University at Buffalo The State University of New York

TNFα: Inflammatory Mediator, Neuromodulator and Therapeutic Target

Tracey A. Ignatowski, Ph.D.
Research Associate Professor
Department of Pathology & Anatomical Sciences

Disclosure Information

Tracey A. Ignatowski, Ph.D.

- No financial interest that could be considered as conflict of interest.

Forging the Future of Nano-medicine™

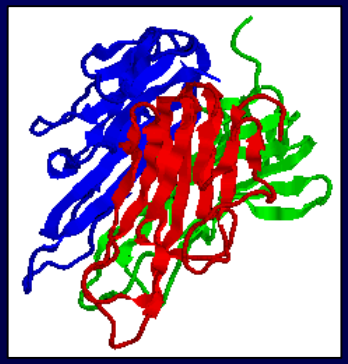
Tumor Necrosis Factor-α

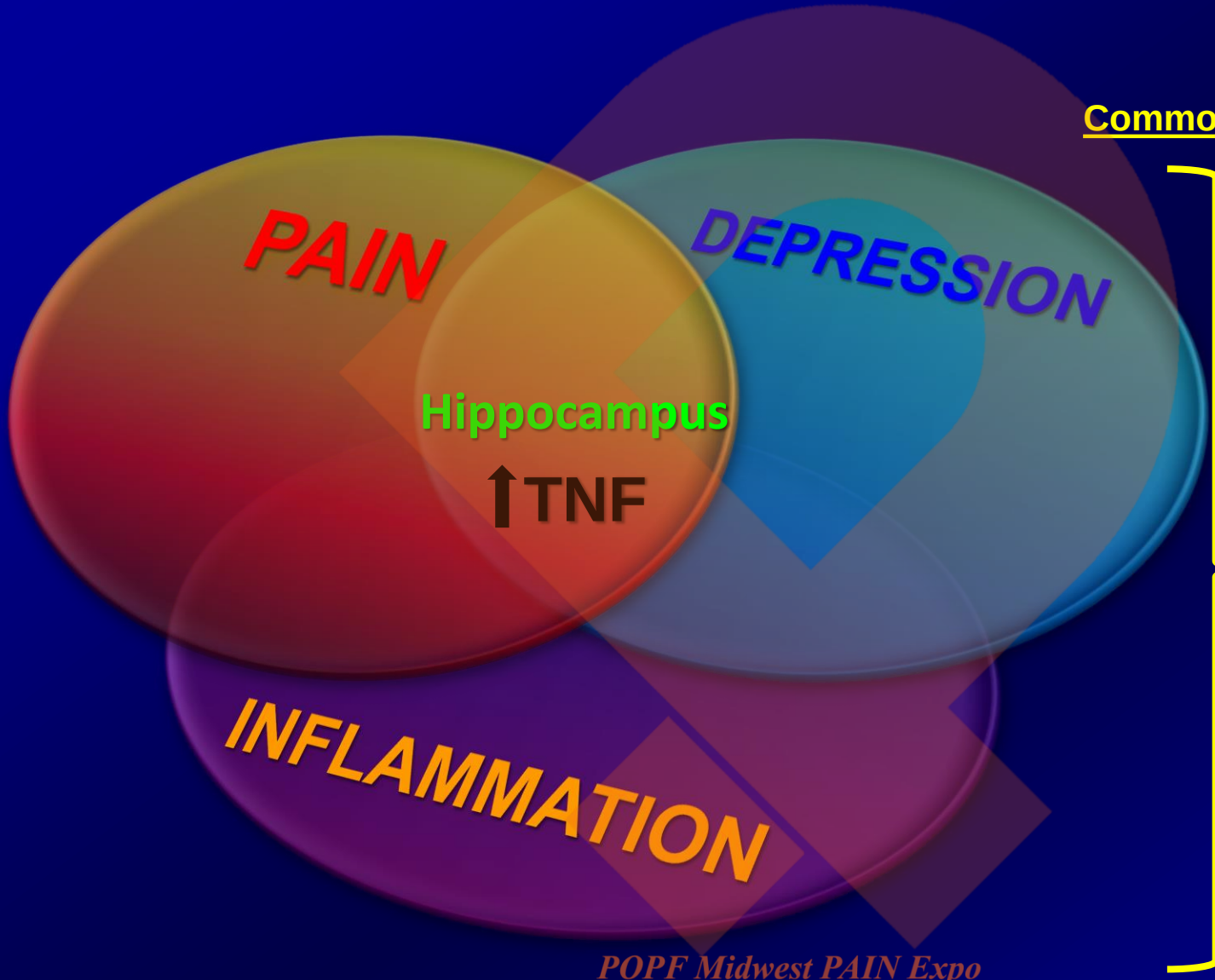
Pro-inflammatory mediator:

- Initiation and coordination of inflammation
- Lethal tissue injury
- Cachexia (chronic starvation state)

Neuromodulator:

- Influences emotional behavior
- Neuroprotection
- Regulation of neurotransmitter release





Commonalities of Disorders

ANATOMY:
Anterior Cingulate Cortex
Prefrontal Cortex
Hippocampus
Amygdala
Thalamus

MOLECULAR DETERMINANTS:
Dysregulated Neurotransmitters
Serotonin
Dopamine
Norepinephrine
Acetylcholine
Glutamate
GABA
Altered Cytokine Profile
Increase proinflammatory cytokines (**TNF**, IL-1, IL-6)
Decrease antiinflammatory cytokines (IL-4, IL-10)

Fasick V, Spengler RN, Samanakan S, Nader ND, Ignatowski TA. The hippocampus and TNF: Common links between chronic pain and depression. Neurosci Biobehav Rev 53:139-159, 2015

Pathologic Disorders

Neuropathic Pain

- Chronic Constriction Injury (CCI)
- Diabetic Neuropathy (STZ)

Depressive Behavior

- Forced Swim Test (FST)
- Novelty-induced Hypophagia (NIH)

