Nano-Pulsed Laser Therapy Selectively Reduces Pro-Inflammatory Microglia in a Rat Model of Traumatic Brain Injury

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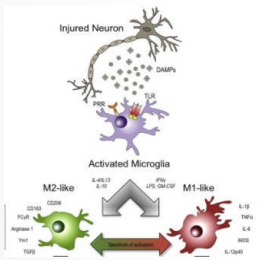
Introduction

- An estimated 2.8 million people sustain a traumatic brain injury (TBI) in the United States each year[1].The neuro-pathologic consequences of TBI persist for years after the initial injury[2].
- Effective therapies addressing the long-term neuro-pathological consequences are not currently available.
- Transcranial Nano-Pulsed Laser Therapy (NPLT) combines near infrared (808 nm) laser light with low-energy, optoacoustic waves[3].
- Microglia are the immune cells of the brain. After neurotrauma they convert to an inflammatory state in an attempt to begin clearing out the damage cells and tissues via phagocytosis. They also release pro-inflammatory cytokines which propagate further microglia activation.



(Loane, 2016)

- The problem is that this activation can persist for months to years after the initial injury [2]. This inflammation is an ongoing secondary damage process which can continue to harm an individual with TBI for years after the initial impact.
- Our previous data showed that NPLT significantly reduced pro-inflammatory microglia (CD68⁺) in the somatosensory cortex of rats subjected to parasagittal fluid percussion injury (FPI)-an established model of TBI.
- **This study was designed to evaluate whether NPLT also reduces the total number of microglia (Iba1⁺) in rats subjected to FPI.**

Experimental Design

- Adult male Sprague-Dawley rats (n= 29) were randomly assigned to receive fluid percussion injury (FPI) or Sham craniotomy surgery
- Half of the TBI rats (8) and SHAM rats (7) received a one time, 5 minute treatment one hour after injury.
- Rats were euthanized after 14 days.
- Brain sections were processed and sent to the NSA (Neuroscience Associates) for IBA1 staining (microglia) and CD68 (activated microglia) staining.

Quantification Methods

- 10x images were taken around a previously defined cavitation region.
- Bregma levels -3.00, -4.44, and -6.36 were imaged and quantified in all 29 rats.
- Stained images were captured using Keyence BZX Analyzer and quantified by Image J area analysis in the area surrounding a previously defined cavitation on the side of the brain ipsilateral to the impact.
- Data was analyzed via Multiple Comparisons T-Test with Tukey's Post Hoc Correction

NPLT Reduces CD68+ Stained Cells

Figure 1.

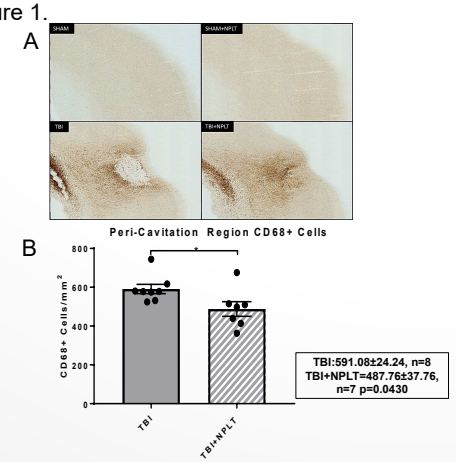
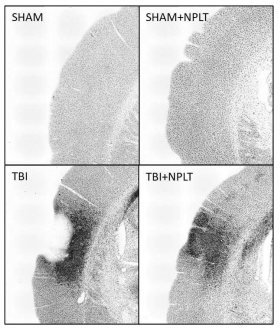


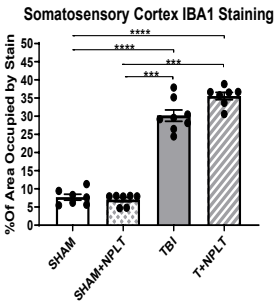
Figure 1. A) Representative images of CD68 immunoreactivity in the cortex of rats.
B) Quantification of CD68+ cells

NPLT Does Not Significantly Reduce IBA1 Stained Cells

Figure 2. A



B



TBI vs SHAM:	Mean Diff=-22.45	p=<0.0001
TBI vs SHAM+NPLT:	Mean Diff=-23.12	p=<0.0001
T+NPLT vs SHAM:	Mean Diff=-27.83	p=<0.0001
T+NPLT vs S+NPLT:	Mean Diff=-28.49	p=<0.0001

C

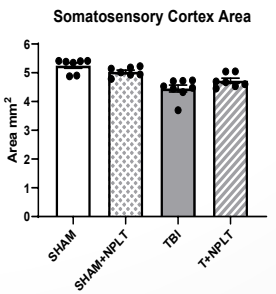


Figure 2. A) Images of IBA1 immunoreactivity in the cortex of rats.
B) Quantification of IBA1 cells
C) Differences in quantification of IBA1 cells as a function of somatosensory cortex area

Results

- Fluid Percussion Injury caused a significant increase in IBA1 staining for microglia in the somatosensory cortex area surrounding the cavitation.
- NPLT caused a significant decrease in CD68 staining but did not significantly change the amount of IBA1 staining.

Conclusions

- This data shows that NPLT applied one hour after injury does not reduce the number of total microglia, however, it does reduce the total number of activated microglia.
- This, in conjunction with other published data analyzing fluid percussion model injuries[4], supports the therapeutic potential of NPLT for TBI.
- Further studies will be needed to evaluate the efficacy of NPLT post TBI.

References

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