Advancements in the Development of Novel Neurosteroids for Treatment of Brain Injury

Arrowhead Conference
June 7, 2022

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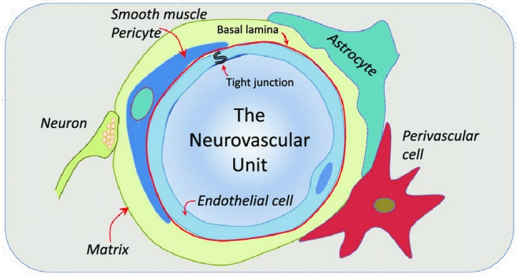
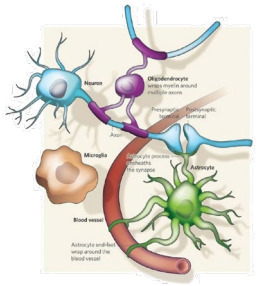
Brain Injury (Stroke and TBI) Triggers Neuroinflammation and Systemic Dysfunction Throughout Acute and Chronic Recovery Phases

Common Brain Pathological Sequelae

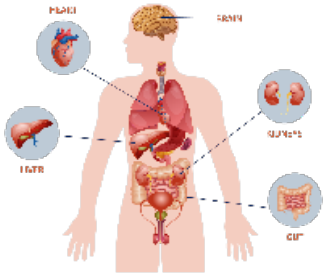


Neurovascular/ BBB Disruption

Neuroinflammation



Cell Death and Dysfunction



Systemic and Peripheral Organ Systems

Stroke: Vascular in origin, affects brain parenchyma and vasculature

TBI: External injury damaging brain and vasculature

Note: Modified based on Maki et al., 2013

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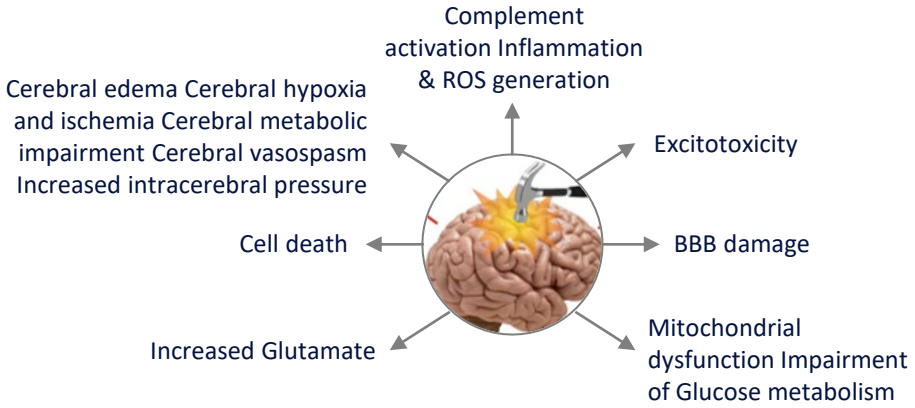
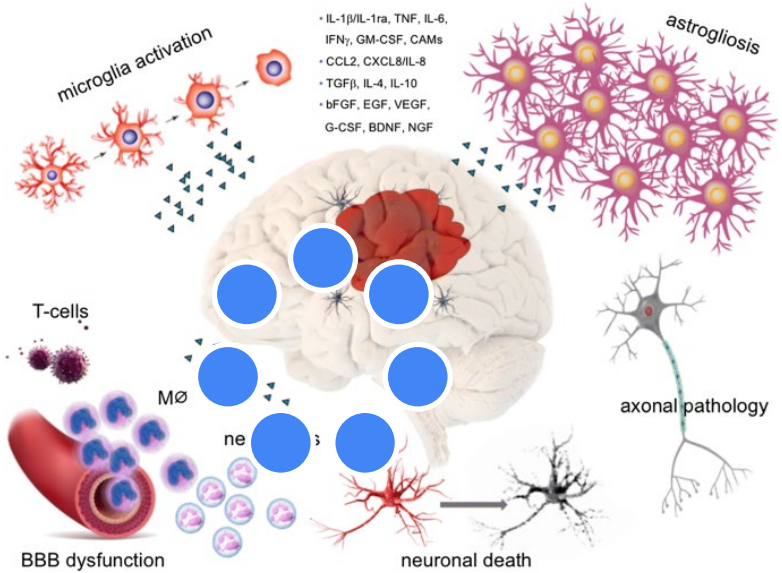
Brain Injury: Multiple Causes / Multiple Effects

