Traumatic Brain Injury and Neuroinflammation

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Consequences of TBI

- Major cause of death and disability
- Physical disabilities
- Neurological deficits
- Visual Dysfunction
- Memory problems
  - Learning disabilities
  - Personality changes
- Military and Civilian relevance

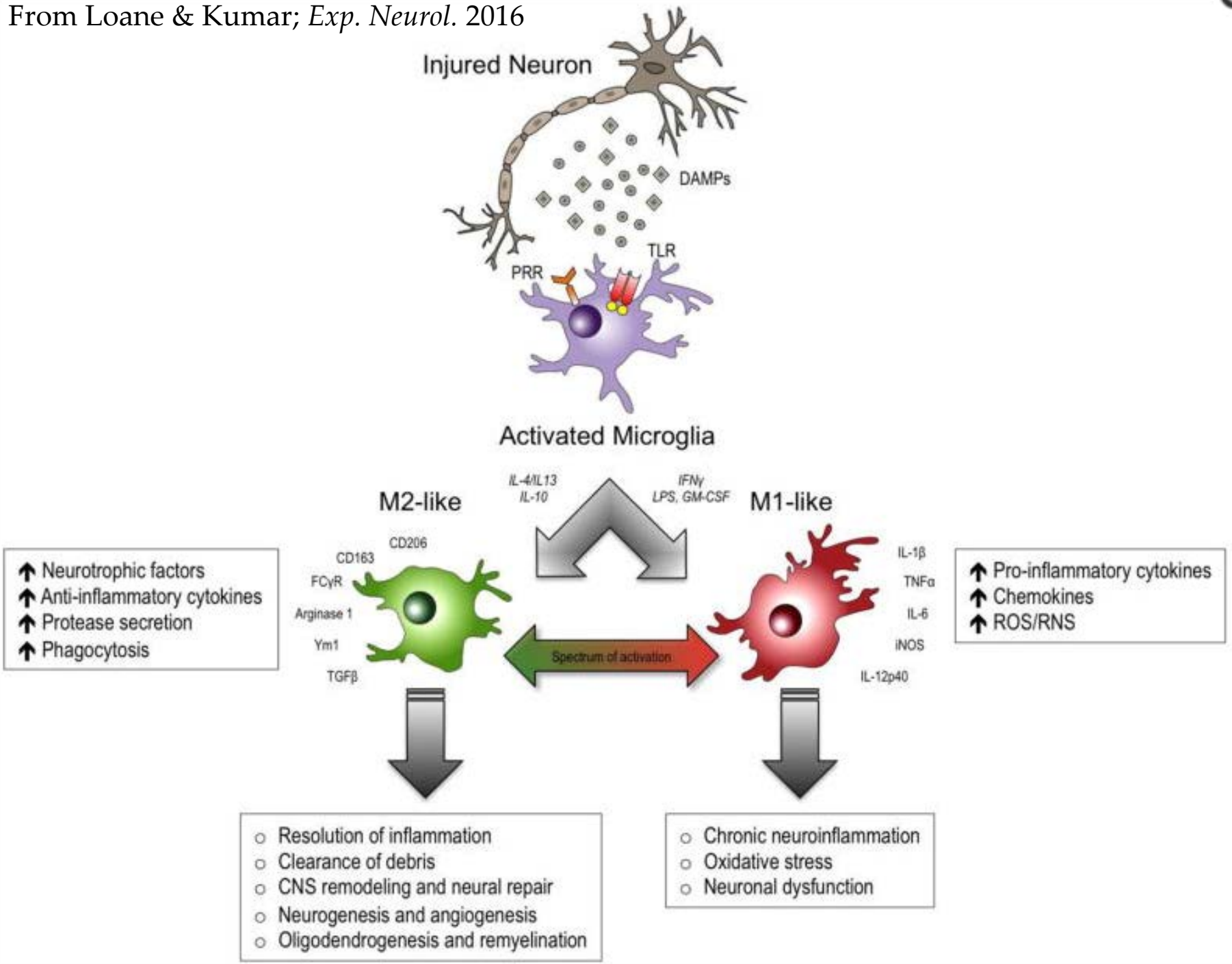
TBI

- Complex multi-modal disease process
- Structural and functional damage
- Primary Injury:
  - Immediate mechanical damage – neuronal/ glial damage, contusion, hemorrhage, axonal sehearing
- Secondary Injury:
  - Cascades of molecular events – calcium homeostasis, glutamate excitotoxicity, mitochondrial dysfunction, free radical generation, apoptosis, neuroinflammation

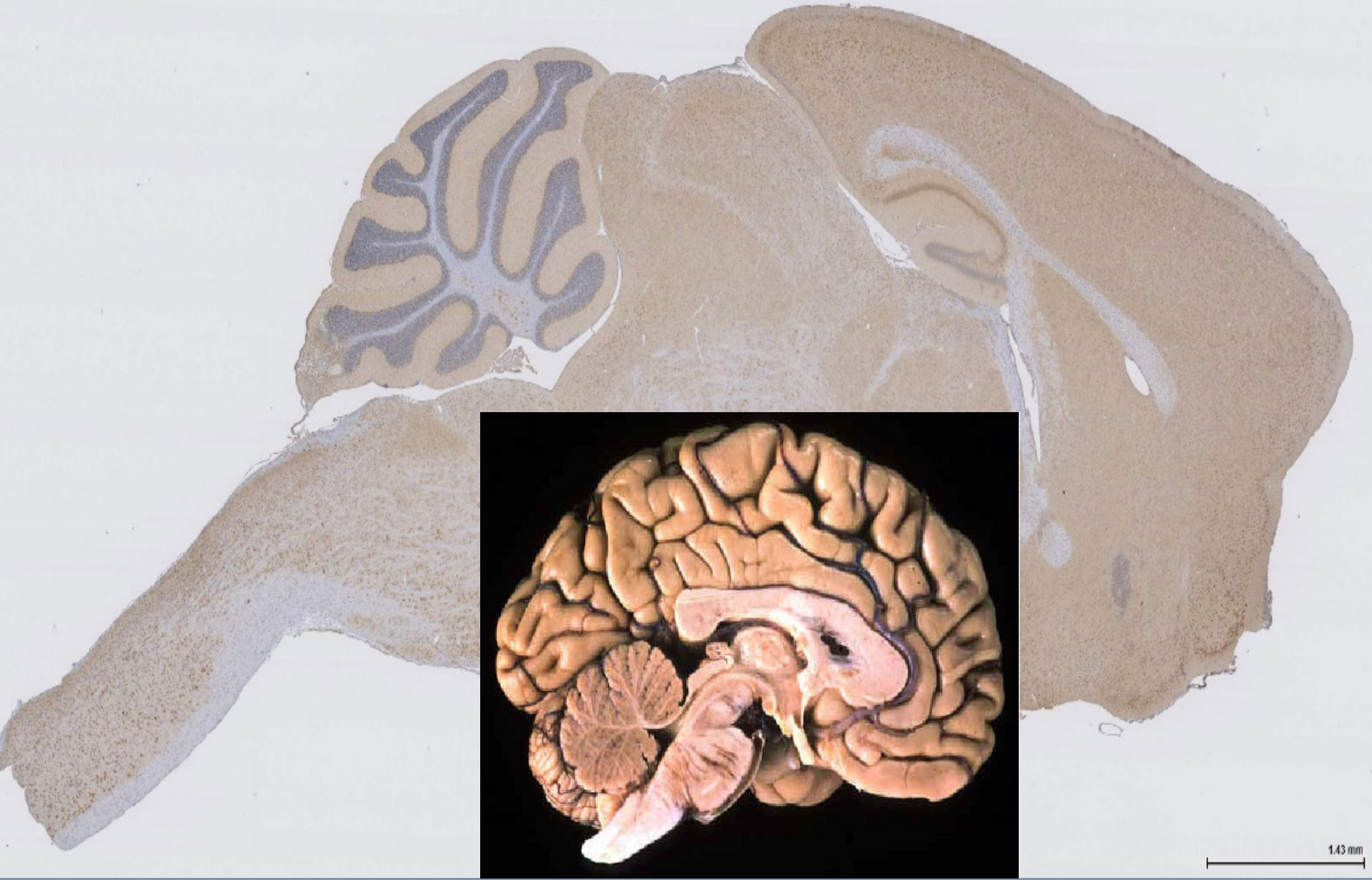
TBI

- Innate and adaptive immune responses
- Cytokines – IL1B, TNFa, IL18…..
- Chemokines – migration of neutrophils, attraction of monocytes/macrophages, recruitment of T-lymphocytes
- Activated macrophages and/or microglia
  - Multiple activation phenotypes
  - “M1-like vs M2-like”

From Loane & Kumar; *Exp. Neurol.* 2016



Pathology: Mouse and Man



TBI models used at Roskamp

- CCI model of TBI in APPsw mouse model of Alzheimer's Disease (22mo old; moderate injury; 1 month timepoint), genomic analyses – significant differences in immune/inflammatory/cell death gene expression

- CCI in APOE transgenic mice (10mo; moderate injury; 1 month timepoint), genomic studies – significantly greater overall response in E3 versus E4 mice – immune/inflammatory/lipid

- CCI in APOE3 and APOE4 proteomic analyses (7mo old; moderate and severe injury; 24hr/1month/3month timepoints) using iTRAQ – immune/inflammatory changes

Other TBI models

- Diversity of preclinical research - Blast, CCI, FPI, Weight Drop, Rotational; animal age/genotype/gender; time of analysis – all demonstrate inflammation

- Closed head impact model of engineered rotational acceleration CHIMERA – 3 hits 24hr apart; Iba1 & GFAP at 6 months post-TBI Chen et al *J Neurotrauma* 2017

- CCI in wild type mice – Microglial activation at 24hrs post-TBI; Microglial-derived microparticles mediating neuroinflammation after TBI; seeding capability – Kumar et al *J Neuroinflammation* 2017

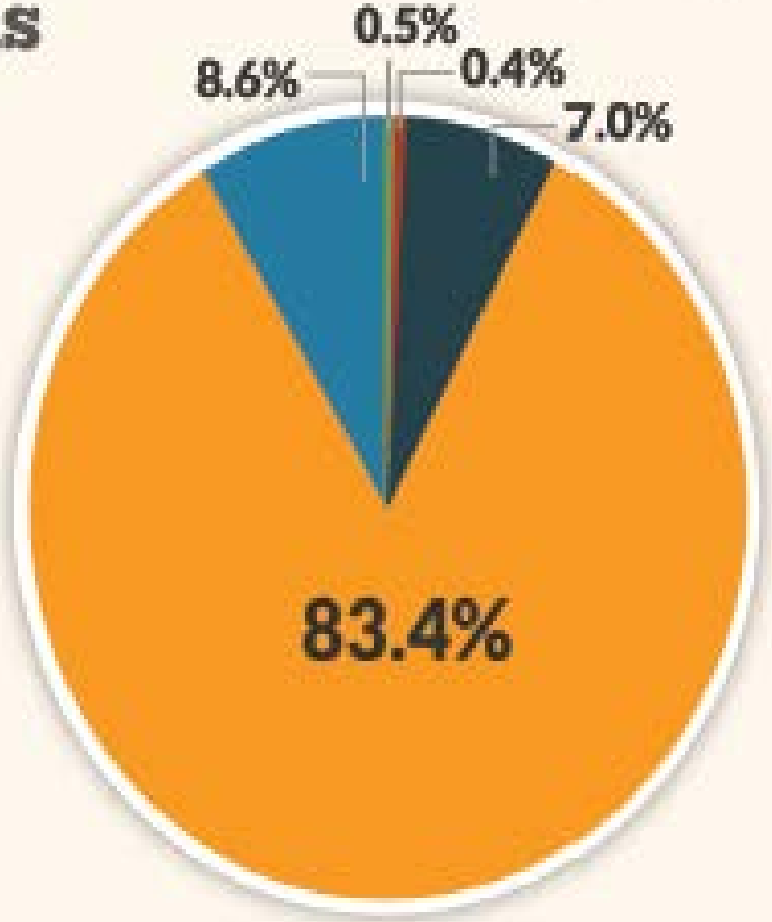
Incidence charts: DoD TBI Numbers

DoD Numbers for Traumatic Brain Injury

Worldwide – Totals

2014 (Q1-Q2)