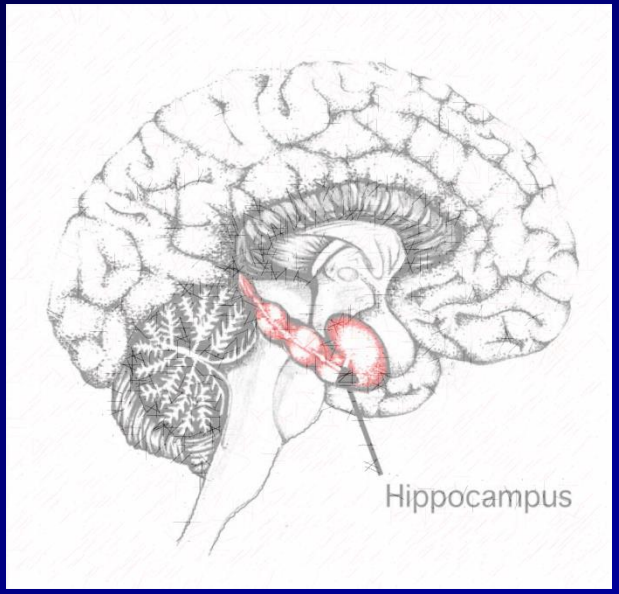
Brain-associated TNF

Contributes to Development of Chronic Pain

Role in Depressive Behavior

Hippocampus

- Primary brain region affected during numerous neurological disorders, resulting in synaptic defects and atrophy
- Effects reversed by antidepressant drugs
  - Induces hippocampal neurogenesis
- Express receptors for TNF



TNF

Neuromodulator

Role in Depressive Behavior

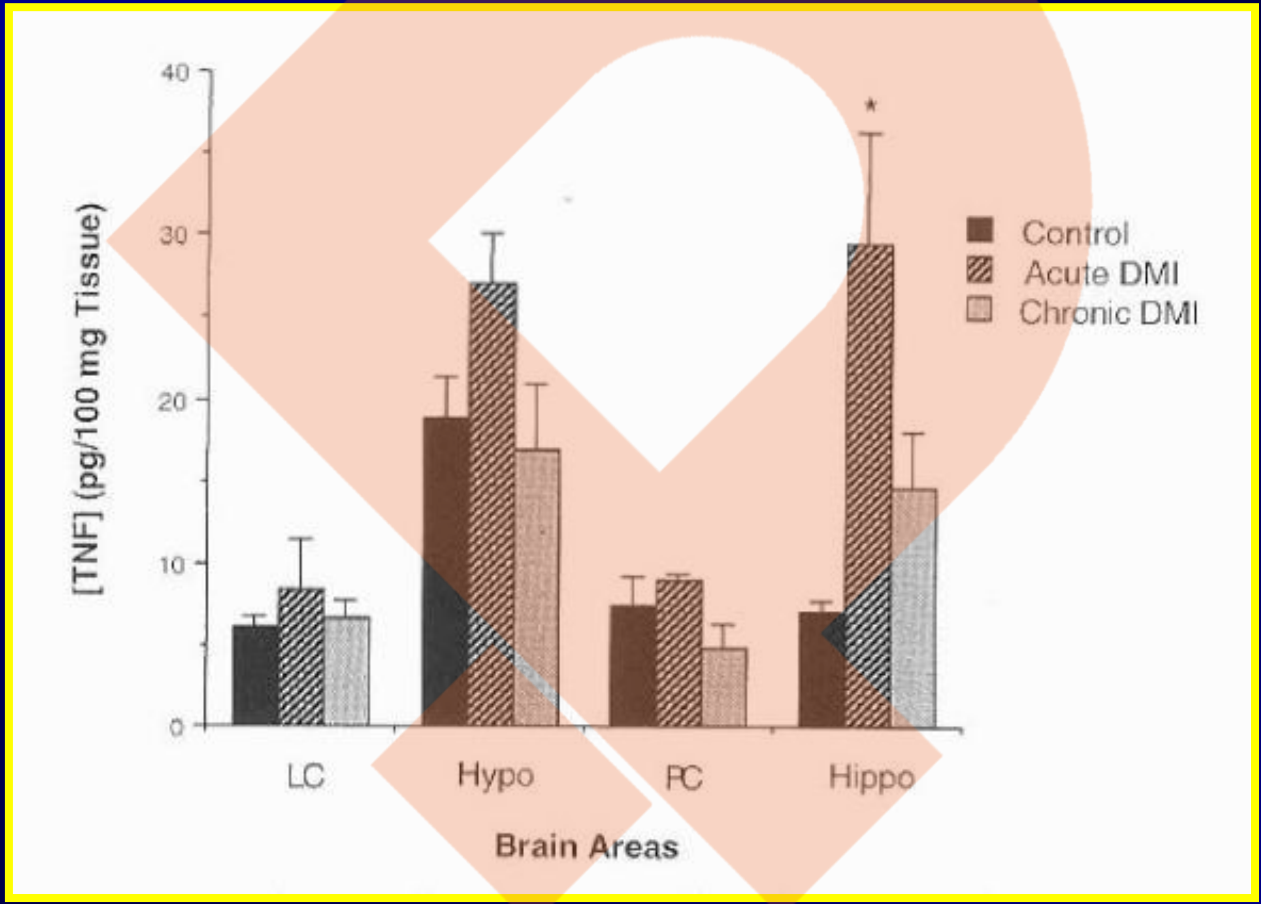
Role in Neuropathic Pain

Methods

Brain TNF Levels

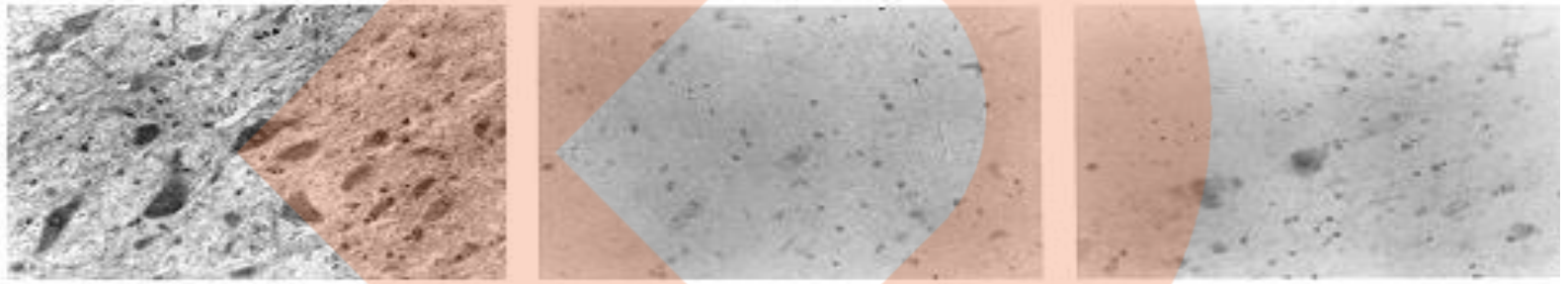
- Brain regions taken
  - Hippocampus
  - Locus Coeruleus
  - Parietal Cortex
  - Medial Frontal Cortex
  - Caudate Nucleus
  - Thalamus
  - Periaqueductal gray
- Weighed and processed for assay - TNF WEHI-13var bioassay
- Immunoperoxidase staining of tissue

Bioactive Levels of TNF



Ignatowski TA, Spengler RN. Tumor necrosis factor-α: Presynaptic sensitivity is modified after antidepressant drug administration. **Brain Res** 665:293-299, 1994.