Causes of Brain Injury

- **Traumatic –** MVA, Sports, Assaults, Falls, Military
- **Non-traumatic –** Stroke, Chemotherapy, Virus, Hemorrhage





Despite the huge unmet need, there are no neuroprotective medications

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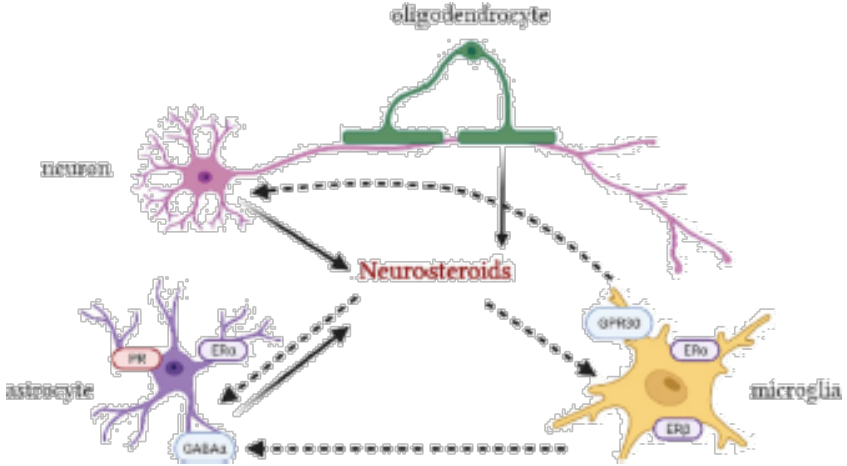
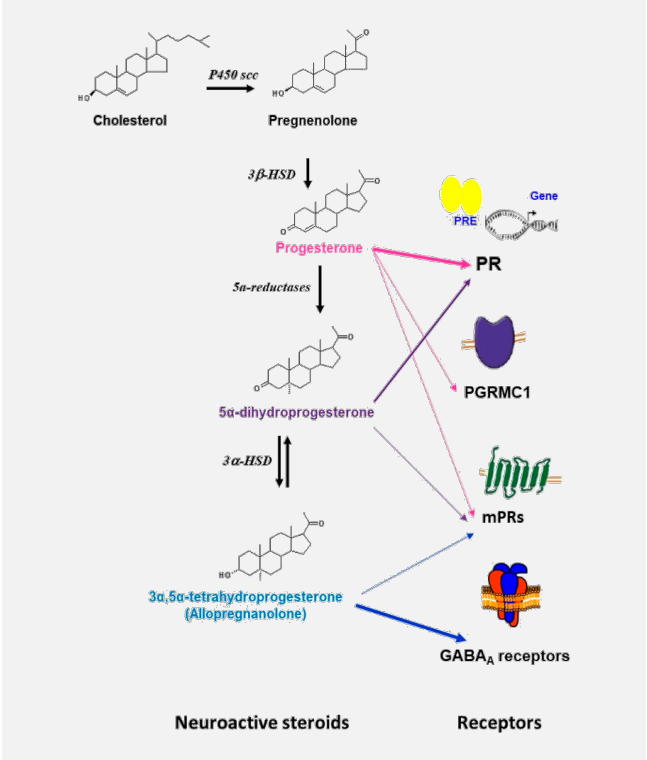
Lessons from Nature: Neurosteroids

Neurosteroids: Endogenous response to brain injury (Guennoun et al., 2015 and 2020)

- Act on multiple receptor types
- Receptor expression changes
- Enzymatic changes





