



Intranasal Insulin Treatment of Traumatic Brain Injury

Fiona Brabazon¹, Guzal Khayrullina¹, William H. Frey², Kimberly R. Byrnes¹

Department of Anatomy, Physiology and Genetics, Uniformed Services University
 Department of Neurology, Oral Biology and Neuroscience, University of Minnesota



Intranasal insulin treatment after moderate controlled cortical impact improves neuronal viability and promotes the anti-inflammatory microglia phenotype

Abstract

Traumatic brain injury (TBI) is a serious health problem that affects approximately 1.5 million people in the United States each year and causes long term cognitive deficits. After injury there is a transient but marked reduction in cerebral glucose uptake. The length and severity of this metabolic crisis is directly correlated with patient outcome. We hypothesized that administration of intranasal insulin, a treatment shown to improve cerebral glucose uptake and memory in Alzheimer's patients, will increase cerebral glucose uptake, neuronal survival and reduce glial mediated inflammation, leading to a reduction in TBI-related histological and functional impairment. To test our hypothesis, adult male Sprague Dawley rats received a moderate brain injury in the left motor cortex using the controlled cortical impact (CCI) model of brain injury. The animals were treated once a day for 7 days with either intranasal insulin or intranasal vehicle (saline) while under isoflurane anesthesia. Intranasal insulin treatment significantly improved the performance of injured animals on a balance beam in comparison to the intranasal saline group. Qualitative assessment of histology showed improved neuronal viability in the hippocampus of the intranasal insulin treated rats. In addition, we examined changes in microglia polarization as a result of treatment. There are 2 dominant phenotypes of microglia, M1 and M2. M1 are classically activated pro-inflammatory cells. They are useful in the initial healing process but persistent activation results in neuronal cell death due to productions of reactive oxygen species. We found that markers of anti-inflammatory, pro-healing M2 microglia/macrophages were significantly increased in the intranasal insulin group in comparison the intranasal saline group. There was no significant increase in expression of M1 markers indicating that the drug treatment is pushing microglia toward an anti-inflammatory phenotype. Microglia are the macrophages of the brain.

Methods

- Adult male Sprague Dawley rats
 - N=8/group for behavior
 - N= 5/group for immunohistochemistry and H&E
 - N= 3/group for Western Blot
- Craniotomy and CCI injury in the left motor cortex with a 4mm impactor, 5m/s impact speed, 200msec dwell time, and 2mm piston depth
- Animals received insulin or vehicle(saline) under isoflurane once a day for 7 days with the first dose 4 hours after injury.
- Motor function testing was performed at 1 and 7 days post injury and compared to baseline measurements.
- 8 days after injury, animals were sacrificed and brain tissue was processed for immunohistochemical labeling of markers for neurons, microglia and H&E staining to quantify hippocampal volume. Protein was also extracted for Western Blot analysis.

