# Mechanisms of recovery from traumatic brain injury in Drosophila melanogaster

**AYURG |** Natural Sciences and Engineering (NSE) **|** *Tags: Lab-based*

*This cover page is meant to focus your reading of the sample proposal, summarizing important aspects of proposal writing that the author did well, or could have improved on.* ***Review the following sections before reading the sample****. The proposal is also annotated throughout to highlight key elements of the proposal's structure and content.*

| Proposal Strengths | Areas for Improvement |
|---|---|
| All field-specific jargon is defined in simple terms. | Section headers are not necessary. |
| The background is based in evidence, but is not simply a summary of what other researchers have said. The author uses past research to tell a story about the topic at hand. | Passive language is used in the methodology rather than active language. Rather than saying something "will be" done, say "I will do…" |
| The author gives us a rough timeline of the various parts of their methodology. | |
| The preparation section includes more than a list of past experiences. Instead the author explicitly tells us the relevant skills they gained from their past experiences. | |

| Other Key Features to Take Note Of |
|---|
| In biology, neuroscience, and related fields, it is important to justify the organism you are using. Also, if you are using one organism, but much of your background evidence uses another organism (as it did in this proposal) you must make an explicit connection supported by evidence. |
| Figures are NOT required in any proposal, however they can be included outside the 2-page limit. You must make sure it is not necessary to see your figure in order to understand your proposal. It should be purely supplemental if it is outside of the 2 page limit. |
| In a lab environment, an independent project is almost always a piece of a larger project/aim of the lab. In your proposal, it can be good to situate your proposed project in terms of what the broader goals of the lab are by including background information from your lab, or showing how the results of your study will apply to larger goals of the lab. |
| It is not strictly necessary to include a hypothesis, however, including something like an expected results section at the end of your methodology shows you will be capable of interpreting the output of your research, and strengthens the argument that your methods help you answer your research question. |
| All Academic Year URGs require a budget. There is no required format; however, we do provide a template on our website. The scope of the proposal should focus on what the funding covers. |

Section Subtitles not necessary

**Introduction:** Traumatic brain injury (TBI) is a common and debilitating condition for which no effective treatments exist. Recent research has led to the implication of central nervous system (CNS) waste clearance – which occurs during sleep – as a potential factor regulating recovery from TBI. We have identified a novel clearance pathway in the fruit fly Drosophila melanogaster. The aim of this project is to use this model organism to further examine the role of CNS waste clearance in recovery from TBI.

**Background/Literature Review:** TBI poses a significant public health burden, with about 50-60 million new cases being reported globally every year (Quaglio et al., 2017). In addition to suffering from a decreased quality of life, TBI patients are at an increased risk of developing dementia and Alzheimer's Disease (Iliff et al., 2014). The most common symptom of TBI is sleep disturbance, which includes insomnia (29% of patients), hypersomnia (28%), increased sleep need, and fatigue (Ouellet et al., 2015). In addition to affecting the patient's life, sleep disturbances negatively impact recovery from TBI. The cause of this disturbance remains unclear, but one of its major consequences is known to be impairment of the glymphatic system, which clears waste and toxins from the brain during sleep (Sullan et al., 2018). One toxin whose clearance is impaired in TBI is the protein tau, the aggregation of which is a hallmark of chronic traumatic encephalopathy (a disease caused by chronic TBI) and Alzheimer's Disease (Sullan et al., 2018). Levels of phosphorylated tau, a form of the protein associated with pathological conditions, are increased after TBI and associated with worse patient outcomes (Zemlan et al., 2002), and tau aggregation has been shown to be a direct consequence of glymphatic system impairment (Iliff et al., 2014).

Defining key terms without jargon

Justifies Organism & Connects human background literature to drosophila literature

TBI also induces tau phosphorylation in Drosophila (Barekat et al., 2016), a common model organism in biomedical research thanks to its short life cycle – which allows for high-throughput experiments – and easily manipulated genome. How invertebrates such as Drosophila – which lack a glymphatic system – clear waste from the brain is unclear, as they lack the closed circulatory system required for glymphatic clearance. The Allada lab is currently investigating an alternate clearance system, wherein the fly uses its proboscis as a pump to increase cycling of hemolymph (the invertebrate homolog of blood). We have previously shown that the spontaneous proboscis extension (PE) reflex occurs mostly during a sleep stage characterized by low brain activity (appendix, Fig. 1). We recently found that wild-type flies dramatically increase PE (Fig. 2A) after TBI (Fig. 2C), and that immobilizing the proboscis with glue decreases post-TBI survival (Fig. 2F-G). In our experiments, TBI reduces 24-hour survival (Fig. 3A) and locomotor activity (Fig. 3B), and induces apoptosis (i.e., cell death) in the Drosophila brain (Fig. 3C-D).

