Dr. Alfred Stracher
was a dedicated biochemist and inventor.
Through his knowledge, innovation, and scientific rigor, a series of novel calpain inhibitors utilizing carriers to localize calpain inhibition at specific tissue sites were developed in collaboration with
Drs. Abraham Shulman and Leo Kesner
at SUNY Downstate Medical Center.
These therapeutics have the potential to change the way we treat various neurodegenerative disorders.

The following is a revised talk on Gabadur, one of the calpain inhibitors developed by Drs. Stracher, Kesner, and Shulma,n presented at the Society for Neuroscience Annual Meeting, 2015

by MD/PhD student, Rachelle Dugue
Ling Laboratory, SUNY Downstate Medical Center

The Effect of the Novel Calpain-Inhibitor *Gabadur* on Traumatic Brain Injury

Rachelle Dugue, Getaw Hassen, Peter Serrano, Hillary Michelson, Abraham Shulman, Jeffrey Goodman, Douglas Ling

Ling Laboratory, SUNY Downstate Medical Center

Goodman Laboratory, Institute for Basic Research

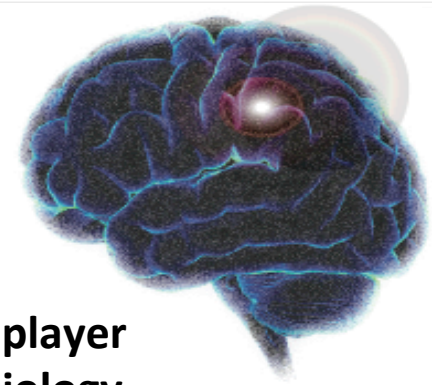
What is Calpain?

- calcium-activated cysteine protease
- Ubiquitously expressed in 2 isoforms (μ-calpain and m-calpain) that target subsets of cytoskeletal, cytosolic and nuclear proteins for proteolysis
- Normal function: to regulate cell migration, cytoskeletal and cell complex rearrangement, implications in synaptic reorganization

Over-activate calpain is implicated in the pathophysiology of many neurodegenerative diseases including Huntington's disease(HD), traumatic brain injury (TBI), stroke, Parkinson's disease(PD), tinnitus and multiple sclerosis.

