

The Impact of GPR4 in Traumatic Brain Injury and Blood Brain Barrier Connection

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Abstract

Alzheimer's Disease is the most prevalent cause of dementia, characterized by cognitive decline and neurodegeneration associated with tau buildup and amyloid beta polypeptides. The Blood Brain Barrier (BBB) is a crucial protective barrier that prevents harmful substances such as amyloid-beta from entering the brain. However, TBI's (Traumatic Brain Injury) can compromise the BBB allowing the entry of neurotoxins. GPR4 (G-protein coupled receptor), lined along the BBB is a receptor that can detect proton change in the blood. Activation of GPR4 leads to the reinforcement and strengthening of the blood brain barrier. We investigated the role of GPR4 in TBI and the blood brain barrier. My hypothesis was that GPR4 knockout mice will show neuroinflammatory responses faster and therefore leading to cognitive decline and worsened motor skills, performing worse in the behavioral tests than the control group.

Method

1. sTBI (Severe Traumatic Brain Injury)

- The mice were all anesthetized and went through a craniotomy (open brain) procedure with the use of a high speed micro drill. The mice then underwent a direct impact on the brain at Bregma - inferior 2.5 mm and lateral 2.5mm, with a velocity at 5 m/s, depth 2 mm, and 180 ms for the impact duration. The injury site was sutured and placed back in their cages for recovery.

2. Behavioral Tests (Performed on Days 0, 1, 3, 7)

- **Rotarod Test (Figure 1a)**
This test involves putting mice on a rotating rod at a linear increase from 4 - 45 RPM and recording how long the mouse is able to stay on for.
- **Foot-Fault Test (Figure 2a)**
Mice are set on elevated hexagonal grids that are connected together. While moving along these grids, each foot fault is counted and recorded at the end.
- **Cylinder Test (Figure 3a)**
Mice are placed in a clear cylinder and the number of times it rears up and presses against the cylinder to climb out are recorded. Additionally, whether the left, right, or both paws are used are also measured because the contralateral side of the mice is typically paralyzed after injury

3. Immunohistochemical Staining

- Samples of brain tissues were collected on day 7
- After washing with PBS, the tissues were incubated in 5% Donkey Serum for 30 minutes
- Brain tissues were incubated in antibodies overnight in 4 degrees Celsius - anti-Glial fibrillary acidic protein (GFAP) antibody, anti-Iba1 antibody
- After rinsing with PBS, the brain sections are incubated with secondary antibodies for one hour at room temperature.

