Immunohistochemical Analysis of Targeted Nanoparticles in Brain Tissue after Traumatic Brain Injury

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Background

- Over 69 million people globally suffer a traumatic brain injury (TBI), which can lead to long-term disability, and cognitive decline[1].
- The blood-brain barrier (BBB) becomes semipermeable after TBI and allows passive drug delivery via nanoparticle (NP) systems[2-3].
- Previous work revealed a targeting peptide specific for acute TBI[4].
- NP decorated with targeting peptides can have increased affinity and retention to specific brain regions[5].
- The project aims to evaluate the targeting and retention of targeting peptide-decorated NPs (T-NPs) in different brain regions one day post-TBI.

Methods

- Female mice received a TBI at 0h. T-NPs were injected 21h post-TBI and allowed to circulate for 3h or 24h to test for targeting or retention, respectively (Figure 1).
- Brains were collected and immunohistochemistry was performed.
- Regions of interest were drawn at the edges of the hippocampus, thalamus, corpus callosum and cortex from the ipsilateral and contralateral sides to the injury region (Figure 2).
- DAPI (cell nuclei), T-NPs, CD31 (blood vessels) and Neun (neurons) percentage area and their colocalization were analyzed via HALO imaging software (Figure 3).
- Pixel intensity was used to customize settings to detect strength of each marker as Weak, Moderate, or Strong (Figure 3).

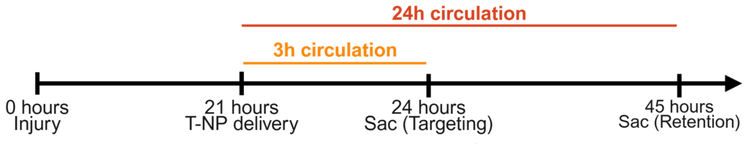


Figure 1: Timeline showing the experimental design for the animal experiments.

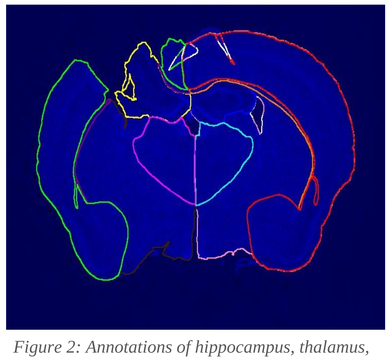


Figure 2: Annotations of hippocampus, thalamus, corpus collosum and cortes on the ipsilateral (left) and contralateral (right) hemispheres to the injury.

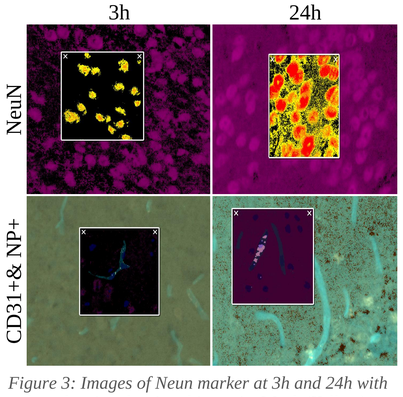


Figure 3: Images of Neun marker at 3h and 24h with box showing the signal intensity Weak (Yellow), Moderate (Orange), and Strong (Red) and box showing CD31+&NP+ colocalization shown in Pink and Purple (Neun+, CD31+, and DAPI+).

NP Fluorescence Percent Area

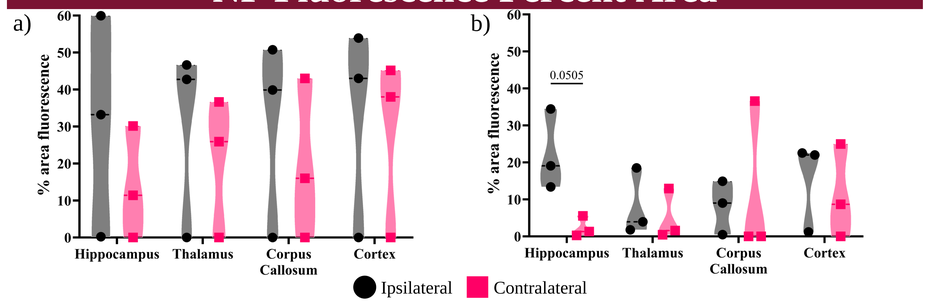


Figure 4: Graphs showing percent area fluorescence of T-NP across different regions after 3h (a) and 24h (b) of NP circulation in ipsilateral and contralateral hemispheres to the injury. (n=3)

Neun Fluorescence Percent Area

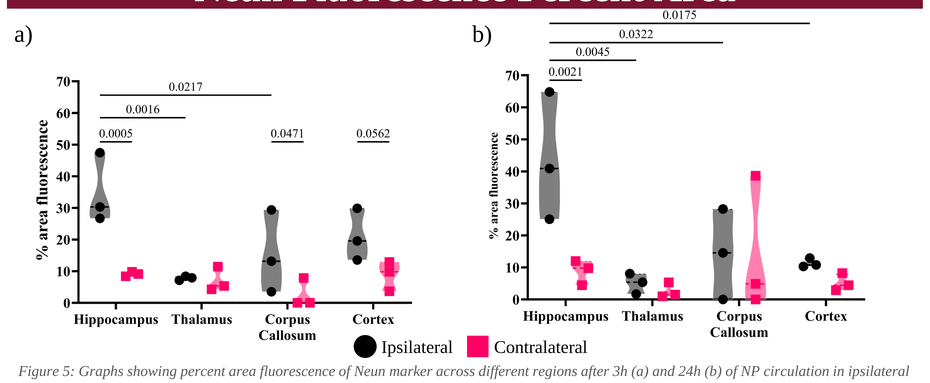


Figure 5: Graphs showing percent area fluorescence of Neun marker across different regions after 3h (a) and 24h (b) of NP circulation in ipsilateral and contralateral hemispheres to the injury. (n=3)