The Pathophysiology of TBI

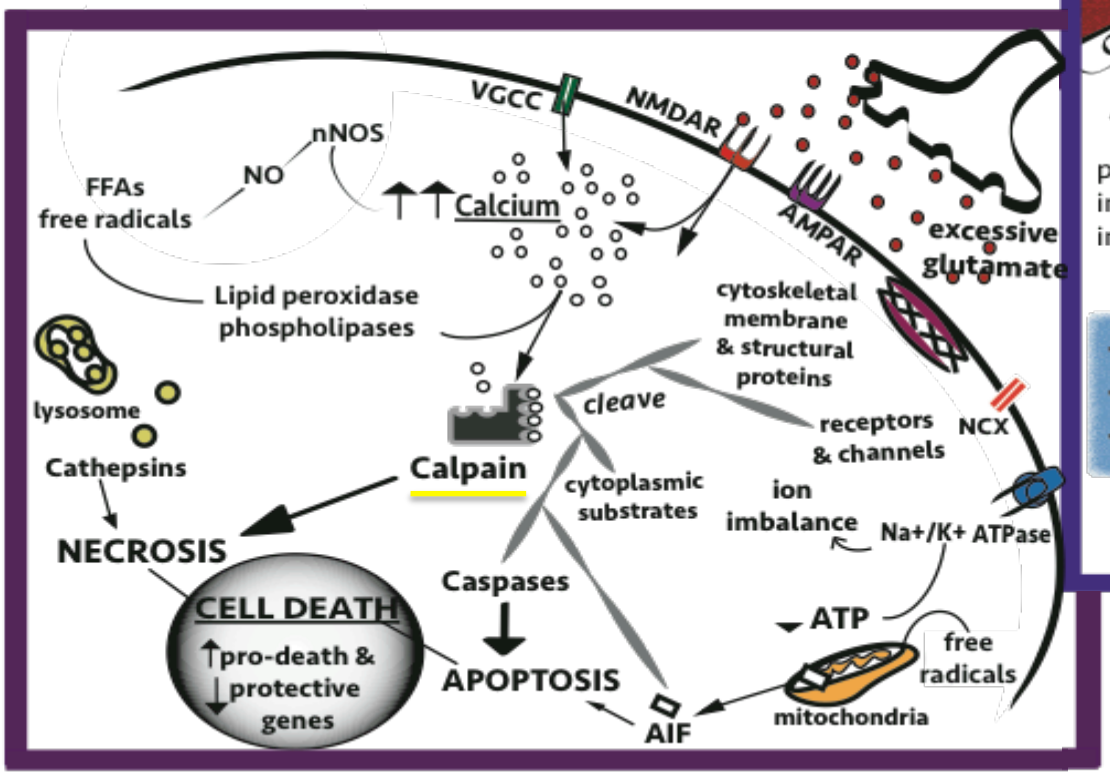
A complex cascade of events

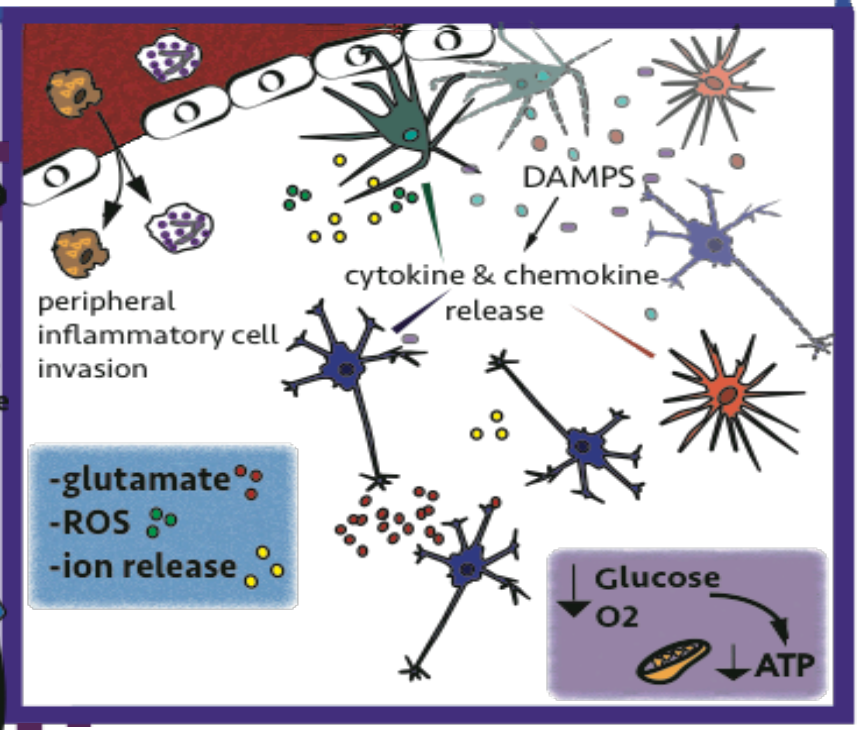


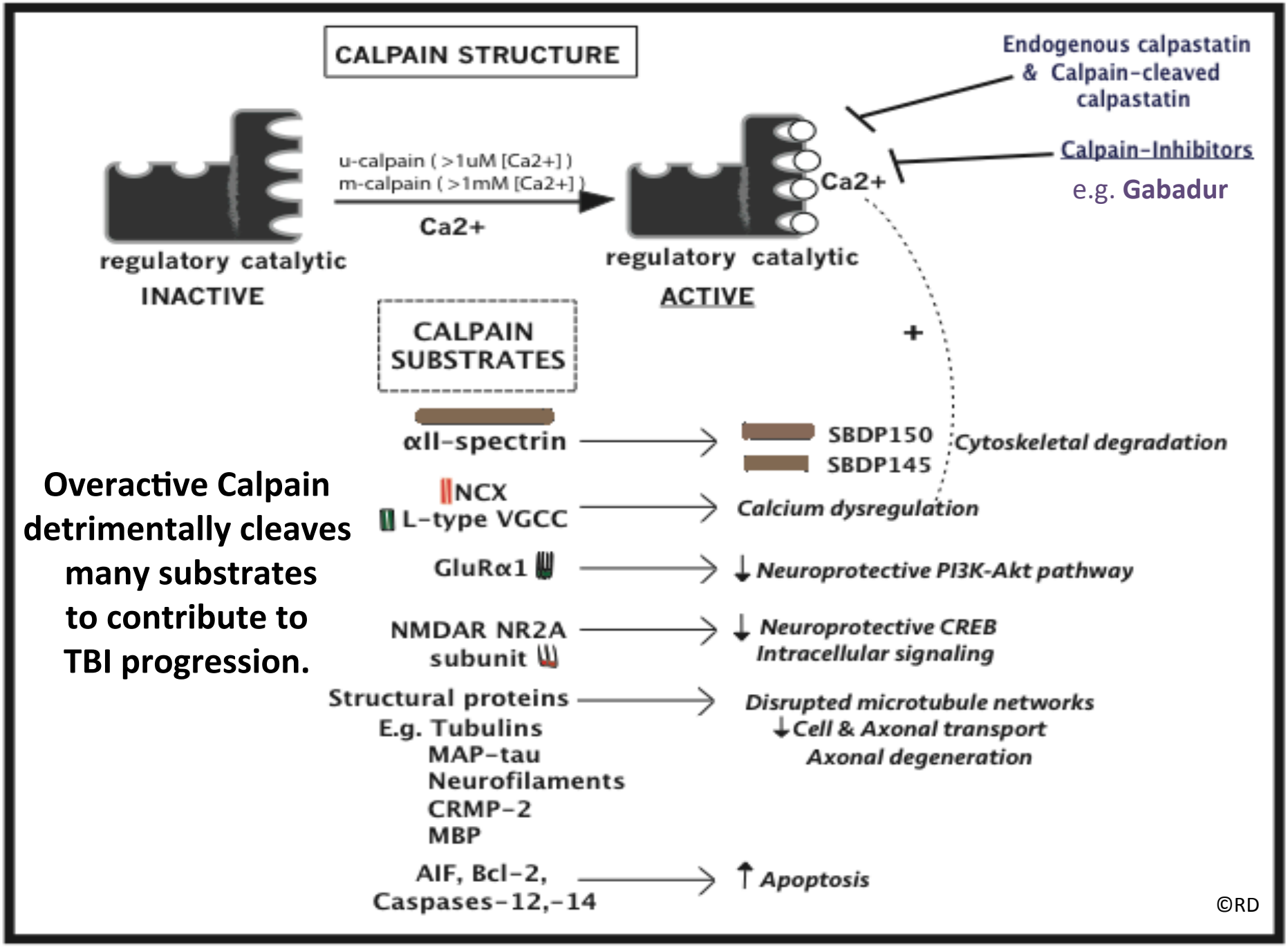
Primary Injury

- -Neural and Glial Injury
- -Blood brain barrier (BBB) disruption
- -Blood Flow dysregulation

Calpain is major player In TBI pathophysiology.