Lab specific background, situates project in larger lab aims

**Research questions:** These findings point to a role for PE-mediated waste clearance in recovery from TBI. To elucidate the involvement of PE in TBI recovery, I will test the effect of both physical (glued proboscis) and genetic inhibition of PE on various post-TBI outcomes. I previously found that constitutive activation of a pair of inhibitory interneurons (NP5137, Flood et al, 2013) through NaChBac, a bacterial sodium channel, reduces spontaneous PE/hour sleep rate and increases sleep need (Fig. 4). I hypothesize that wild-type flies increase PE after TBI (Fig. 2C) to pump debris and toxins such as tau out of the brain. If NP5137>NaChBac flies show a diminished increase in PE in response to TBI, then their recovery from TBI should be impaired. To test this hypothesis I will measure post-TBI mortality rate in NP5137>NaChBac flies and parental controls. If genetic inhibition of PE has the same effect as physical inhibition, survival of NP5137>NaChBac flies should be similar to that of glued proboscis flies (Fig. 2F-G), which, importantly, do not show deficits in sleep or food intake (data not shown).

Hypothesis not necessary, but shows deep understanding of the project in this case

I will also examine the effect of PE on more specific cellular and molecular responses to TBI. Specifically, I will explore the impact of physical and genetic inhibition of PE on TBI-induced apoptotic cell death. Fly brains that cannot clear the toxins and debris generated in response to TBI should show an increase in apoptotic cells. I will also examine levels of tau, a likely contributor to post-TBI cell death, in the brain and hemolymph of NP5137 and glued-proboscis

flies. If PE mediates toxin clearance, then NP5137 and glued-proboscis flies should show increased tau levels in the brain as well as a lower proportion of tau in the hemolymph – the most likely destination for waste cleared from the brain – compared to parental/wild-type controls.

**Methodology:** We have developed a method to reliably induce TBI in unanesthetized flies by targeted strikes to the head (Fig. 5). In all flies, TBI will be induced by five consecutive strikes to the head. Experimental NP5137 lines will be generated by crossing (i.e., mating) NP5137-Gal4 flies with UAS-NaChBac flies, and using the progeny, which express NaChBac in the NP5137 neurons, for experiments. NP5137-Gal4 and UAS-NaChBac parental crosses will be generated by crossing flies of both parental genotypes with wild-type flies. Post-TBI survival in NP5137 flies will be determined as follows: TBI will be induced in 50 flies per group (i.e., the experimental cross and the two parental crosses) and surviving flies will be counted 24 hours later. Data will be expressed as the percent of flies surviving at 24 hours, and survival rates of the three genotypes will be compared/graphed. This experiment will take only a few days. To measure post-TBI apoptosis, TBI will be induced in NP5137 and control flies (10 per group), and 24 hours later the brains of surviving flies will be dissected out, sectioned (i.e., sliced), and stained with the TUNEL (terminal deoxynucleotidyl transferase dUTP nick-end labeling) immunofluorescence kit (Sigma-Aldrich) – a standard assay for measuring apoptosis. Sections will be imaged with fluorescence microscopy and TUNEL-positive (i.e., fluorescent) cells will be counted. This experiment will be repeated in wild-type glued-proboscis (Fig. 2E) and control flies.

I will determine tau levels by inducing TBI in NP5137 and control flies (10 per group). 24 hours later brains will be dissected out, and hemolymph extracted. Brain and hemolymph extracts will be separated by gel electrophoresis and stained with an antibody against phosphorylated tau, using a standard Western Blotting procedure. Blots will be imaged by autoradiography and analyzed with the ImageJ program. Total tau levels in the brain and hemolymph, as well as brain-to-hemolymph ratio, will be compared to control conditions. This experiment will also be repeated in glued-proboscis flies. All data will be graphed as mean +/- SEM, and ANOVA will be used to determine significance. The TUNEL and tau experiments will represent the bulk of my research time.

**Preparation:** My coursework as a Neuroscience major has taught me the basic principles of neuroscience, and more importantly how to apply them in practical settings – how to design experiments from hypotheses based on existing scientific knowledge, and how to critically analyze and interpret results. My past research experience – academic years in the Allada lab since January 2016 and the past three summers in the Sol Snyder lab at Johns Hopkins – has given me much practice in designing and conducting experiments, analyzing data, and re-evaluating my hypotheses when they are disproved by data. In both labs I have been given much intellectual and experimental freedom to grow as a scientist – through asking questions of my mentors, trying new techniques, and often by making mistakes. I feel that this freedom has allowed me to develop a maturity that would not be present had I spent the last three years blindly following the orders of my superiors. I feel especially well-prepared to handle the experimental strategies this project entails, as my work in the Allada lab has provided me a great knowledge of Drosophila genetics, and my time in the Snyder lab has equipped me with proficiency in the techniques of molecular biology, including those described above.

