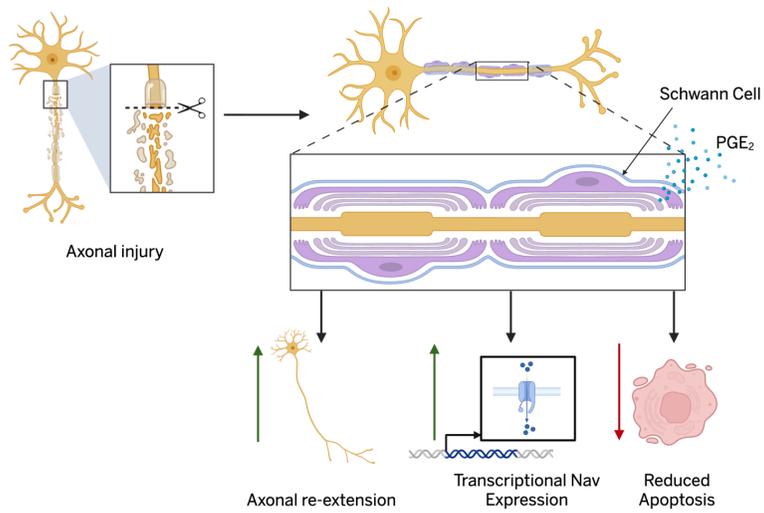## VISUAL ABSTRACT

Axonal injury

Schwann Cell

PGE₂

Axonal re-extension

Transcriptional Nav Expression

Reduced Apoptosis

Figure 1: Visual Abstract. *Image created by author using Biorender, 2025*

Background
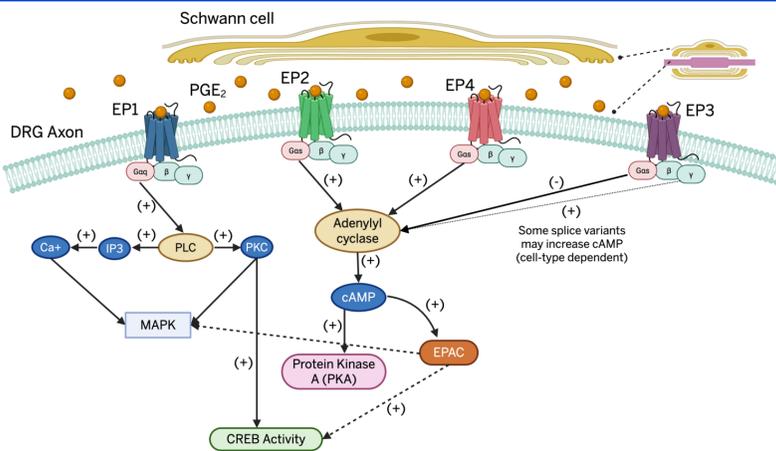
Figure 3: PGE2 Signaling Schematic. *Image created by author using Biorender, 2025*
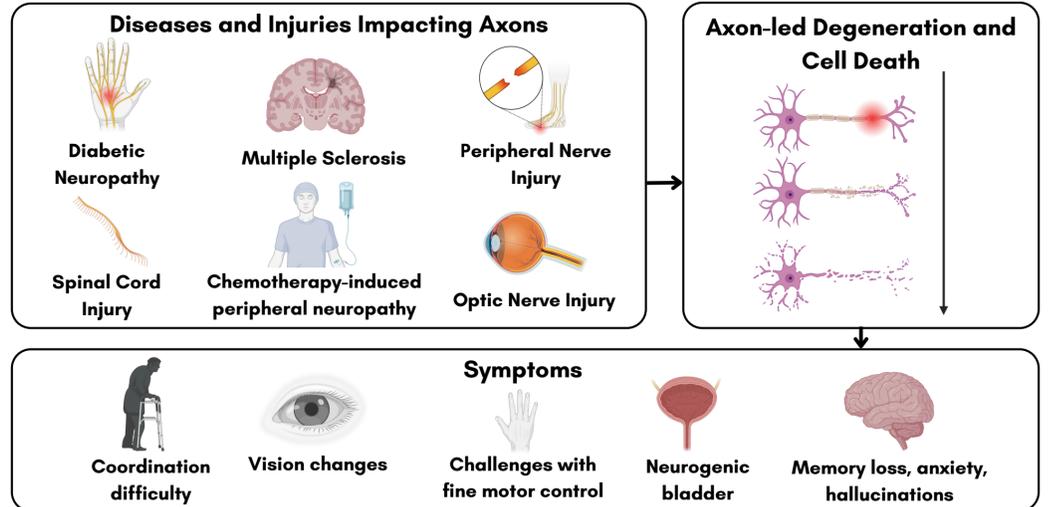
INTRODUCTION