Behavioral Tests

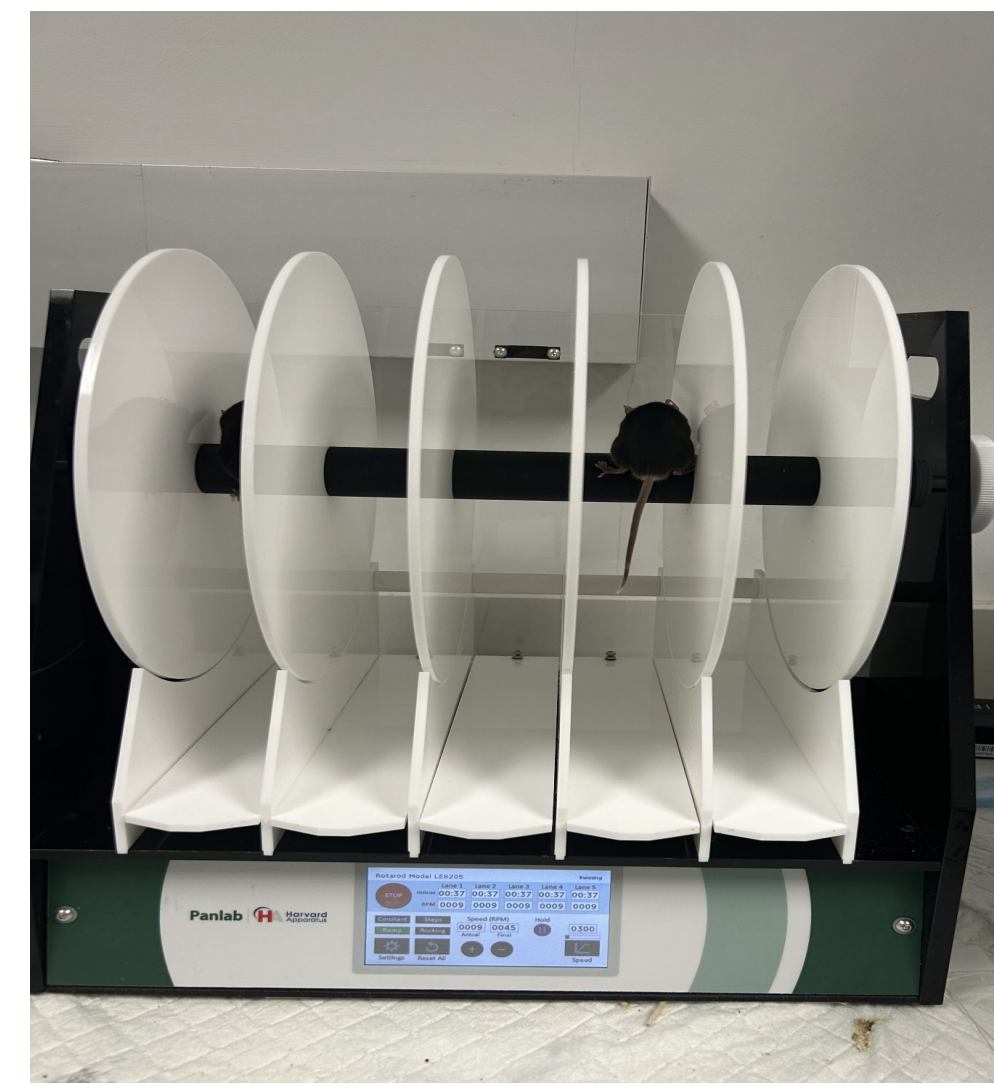


Figure 1a: Rotarod Setup

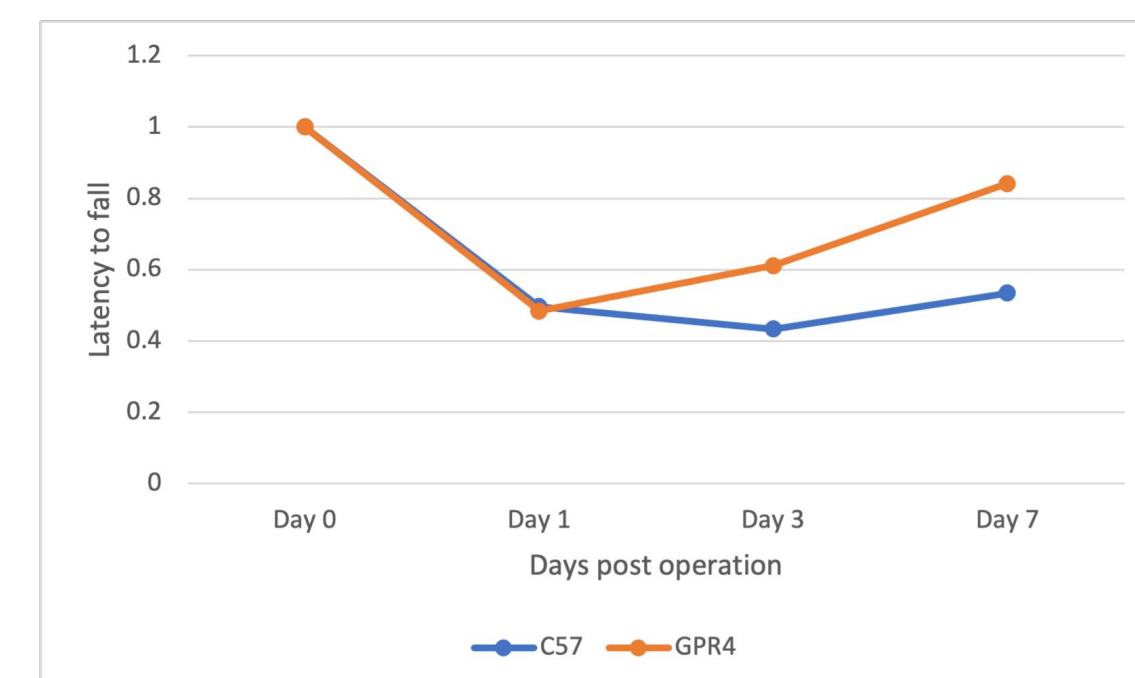