NP Retention in Blood Vessels

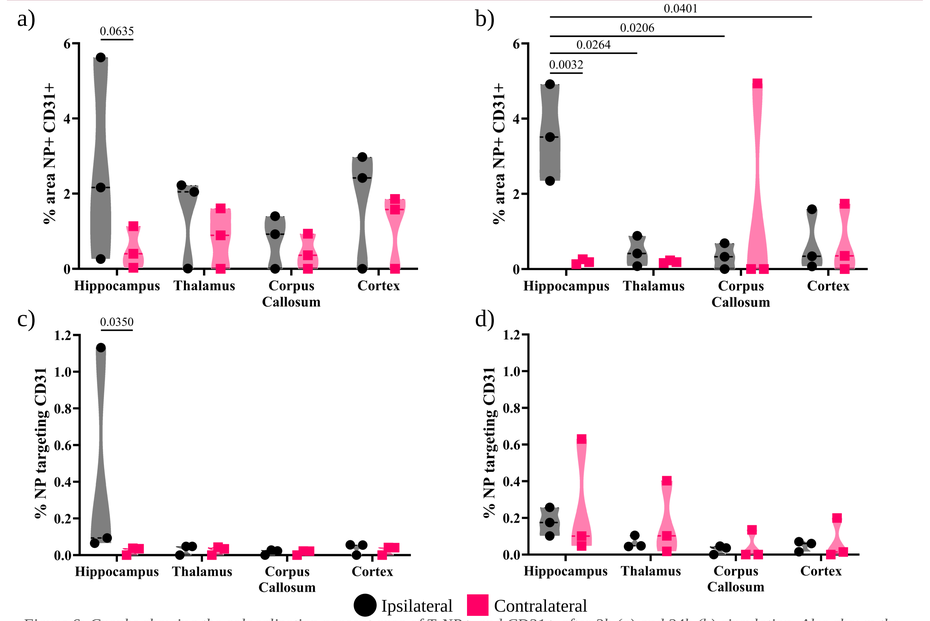
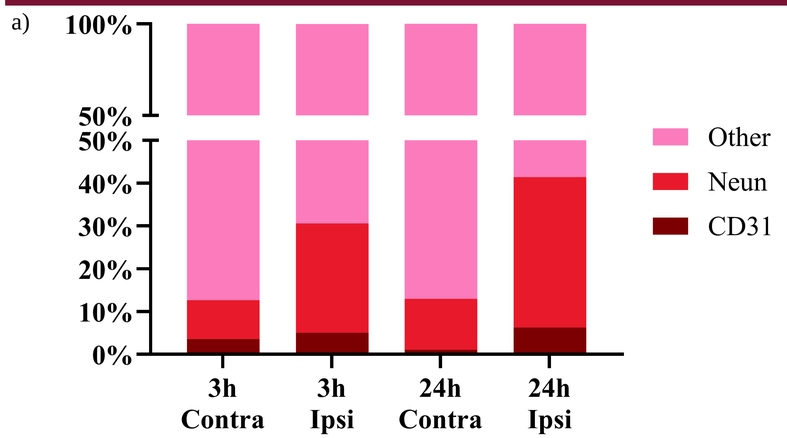


Figure 6: Graphs showing the colocalization percent area of T-NP+ and CD31+ after 3h (a) and 24h (b) circulation. Also shown the percentage of NP targeting CD31 (NP+CD31+/NP) across different regions after 3h (c) and 24h (d) of NP circulation in ipsilateral and contralateral hemispheres to the injury. (n=3)

NP Colocalization with Markers



b)

	3h Contra	3h Ipsi	24h Contra	24h Ipsi
Other	87.4	69.4	87.0	58.6
Neun	9.1	25.6	11.9	35.1
CD31	3.6	5.0	1.1	6.3

Figure 7: (a) Graph showing total T-NP area with colocalization with Neun and CD31. (b) Table depicting average percentages of T-NP signal by itself and colocalization with Neun and CD31. (n=3)

Conclusions

- No significant difference in T-NP percent area fluorescence was observed in targeting but near significant retention (0.0505) was observed in the ipsilateral hippocampus.
- There is an increased Neun signal in the 3h and 24h circulation.
- NPs have a high colocalization with blood vessels in the ipsilateral hemisphere to the injury.

Future Work

- Examine the remaining groups for the study which include male cohorts, additional cell markers, and NPs decorated with control peptides.
- Literature research regarding Neun staining and neuron morphology after TBI and its contribution to increased Neun signal on our study.

Acknowledgments

Thanks to Dr. David Flores-Prieto and Dr. Sarah Stabenfeldt for their mentorship and guidance. The Regenerative Medicine Core and the Eyring Materials Center for the images used in this study. Grant R01NS116657 for helping fund this project. Finally, I would also like to thank the Stabenfeldt lab for their advice and feedback throughout this project.

References

[1] Michael et al 2023 BMC Emergency Medicine
[2] Bharadwaj et al 2018 Advanced Healthcare Materials
[3] Bharadwaj et al 2016 Scientific Reports
[4] Martinez et al 2022 Science Advances
[5] Flores-Prieto & Stabenfeldt 2024 Journal of Neural Engineering