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# Diacylglycerol Lipase- $\beta$ Knockout Mice Display a Sex-Dependent Attenuation of Traumatic Brain Injury-Induced Mortality with No Impact on Memory or Other Functional Consequences

O'Brien et al.

Jefin Jose

MJC Presentation

7/15/21

# Traumatic Brain Injury (TBI)

- Traumatic brain was once thought of as a simple acute injury, but more recently, even minor injuries to the brain are perceived as potentially life-threatening
- Even long-term, Mohammed Ali and Aaron Hernandez serve as testaments to the severity of long-term concussive injuries to the brain
- The awareness surrounding brain injury has prompted stricter medical controls in sports and the recognition of traumatic brain injury as a life-altering event
- Around 1.5m Americans sustain a TBI each year, of which 50,000 die



# TBI at the cellular level

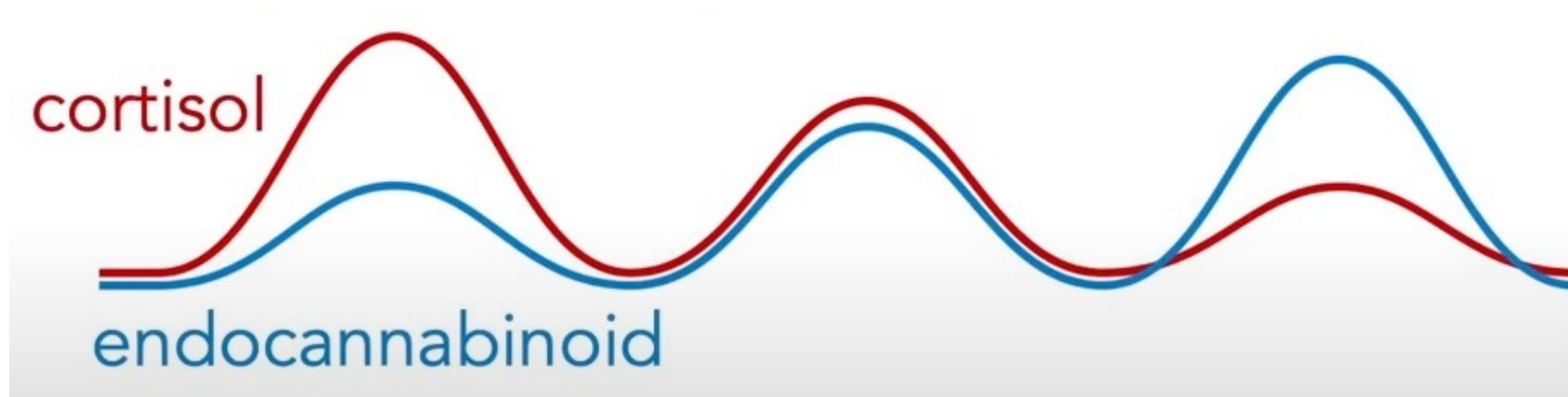
- Beginning immediately, traumatic brain injury leads to an immune response, triggering immunomodulatory regulators such as cytokines to be released and leads to inflammation
- Even chronic neuroinflammation can result from TBI
- The inflammatory response in the brain leads to impaired memory and learning
  - Changes in composition of AMPA receptors (similar to NMDAR)
  - Excitotoxicity

# Endocannabinoid System

- Our body has an endogenous cannabinoid system independent of external cannabinoids such as THC
- The endocannabinoid system oversees the development of chemicals such as eicosanoids that stimulate an immune response, including inflammation in the brain
  - Thus, we can conclude that increasing the activity of the endocannabinoid system leads to inflammation
  - Especially if this inflammation is centralized in the brain (as in the case of TBI), we can conclude that more inflammation would

# Endocannabinoid System

- However, the endocannabinoid system also has positive effects within the body
- The endocannabinoid system is very important for brain development (synaptogenesis, synaptic pruning)
- When exposed to stress, the endocannabinoid system leads to relaxation
  - Leads to hunger
  - Pain relief