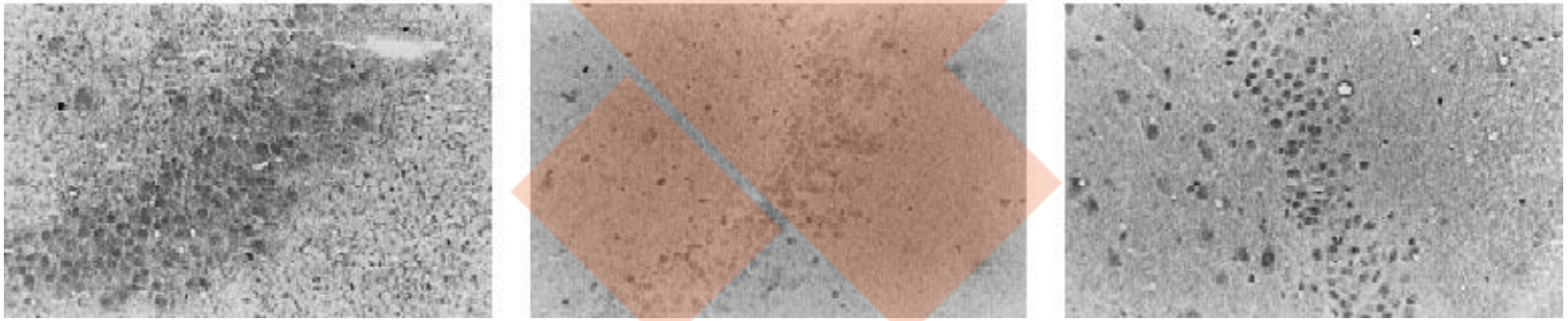
Immunoperoxidase Staining for TNF



Locus coeruleus

Immunohistochemistry (25X)

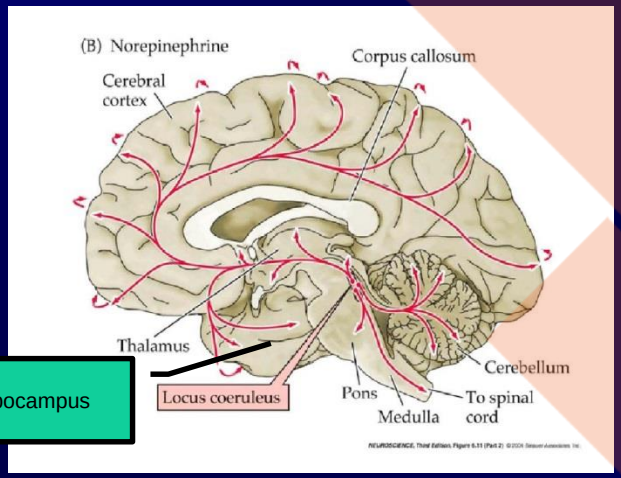
Hippocampus

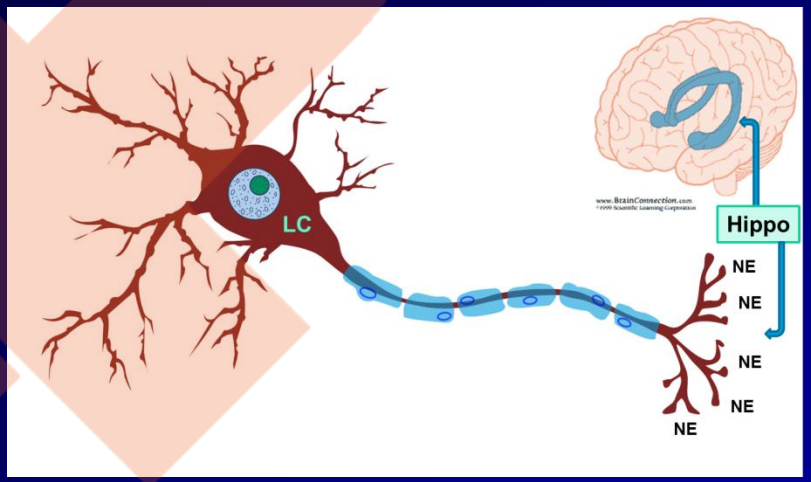


Ignatowski TA, Noble B, Gorfien J, Wright JR, Spengler RN. Neuronal-associated tumor necrosis factor (TNFα): Its role in noradrenergic functioning and modification of its expression following antidepressant administration. **J Neuroimmunol** 79:84-90, 1997.