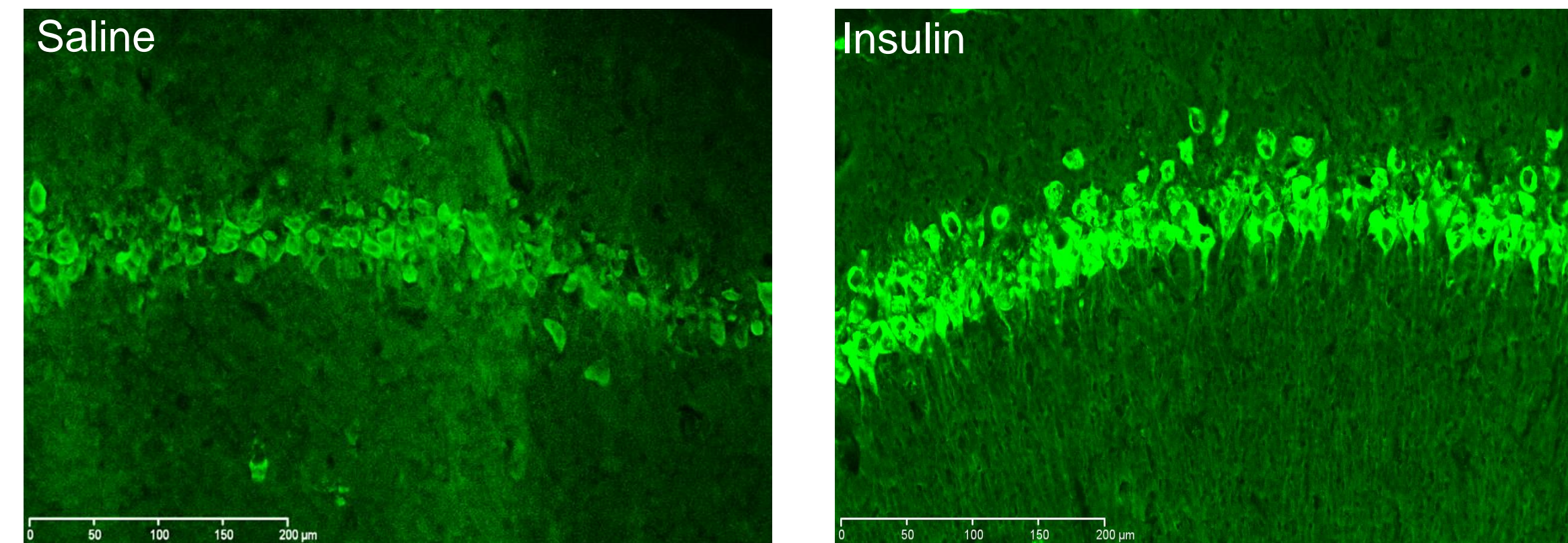


Figure 1. NeuN staining was increased with intranasal insulin treatment

TBI results in neuronal cell death. Neuronal cell death in the hippocampus impairs memory function. NeuN, an immunohistochemical marker of neurons, was used to examine the effect of intranasal insulin on neurons after injury. Qualitative assessment of histology showed improved neuronal viability in the hippocampus of the insulin treated rats.

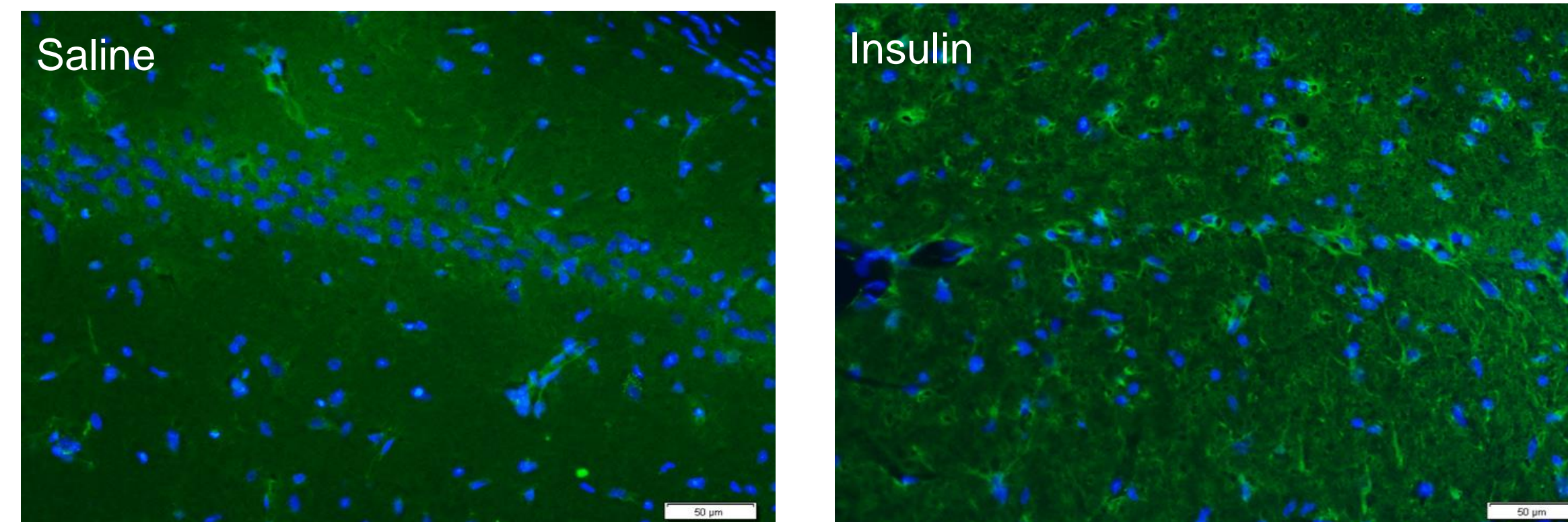


Figure 2. Intranasal Insulin increases the expression of anti-inflammatory microglia in the hippocampus