# Endocannabinoids

- Diacylglycerol Lipase ( $\alpha$  and  $\beta$ ) is responsible for generating the most abundant endocannabinoid in the body, 2-arachidonylglycerol (2-AG)
- DAGL- $\beta$  makes endocannabinoids, which plays a role in neuroinflammation
- In particular, DAGL- $\beta$  is highly expressed by microglial cells and macrophages, making them a target for neuroimmunological research
- Hypothesis: If we block what makes endocannabinoids, we can prevent the brain impairment of traumatic brain injury

# Sphingolipids

- Sphingolipids are used to insulate axons in the brain and spinal cord
- However, sphingolipids are used to make harmful compounds like ceramides, which leads to cell death
- Furthermore, ceramide can be broken down into sphingosine which leads to mitochondrial dysfunction and brain impairment
- This is especially common in the case of TBI
- The study also wanted to see if TBI causes a change in the level of sphingolipids/ceramides



*Axonal insulation leads to faster cell signaling in the nervous system*



# Knockout Mice

- Unless you remove all the DAGL- $\beta$  protein in the body, it is hard to measure any difference
- Thus, you change the genotype of the mice in their embryonic form
- DAGL- $\beta$  knockout mice

*DAGL –  $\beta^{+}/+$*

*Both alleles for the DAGL- $\beta$  protein are kept (normal mice)*

*DAGL –  $\beta^{-}/-$*

*Both alleles for the DAGL- $\beta$  protein are "knocked-out"*

# Procedure

- In roughly half of the brain, the researchers caused TBI
  - Craniotomy
  - Percussion (like a concussion)
- Mice without TBI were denoted “Sham”
- Half of the mice (irrespective of their phenotype) were DAGL- $\beta$  knockout mice
- All the mice that died in the study died within 2 minutes (removed from the study)

<i>DAGL</i> – $\beta^{-/-}$ Sham n = 15	<i>DAGL</i> – $\beta^{-/-}$ TBI n = 16
<i>DAGL</i> – $\beta^{+/+}$ Sham n = 12	<i>DAGL</i> – $\beta^{+/+}$ TBI n = 12

# Discussion Question

- Do you agree with the procedure so far?
  - Changed the genes of the mice before birth
  - Induced TBI
  - Some mice died
- Are these just some of the costs that come with research, or should we switch to more humane methods that would not involve the harm of animals?

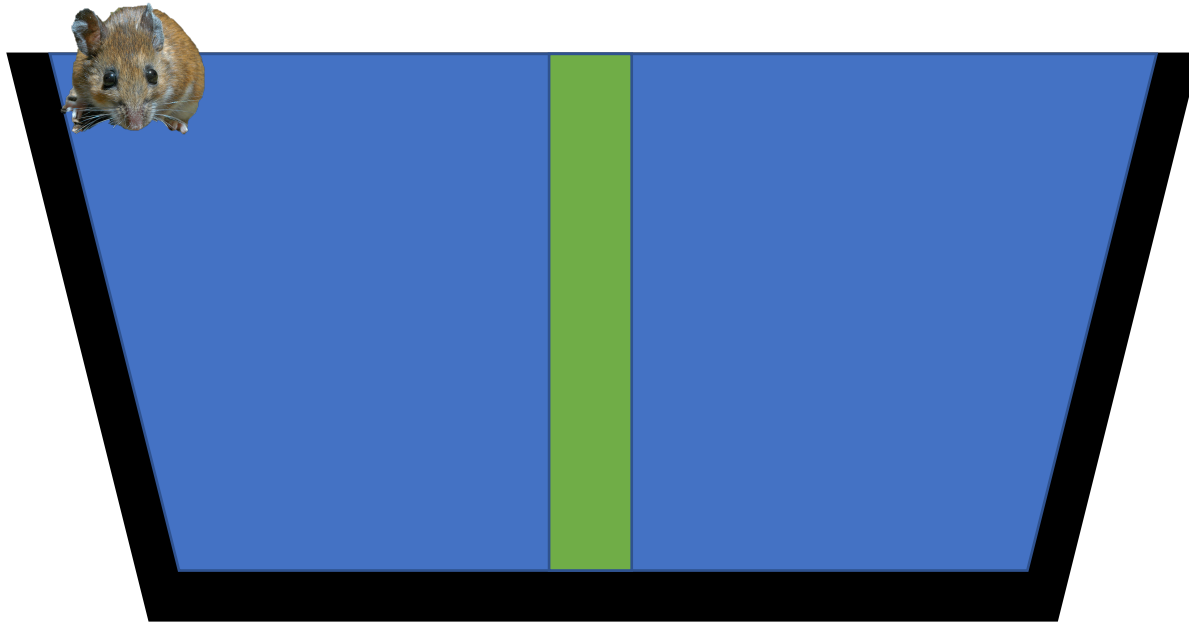


Is this research ethical?