**Conclusion:** Through this project I hope to gain experience in using techniques from a variety of different levels of basic science (behavioral, genetic, molecular) to tell a story with translational relevance. My hope is that this research will provide some insight into the nature of TBI, and help develop a high-throughput model that can be used to study the role of sleep-triggered CNS waste clearance in a variety of neurological diseases. Personally, I am not conducting a senior thesis, so I feel that this project, as part of my NEUROSCI 399 research, would be a very satisfying and rewarding way to finish my undergraduate scientific experience.

Too much passive language such as "will be induced" and "will be determined". Instead use active "I will___" language.

Timeline indicators help to show your project is feasible in the time you are proposing to do it.

Shows what will be done with the data once collected.

Goes beyond listing qualifications, explicitly identifies skills gained in past experience

Conclusions not needed. This is more like a personal future directions though, which should be included

## APPENDIX: REFERENCES AND FIGURES

### References

Barekat, A., Gonzalez, A., Mauntz, R.E., Kotzebue, R.W., Molina, B., El-Mecharrafie, N., Conner, C.J., Garza, S., Melkani, G.C., Joiner, W.J., et al. (2016). Using Drosophila as an integrated model to study mild repetitive traumatic brain injury. Sci. Rep. *6*.

Flood, T., Iguchi, S., Gorczyca, M., White, B., Ito, K., and Yoshihara, M. (2013). A single pair of interneurons commands the Drosophila feeding motor program. Nature *499*, 83–87.

Iliff, J.J., Chen, M.J., Plog, B.A., Zeppenfeld, D.M., Soltero, M., Yang, L., Singh, I., Deane, R., and Nedergaard, M. (2014). Impairment of Glymphatic Pathway Function Promotes Tau Pathology after Traumatic Brain Injury. J. Neurosci. *34*, 16180–16193.

Ouellet, M.-C., Beaulieu-Bonneau, S., and Morin, C.M. (2015). Sleep-wake disturbances after traumatic brain injury. Lancet Neurol. *14*, 746–757.

Quaglio, G., Gallucci, M., Brand, H., Dawood, A., and Cobello, F. (2017). Traumatic brain injury: a priority for public health policy. Lancet Neurol. *16*, 951–952.

Sullan, M.J., Asken, B.M., Jaffee, M.S., DeKosky, S.T., and Bauer, R.M. (2018). Glymphatic system disruption as a mediator of brain trauma and chronic traumatic encephalopathy. Neurosci. Biobehav. Rev. *84*, 316–324.

Zemlan, F.P., Jauch, E.C., Mulchahey, J.J., Gabbita, S.P., Rosenberg, W.S., Speciale, S.G., and Zuccarello, M. (2002). C-tau biomarker of neuronal damage in severe brain injured patients: association with elevated intracranial pressure and clinical outcome. Brain Res. *947*, 131–139.

### FIGURES

As noted previously, figures are not required. If you choose to include them, they should not be required to understand your proposal; rather, they should supplement your background or methods. We removed most figures included in this proposal for data confidentiality; the remaining figures are already published.