Penetrating	66
Severe	53
Moderate	849
Mild	10,081
Not Classifiable	1,034
Total - All Severities	**12,083**



Source: Defense Medical Surveillance System (DMSS), Theater Medical Data Store (TMDS) provided by the Armed Forces Health Surveillance Center (AFHSC)

Prepared by the Defense and Veterans Brain Injury Center (DVBIC)

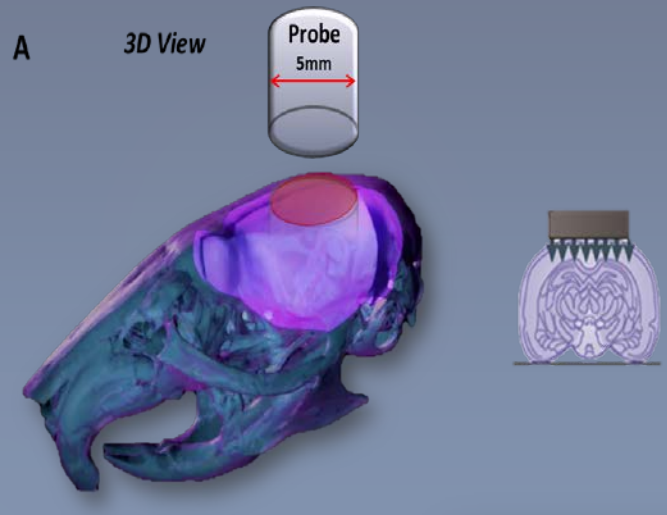
2014 (Q1-Q2), as of Aug 19, 2014

Animal model of TBI:

Electromagnetic impactor

Focal injury with a 5 mm tip

Modified CCI apparatus

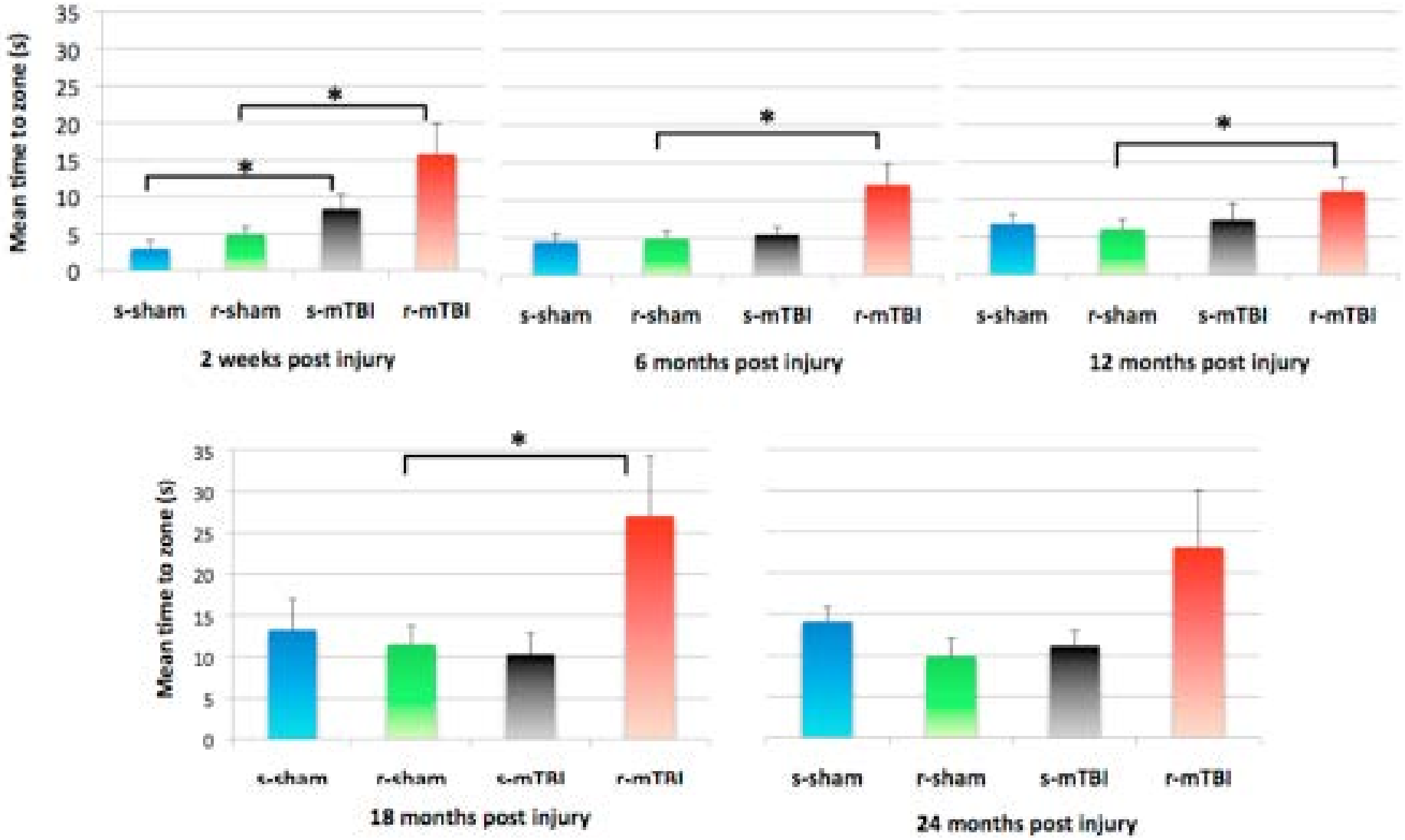


Roskamp mTBI model

- Closed head injury - 1mm depth midline hit
- no fracture, no bleed
- single hit (s-mTBI) and relevant sham (s-sham)repetitive hit (r-mTBI) - 5 hits with an inter-injury interval of 48 hrs - and relevant sham (r-sham)
- Neurobehavioral and neuropathological characterization to 24 months post-injury