# Testing

- The researchers tested the mice in cognitive/motor/sensory domains
  - Cognitive (related to mental function)
    - Morris water maze platform acquisition
  - Motor (related to speed and distance)
    - Rotarod
    - Neurological Severity Score
  - Sensory (related to time)
    - Righting reflex
    - Light/dark box test
    - Elevated plus maze
- The researchers also tested for lipids using “chromatography–electrospray ionization–tandem mass spectrometry”
  - Used to determine the presence of lipids in moles/mL

# Morris Water Maze



- Mouse first had to find the platform when elevated 1 in above the water (the mice found it, or the researchers showed them)
- After being trained, the water level was raised to occlude the platform and the mice had to find the platform on their own
- Mice are expected to linger at the side of the bowl at first
- This is a test of reference memory

# Rotarod



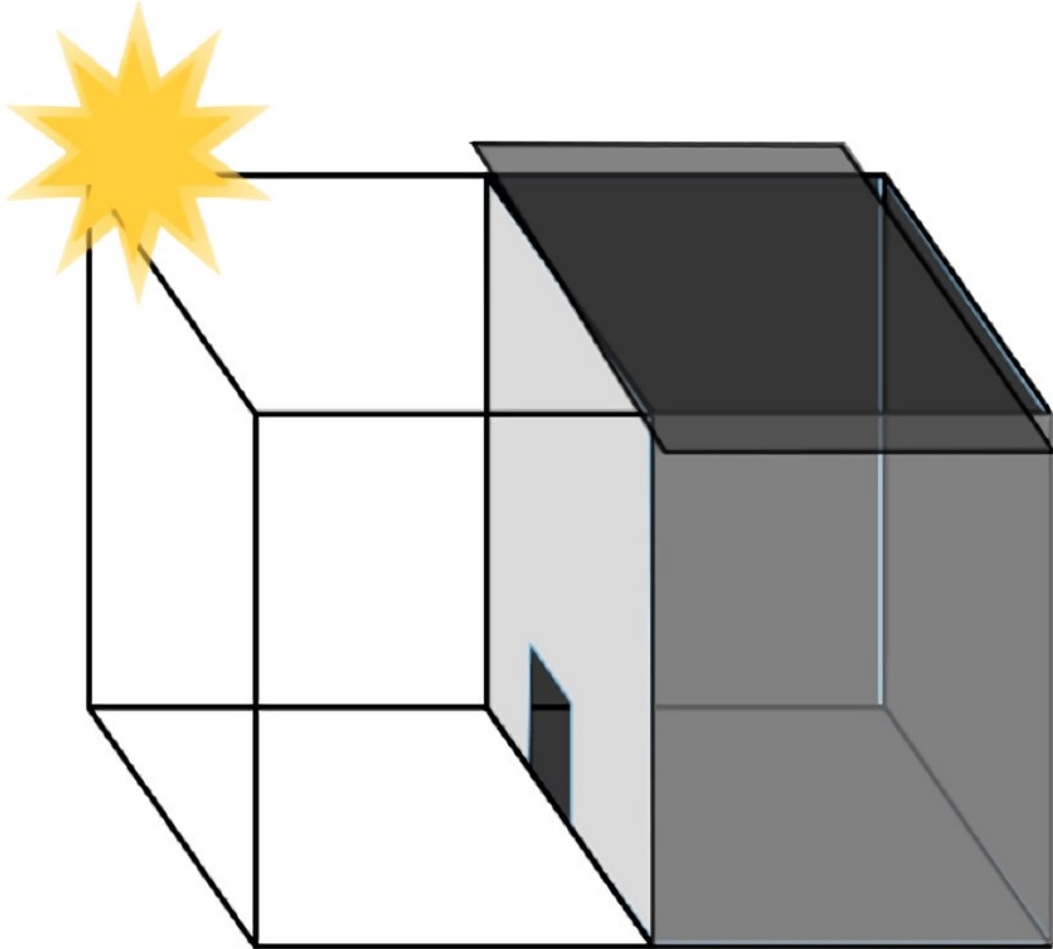
- Mice must maintain their position on the rotating rod
- Eventually, they fall
  - At which point, the researchers put them back on the rotating rod
- This is a test of their motor skills