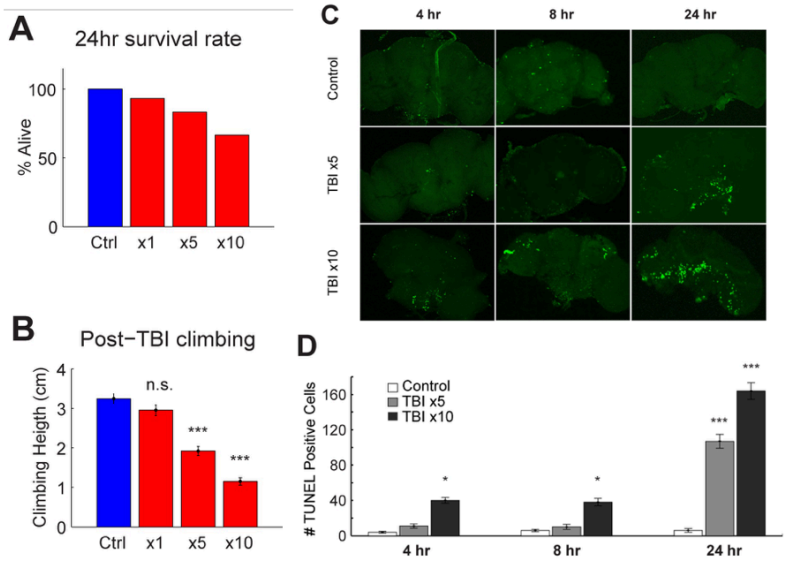


**Figure 3. TBI causes cell death, mortality and impaired climbing in a dose-dependent manner**. Male w1118 flies were exposed to either 1, 5 or 10 strikes to the head, delivered at 1 strike per second (n=32 per group). (**A)** 24 hour survival rate decreased with increased number of strikes. (**B)** in surviving flies, climbing behavior was quantified and compared to sham-treated controls 24 hours after TBI. Climbing behavior became more impaired with increased TBI severity (n.s = not significant, *** $p < 0.001$, t-test with Bonferonni correction). Cell death following TBI was quantified with a TUNEL assay (C) Representative images of TUNEL staining at different time points in control and post TBI flies (D) Histogram showing significantly increased TUNEL positive cells post TBI in a dose dependent manner (n = 7-10 per group). * $p < 0.05$, *** $p < 0.001$ t-test. Error bars indicate SEM.

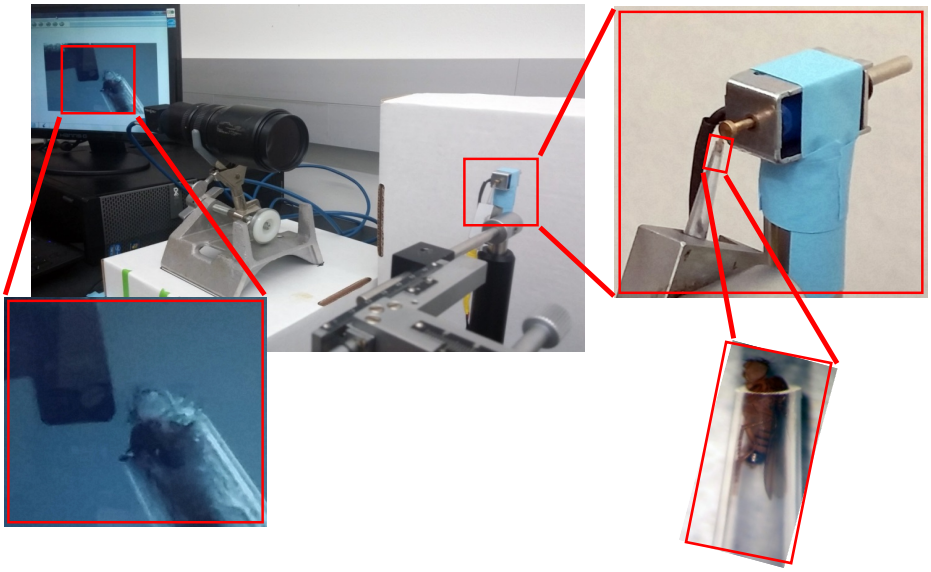

**Figure 5. Setup for induction of TBI in individual unanesthetized *Drosophila*.**

# BUDGET FORM

## A. Research-Related Expenses (Data Collection; Analysis)

| TYPE | COST | NOTES |
|---|---|---|
| 1. Consumable Materials | $1,000.00 | In Situ Cell Death Detection Kit ("TUNEL kit" - from Sigma Aldrich) - $480; phospho-tau antibody (from Cel Signaling) - $300; immunofluorescence/western blot reagents - $220 |
| 2. Non-Consumable Materials | n/a | |
| 3. Equipment/Durable Goods | n/a | |
| 4. Research Subject Compensation | n/a | |
| 5. Fees | n/a | |
| 6. Transcription Services | n/a | |
| 7. Tuition/Mandatory Fees | n/a | |
| 8. Instructional Materials | n/a | |
| 9. Living Expenses | n/a | |
| 10. Other | n/a | |

## B. Travel-Related Expenses

| TYPE | COST | NOTES |
|---|---|---|
| 1. Airfare (round trip) | n/a | |
| 2. Housing | n/a | |
| 3. Food | n/a | |
| 4. Local Travel Expenses | n/a | |
| 5. Other | n/a | |

## C. International-Related Expenses

| TYPE | COST | NOTES |
|---|---|---|
| 1. Entry Visa or Visas | n/a | |
| 2. Required Vaccines | n/a | |
| 3. Recommended Vaccines | n/a | |

| | | |
|---|---|---|
| 4. Travel Health Insurance (HTH) | n/a | |
| 5. Passport | n/a | |
| 6. Other | n/a | |

## TOTAL EXPENSES

| TYPE | COST | NOTES |
|---|---|---|
| Total Research Expenses (A) | $1,000.00 | |
| Total Travel Expenses (B) | $0.00 | |
| Total International Expenses (C) | $0.00 | |
| **TOTAL EXPENSES** | **$1,000.00** | |

## D. POTENTIAL FUNDING

| SOURCE | AMOUNT | NOTES |
|---|---|---|
| Academic Year URG funding | $1,000.00 | |
| | | |
| | | |
| | | |
| **TOTAL FUNDING** | **$1,000.00** | |