Note: Xu et al., J. Neurochem. 2021; Guennon, Int. J. Mol. Sciences 2020

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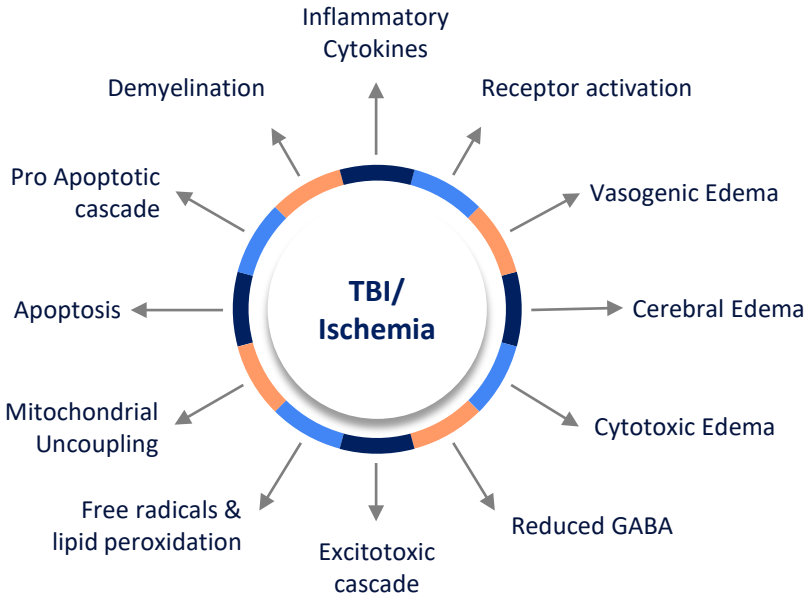
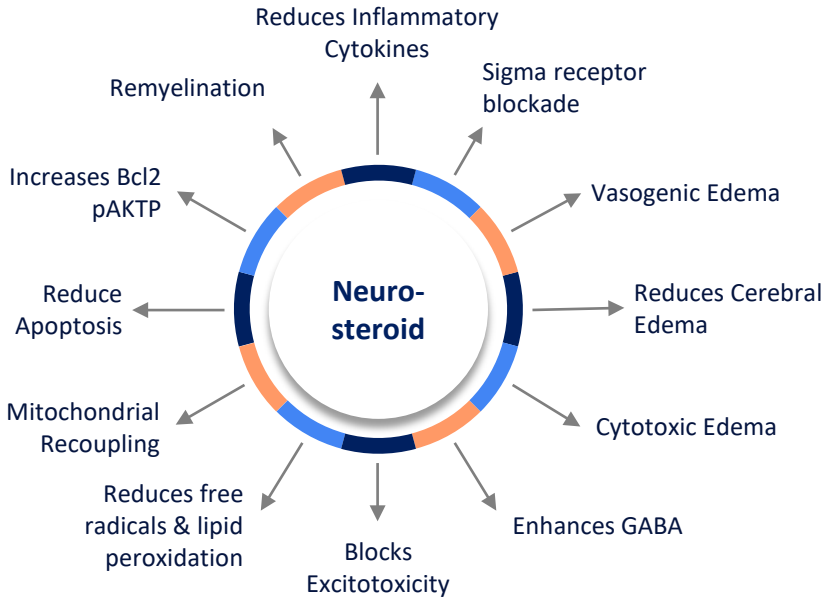
Neurosteroids And Neuroprotection

Pathophysiology of Brain Injury



Neurosteroid Mechanisms in Brain Injury



Strong potential to improve function in **acute through chronic** phases of recovery

Note: Adapted from Sayeed and Stein, 2009

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Neurosteroids gaining traction as CNS therapeutics

Recent approvals

- **Zulresso™ (brexanolone IV form of allopregnanolone)** for PPD (March, 2019)
- **Ztalmy™ (ganaxolone)** for a rare epilepsy disorder (March 2022)

Others in development

- Zuranolone Phase 3+ for Major depressive Disorder and other forms of depression
- PH94B in Phase 3 for social anxiety disorder
- SAGE-324 in Phase 2 for essential tremor
- SAGE 718 in Phase 2 for neurodegenerative disease cognitive impairment

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Progesterone Represented A Compelling Approach For Neuroprotection

Progesterone was the prototype of a multi-targeted neuroactive, brain-penetrant steroid

Decades of published evidence

- >300 preclinical reports in TBI and stroke pointed to therapeutic potential
- Known U-shaped dose-response relationship (i.e. higher doses not efficacious)

Pivotal human trials held in **severe TBI**--ProTECT and SYNAPSE[1] – did not succeed for many addressable reasons[2]

Repurposing did not follow standard drug development tenets:

- **Solubility and formulation** for IV product
- Dose selection & regimen not based on PKPD
- Early use of biomarkers

1. ProTECT and SYNAPSE study publications; 2. Stein, Brain Injury 29: 1259-1272, 2015; Schumacher et al., J. Steroid Biochem. Mol Biol. 160: 53-66, 2016; ce: See references

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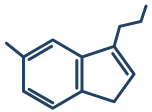
A Superior Multi-targeted Drug Would Be A Major Advancement For Treating Brain Injury

Emory neuroscientists challenged their world-class medicinal chemistry colleagues at the Emory Institute of Drug Development (EIDD) to engineer a solution



Active Novel Neurosteroid (EIDD-036 or NTS-105)
High potency

Novel Prodrug (EIDD-1723 or NTS-104) with aqueous solubility

Important Leads are now in development at NTS

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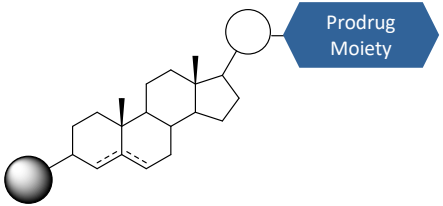
Two Novel Chemical Entities With Prodrug – Neuroactive Drug Relationship