TNF

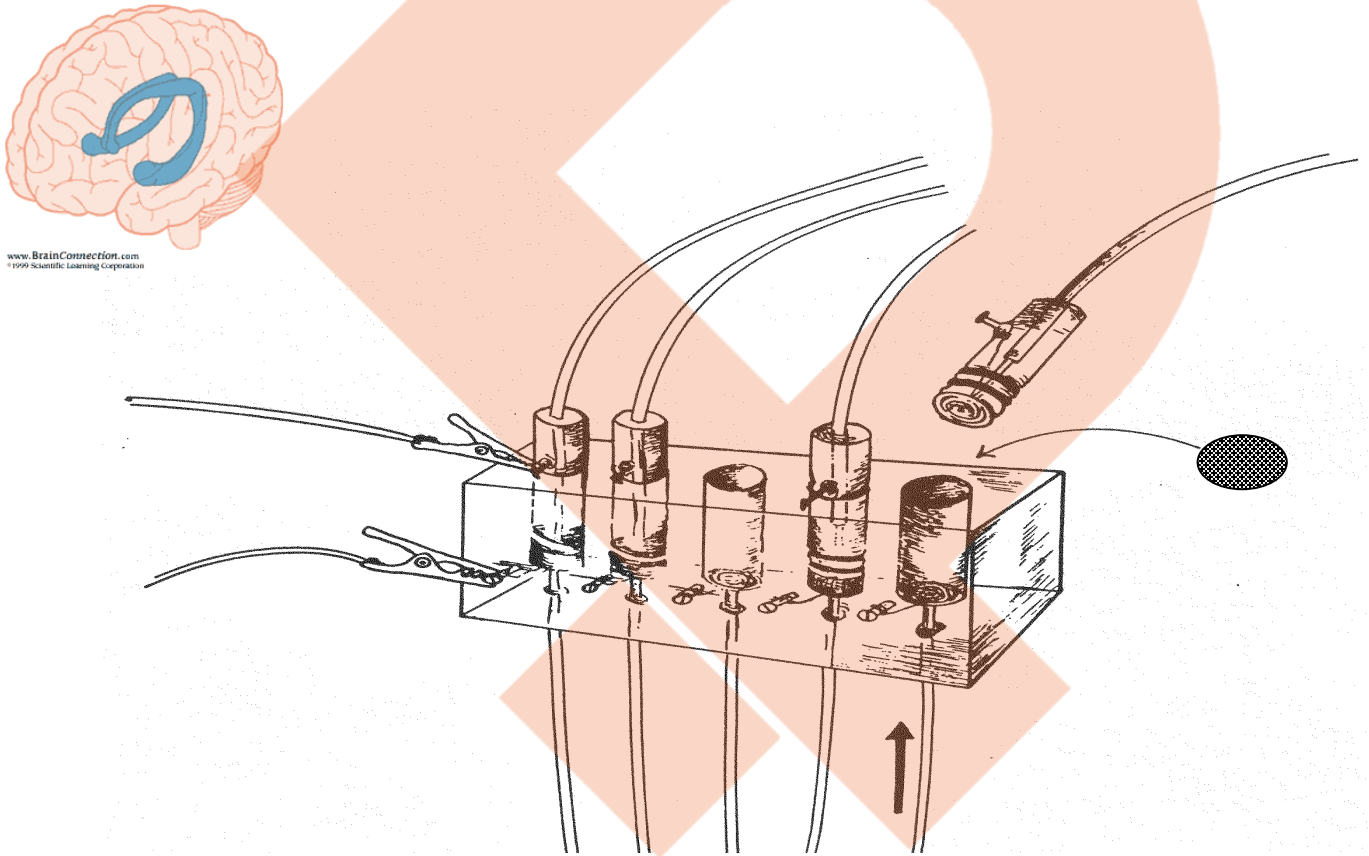
Neuromodulator



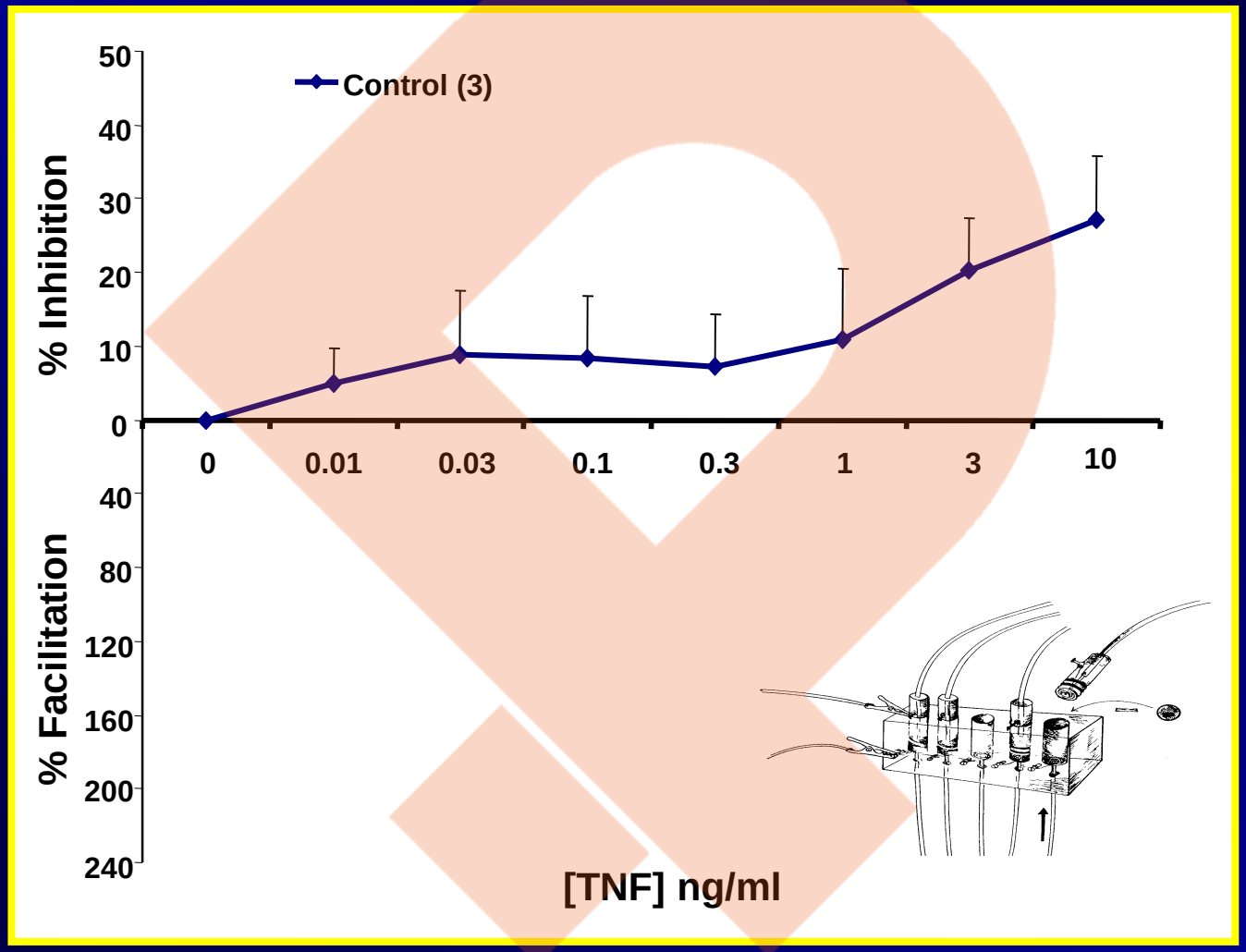