Figure 1b: Rotarod Results

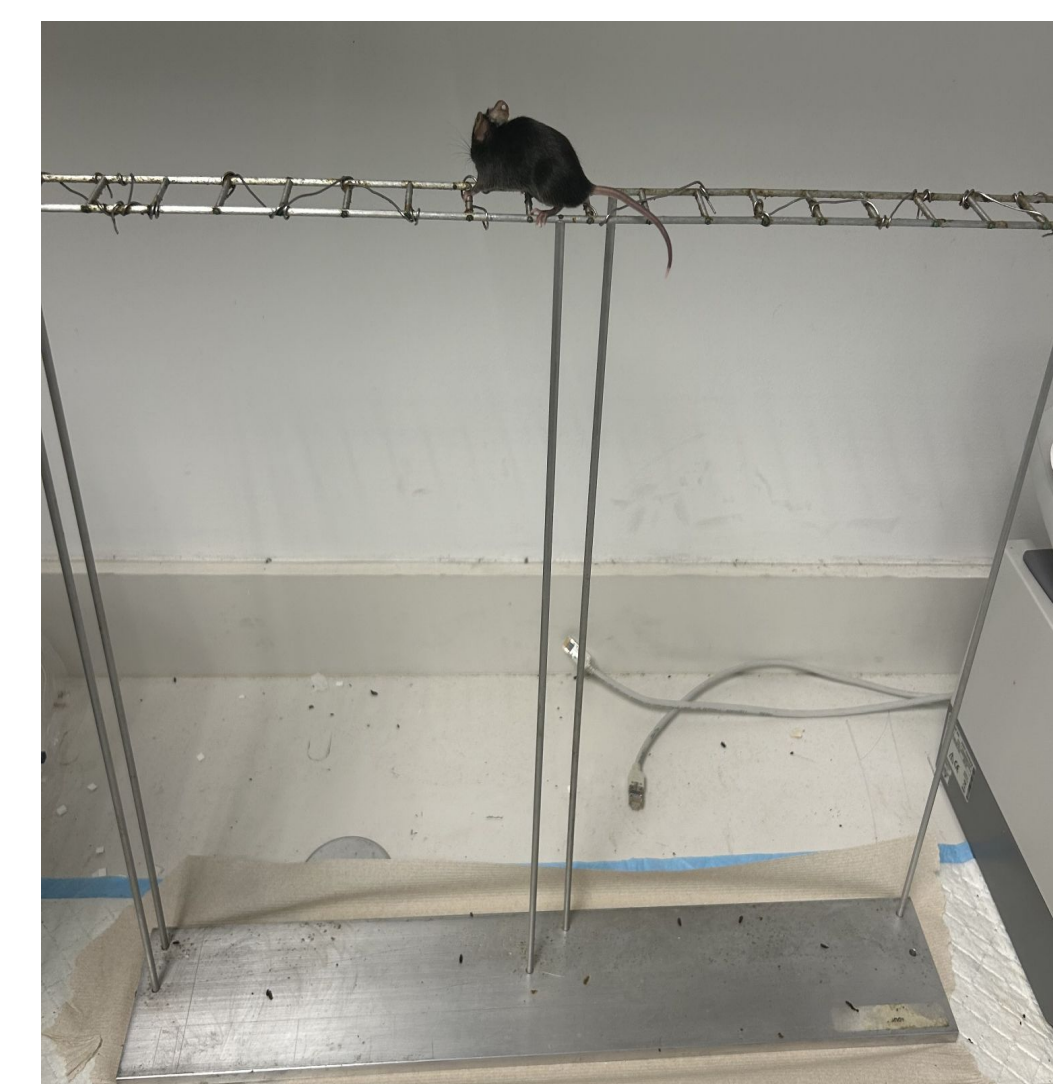


Figure 2a: Foot-Fault Test Setup