Prodrug

- High aqueous solubility and stability
- Converts rapidly to NTS-105 in plasma
- Suitable for IV dosing



NTS-104

Conversion

NTS-105

- Enhanced pharmacologic properties relative to progesterone
- Crosses BBB and achieves pharmacologically important brain concentrations
- Suitable for parenteral and non-parenteral dosing
- Activates PR nuclear hormone receptor at nanomolar potency

Neuroactive Drug

Source: NTS internal data

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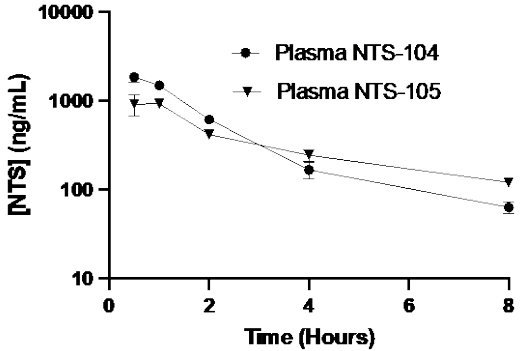
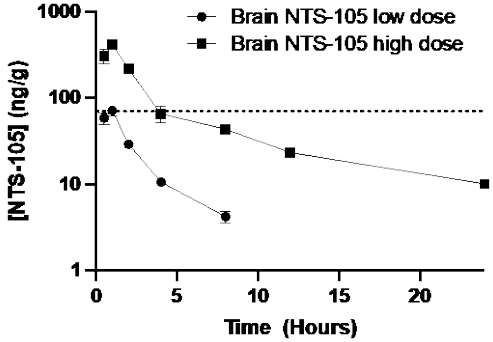
Brain NTS-105 Concentrations Engage Molecular Targets After Systemic Administration Of Efficacious Doses

Plasma concentrations



Brain concentrations



50% target engagement level based on free NTS-105 concentration

Protein binding >99%

Brain concentrations of free NTS-105 after IM dosing of effective NTS-104 doses are sufficient to bind to and activate progesterone receptors

Interactions evaluated against a broad range of safety-related receptors, enzymes and ion channels

Systemic and brain metabolism mediated by neurosteroid enzymatic cascade

Major metabolites of NTS-105 have been identified

Study CEAA-0026 CRL

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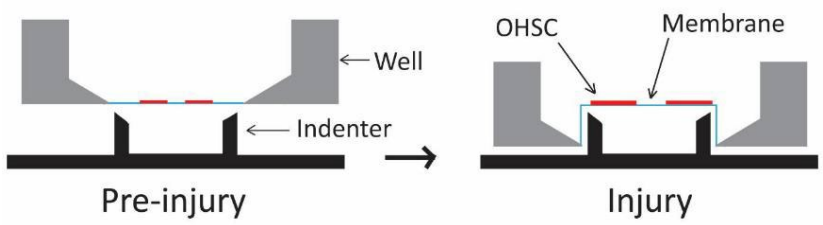
NTS-105 Reduces Cell Death In An *Ex Vivo* Model Of TBI

Substrate Strain Model
(Morrison Laboratory, Columbia U.)