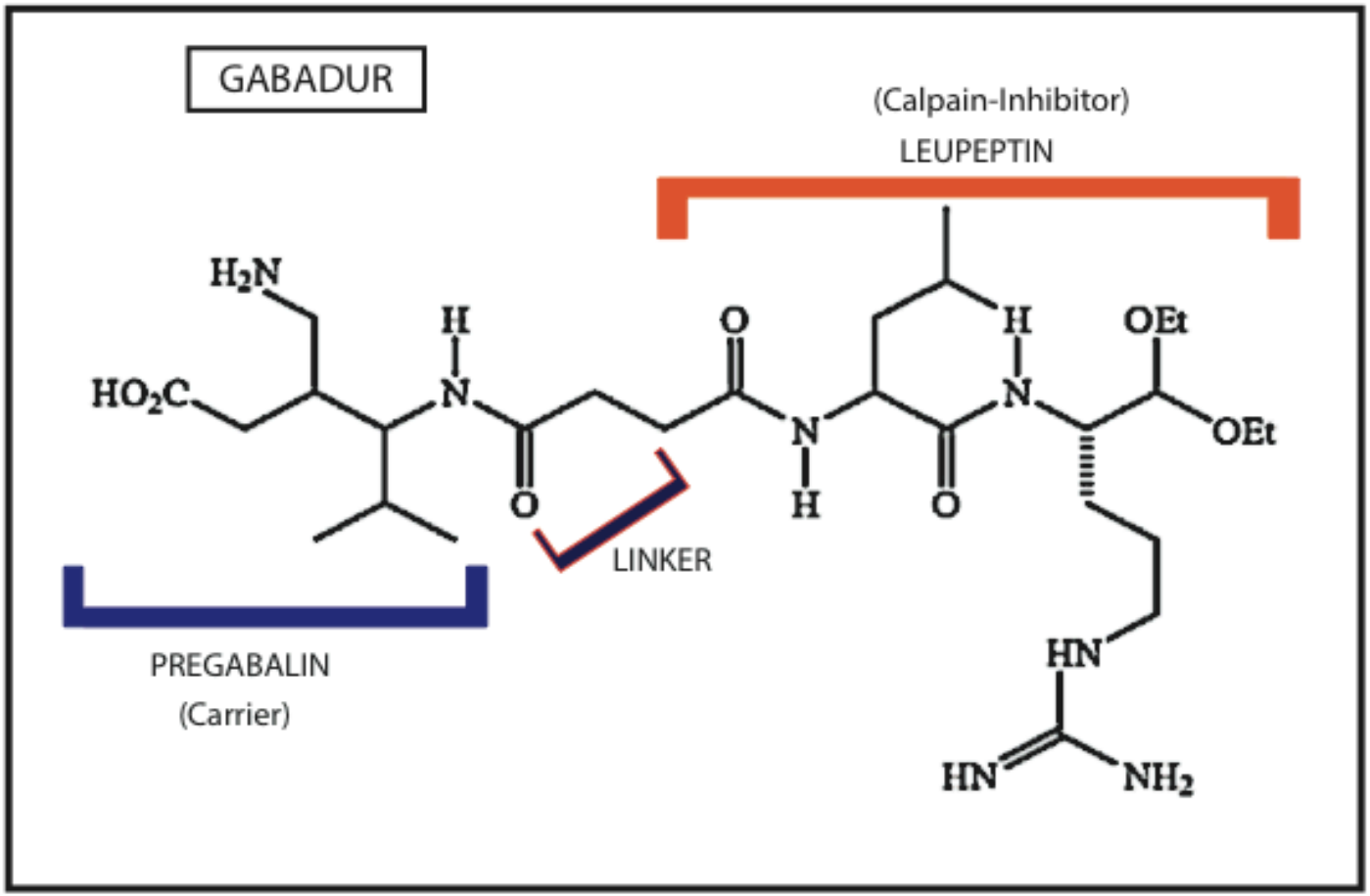





CALPAIN STRUCTURE
Endogenous calpastatin & Calpain-cleaved calpastatin
Calpain-Inhibitors
e.g. Gabadur
u-calpain (>1uM [Ca2+])
m-calpain (>1mM [Ca2+])
Ca2+
Ca2+
regulatory catalytic
INACTIVE
regulatory catalytic
ACTIVE
+
CALPAIN SUBSTRATES
αII-spectrin
SBDP150
SBDP145
Cytoskeletal degradation
NCX
L-type VGCC
Calcium dysregulation
GluRα1
↓Neuroprotective PI3K-Akt pathway
NMDAR NR2A subunit
↓Neuroprotective CREB Intracellular signaling
Structural proteins
E.g. Tubulins
MAP-tau
Neurofilaments
CRMP-2
MBP
Disrupted microtubule networks
↓Cell & Axonal transport
Axonal degeneration
AIF, Bcl-2, Caspases-12,-14
↑Apoptosis
Overactive Calpain detrimentally cleaves many substrates to contribute to TBI progression.
©RD

Gabadur- a novel calpain inhibitor

- leucyl-argininal calpain inhibitor (Leupeptin) bound to the FDA-approved pregabalin (Lyrica) as a carrier for transport **across the blood brain barrier**



A new form of "combination therapy"

Stracher A, Inventor. Kesner L, Shulman A, collaborators

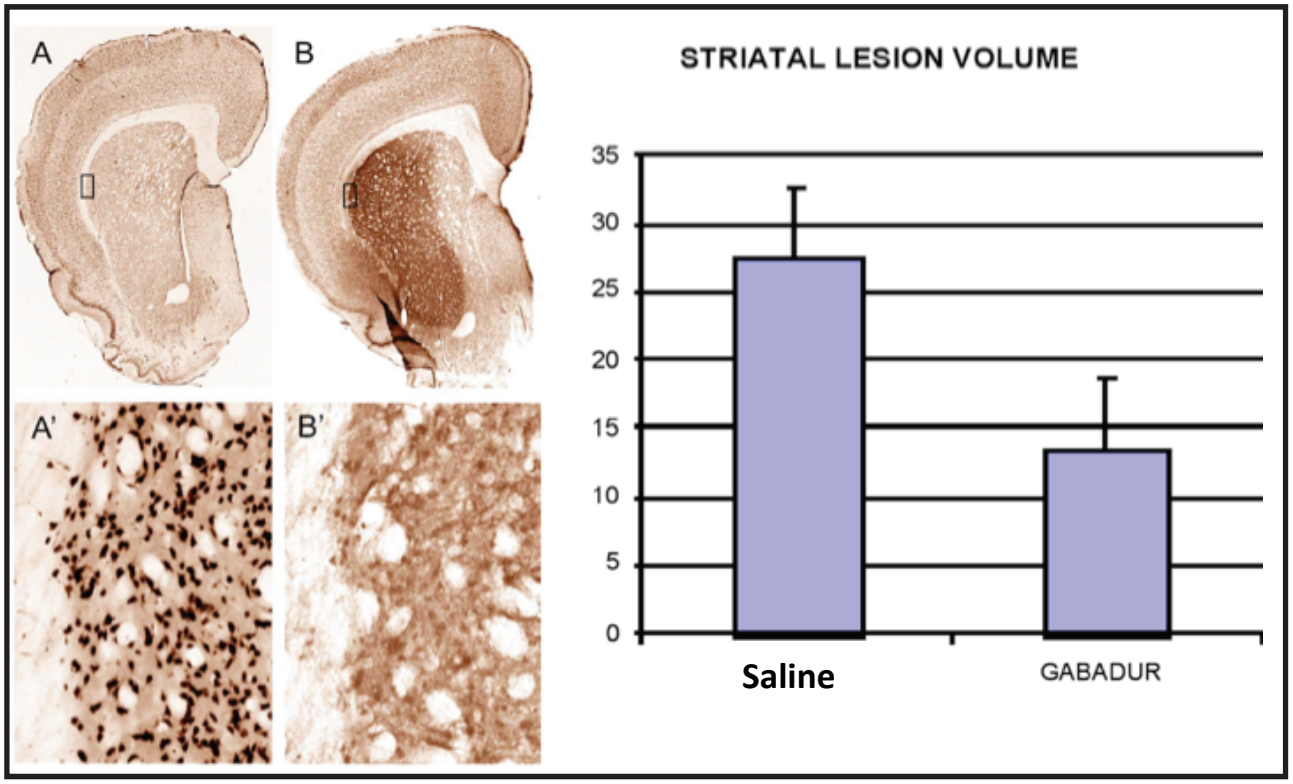
The Need for Effective Therapeutics

- Current clinical TBI therapy is limited to skull fracture repair, hematoma evacuation and supportive treatment
- The characteristics of an ideal treatment
  - Neuroprotective
  - Easily Administered with limited toxicity and side effects
  - Crosses the blood brain barrier to achieve effective concentrations at the site of injury
  - Large time window for administration