The Problem

Diseases and Injuries Impacting Axons

Diabetic Neuropathy • Multiple Sclerosis • Peripheral Nerve Injury • Spinal Cord Injury • Chemotherapy-induced peripheral neuropathy • Optic Nerve Injury

Axon-led Degeneration and Cell Death

Symptoms

Coordination difficulty • Vision changes • Challenges with fine motor control • Neurogenic bladder • Memory loss, anxiety, hallucinations

Figure 2: Problem Statement. *Image created by author using Biorender and Canva, 2025*

METHODS

1 *In vitro* Axotomy
- Effects of PGE2 signaling on axonal growth off of cut
- **Determining if PGE2 promotes axonal re-extension**

2 RNAScope
- Voltage-gated sodium channels
- **Seeing if treatment increases excitability after injury**

3 Annexin-V
- Annexin-V protein stains dead cells
- **Determine the best dosage of PGE2 to promote cell survival**

4 Brainbow
- Visualization of individual cells
- **Validation of increased axonal growth**

5 Molecular Docking
- Dock transport protein to prodrug
- **Understand potential to reduce off-target effects**

6 Transcriptomics
- Pathway analysis of differentially expressed genes in injury model
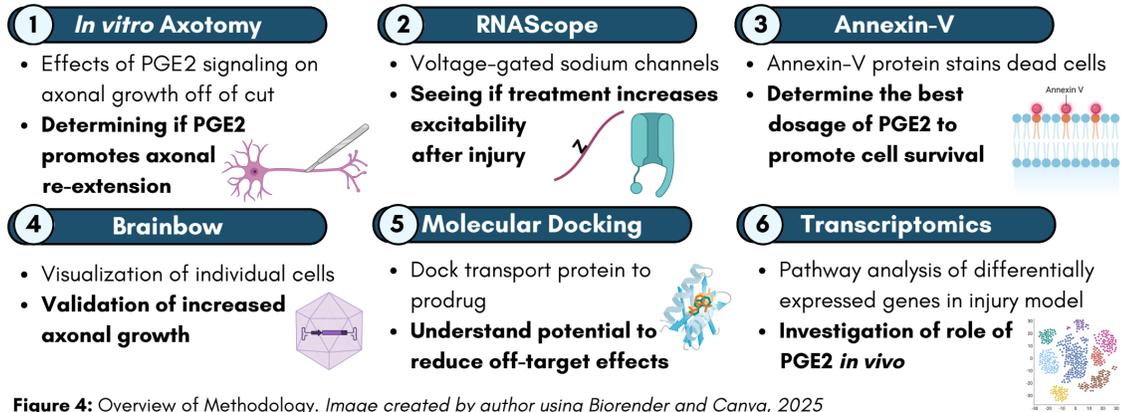- **Investigation of role of PGE2 *in vivo***

Figure 4: Overview of Methodology. *Image created by author using Biorender and Canva, 2025*

Research Questions

- Can **PGE₂ promote axonal re-extension** and **cell survival** after injury?
- What is the optimal dosage of **PGE₂** to **prevent toxicity** and maximize protective effects?
- How does PGE2 impact neuronal excitability **after injury**?
- How do PGE2 downstream signaling molecules and EP Receptors affect the **efficacy of PGE2** in promoting a repair state?
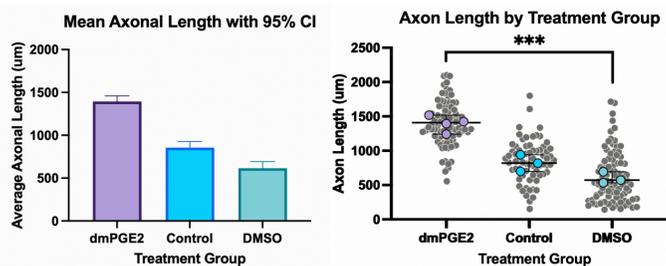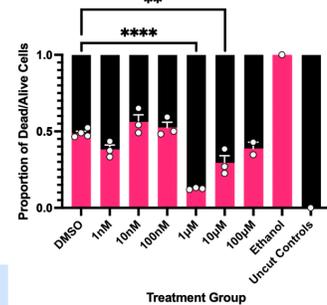
RESULTS

dmPGE2 Promoted Re-extension

Figure 5A-B: dmPGE2 boosts re-extension. *Image created by author using Prism, 2025*