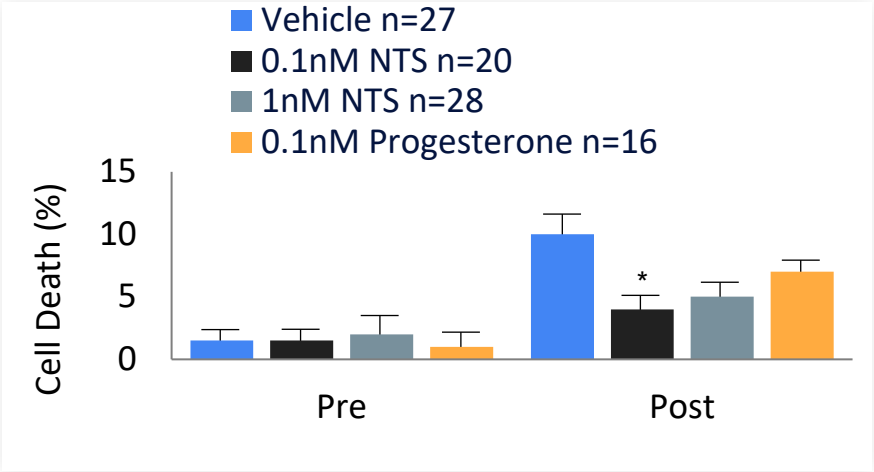
Equibiaxial, homogeneous strain field

Verifiable tissue biomechanics with high-speed video

Continuous exposure starting at 1 h

PI cell death endpoint at 96 h

High Potency Neuroprotection



* $p < 0.05$

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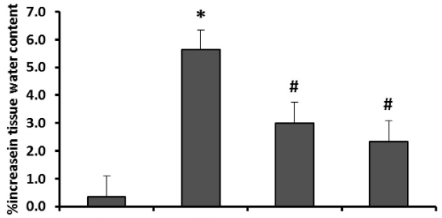
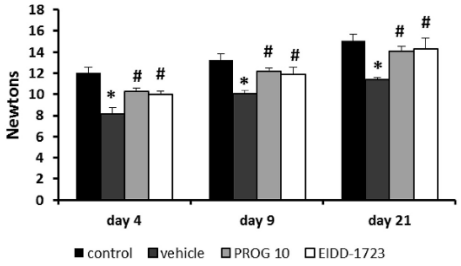
NTS-104 (EIDD-1723) Treatment Improves Functional Outcomes Following TBI

CCI In Rat
Wali et al., 2016

Edema level at 24th post TBI

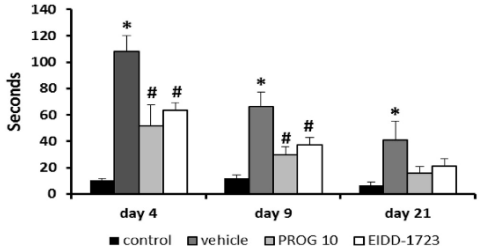


Grip strength



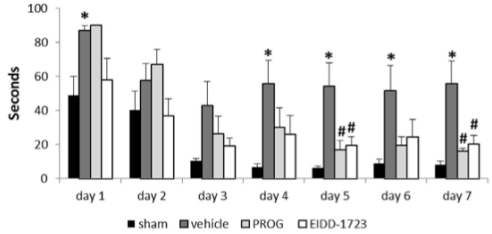
n = 8 per treatment group

Latency to remove sticker

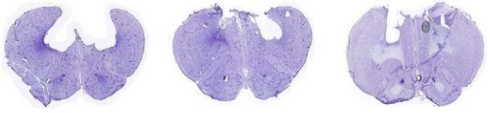


n = 8 per treatment group

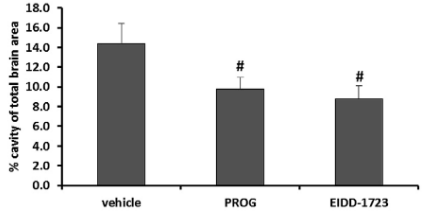
Latency to reach the platform in trial 2



n = 8 per treatment group



Necrotic cavity measurement



n = 8 per treatment group

*p<0.05 CCI veh vs Sham; #p<0.05 drug vs veh

NTS-104 dosing post-TBI
1 h, 6h, 24 h, then once daily to 7 days (last 2 days tapered)

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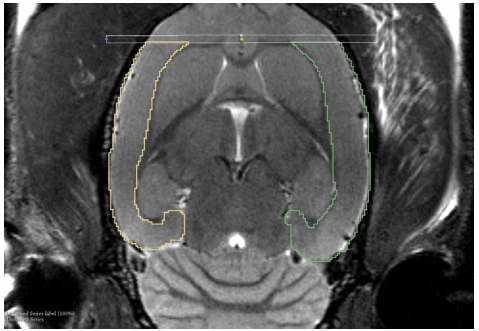
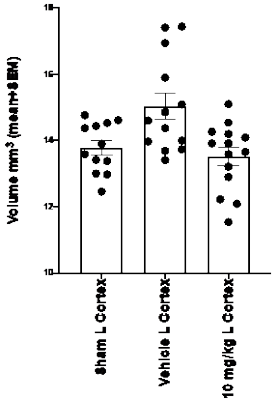
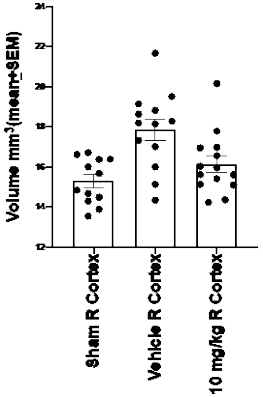
Moderate-Severe TBI Pilot Study: NTS-104 Reduced Edema and Improves Cognitive Outcome (Lateral Fluid Percussion In Rat)