Mouzon et al. 2012, 2014

Persistent cognitive deficits in wild type mouse model of mild TBI



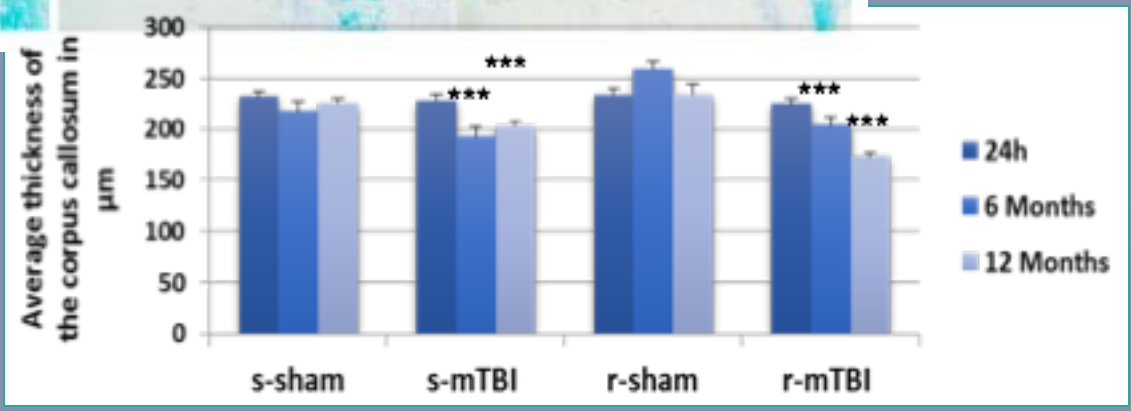
Mouzon *et al.* 2012, 2014

White matter changes in wild type mouse model of mild TBI

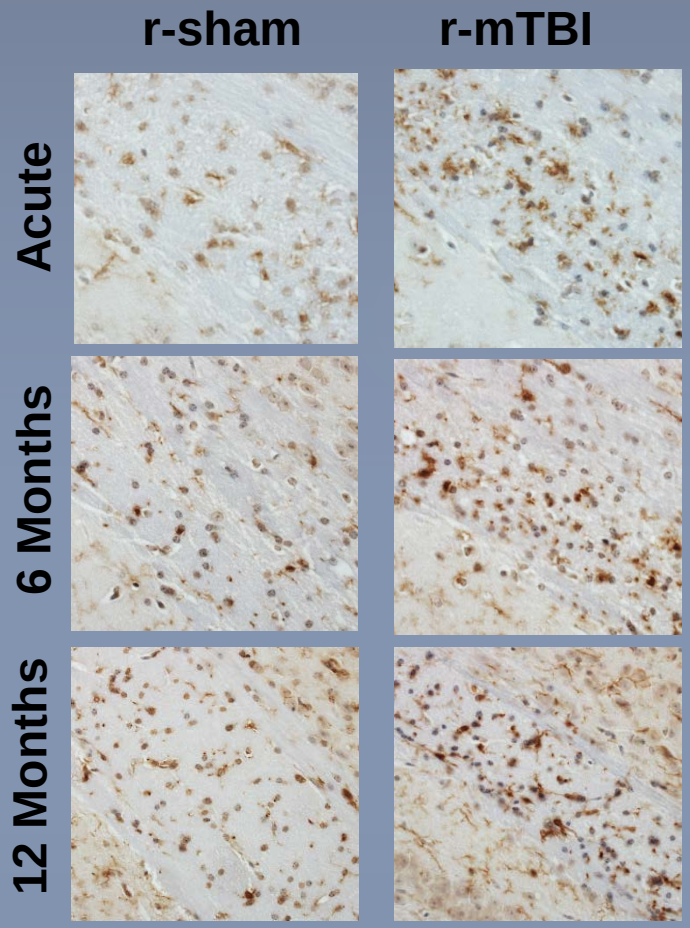
s-sham s-mTBI r-sham r-mTBI

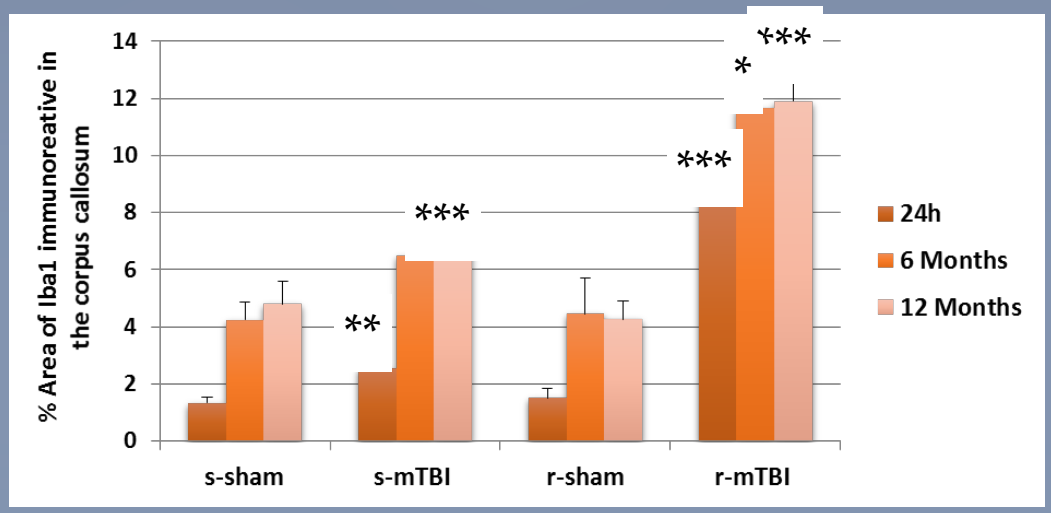
12 months

6 months

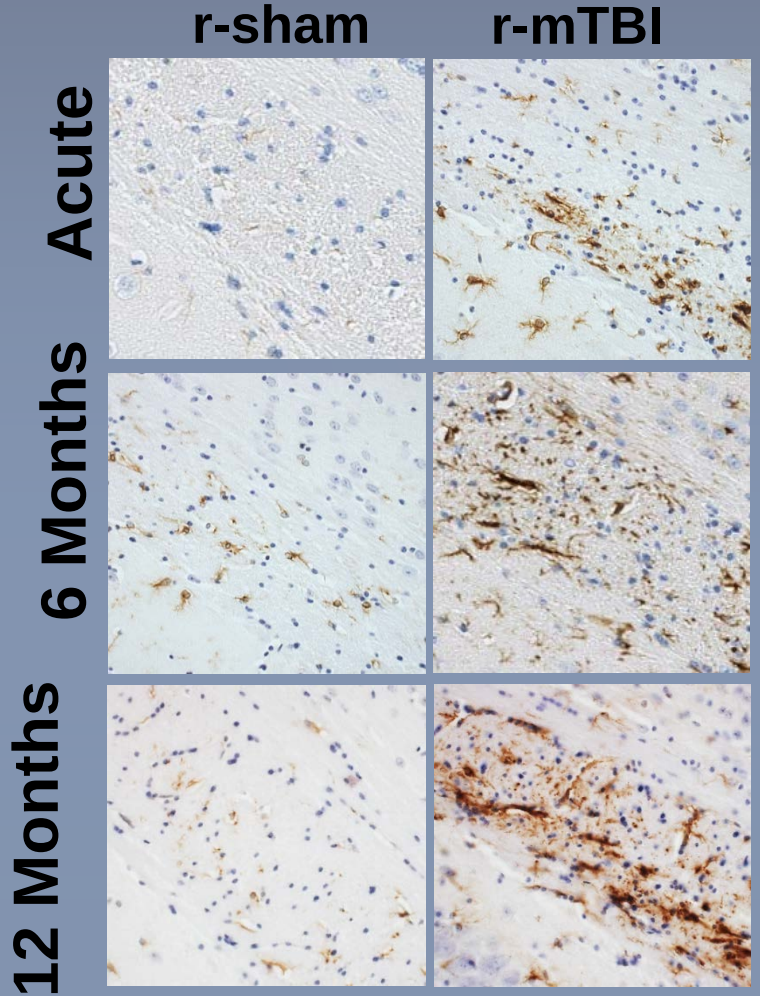


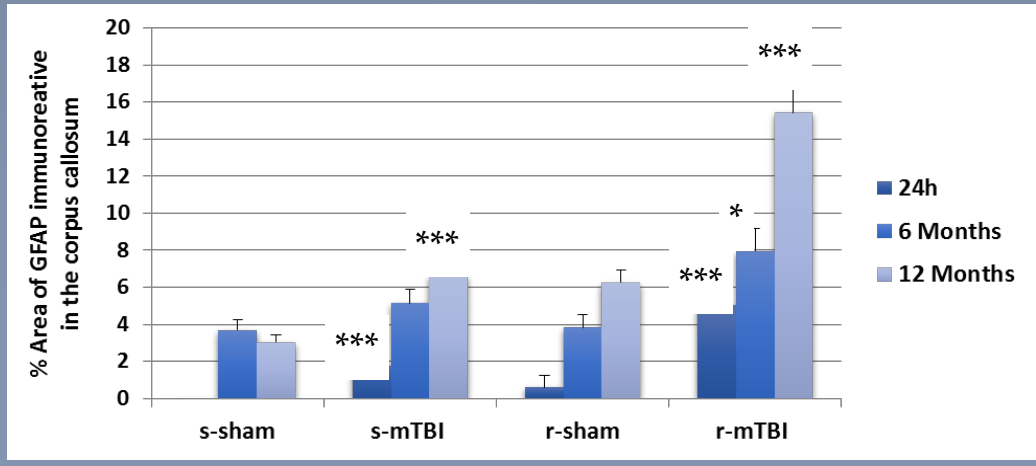
Progressive neuroinflammation - Iba1 in wild type mouse model of mild TBI