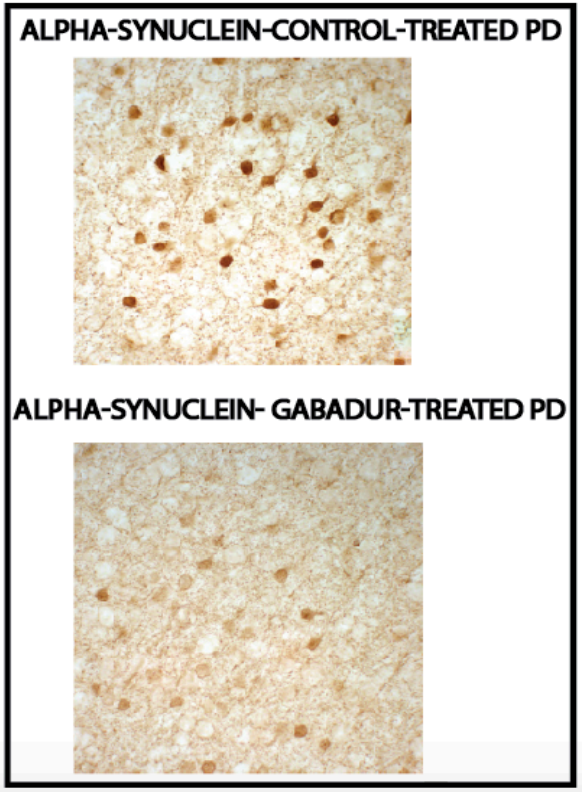
Gabadur fulfills many of these criteria!

Gabadur as a treatment for Neurodegenerative Diseases

Gabadur in a 3-nitropropionic Model of Huntington's Disease decreased striatal lesion volume by 50% :



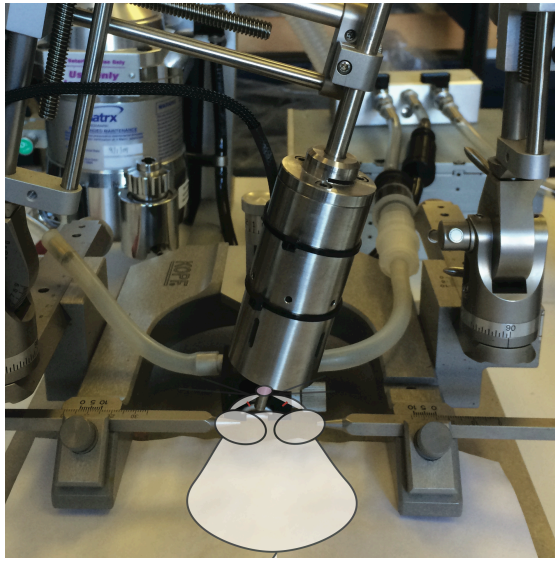
Gabadur in a transgenic mouse model of Parkinson's disease decreased harmful deposits by 60%:

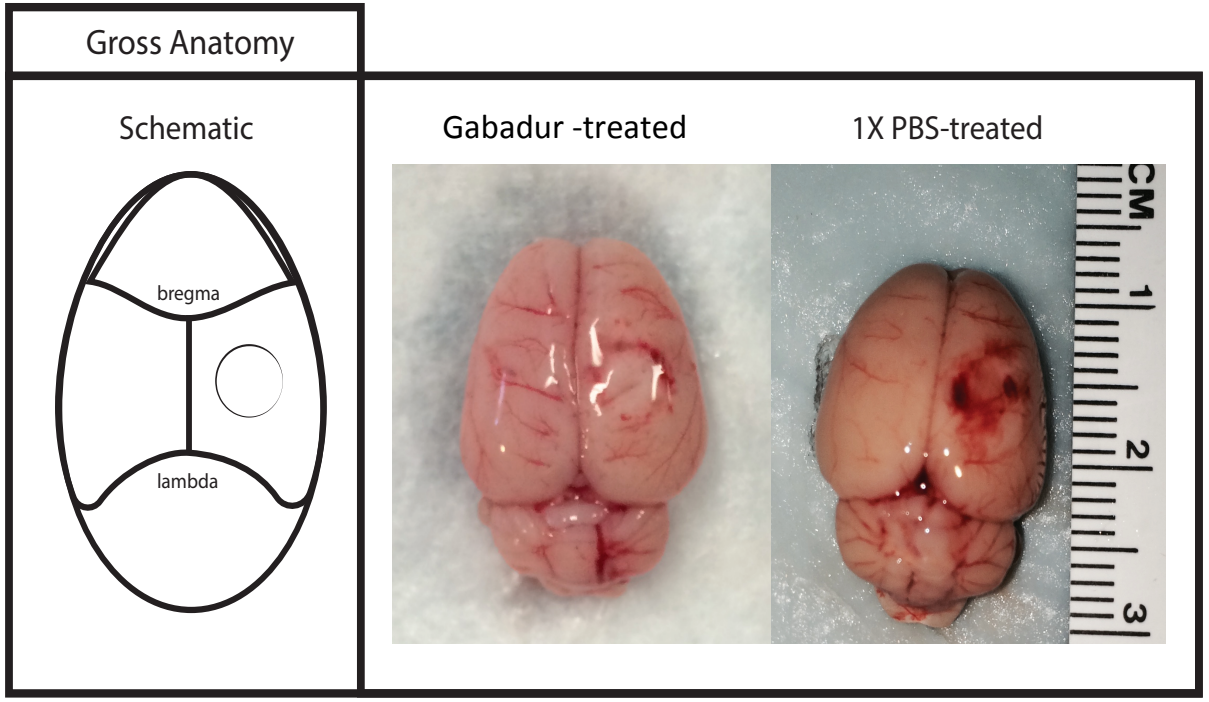


Kesner et. al. New Calpain inhibitor preserves brain architecture in 3-NP Model of Huntington's Disease