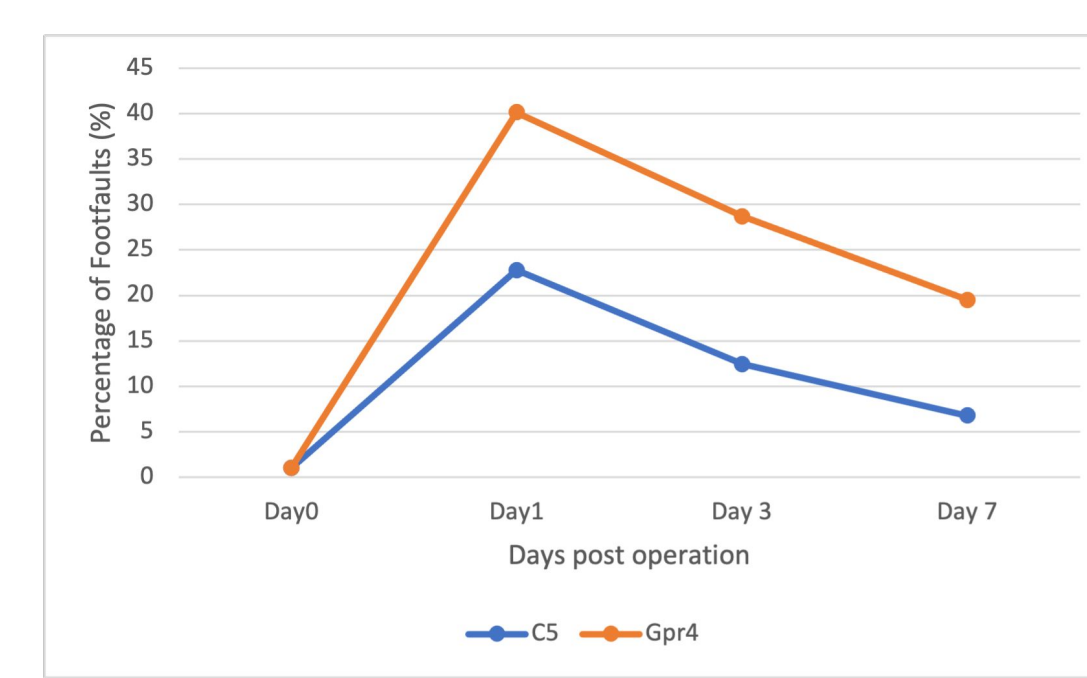


Figure 2b: Foot-Fault Test Results

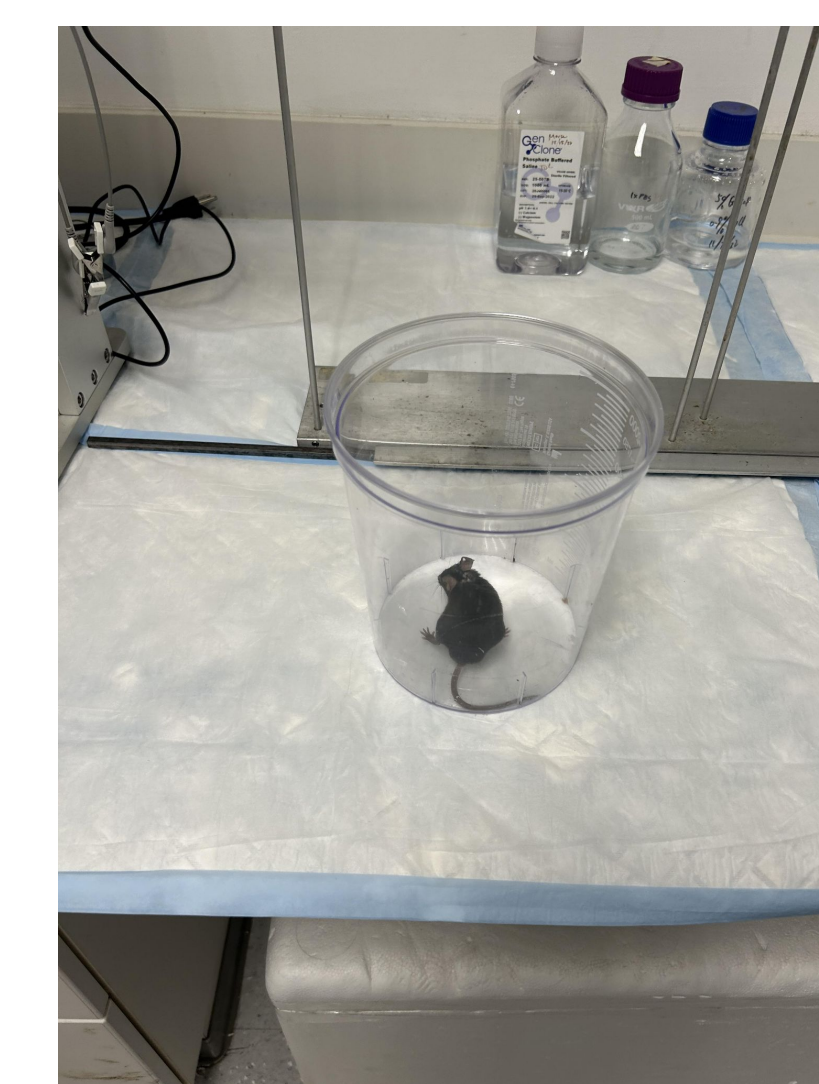


