

Problem & Rationale

Traumatic Brain Injury (TBI)

- America’s leading cause of disabilities and deaths (Taylor, 2024).
- 2.5M hospitalizations and deaths; \$19B in economic losses (Johnson and O’Brady, 2024).
- Affects 67% of veterans and military personnel (Brain Injury Assoc., 2024).
- Increases risk of **neurodegenerative diseases** ex. Parkinson’s, Alzheimer’s, dementia, CTE (Ferrazzoli et al., 2024).
- Highest risk: age 65+, military/veterans, professional athletes (Ryan, 2026; Mullins, 2026).
- Research gap:** While the *injury* is well studied, *treatments* for progressive, secondary TBI that occurs over hours and days *lag behind* (Fig. 1) (Henry, 2026; Ryan, 2026).

➡ **Goal:** Determine if the antioxidant, Methylene Blue (MB), is *neuroprotective*, thus mitigating secondary TBI.

Primary Brain Injury

Secondary Brain Injury

Cognitive Problems

Inflammation

Protein Build-up

Oxidative Stress

Apoptosis

Neurodegeneration

Stretching, compression, tearing

Fig. 1. The Two Stages of TBI (Original image from Ikram et al., 2021, doi.org/10.2147/IJR.S334423. Modified by Finalist using MS Word, 2025)

Project Origin

I was motivated by my brother’s TBI experience after he was violently assaulted. He suffered long-term memory, thinking and brain fatigue issues affecting his work and daily life. Once I learned that TBI is so common among our veterans, I was passionate about finding solutions since my cousin and so many of our family and friends are veterans who deserve to have healthy lives after serving our country.

Background Research

Fruit flies (*Drosophila hydei*)

- 75% of their genes are homologous to humans’ (Barekat et al., 2016).
- Similar organ systems and TBI mechanisms as humans can advance our understanding of TBI treatment (Figs. 2, 3).

Methylene Blue (MB)

- Antioxidant; counteracts protein build-up and over-production of reactive oxygen species (ROS).
- Helps mitochondria generate energy; regulates apoptosis (cell death) (Buhlman et al., 2021).
- Treats similar protein build-up in Alzheimer’s, Parkinson’s (Hashmi et al., 2023).
- Low-cost:** \$5-10/month; **well-tolerated**; **available worldwide** (Roldan, 2026; Ginimuge, 2010).

Human

Aggression, depression, poor memory

Loss of consciousness

Fencing (extending arm)

Ataxia (confusion, loss of coordination)

Hypoactivity

Drosophila

Aggression

Loss of consciousness

Fencing (playing wings)

Ataxia (confusion, loss of coordination)

Hypoactivity

Fig. 2. Analogous TBI Symptoms in Humans vs. Flies. (Original image from Buhlman et al., 2021, doi.org/10.1016/j.biopha.2021.112079. Modified by Finalist using Adobe Express and MS Word, 2025)

Homologous genes & TBI mechanisms

Treatment Modeling

Drug discovery

Fig. 3. Fruit Flies as a Novel TBI Treatment Model. (Original image from Yamamura, Ooshio & Sonoshita, 2021, doi.org/10.1111/icas.14747. Modified by Finalist using MS Word, 2025)

Interview Spotlight:

Dr. Kelly Ryan, Neurologist, Baltimore Orioles

Dr. Ryan specializes in TBI from sports injuries in professional baseball and horse racing. “Quick treatment is essential – the sooner you treat a patient after primary injury, the less severe secondary injuries will be” (Personal Interview with Alekha Goldberg, 2026), corroborating research on urgency for treatment after primary TBI (Johnson and O’Brady, 2024). Moreover, she indicated that there is **no certain method yet to prevent or decrease the effects of TBI**, and that **this is a new area of research urgently needing further exploration**.

Research Questions

- RQ1) Will MB mitigate secondary brain injury in fruit flies following TBI?
- RQ2) If so, which dosage will cause the most flies to pass the “finish line” of the climbing assay and survive for 24 hours after TBI: 2 mg/kg, 4 mg/kg, 6 mg/kg, or 8 mg/kg?