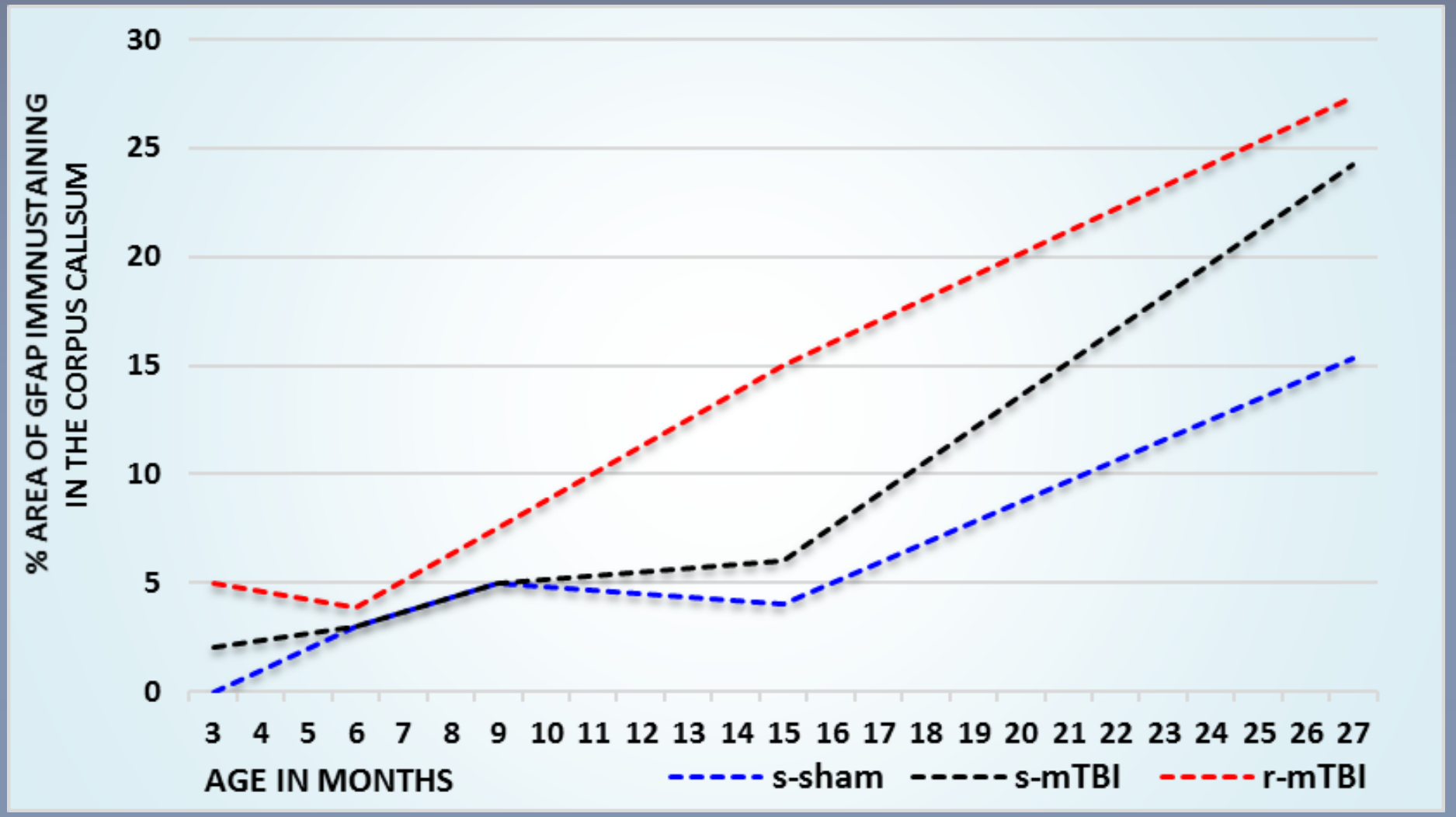


Progressive neuroinflammation - GFAP in wild type mouse model of mild TBI

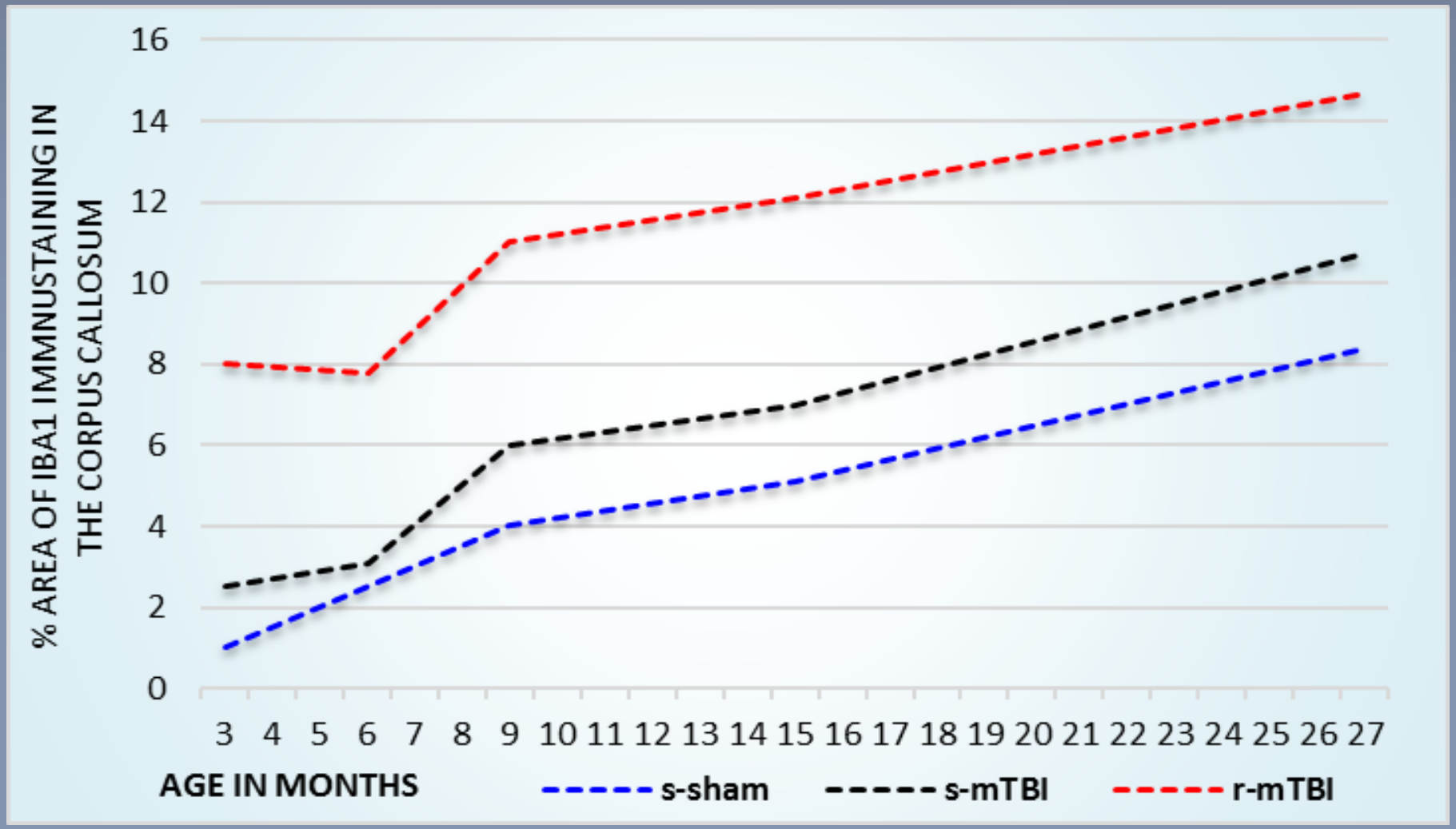




Lifelong changes in GFAP – astrocyte activation - in wild type mouse model of mild TBI



Lifelong changes in Iba1 – microglial activation - in wild type mouse model of mild TBI



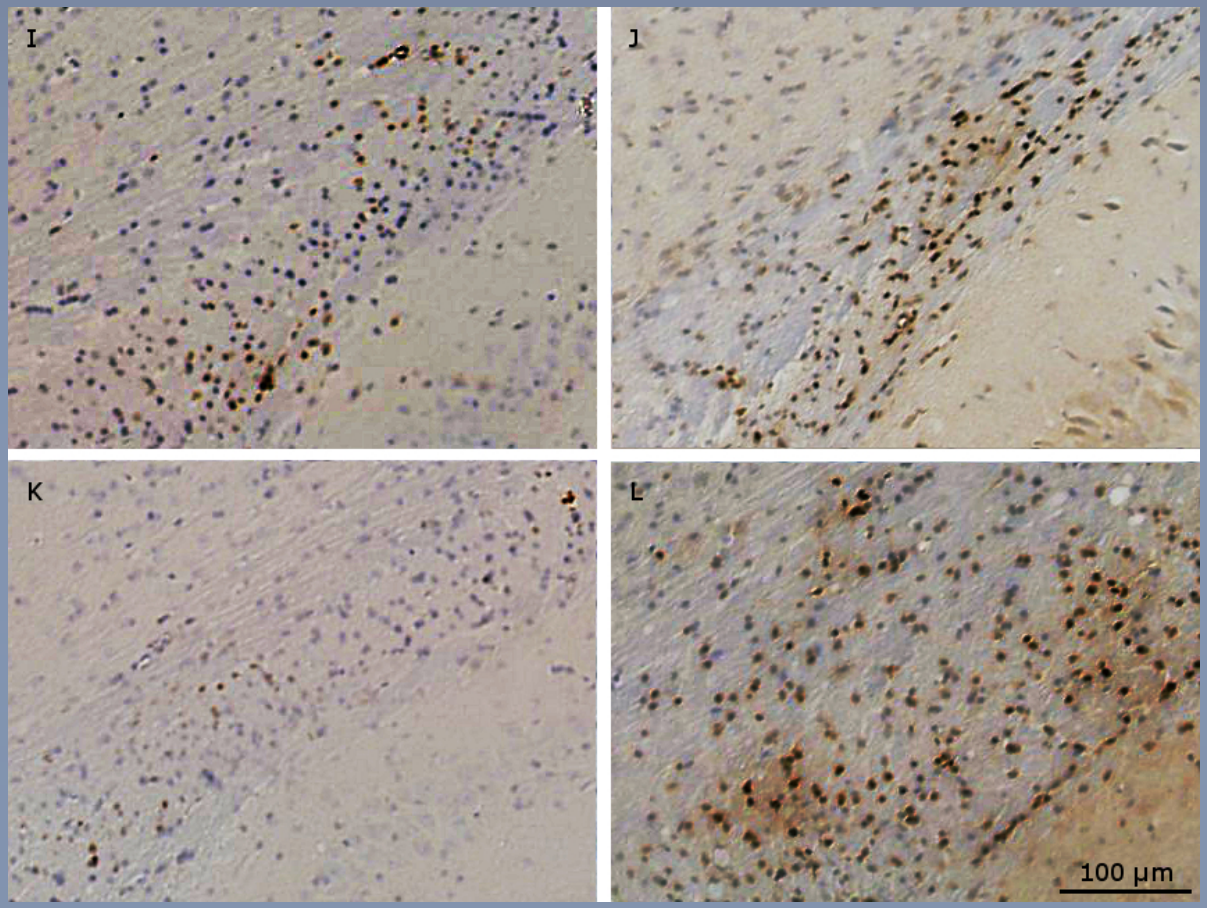
Progressive neuroinflammation – Phospho-STAT3