# Elevated Plus Maze



- Mice are first added to the very center of the “maze”
- In fear, the mice tended to creep into the parts of the maze enclosed by the barriers
- After some time, the mice developed courage to go onto the open arms
- This was a test of mouse anxiety

# Light/dark Box



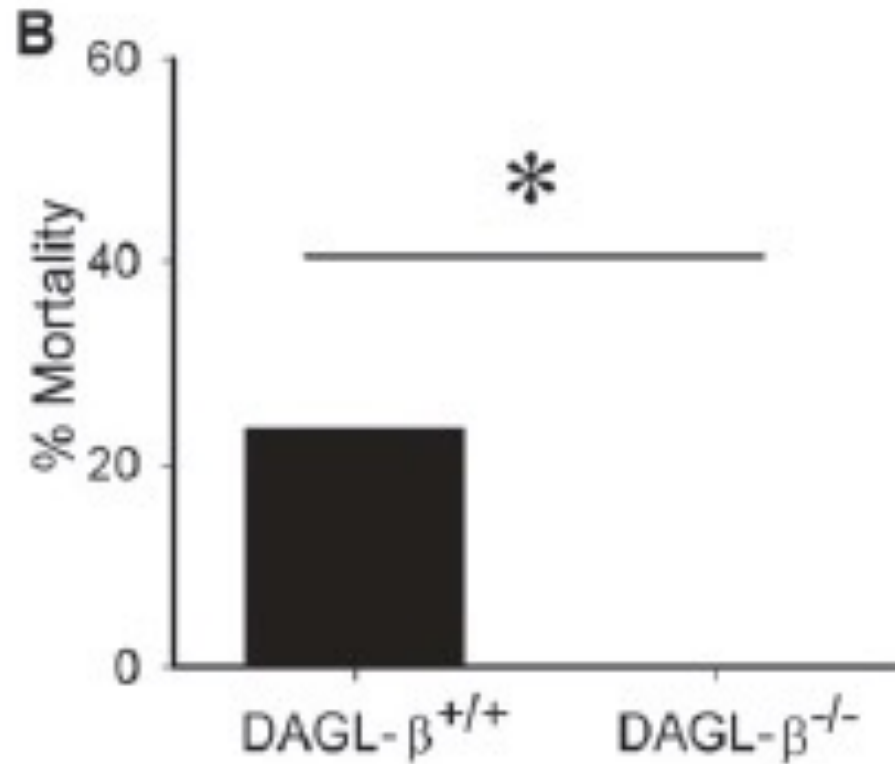
- Mice prefer to be in the dark
- Mice were first introduced to the light environment and explore it
- After being introduced to the light/dark box, mice had up to 5 minutes to explore
- Longer time spent in the light environment meant more willingness to explore