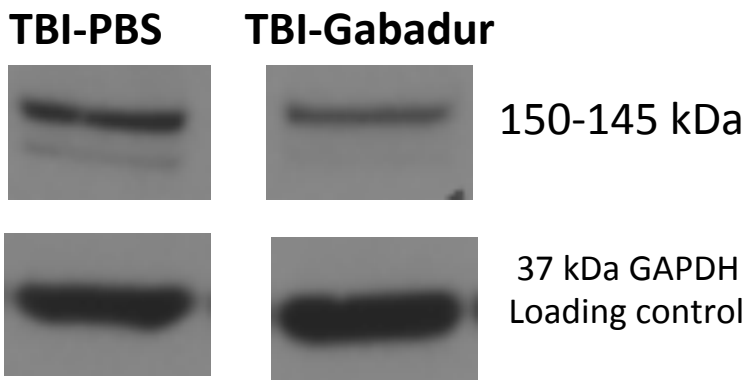
Methods

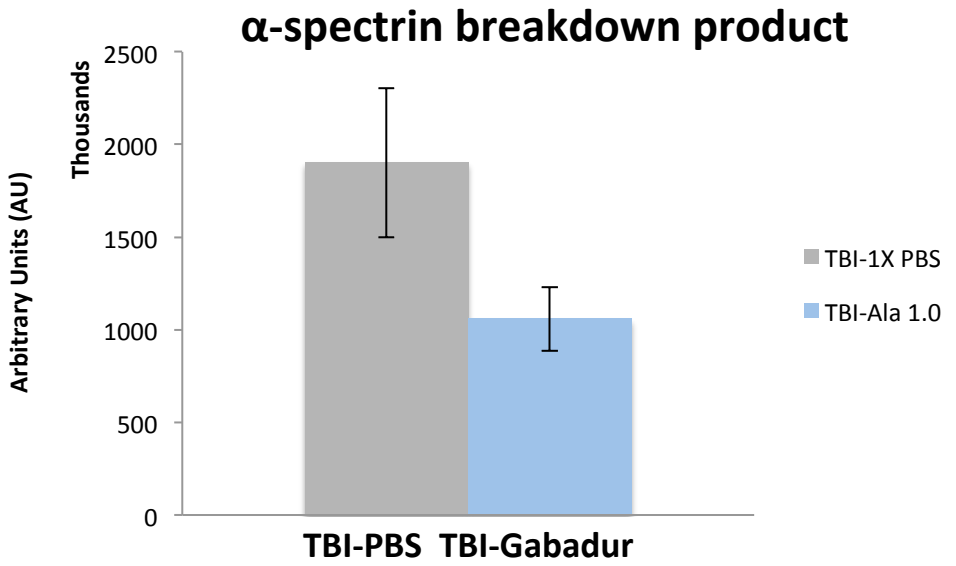
- Controlled cortical impact model of TBI-targets the cortex, the primary location of TBI-induced damage and neurological dysfunction.
- A “moderate” CCI injury was produced-5.0 mm diameter electromagnetic impactor/depth of 2.0 mm at 4m/s.
- Rats were given a single dose of 1X PBS or 80mg/kg of Gabadur intraperitoneally within 5 mins post-impact
- Rats were sacrificed at 48 hours post-TBI
  - Western blot analysis
  - Fluoro-Jade B stain