12 months post-r-mTBI

24 months post-r-mTBI

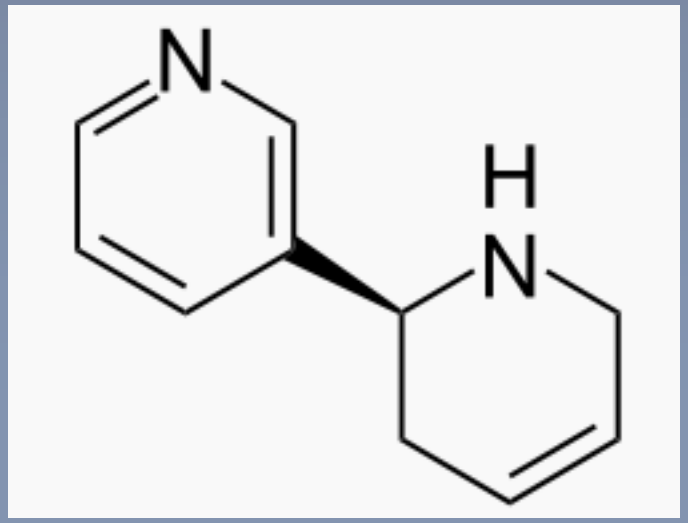
r-mTBI

r-sham



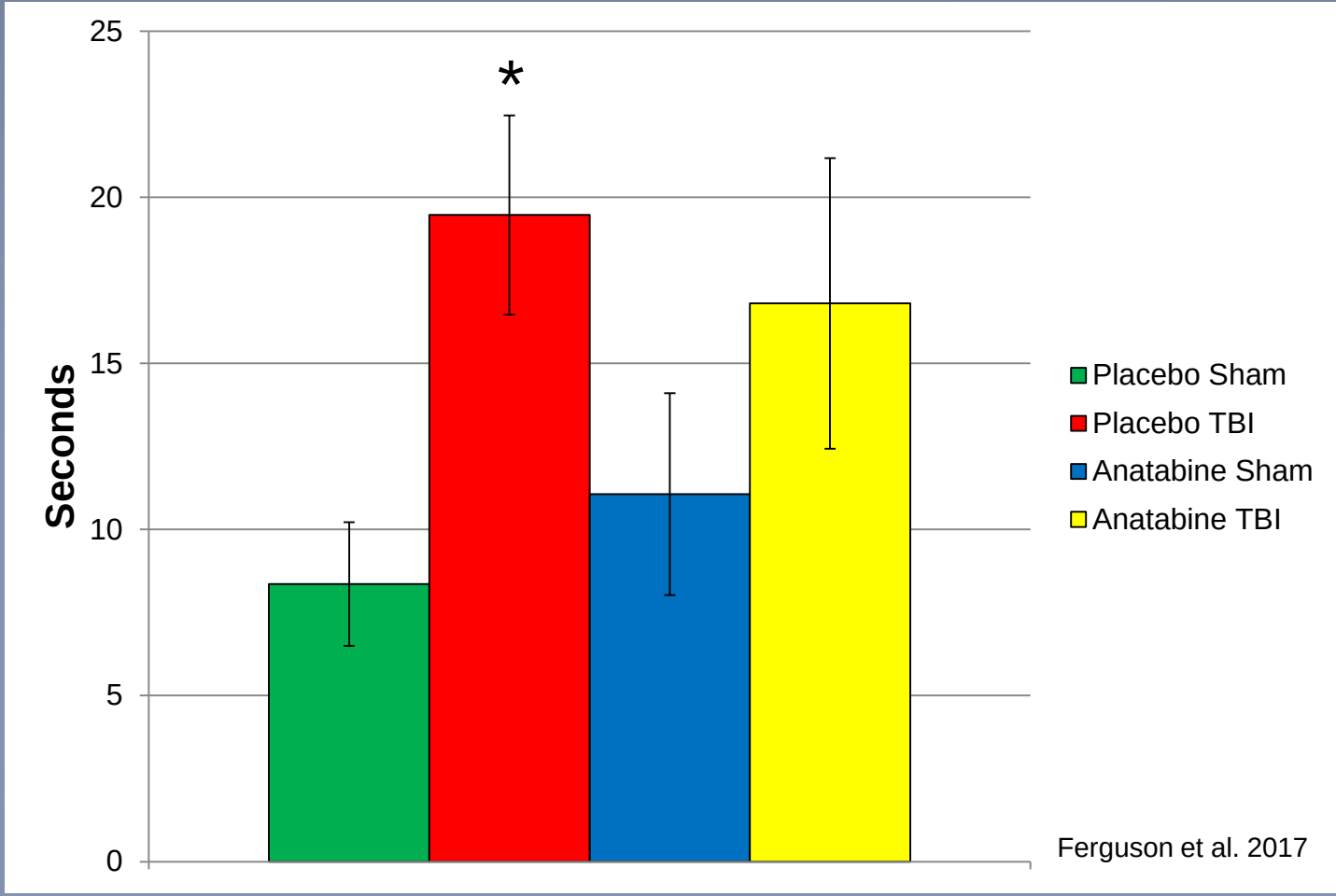
Targeting Inflammation

Anatabine

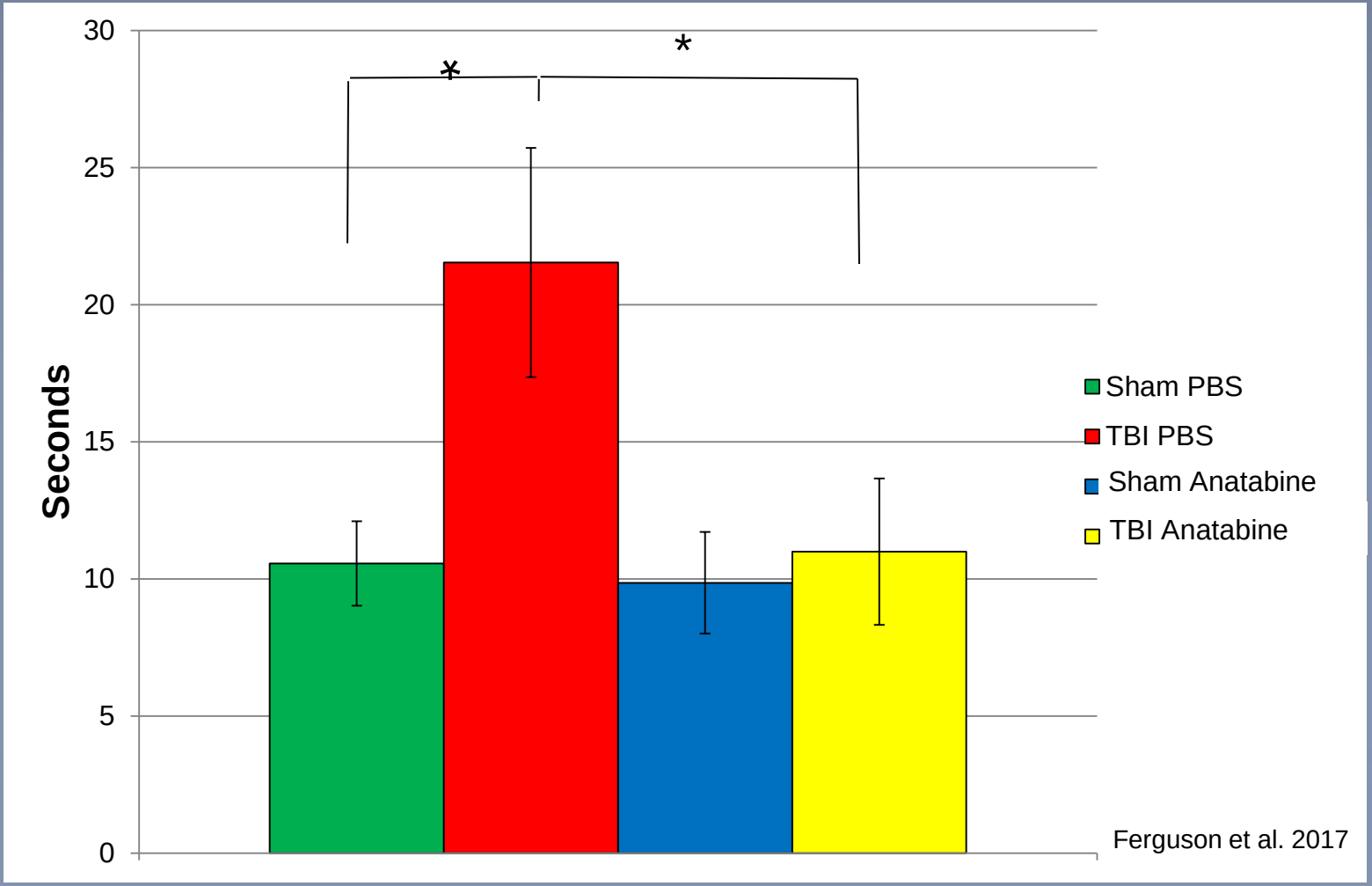


- Alkaloid derived from tobacco and plants of solenaceae family
- 3 year history of safe use as a dietary supplement
- Potent anti-inflammatory – inhibitor of STAT3 and NFkB
- Efficacy in mouse models of AD, EAE model of Multiple Sclerosis, Tauopathy

Acute effects of anatabine on Barnes Maze performance in the r-mTBI model



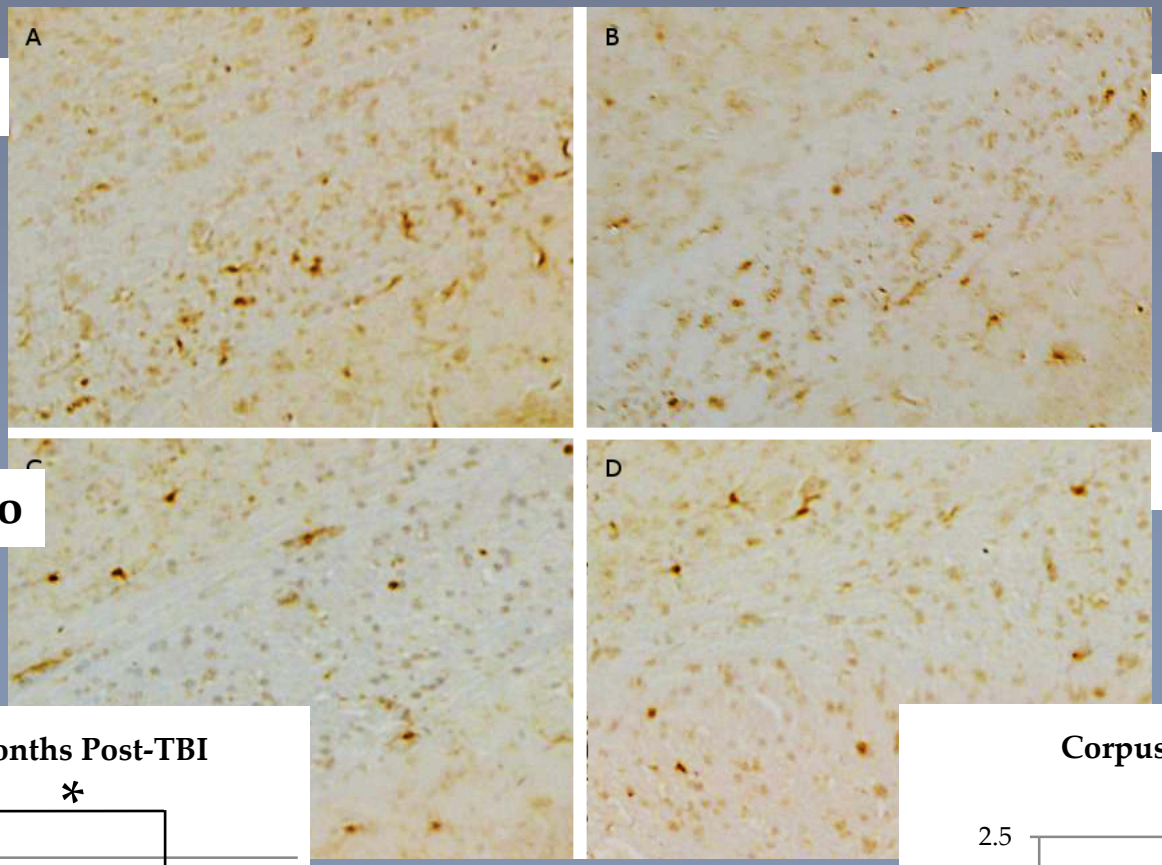
Chronic effects of anatabine on Barnes Maze performance 6 mo. after r-mTBI