POPF Midwest PAIN Expo

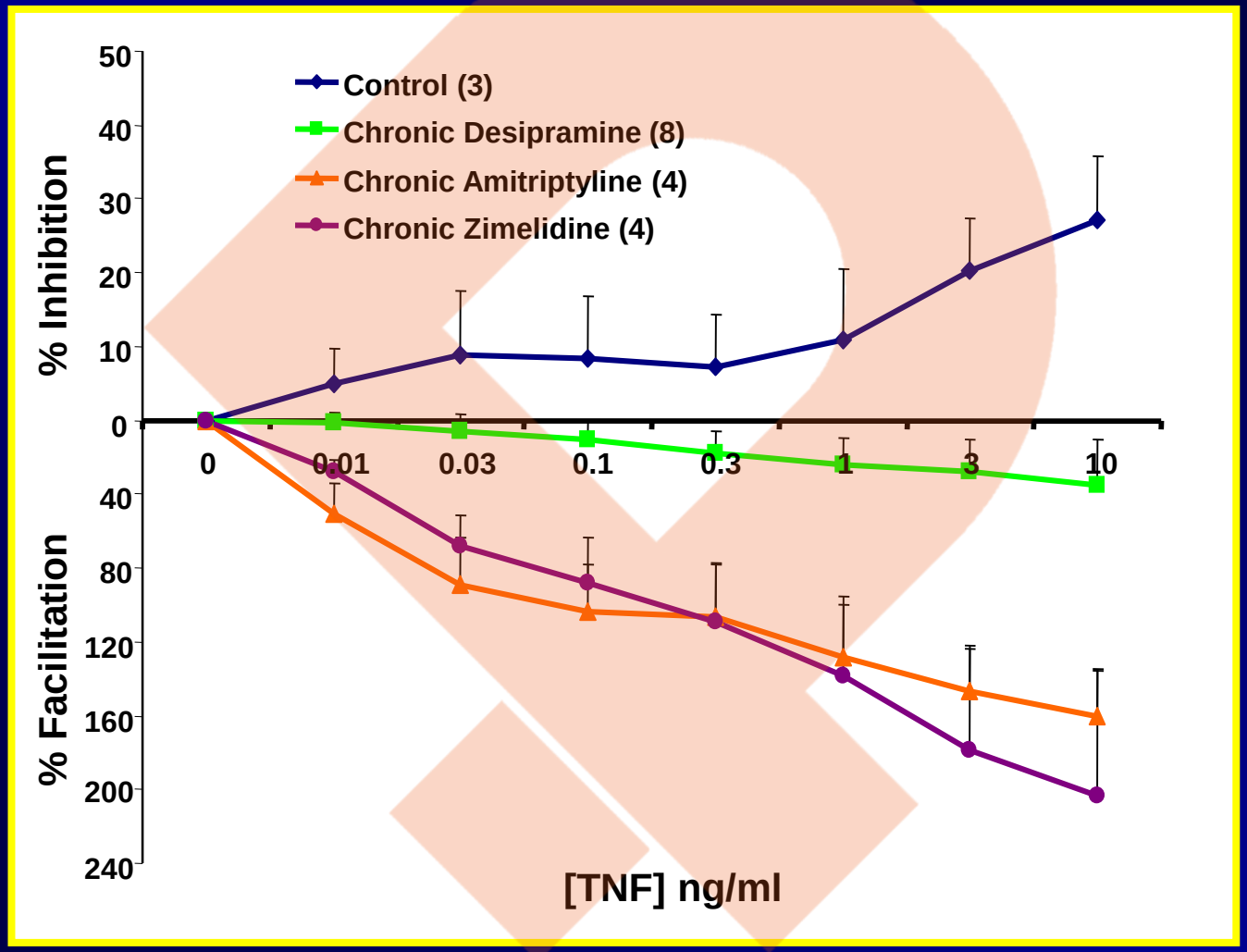
Field Stimulation and Superfusion of Hippocampal Brain Slices