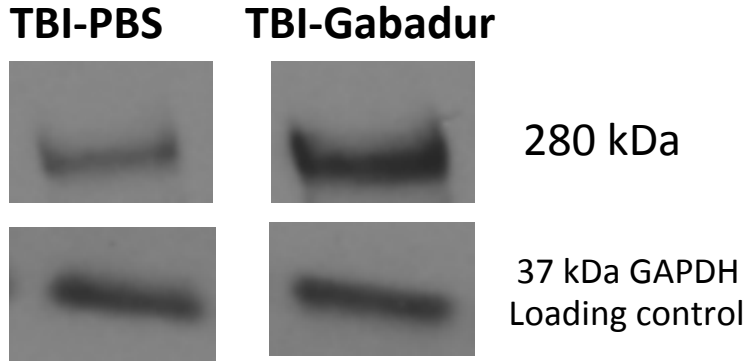


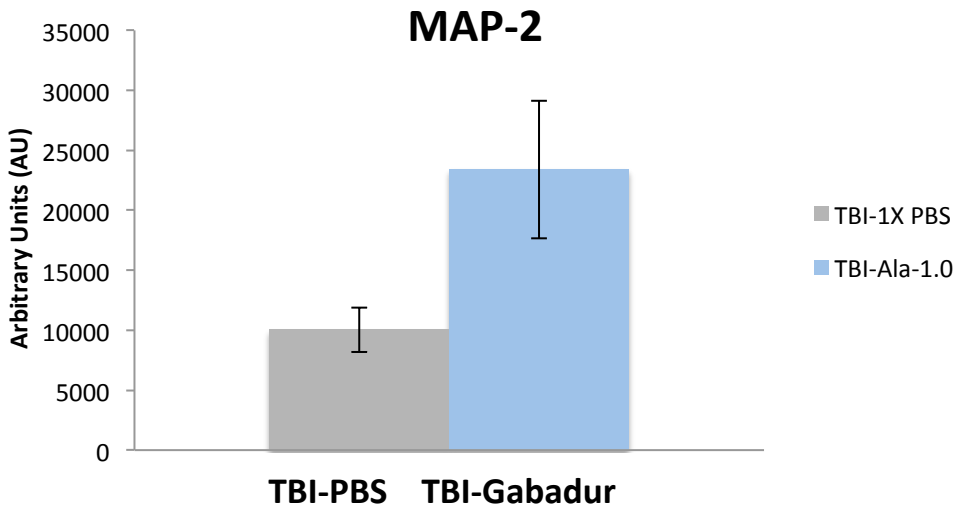


Cytoskeletal proteins indicate Gabadur's ability to cross the BBB & inhibit calpain