Effects of anatabine on Iba1 staining 9 months after r-mTBI

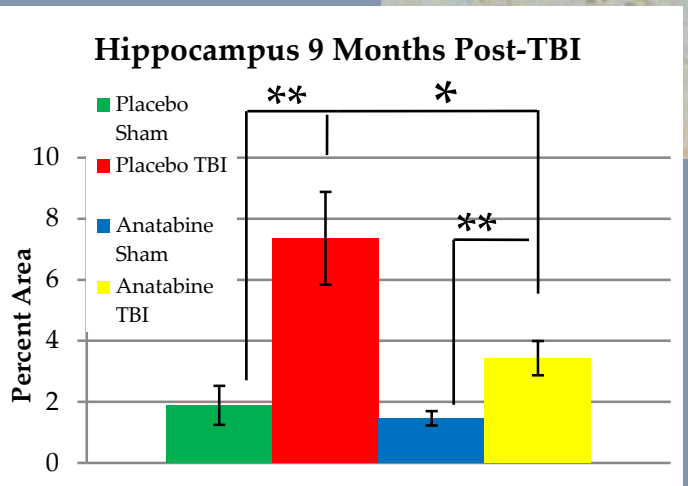
TBI-Placebo

TBI-Anatabine

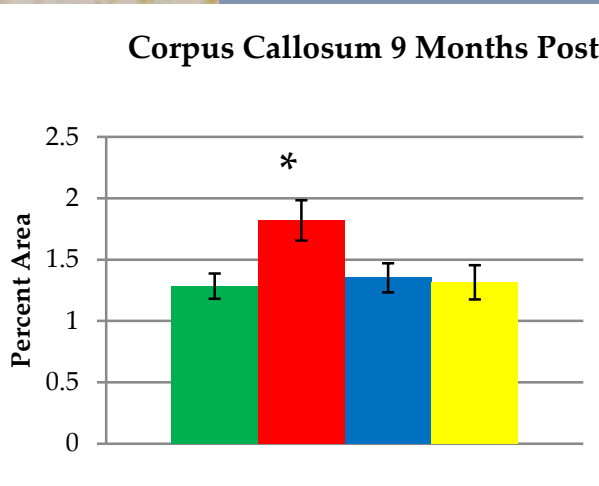


Sham-Placebo

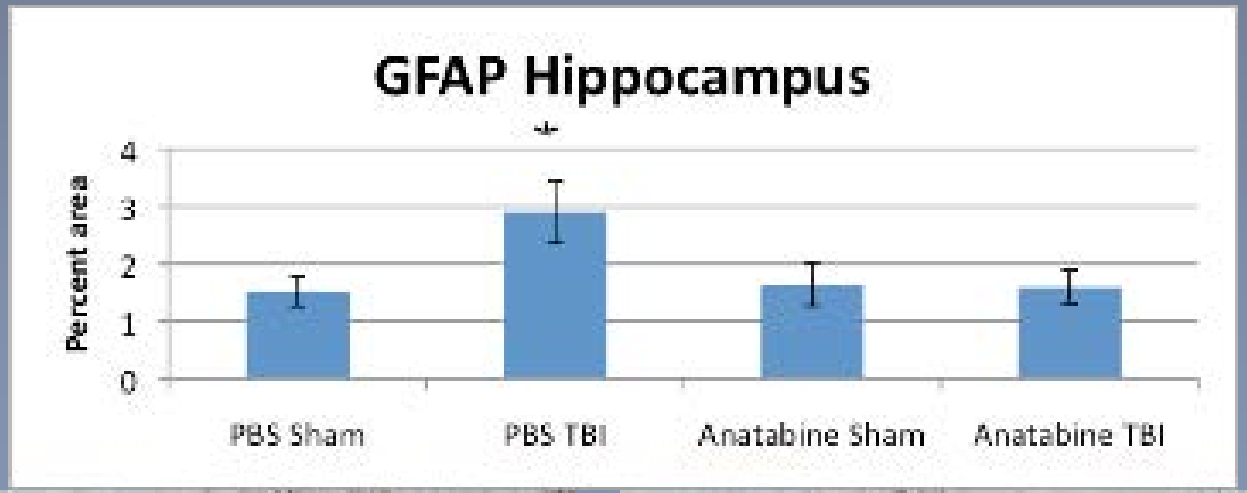
Sham-Placebo

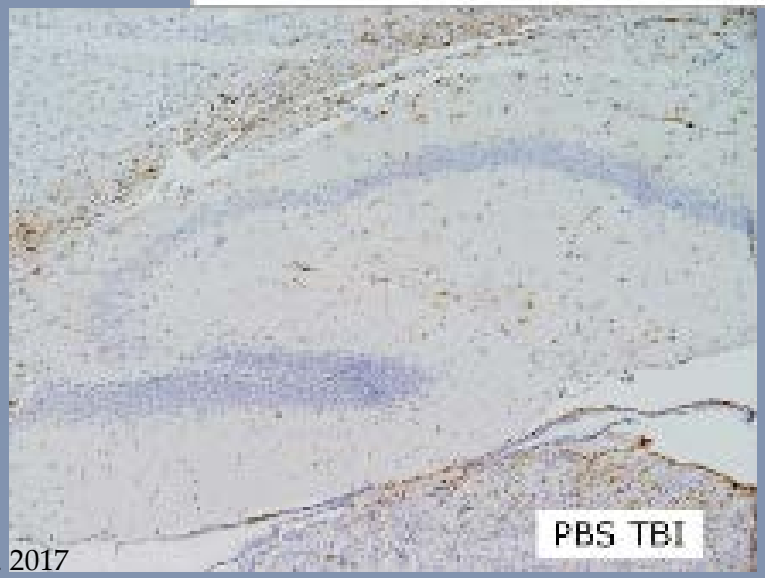


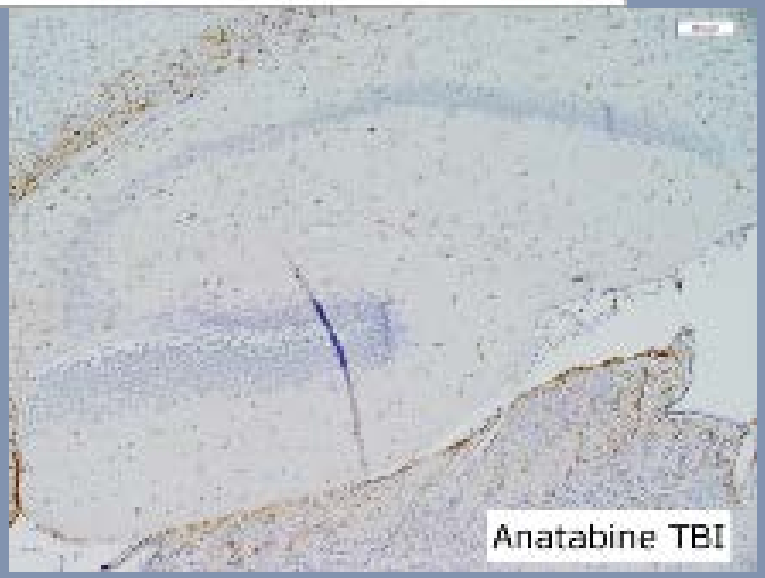
Ferguson et al. 2017



Effects of anatabine on GFAP staining 9 months after r-mTBI

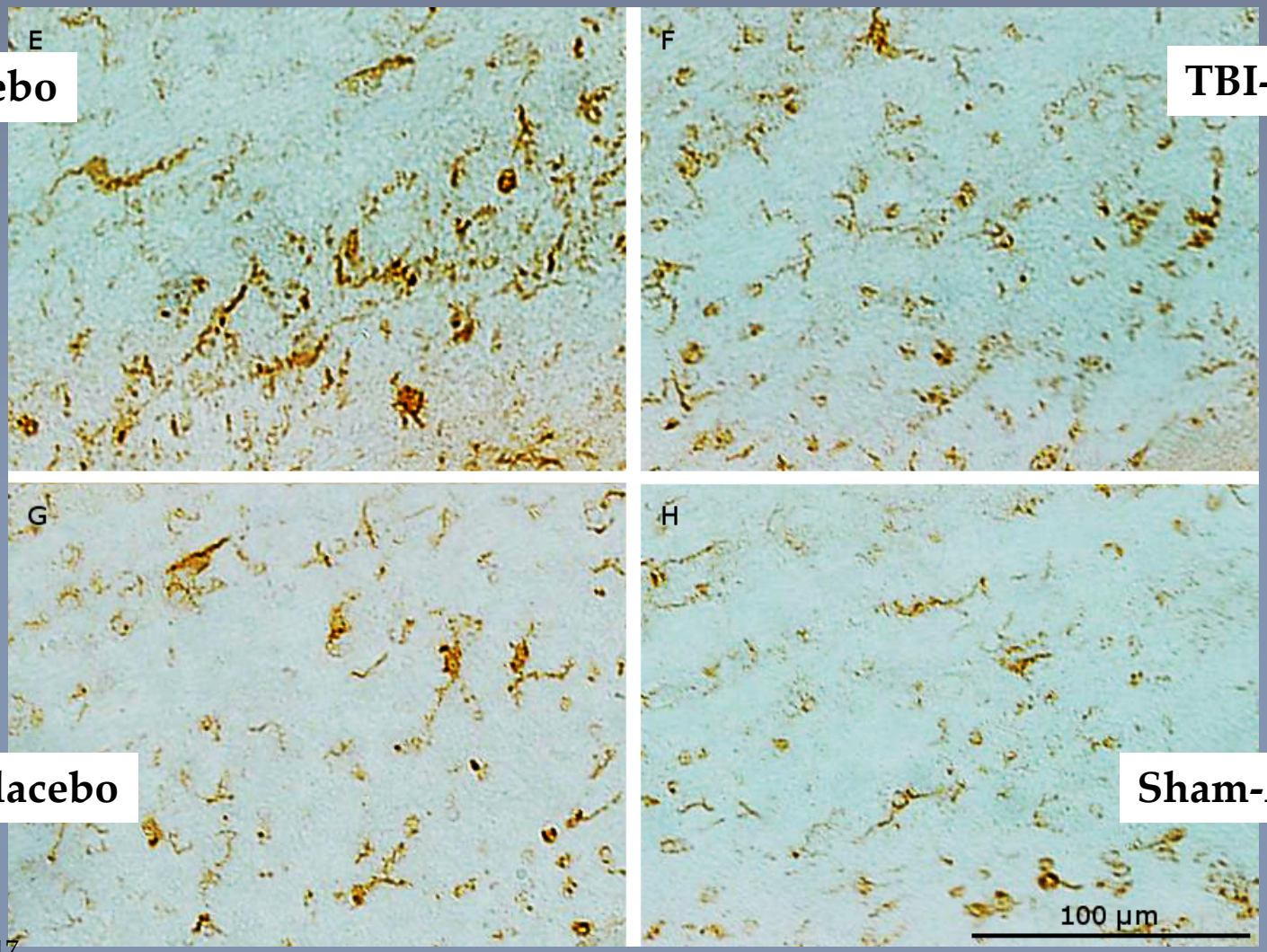






Ferguson et al. 2017

Anatabine crossover at 9 mo after r-mTBI; Iba1 in CC at 18 mo



TBI-Placebo

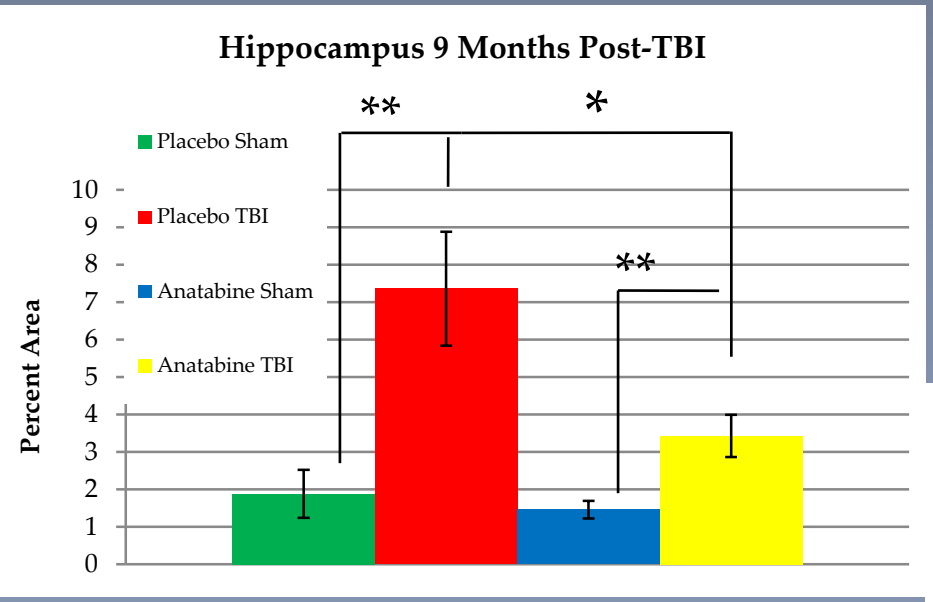
TBI-Anatabine

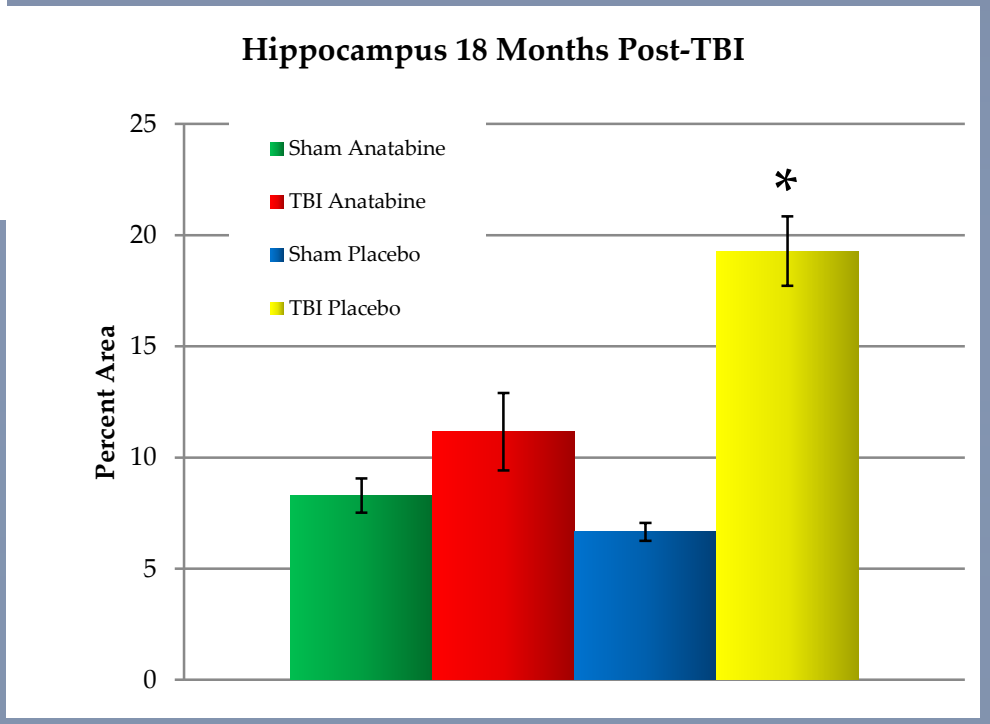
Sham-Placebo

Sham-Anatabine

Ferguson et al. 2017

Delayed treatment with Anatabine mitigates microgliosis (Iba1)