TNF Concentration-Effect Curves



TNF Concentration-Effect Curves



TNF

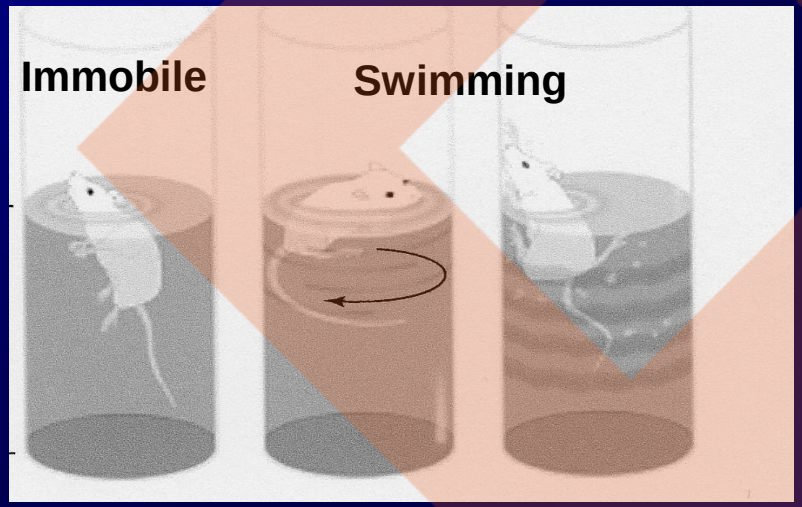
Neuromodulator

Role in Depressive Behavior

Role in Neuropathic Pain

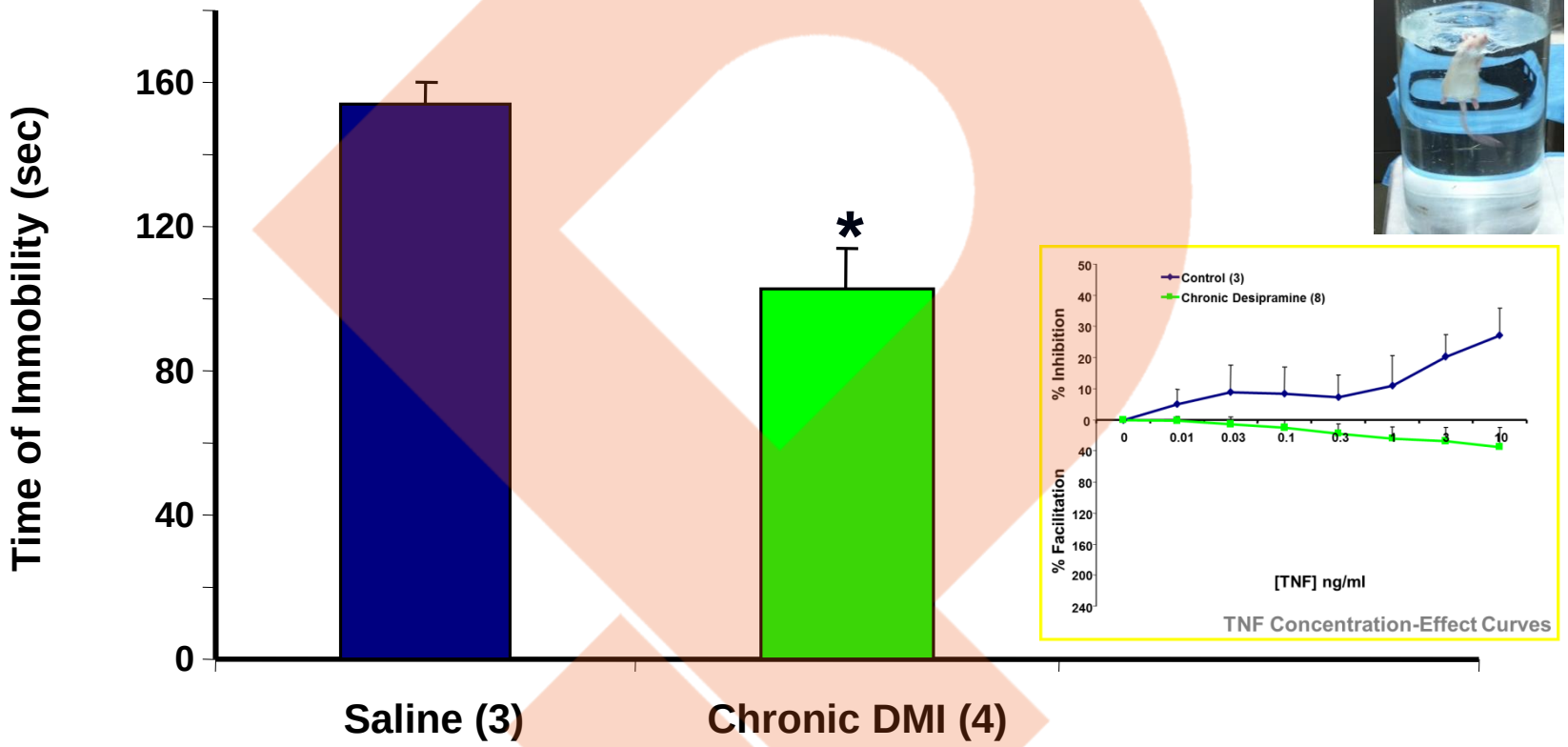
POPF Midwest PAIN Expo

Forced Swim Test

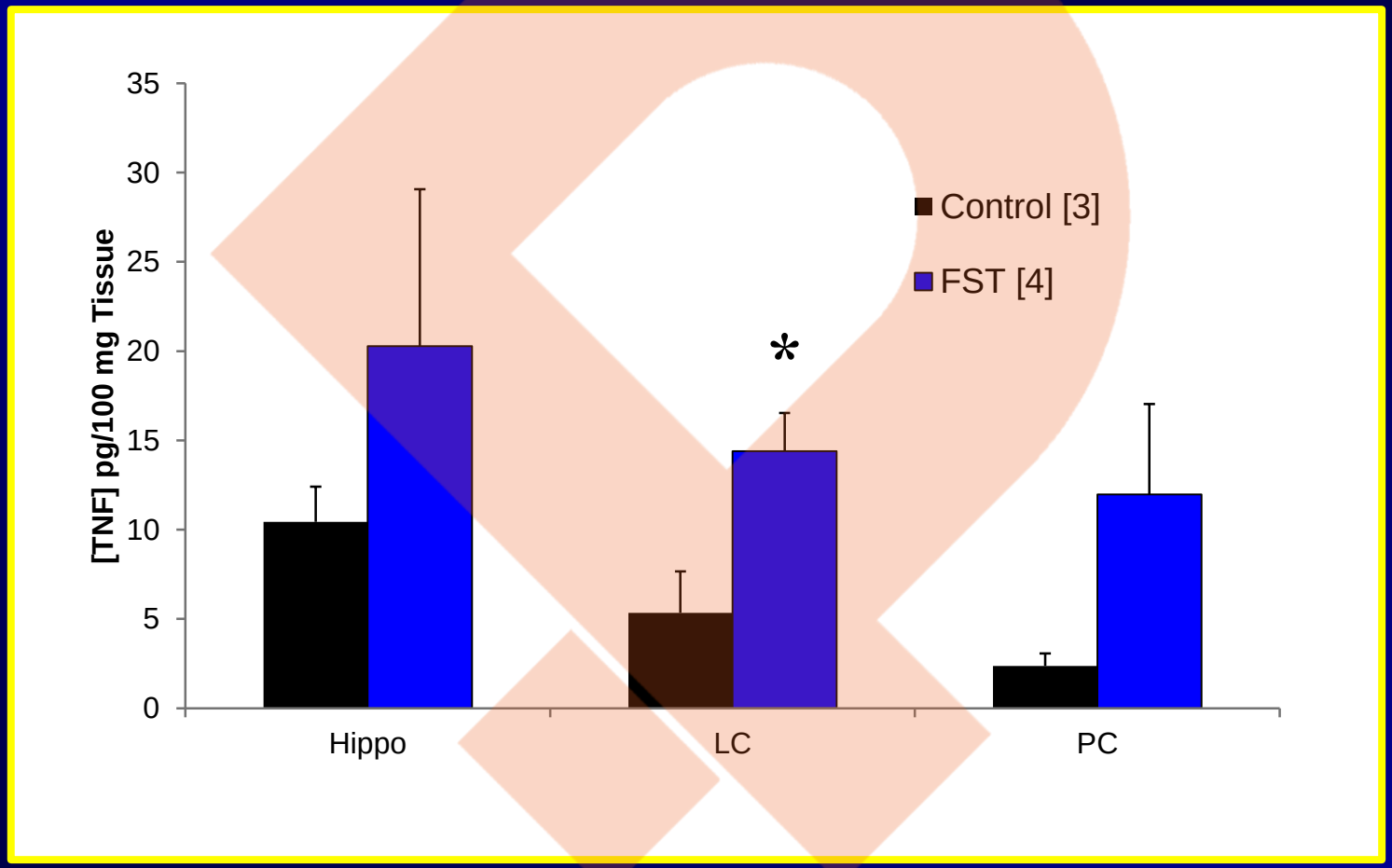


- Used for antidepressant effectiveness
- Stressor – behavioral despair/learned helplessness
  - 15 min pre-test swim
  - 5 min test swim