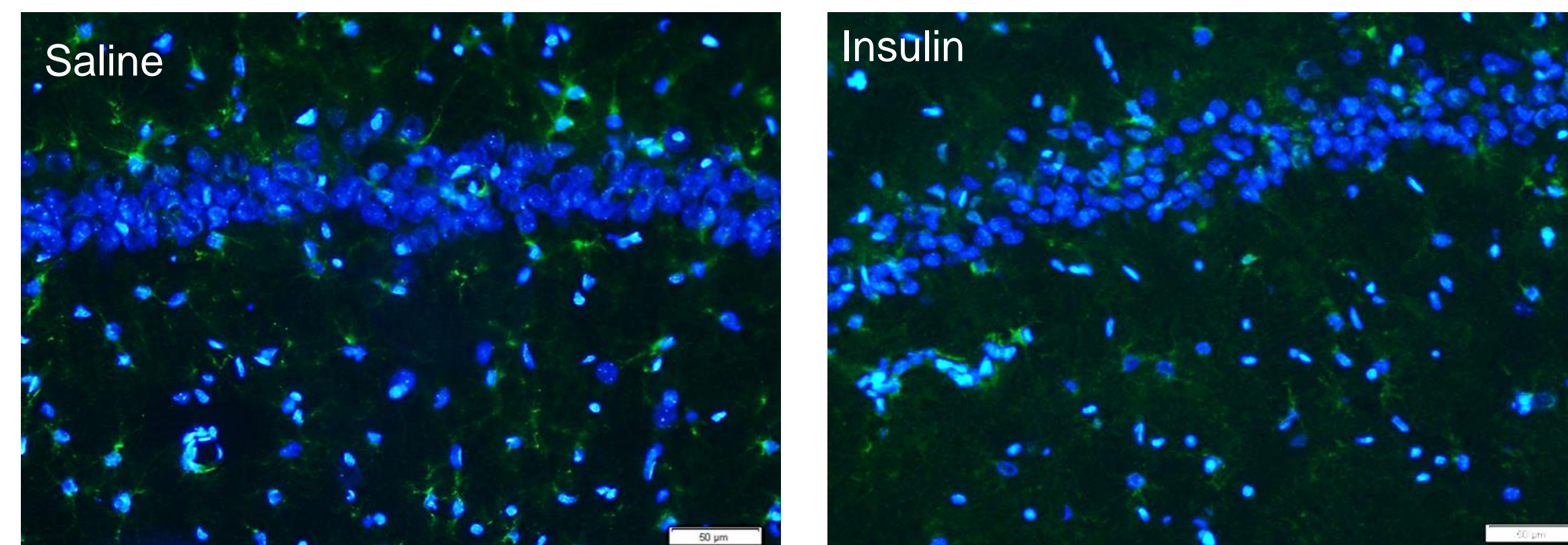


Figure 3. Intranasal does not alter the expression of pro-inflammatory microglia in the hippocampus

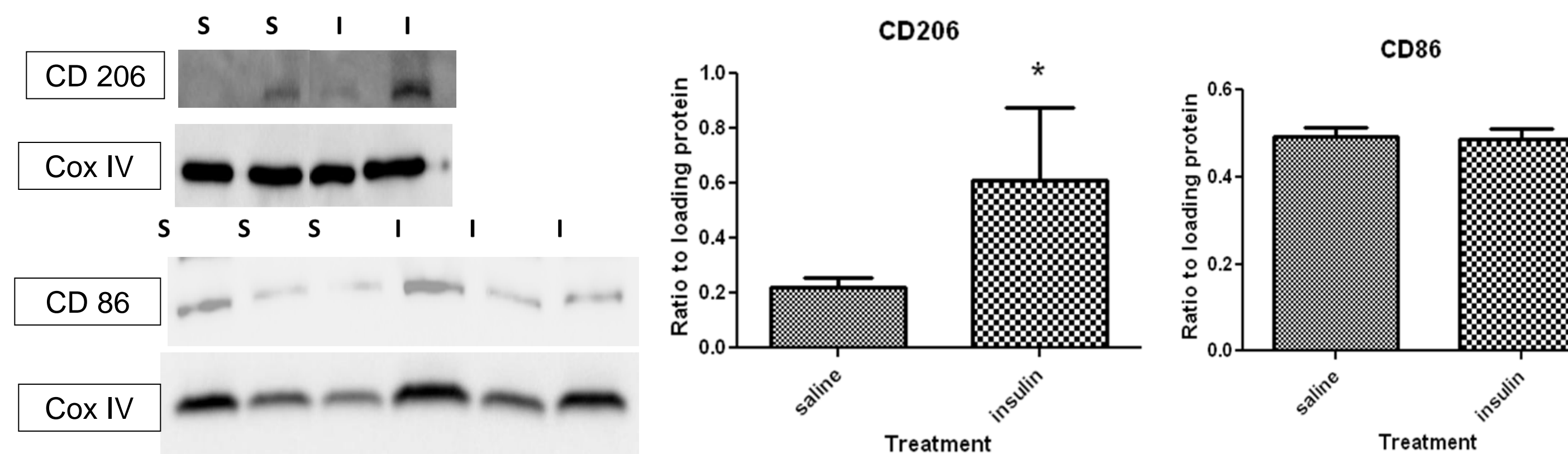


Figure 4. Intranasal Insulin Treatment Significantly alters Microglia Polarization