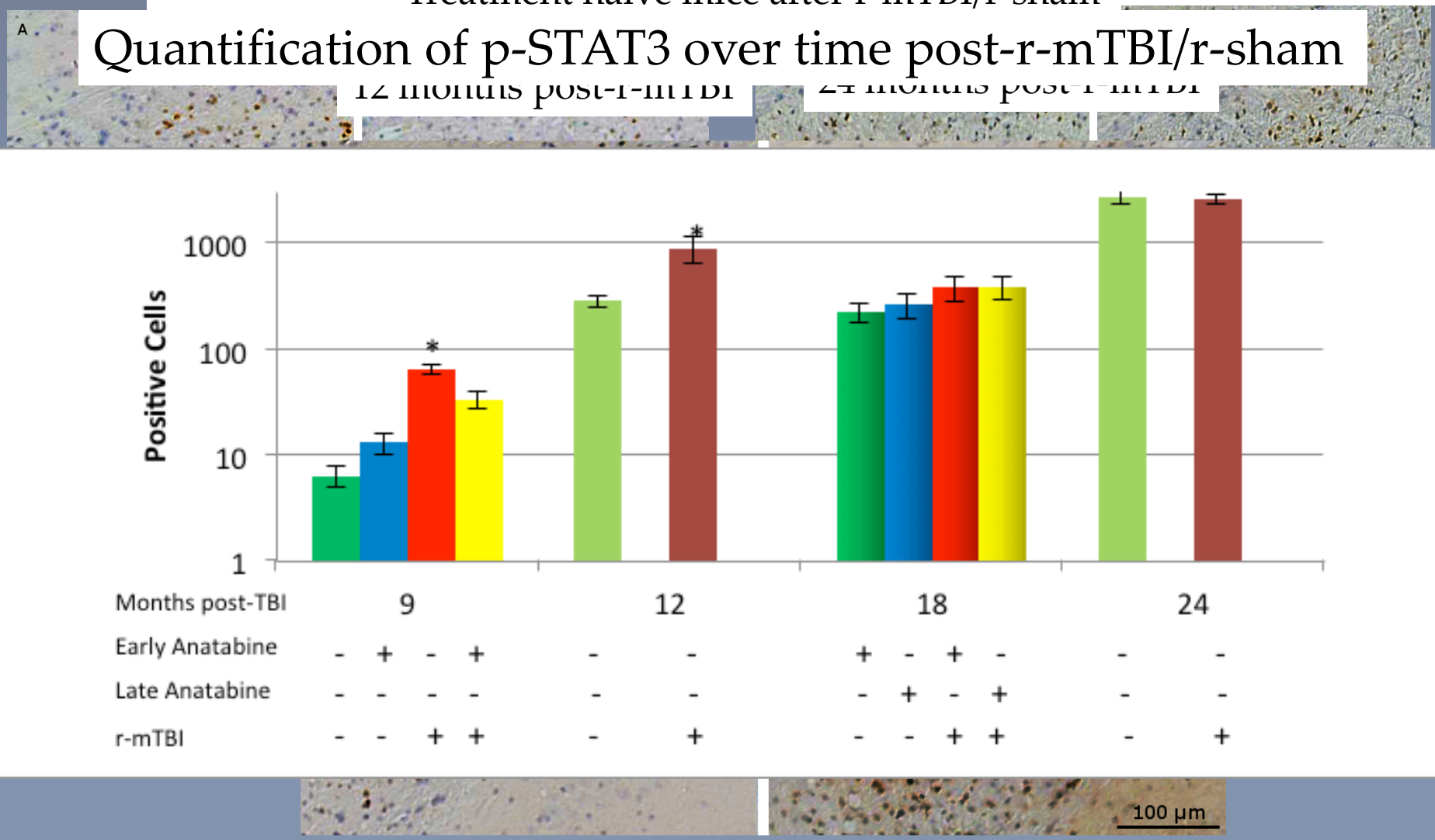


Ferguson et al. *J Neurotrauma* 2017

Effects of anatabine on STAT3 after r-mTBI

9 months after Treatment naïve mice after r-mTBI/r-sham mo after crossover)

Quantification of p-STAT3 over time post-r-mTBI/r-sham



Tau in TBI models

- Published difficulties in recapitulating TBI-dependent tau pathology in mouse models
- Variables influencing tau pathology in preclinical models
  - Nature of the injury – *e.g. Goldstein 2012, Huber 2013*
  - Site of the injury – *e.g. Luo 2014*
  - Severity of injury
  - Frequency of injury – *e.g. Petraglia 2014*
  - Other injury paradigms
  - Age at injury – existing pathology *e.g. Ojo 2014*

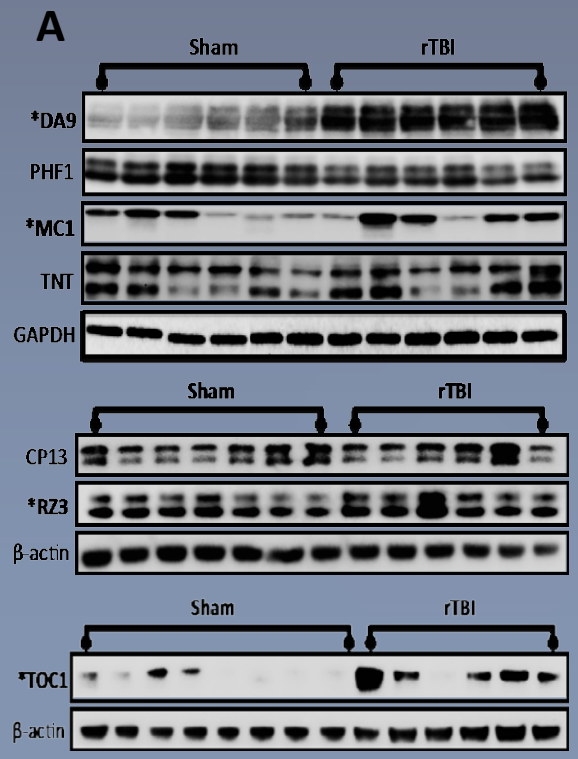
Exploring additional r-mTBI paradigms

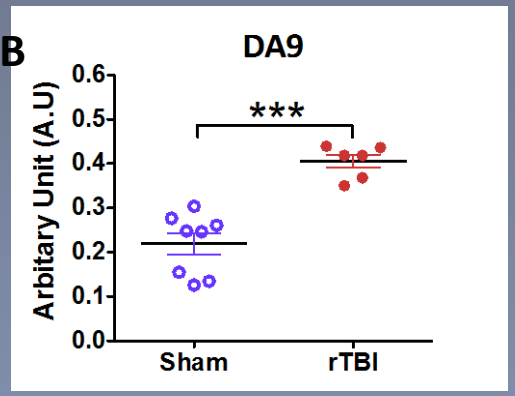
Same mild injury but administered in different paradigms

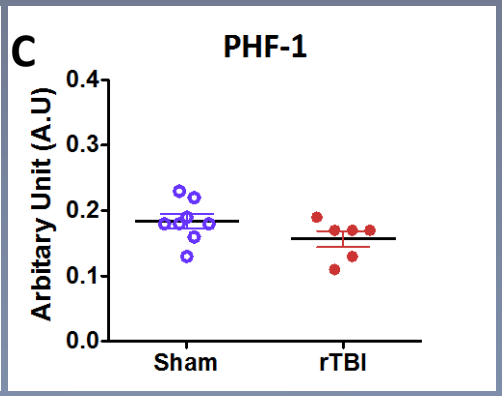
Roskamp Chronic r-mTBI

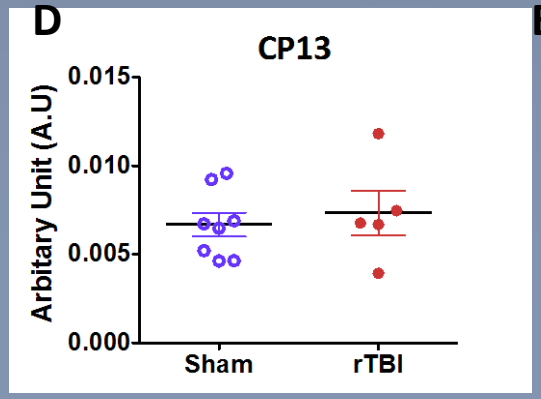
- 2 hits/week for 2-3 months
- Euthanasia at 6 months post-start of r-mTBI paradigm
- Relevant sham/anesthesia controls
- hTau transgenic mice, 10 r-mTBI, 12 r-sham