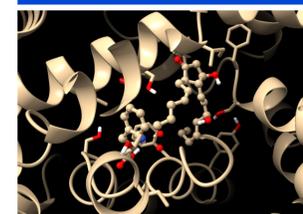**126.29%** more growth in dmPGE2 wells compared to DMSO control

Optimal Dosage of PGE₂ Improves Survival After Injury

1uM and **10uM** dosages significantly improved survival

Figure 6: Annexin-V Staining results. *Image created by author using Prism, 2025*

Molecular Docking

Binding Affinity
-8.28 kcal/mol

Table 1: Binding Affinity. *Created by author using Canva, 2025*

LAT1 + prodrug shows potential for delivery

Figure 7: Prodrug docked to LAT1. *Image created by author using ChimeraX, 2025*
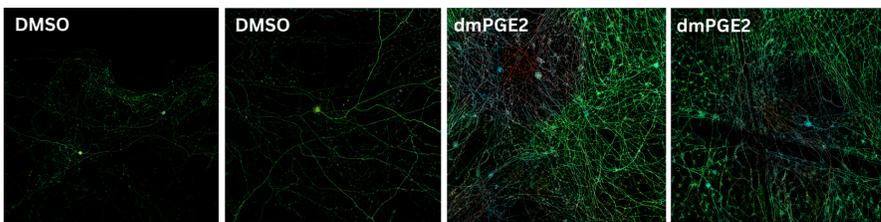
Brainbow AAV: More Axonal Regrowth Following Axotomy

DMSO | DMSO | dmPGE2 | dmPGE2

Figure 8: Brainbow sample images. *Image created by author using Fiji, 2025*

PGE2/cAMP/MAPK Differences Across Injury and Naive

Schwann Cell Upregulated Pathways — MAPK Signaling, cAMP

Mrgprd/Gm7271 Downregulated Pathways — cAMP

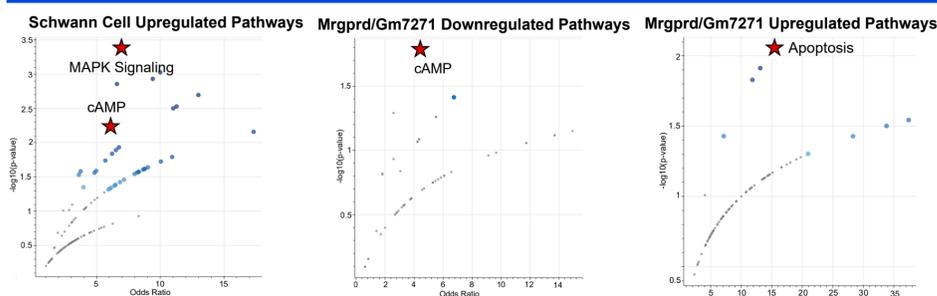Mrgprd/Gm7271 Upregulated Pathways — Apoptosis

Figure 10: DEG and Pathway Analysis Results. *Image created by author using R (GGPlots) and Appyter, 2025*

cAMP downregulated in apoptotic neurons, **MAPK and cAMP** upregulated in Schwann cells after injury

Increased Voltage-Gated Sodium Channel Activation in dmPGE2-treated Cells

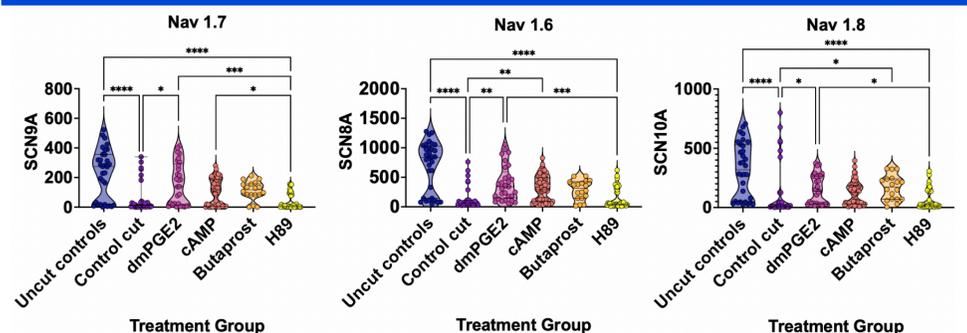Nav 1.7 | Nav 1.6 | Nav 1.8

Figure 9: RNAScope Quantification Results. *Image created by author using Prism, 2025*

IMPACT

- Offers direction for future treatment - PGE2 may be a useful **therapeutic target** to promote the re-extension of axons following injury
- Axonal re-extension can not only **prevent cell death,** but also lead to a **restoration of function** for millions of patients

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