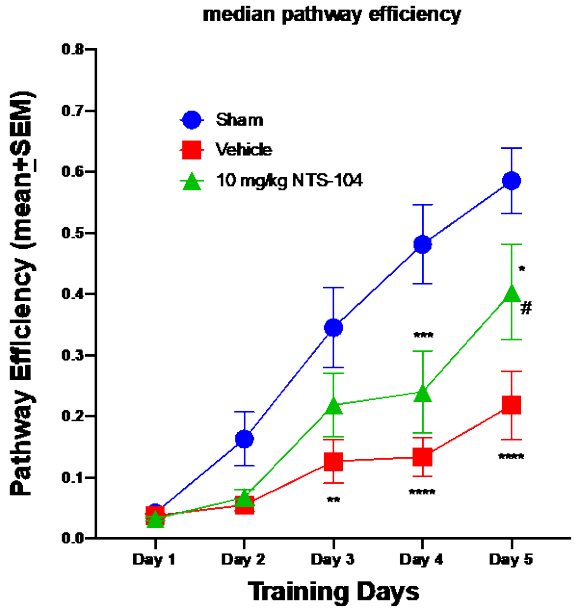
Contralateral Cortex Volume



Ipsilateral Cortex Volume



Effect of NTS-104 administration
(IM 4, 10, 24, 48 hours after injury, n = 12)



* = relative to sham
\# = relative to vehicle

Note: SUNY study – pilot TBI 090820

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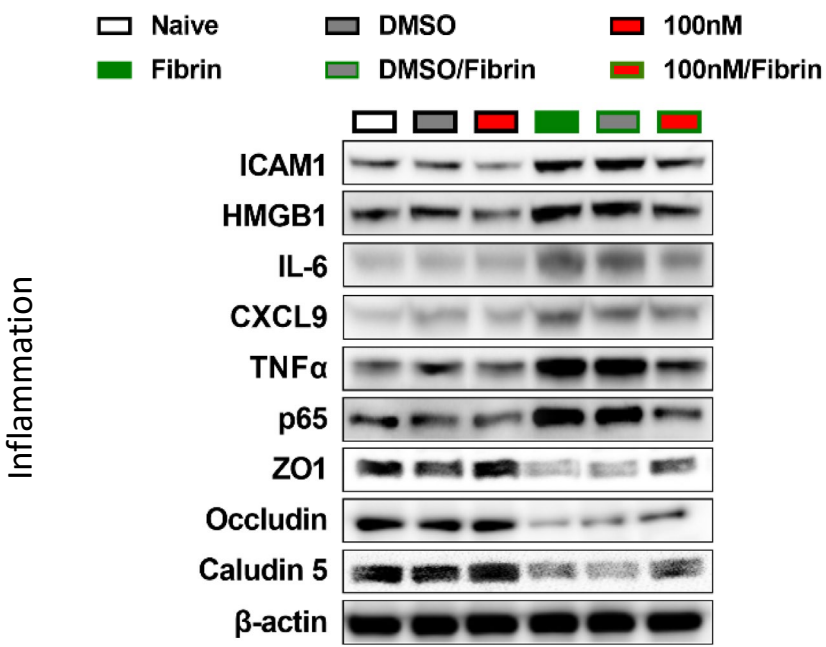
Cerebrovascular Fibrin Exposure: NTS-105 Reduced Markers Of Cerebrovascular Endothelial Cell Damage And Inflammation

Single mild TBI in SHR Rats—persistent fibrin accumulation, disrupted neurovascular function and neuroinflammation (Szarka et al., Int J Mol Sci, 2019)



In cultured cerebrovascular endothelial cells

- Fibrin stimulates inflammatory marker and reduces tight junction protein expression
- NTS-105 reduces inflammation induced by fibrin
- NTS-105 increases expression of tight junction markers reduced by fibrin