# Results

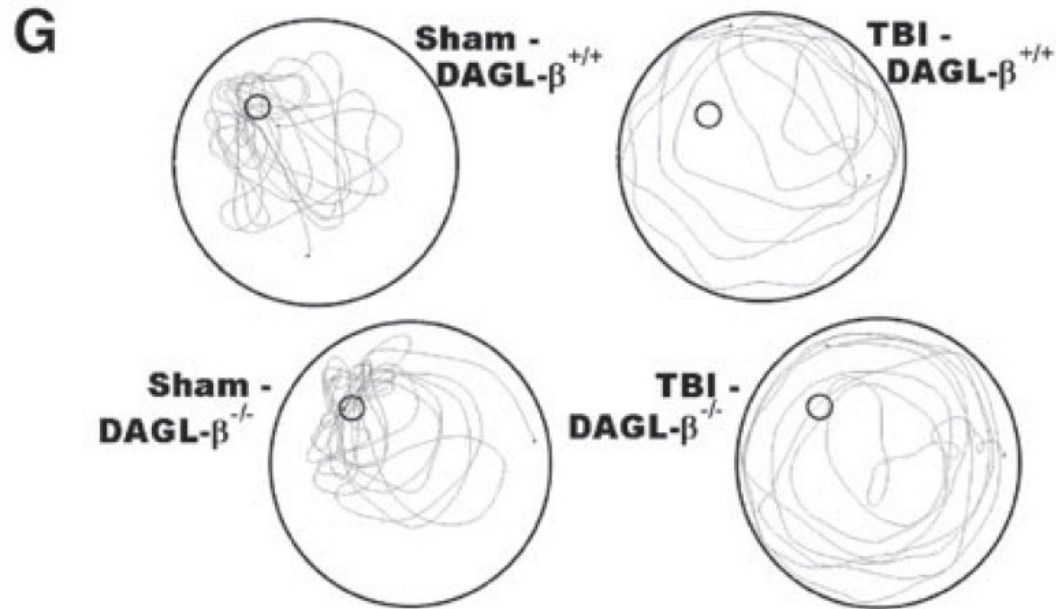
- The research tested for variety of different variables within the study including baseline temperature, baseline weight, and temperature during the swim in the Morris water maze
  - None of these variables had to do with TBI or DAGL- $\beta$
- However, the researchers did find differences in mortality and motor ability
- All in all, the functional characteristics of the mice did not change with TBI or DAGL- $\beta$  knockout

# Mortality



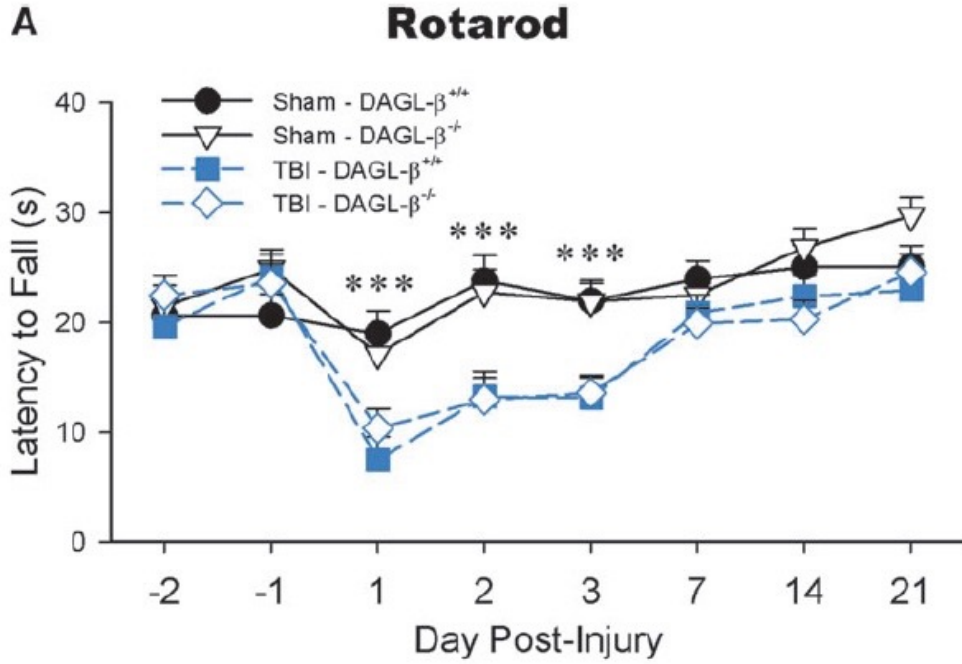
- The mortality rates were 0% in DAGL- $\beta$  knockout mice
- However, the mortality was significantly higher in DAGL- $\beta$  positive mice
- This would suggest the DAGL- $\beta$  removal has a protective effect
- Moreover, only male mice died
- No female mice died during experimentation
- However, being DAGL- $\beta$  could lead to greater stress long-term due to the lack of endocannabinoid reception