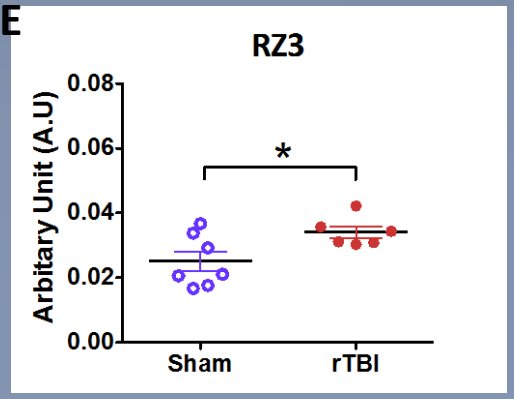
Cortical Tau Immunoblotting

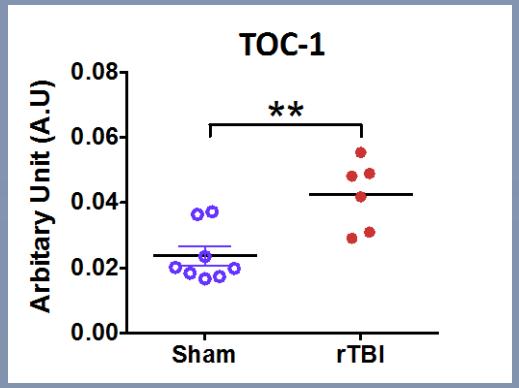


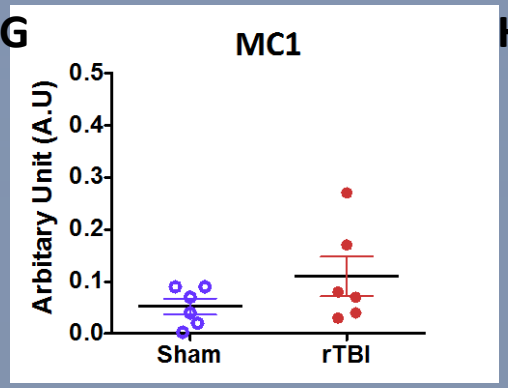


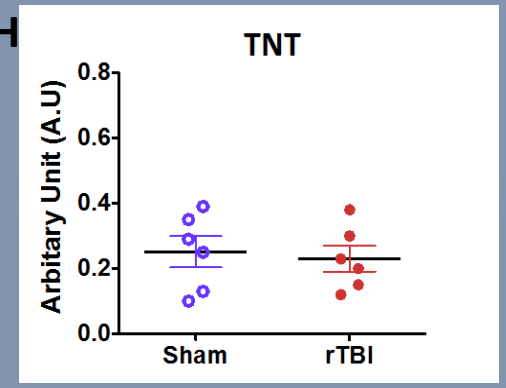




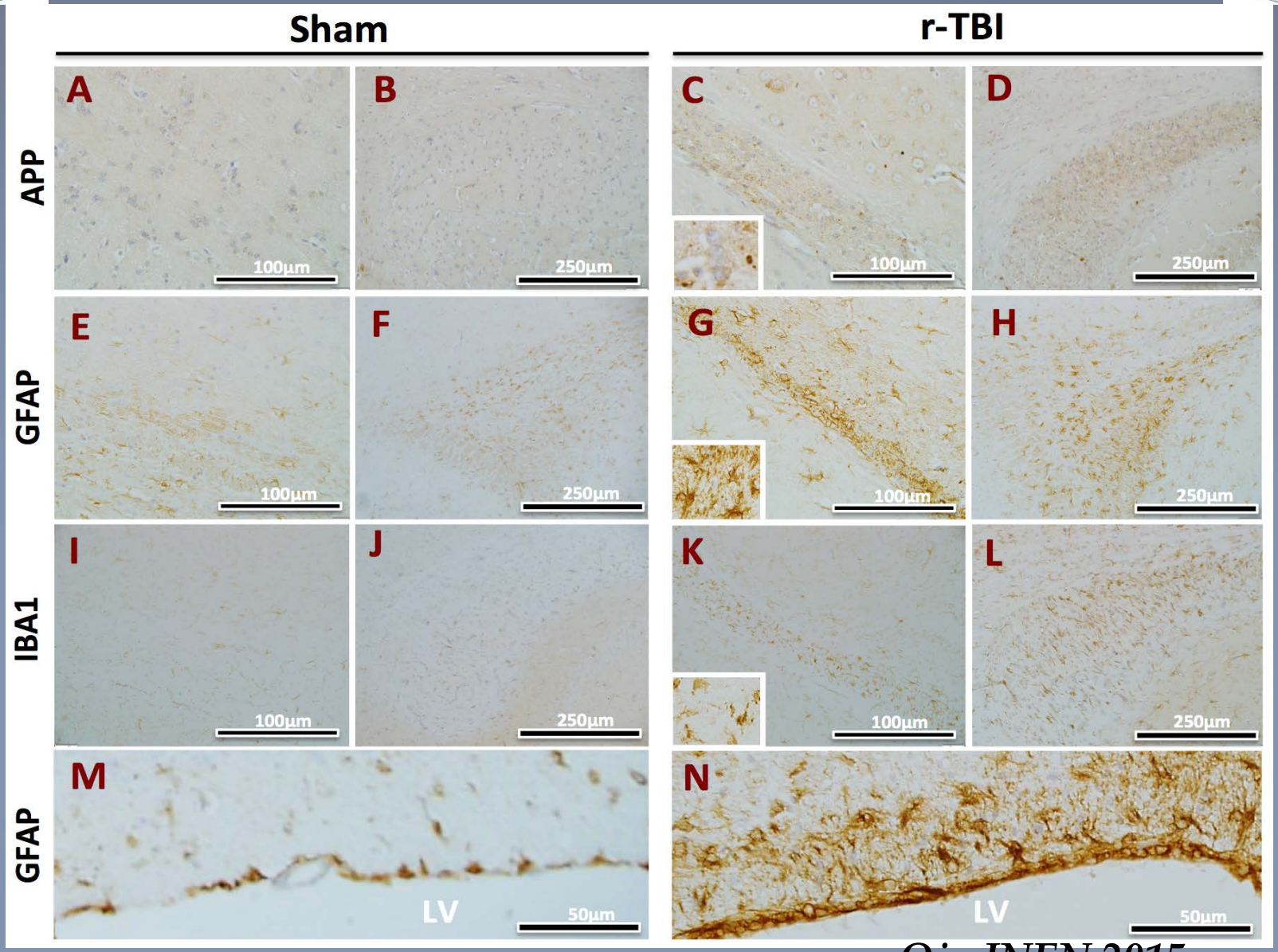






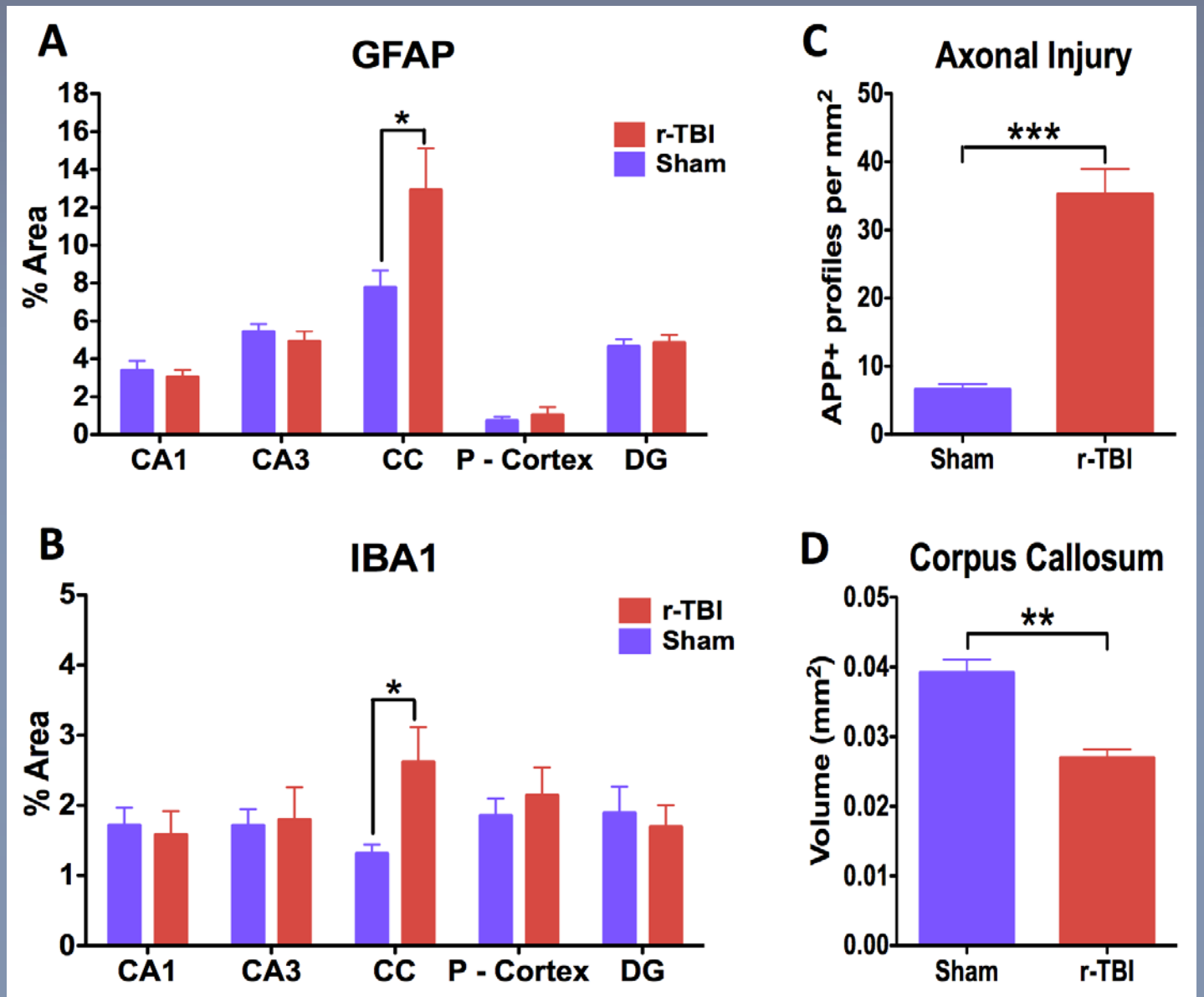


Neuropathology in chronic r-mTBI

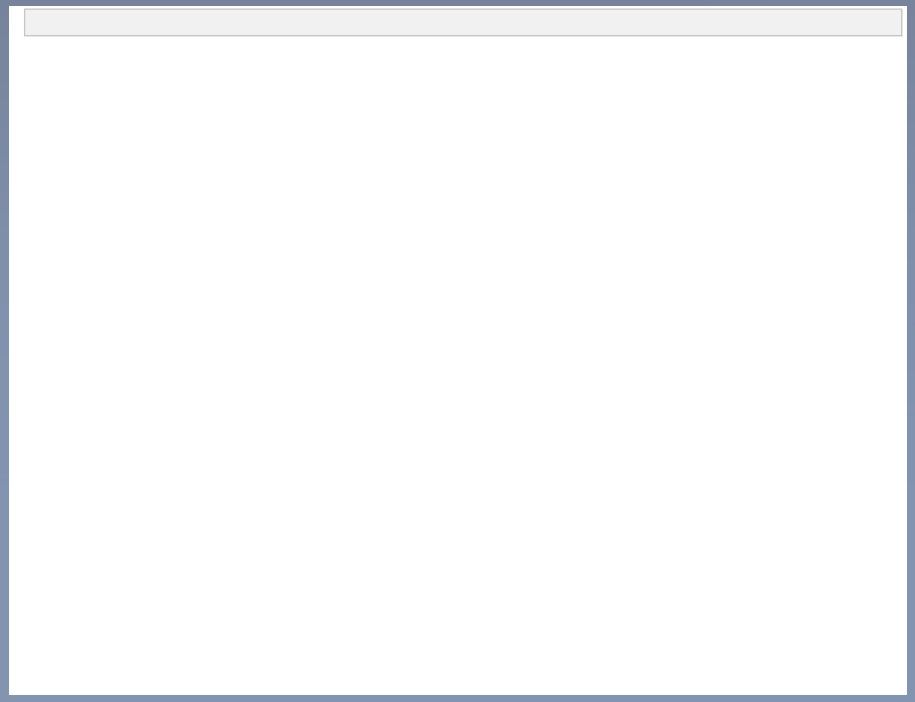


Ojo JNEN 2015

Neuropathology quantification



Mitigation of Tau pathology (PHF1) in CD40-deficient P301S mice



Conclusions

- Many variables influence TBI sequelae –
  - Nature and severity of injury, genotype, diet
- Inflammation represents a common denominator across all human TBI cases and all preclinical models of TBI
- Heterogeneity of human TBI
  - Requires investigation of multiple models of TBI
- The therapeutic window may be more favorable than originally anticipated
- Anatabine show promise as a TBI therapeutic - much more preclinical work to do for effective clinical translation

Conclusions & Future Directions

Targeting specific aspects of neuroinflammatory pathways with compounds demonstrating efficacy in multiple preclinical models

Understanding the mechanisms responsible for the persistence of neuroinflammation after TBI, and the timing and context of those responses, is critical to development of TBI therapeutics

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