Figure 3a: Cylinder Test Setup

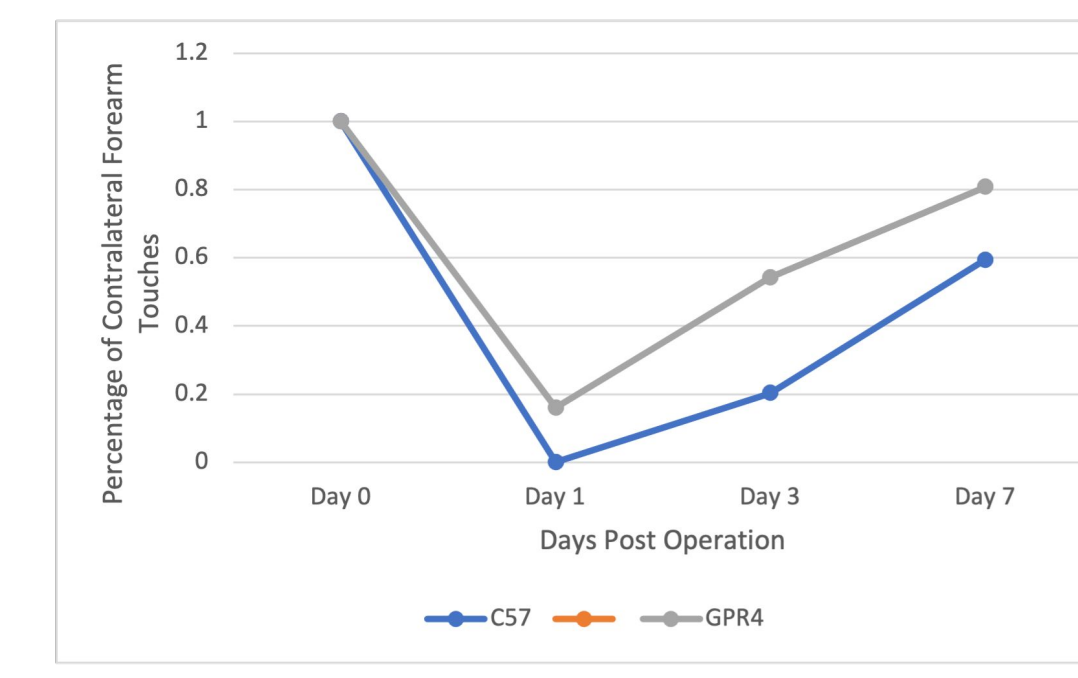
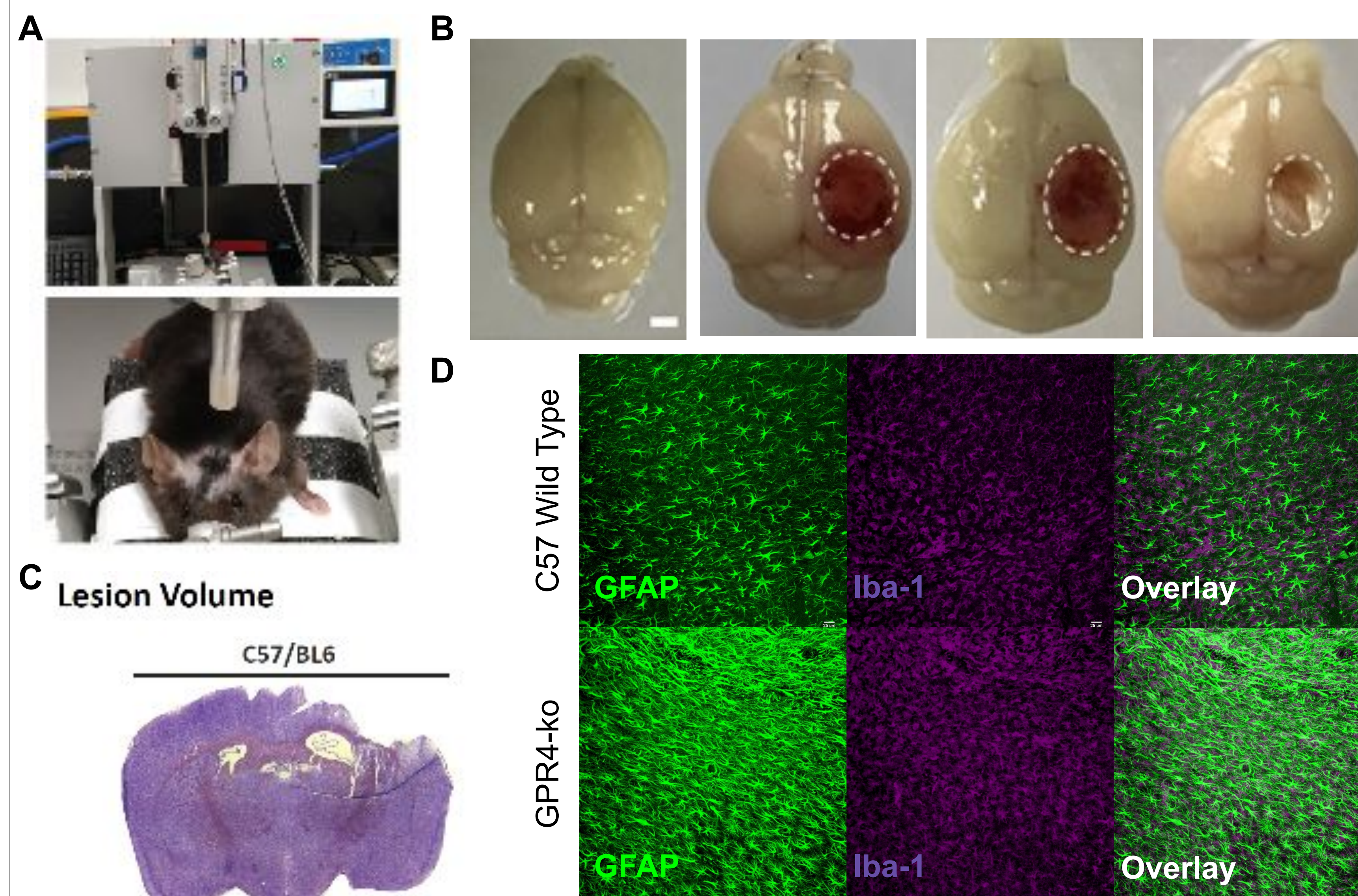