# Morris water maze



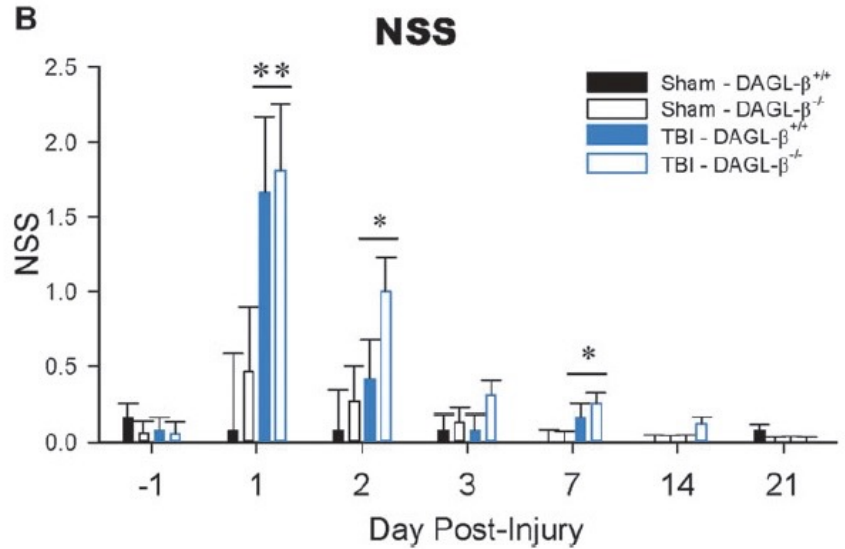
- There was no observed difference between the maze paths or speed between DAGL-β knockout mice and DAGL-β positive mice
- However, TBI lead to significant differences in path length and course time
  - TBI mice spent significantly longer looking for the platform
  - TBI mice were also slower in looking for the platform
  - TBI mice also spent more time on the sides of the tank, suggesting reluctance to search
- This result undermines the role of DAGL-β in cognition but exemplifies the effect of TBI on motor function