Source: NTS Study, M. Chopp

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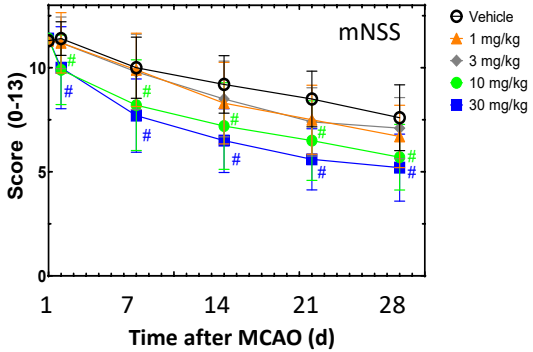
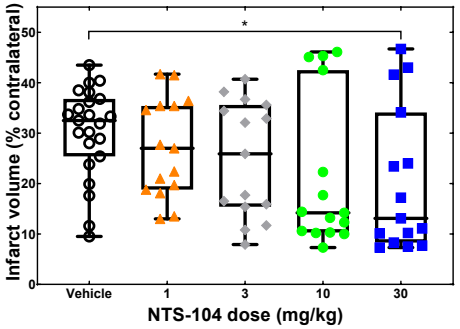
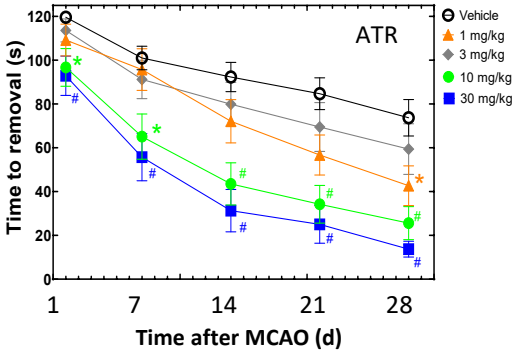
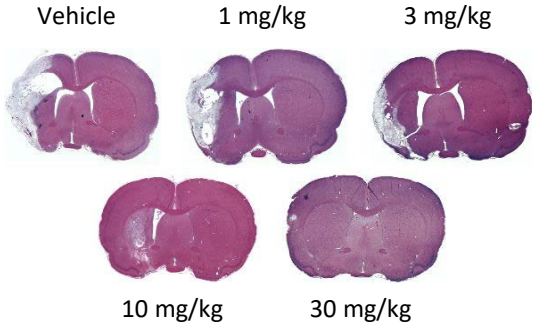
NTS-104 Improves Neurological Function After Ischemic Stroke

- Dose ranging effects of NTS-104 in experimental stroke (Chopp Laboratory)
- Study Design
  - Embolic MCAo model
  - NTS-104 dosed at 1, 3, 10, and 30 mg/kg
- Dosing schedule: 4, 10, 24, 48, doses halved at 72, and again 96 h
- 15 rats per group