Hypothesis

IF fruit flies consume Methylene Blue pre- and post-traumatic brain injury, ***THEN*** it will mitigate secondary brain injury, with the highest dosage being most effective, ***BECAUSE*** Methylene Blue is neuroprotective, thus decreasing oxidative stress and regulating cell processes.

Blue-reka!

Investigating Methylene Blue as a Neuroprotectant for Traumatic Brain Injury Using a Novel *In Vivo* Drosophila Model

Variables

Control: Flies not consuming MB	Constant Variables: # of days flies consume Methylene Blue # of hits to inflict TBI - HIT (High Impact Trauma) Device retraction angle - Rest time before and after strikes - Distance and time to reach finish line - Flies’ living conditions (food, temperature, light)
Independent Variable: MB dosage	
Dependent Variables: Climbing assay (# flies passing finish line) 24-hour survival count (number of flies surviving after 24h)	

Methodology

Fig. 4. MB into Food. (Photo by R. Goldberg, 2025)

Fig. 5. HIT Device Inflicting TBI. (Photo by R. Goldberg, 2025)

Fig. 6. Striking Vial. (Photo by Finalist, 2025)

Fig. 7. Climbing Assay. (Photo by Finalist, 2025)

- Treatment (Fig. 4):** Prepare flies’ treatment vials by mixing 2, 4, 6, or 8 mg/kg MB with water and food medium (control gets no MB). Place 10 flies in each vial to feed for 5 days.
- Inflict TBI (Fig. 5):** Transfer a given dosage’s flies into HIT Device’s striking vial (Katzenberger et al., 2015). Administer 3 hits from 90° retraction angle.
- Assessment after TBI (Figs. 6-7):**
 - Climbing assay** (5 min post-TBI): # of flies passing 2.5 cm “finish line” in 20 seconds. Return flies to treatment vial for 24 hours.
 - 24-hour survival count:** # flies surviving.

Observations & Data

BEFORE TBI

- Active. Reflexively climbed walls upward, quickly, in a straight line (Fig. 8).
- Ability to walk upside down along the stopper bottom.
- Self-righting if they fell when reaching the stopper.
- Wings mostly closed, intermittent fanning (Fig. 9).
- Minimal interaction with each other.

AFTER TBI

- Hypoactive or unconscious. Walked sluggishly - diagonally/sideways (Fig. 10).
- Falling before reaching the stopper. Walking along floor rather than walls.
- Ataxia:** turning in a circle, walking backwards, falling, inability to right themselves (Fig. 11).
- Fencing:** splayed wings (Figs. 12-13)
- Aggression:** head butting, boxing (Fig. 14), chasing (Fig. 15), lunging.

Observations Pre- and Post-TBI (Graphic created by Finalist using MS Word, 2026)

Before TBI: Figs. 8-9, at left:
Fig. 8. Walking straight up the walls of the vial.
Fig. 9. Wings closed.

After TBI: Figs. 10-15, at right:
Fig. 10. Walking diagonally and sideways, 2 mg/kg group.
Fig. 11. Ataxia and inability to right themselves, 4 mg/kg group.
Figs. 12 - 13. Fenced wings in 8 mg/kg (Fig. 12), and Control groups (Fig. 13).
Fig. 14. Aggression - boxing, 2 mg/kg group.
Fig. 15. Aggression - chasing, 6 mg/kg group.

(Photos by Finalist and modified by Finalist using MS Word, 2025)

Analysis & Discussion

Statistically Significant Results

Statistical analysis demonstrated that **Methylene Blue significantly mitigated secondary TBI** (Fig. 17, Table 4).