# Rotarod & NSS

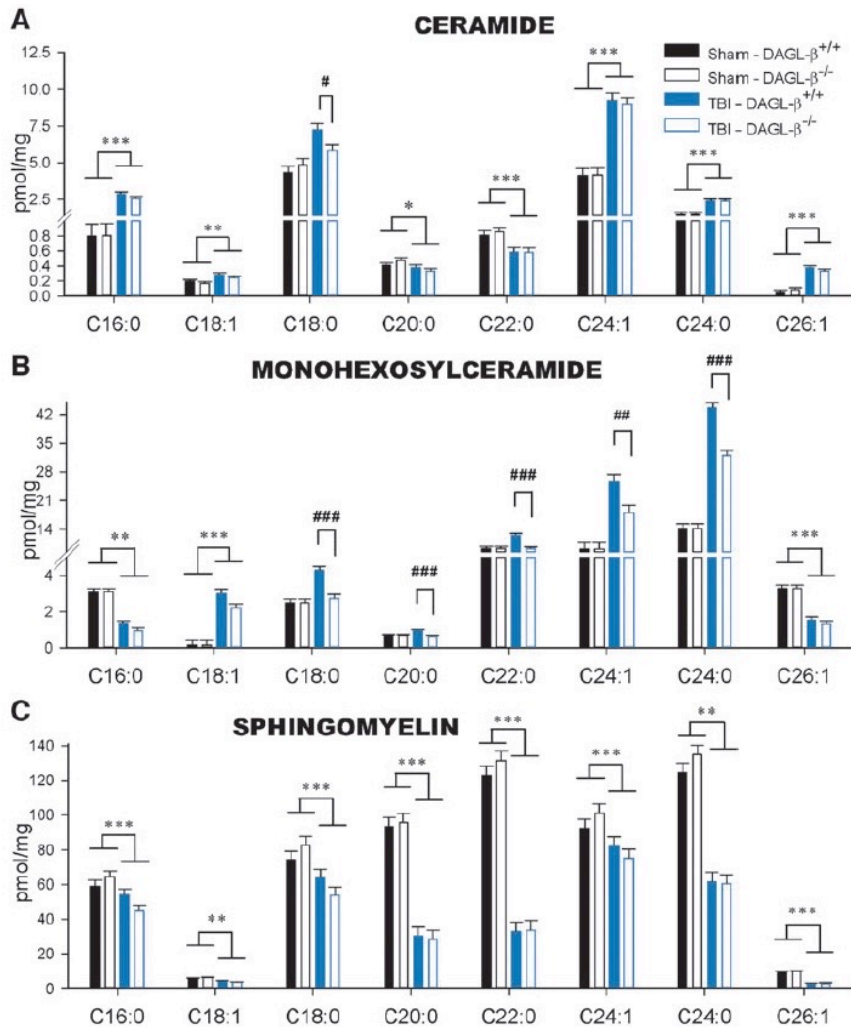


- NSS: lower scores are better
- The TBI mice did significantly worse on NSS tests immediately following injury

- Immediately following injury, TBI mice fell more easily from the rotating rod
- These mice exhibited poor motor control



# Sphingolipid Profiles



- TBI dramatically changed the sphingolipid concentrations
- Increased concentrations of ceramide/monohexosylceramide means more distress for the mitochondria
- However, lower levels of sphingomyelin (insulates brain cells) is tied to low-speed communication

# Discussion

- One of the most interesting results from the paper was that female mice died significantly less frequently than male mice
  - 1. Females tend to experience an immune response in phases whereas males experience one increase in immune mediators (more immune responder available)
  - 2. The decline in immune response is also slower in female mice (immune response is greater on onset, and the immune response lasts longer)
  - 3. Estrogen and progesterone leads to more neuroprotection, leading to greater survivability.
- This finding warrants further study as the reason is not understood

# Discussion

- TBI injury largely did not lead to any functional deficits
  - However, there was an increase in bad lipids and a decrease in good lipids, suggesting an altered lipid profile in the brain
  - The speed of neural communication was reduced, however (slower detection and speed in Morris water maze)
- DAGL- $\beta$  largely did not lead to any changes in the cellular environment
  - However, for none of the male DAGL- $\beta$  knockout mice died, suggesting a protective effect for the male DAGL- $\beta$  knockout mice

# Discussion

- “it is well established that the respective brain accumulation of glucosylceramide and galactosylceramide in Gauche and Krabbe disease patients leads to massive neuronal cell death”
  - When introduced to TBI, mice face the following:
    - Pain
    - Mitochondrial damage from the ceramide
    - Axonal injury from the physical procedure
    - Unsheathing of the insulating layer around axons
    - Poorer performance in all neurocognitive/motor/sensory areas
- However, DAGL- $\beta$  can prevent mortality



# Discussion Questions

- Why do you think it is that female mice did not die shortly after TBI? Should disparities between the genders be significant enough to look into for non-reproductive-related disorders like TBI?
- TBI applies a lot of cellular stress to the brain beyond the sheer physical stress. Why do you think it is that the researchers looked into the outward signs of TBI such as motor control as opposed to cellular components such as DAGL- $\beta$  expression?
- Why do you think it is that the brain of these mice demonstrated ability in these tests (perhaps slower, but sufficient long-term) despite TBI? (Why did TBI not affect the functional components of these rats?)

# Discussion Questions

- Mice who died only died within the first 2 minutes of the TBI. Why do you think it is that no mice died under long-term immunological stress?
- Should greater attention be given to percussive injuries, or is this study proof that there are no functional differences in people with TBI opposed to those who do not have TBI?
- What kinds of follow-up studies would be useful to build upon this research?

# Citation

O'Brien LD, Smith TL, Donvito G, Cravatt BF, Newton J, Spiegel S, Reeves TM, Phillips LL, Lichtman AH (2021) Diacylglycerol lipase- $\beta$  knockout mice display a sex-dependent attenuation of traumatic brain injury-induced mortality with no impact on memory or other functional consequences, Cannabis and Cannabinoid Research X:X, 1–14, DOI: 10.1089/can.2020.0175.