Figure 3b: Cylinder Test Results

Histological Data



- A.** The controlled cortical impact (CCI) model of TBI in mice.
- B.** Representative images of whole brains in the sham and different TBI groups (at 1 day, 3 days, and 7 days post-insult, respectively)
- C.** Representative images of TBI-induced lesion volume post injury.
- D.** Astrocyte (GFAP) and microglial (Iba-1) activation around the injury site for C57 and GPR4-ko mice after severe Traumatic Brain Injury

Results

- All of the behavioral tests were done but we are currently increasing our sample size.
- IHC (immunohistochemistry) staining show that GPR4 knockout mice have greater activation of the microglia and astrocytes through the IBA1 and GFAP antibodies respectively.
- Both the behavioral tests and the histological evidence from staining show that the GPR4 knockout mice have worsened motor skills and cognitive function compared to the control group

Summary

My hypothesis states that the GPR4 knockout mice will exhibit accelerated neuroinflammatory responses, ultimately leading to cognitive decline and impaired motor skills. To do this, all the mice underwent a sTBI, carried out several behavioral tests that would measure cognitive abilities and motor skills, and lastly sacrificed the mice for staining. Preliminary evidence points towards the fact that GPR4 knockout mice have a cognitive deficit and further testing has to be done with a wider sample size. Additionally, evidence with IHC staining also support this claim as it reveals heightened activation of the microglia and astrocyte in GPR4 knockout mice.

Reference

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