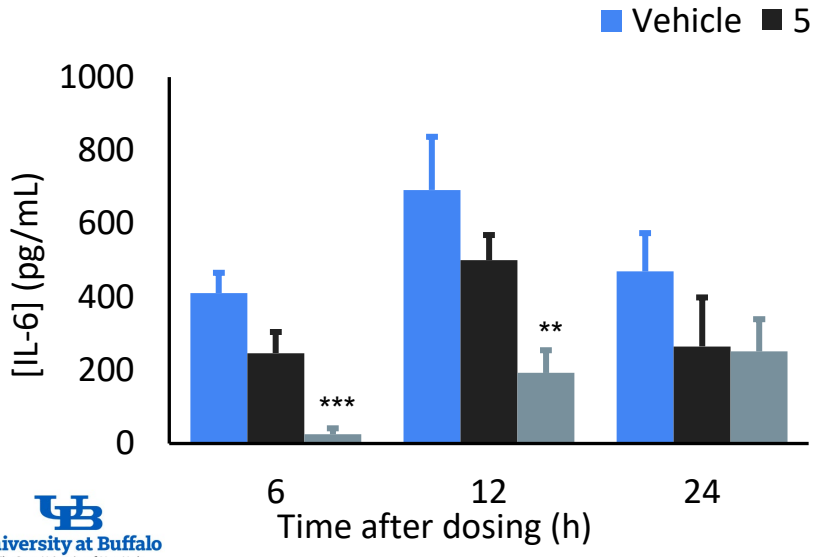




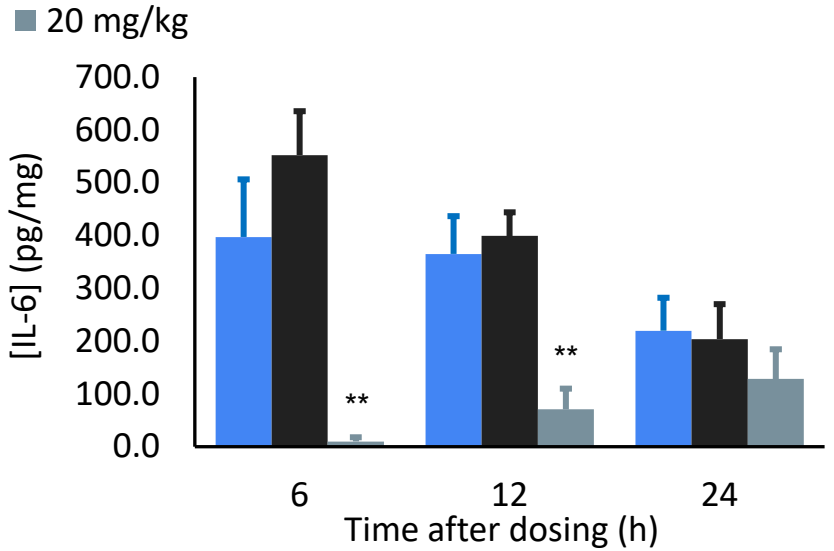


NTS-104 Treatment Dose-Dependently Reduces Circulating And Cortical IL-6 Acutely Following Ischemic Stroke

IL-6 concentration in plasma



IL-6 concentration in cerebral cortex



University at Buffalo
The State University of New York

Modulates a host of proinflammatory cytokines as well as complement proteins

n = 5/group; Dosing at 6 h after initiation of ischemia
2. $p < 0.01$ vs. vehicle; 3. $p < 0.001$ vs vehicle

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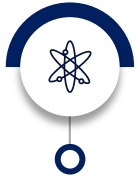
Conclusions/Future Objectives

NTS-104 is a novel water-soluble prodrug that converts rapidly to NTS-105 in plasma

NTS-105 is a novel, potent neurosteroid that crosses the BBB



NTS-105 has nanomolar affinity for PR nuclear hormone receptors

Administration of NTS-104 following ischemic stroke or TBI in rats improves functional outcomes and lesion volume

NTS-104 administration reduces acute inflammatory mediators and inflamed cerebrovascular endothelial cell markers

Efficacy demonstrated in multiple independent laboratories and models

NTS-104 is being advanced into Phase 1 clinical trials in AIS and TBI (2022)

NTS-104 has an issued composition of matter patent

NTS-104 and NTS-105 have issued method of treatment patents

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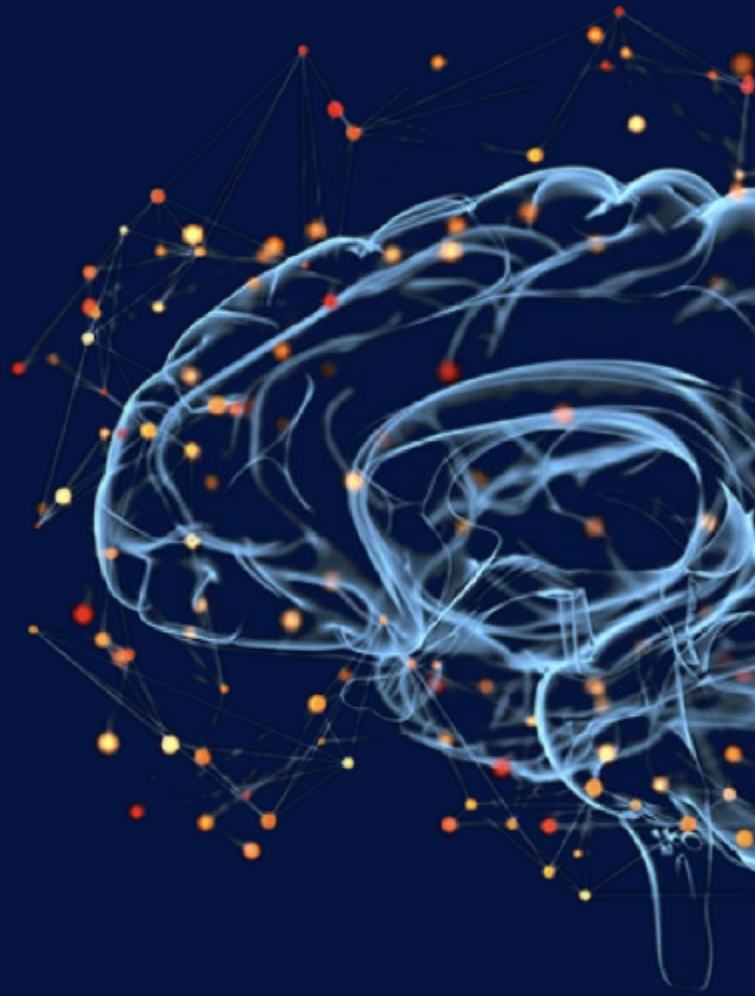
THANK YOU



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Acknowledgements

Don Stein

Michael Chopp

David Poulsen

Barclay Morrison

Dennis Liotta

Zhenggang Zhang

Emory

HFHS

SUNY Buffalo

Columbia University

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