H₀ is rejected
p = 0.0008359
F = 3.95
Effect size = 0.3448 (large)

Fig. 17. One-way MANOVA of Climbing Assay and 24-Hour Survival Count Data (Graphic created by Finalist using MS Word, 2026)

Dosage	N	Mean	Std. Dev.	Std. Error
Control	7	0.91	0.3532	0.1335
2 mg/kg	7	1.24	0.2637	0.0997
4 mg/kg	7	1.47	0.3885	0.1469
6 mg/kg	7	1.71	0.1574	0.0595
8 mg/kg	7	1.90	0.0579	0.0219

p < 0.001
F = 14.09

Table 4. ANOVA of CSI (Table created by Finalist using MS Excel, 2026)

Why Might Methylene Blue Have Been Effective?

- Keeps mitochondria functioning efficiently.
- Counteracts over-production of ROS, thereby decreasing oxidative stress.
- Averts protein building up in the brain, reducing neurodegeneration.
- Regulates the immune response, protecting against prolonged neuroinflammation.

Anomaly: Smaller avg. survival increase from 2 mg/kg to 4 mg/kg; Revisit with further trials and by addressing possible experimental errors, below:

Error & Impact	Potential Solution
1. Age variation from supplier: Larvae may hatch during transit. ➢ Older flies may suffer more severe TBI.	Cultivate a new batch of flies to guarantee uniform ages.
2. Baseline health variations: Natural or shipping stress impacts baseline health. ➢ Responses may vary regardless of Methylene Blue treatment.	Source all flies from a single batch; increase sample size.
3. Inconsistent MB intake: Varying consumption rates between flies. ➢ Introduces dosage variations within the same treatment group.	Conduct more trials for a larger sample size.
4. Fly position in HIT Device: Varying orientation inside the vial upon impact. ➢ Alters how impact distributes across head and internal organs.	Conduct more trials for a larger sample size.

(Table created by Finalist using MS Word, 2026)

Conclusion

Research Questions Answered

- RQ1) Methylene Blue successfully **mitigated** secondary traumatic brain injury in fruit flies.
- RQ2) The **highest dosage** demonstrated the **largest mitigating effect**.

Hypothesis Supported

- Data strongly supports the hypothesis that Methylene Blue is neuroprotective, mitigating TBI, likely by decreasing oxidative stress and regulating cell processes.
- All treatment groups yielded statistically significant improvements compared to the control group.

Contributions to the Field

Methylene blue: Effective neuroprotectant in a dose-dependent manner.

Innovative CSI Biomarker: Immediate + delayed = TBI progression

Improved TBI outcomes

Confidence in Results:

- Large sample N=350
- 7 replications
- Statistical significance (large effect size, p<0.001)

Novel In Vivo Model: Extend drosophila beyond injury models into treatment studies.

(Graphic created by Finalist using MS Word, 2026)

Real-World Impacts on Medical Sciences, Economy & Society

- Findings reinforce the potential of Methylene Blue as an **accessible, well-tolerated, and low-cost neuroprotectant for the prevalent injury of TBI**.
- This study’s **Climbing-Survival Index (CSI)** biomarker can be adapted to human indicators of secondary injury progression (ex. mood disorders, confusion, etc.) to provide a more **holistic** assessment of TBI in patients.
- This study has strong potential for the **military, healthcare, drug development, and treatment studies** to improve TBI outcomes.

Methylene Blue’s Potential (Graphic created by Finalist using MS Word, 2026)

Future Directions for Research

- Dissecting flies’ brains after MB treatment (ex. study protein build-up) to investigate how MB may be working.
- Isolating treatments to pre- vs. post-injury to know the exact stage for MB therapy (ex. prophylaxis could help those in high-risk professions).
- Measuring aggression in flies. This is a progressive TBI symptom in humans, so it may allow adapting CSI to human healthcare contexts.
- Exploring other accessible antioxidants that may further treat TBI.
- Scaling up research into clinical trials to explore MB’s effectiveness in humans, and to target drug development.

➡ By honing methodology and expanding scope, this project can promote the development of innovative TBI treatments, thus improving patient outcomes and quality of life.