Our study shows a significant increase in M2 microglia, characterized by expression of CD206, in the intranasal insulin group compared to intranasal saline. There was no significant increase in expression of M1 markers indicating that the drug treatment is pushing microglia toward an anti-inflammatory phenotype.

Conclusion In conclusion our studies indicate that intranasal insulin, a clinically proven treatment for Alzheimer's disease, increases neuronal viability, M2 activation and functional recovery following TBI.

Intranasal Insulin Improves Performance in Motor Function Test

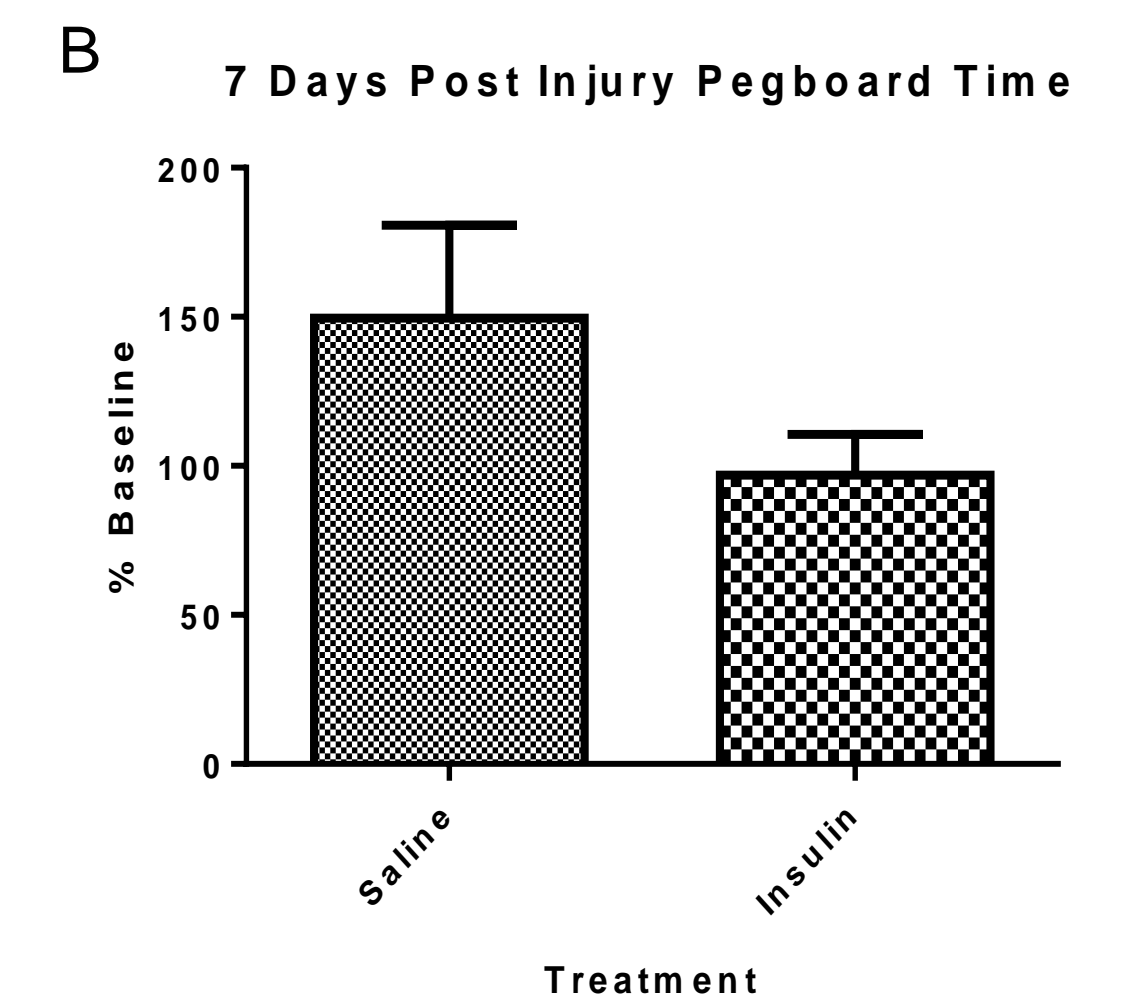
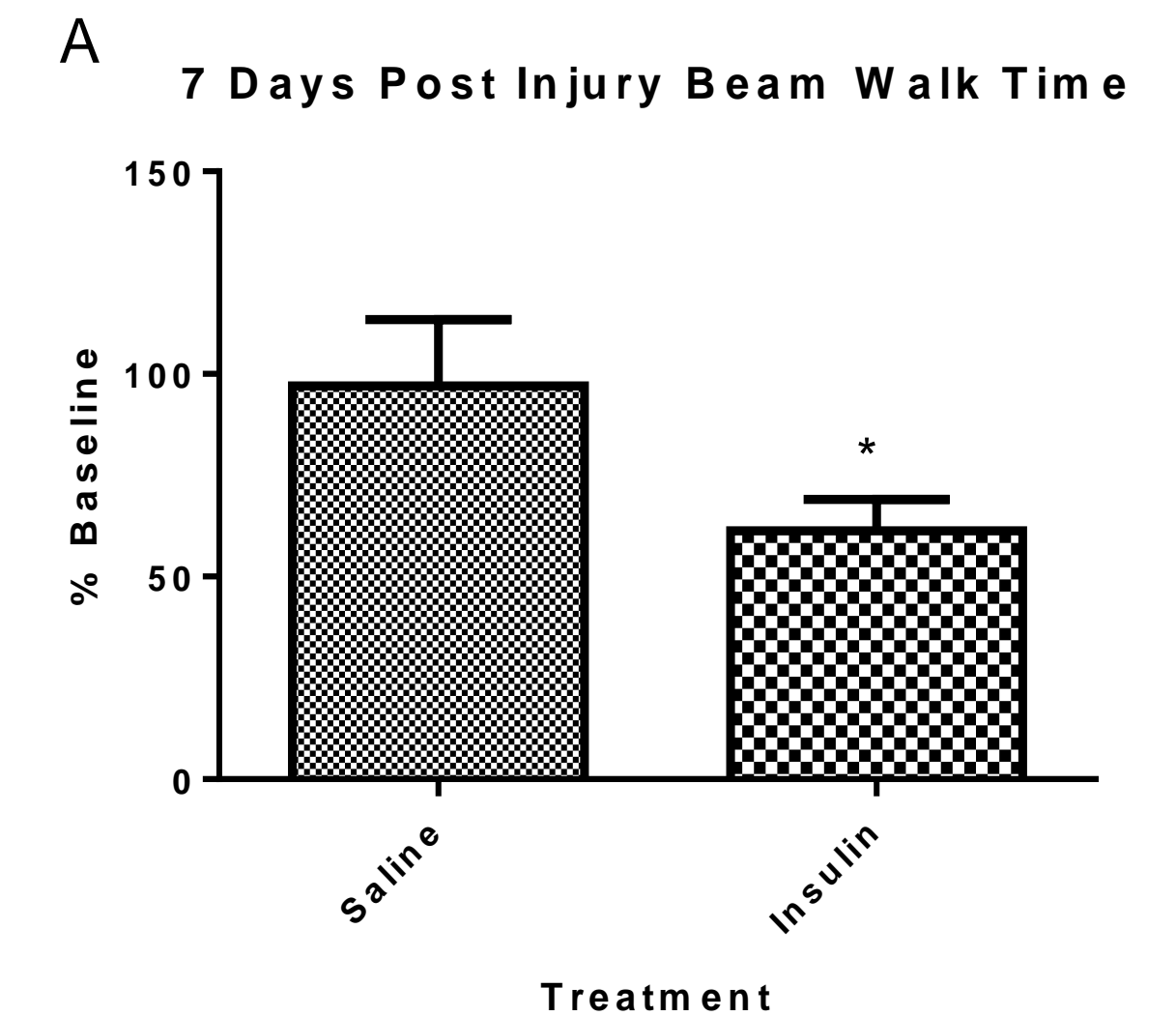


Figure 4. Intranasal Insulin Improves performance on Motor Function Test

Significant increase in time to cross the beam in beam walk and non-significant trend in peg walk. This may indicate improvement in learning or memory of how to do the task. Future studies will also utilize Morris water maze as a better measure of cognitive function and efficacy of the treatment.

**unpaired t-test
 *=significant p value > 0.05

Acknowledgements

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