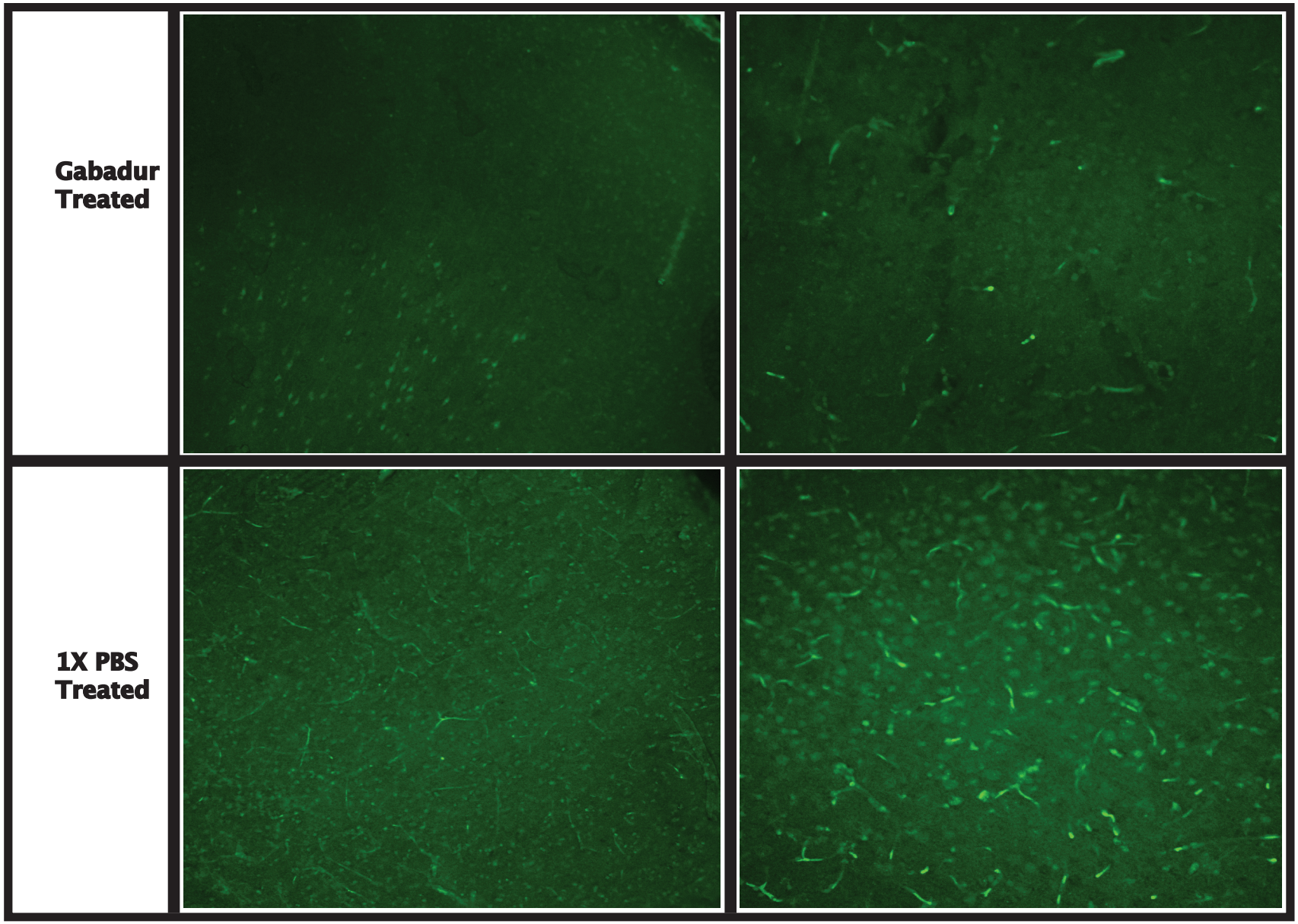




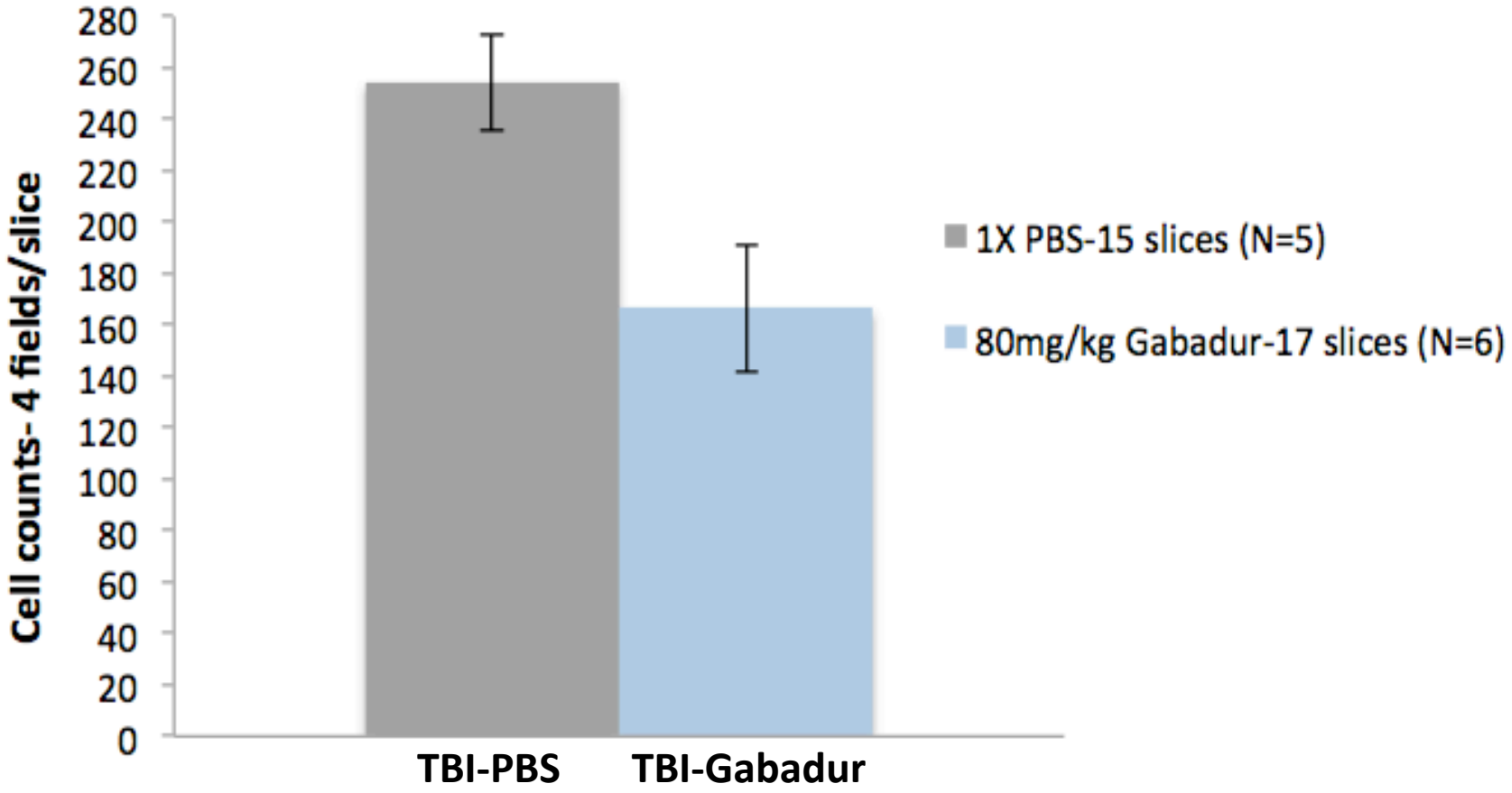


N=3/group

Gabadur crosses the BBB and is neuroprotective



FJB-positive cells in PBS- versus Gabadur-treated rats



Conclusions

- A **single** 80mg/kg intraperitoneal dose of Gabadur
  - Crosses the blood-brain barrier to inhibit calpain.
  - Protects cytoskeletal membrane proteins αII spectrin and microtubule-associated protein 2
  - Decreases neurodegeneration in the cortical region of injury by 40%.

In summary..

- Calpain plays a major role in TBI and neurodegenerative disease pathology.
- Gabadur crosses the blood brain barrier to target the site of neuronal injury.
- Gabadur inhibits calpain.
- Gabadur is neuroprotective.

All with a single 80mg/kg i.p injection within 5 mins post-TBI

Next steps...

Evaluate the ability of Gabadur to improve neurological function when administered 5 mins post-TBI and at later, more clinically relevant time-points through the evaluation of histological, biochemical, and behavioral outcome measures.

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& YOU for your attention!
There is more to come!

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