Key References

Barekat, A., Gonzalez, A., Maunz, R. E., Kotzbus, R. W., Molina, B., El-Mechattari, N., Conner, C. J., Garza, S., Mellani, G. C., Joiner, W. J., Lipinski, M. M., Finley, K. D., & Ratliff, E. P. (2016). Using *Drosophila* as an integrated model to study mild repetitive traumatic brain injury. *Scientific Reports*, 6(1), Article 25252. <https://doi.org/10.1038/srep25252>

Brain Injury Association of America. (2024, December 17). Incidence of traumatic brain injury in military service members receives widespread media coverage. <https://braininjury.org/public-affairs/public-awareness/news/incidence-of-traumatic-brain-injury-in-military-service-members-receives-widespread-media-coverage>

Buhlman, L. M., Krishna, G., Jones, T. B., & Thomas, T. C. (2021). *Drosophila* as a model to explore secondary injury cascades after traumatic brain injury. *Biomedicine & Pharmacotherapy*, 142, Article 112079. <https://doi.org/10.1016/j.biopha.2021.112079>

Ferrazzoli, D., Ortolini, P., Verace, V., Stolz, J., Deri, S., Vos, P., Giladi, N., Salta, L., & Sebastianelli, L. (2024). Post-traumatic parkinsonism: The intricate twist between trauma, inflammation and neurodegeneration. A narrative review. *Journal of the Neurological Sciences*, 466, Article 123242. <https://doi.org/10.1016/j.jns.2024.123242>

Ginimuge, P. R., & Jyothi, S. D. (2010). Methylene blue: Revisited. *Journal of Anaesthesiology Clinical Pharmacology*, 26(4), 517-520. <https://pmc.ncbi.nlm.nih.gov/articles/PMC3087269/>

Hashmi, M., Ahmed, R., Mahdi, S., Ahmed, K., Boudra, N. M., Ahmed, A., Elwadai, B., Madni, A., Saad, A., & Abdelrahman, N. (2023). Exploring methylene blue and its derivatives in Alzheimer’s treatment: A comprehensive review of randomized control trials. *Cureus*, 15(10), Article e46732. <https://doi.org/10.7759/cureus.46732>

Ikram, M. U.S. Department of Veterans Affairs (VA) Office of Research. (2026, March 17). *Personal interview by Alekha Goldberg. Email.*

Henry, M., Park, H. Y., Ali, T., & Kim, M. O. (2021). Melatonin as a potential regulator of oxidative stress, and neuroinflammation: Mechanisms and implications for the management of brain injury-induced neurodegeneration. *Journal of Inflammation Research*, 14, 6251-6264. <https://doi.org/10.2147/IJR.S334423>

Johnson, T., & O’Brady, S. (2024). The platinum 10 and the golden hour: An interdisciplinary approach. *The Surgical Technologist*, 56(4), 156-162. <https://www.ast.org/eonline/articles/486/486.pdf>

Katzenberger, R. J., Loewen, C. A., Bookstuck, R. T., Woods, M. A., Garetzky, B., & Wasserman, D. A. (2015). A method to inflict closed head traumatic brain injury in *Drosophila*. *Journal of Visualized Experiments*, 1009, Article e52905. <https://doi.org/10.3791/52905>

Mullins, B. Case Manager, Wounded Warriors Project. (2026, March 10). *Personal interview by Alekha Goldberg. Email.*

Roldan, C. (2026, March 3). Methylene blue: Should you take it? MD Anderson Cancer Center. <https://www.mdanderson.org/cancerwise/methylene-blue-should-you-take-it-80615985767.html>

Ryan, K., MD, Neurologist. (2026, January 9). *Personal interview by Alekha Goldberg. FaceTime.*

Taylor, S. (2024, February 20). Methylene blue and brain injury. Fagron Academy. <https://www.fagronacademy.us/blog/methylene-blue-and-brain-injury>

Yamamura, R., Ooshio, T., & Sonoshita, M. (2021). Tiny *Drosophila* makes giant strides in cancer research. *Cancer Science*, 112(2), 505-514. <https://doi.org/10.1111/icas.14747>

Alekha Goldberg