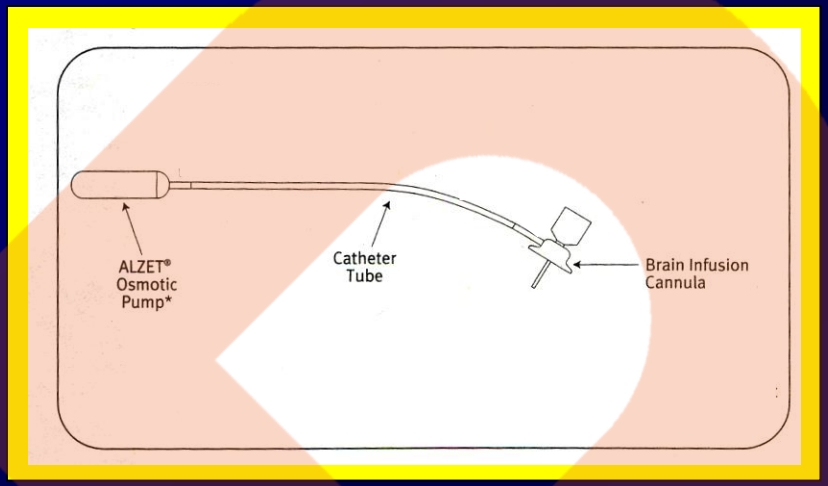
FST-induced Immobility



FST-induced TNF Levels

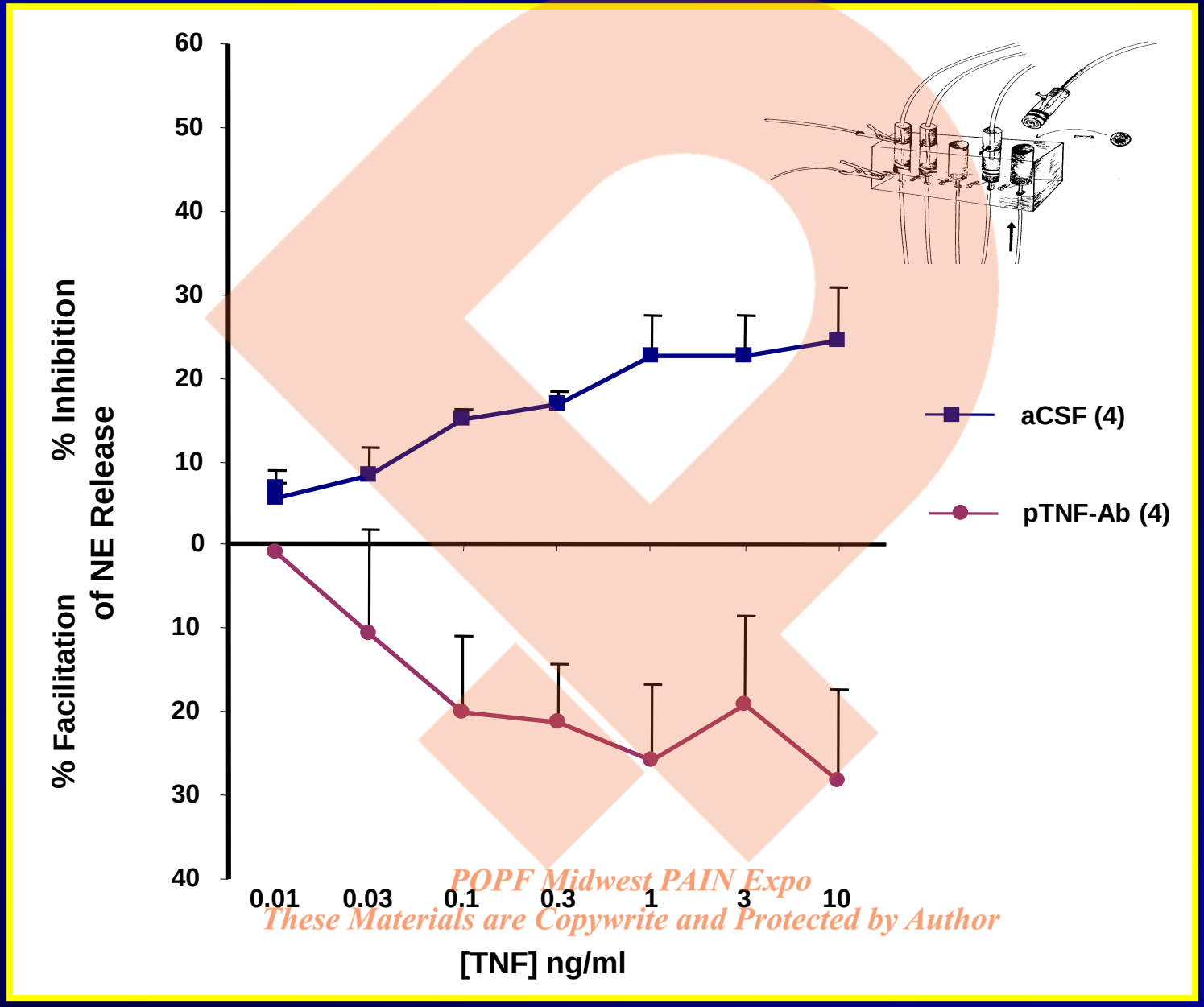


Intracerebroventricular Microinfusion



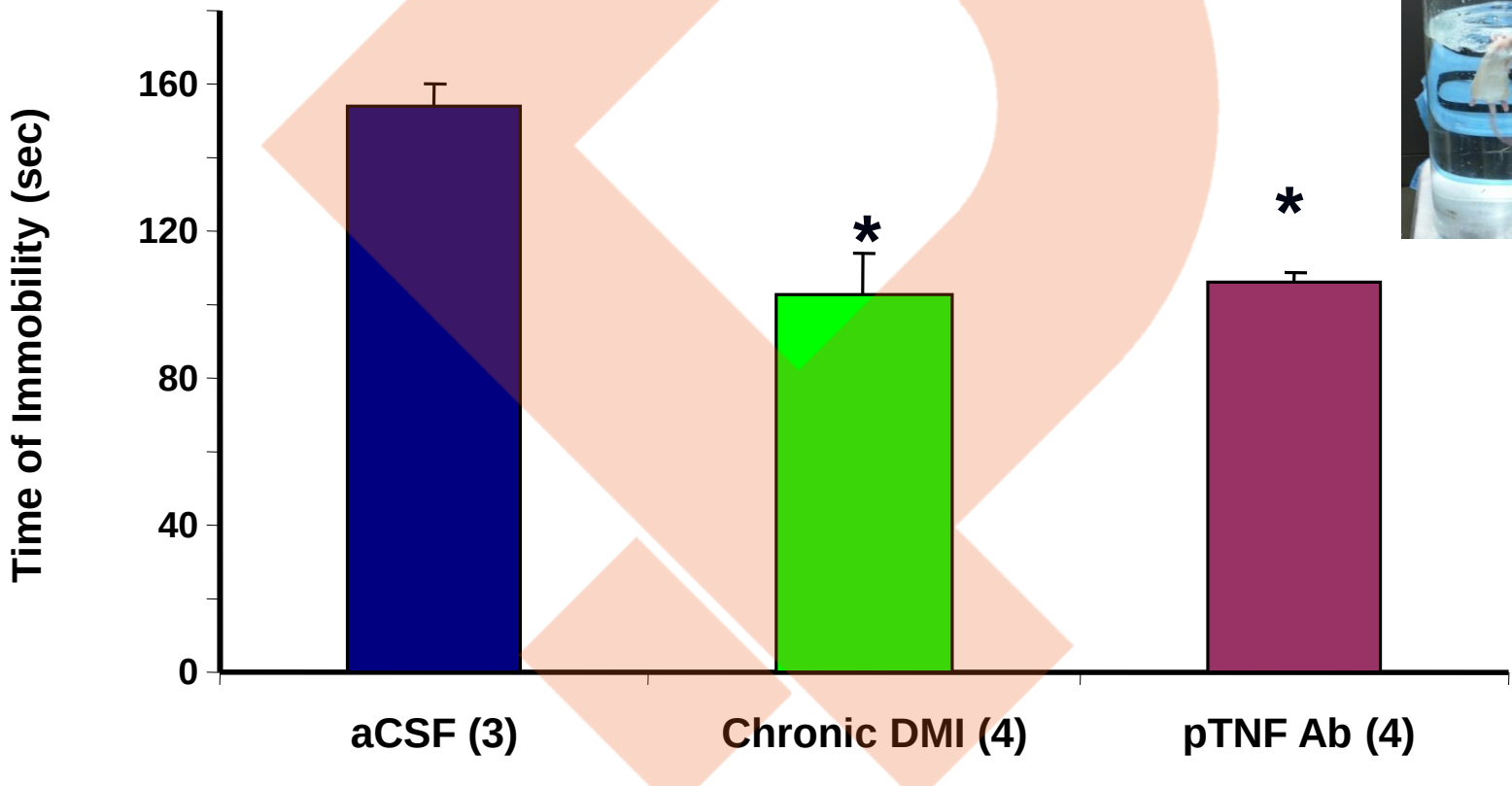
- Microinfusion of compound into right lateral cerebral ventricle for 14 days

- Compounds Infused:
  - Artificial Cerebral Spinal Fluid (aCSF)
  - polyclonal TNFα Antibody (pTNF-Ab)



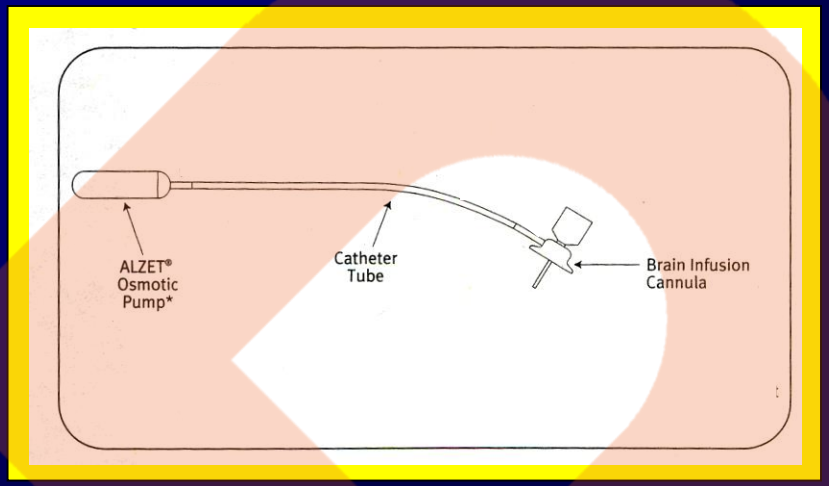
Reynolds JL, Ignatowski TA, Sud R, Spengler RN. Brain-derived tumor necrosis factor-α and its involvement in noradrenergic neuron functioning involved in the mechanism of action of an antidepressant. **J Pharmacol Exper Ther** 310:1216-1225, 2004.

FST-induced Immobility



Reynolds JL, Ignatowski TA, Sud R, Spengler RN. Brain-derived tumor necrosis factor-α and its involvement in noradrenergic neuron functioning involved in the mechanism of action of an antidepressant. **J Pharmacol Exper Ther** 310:1216-1225, 2004.

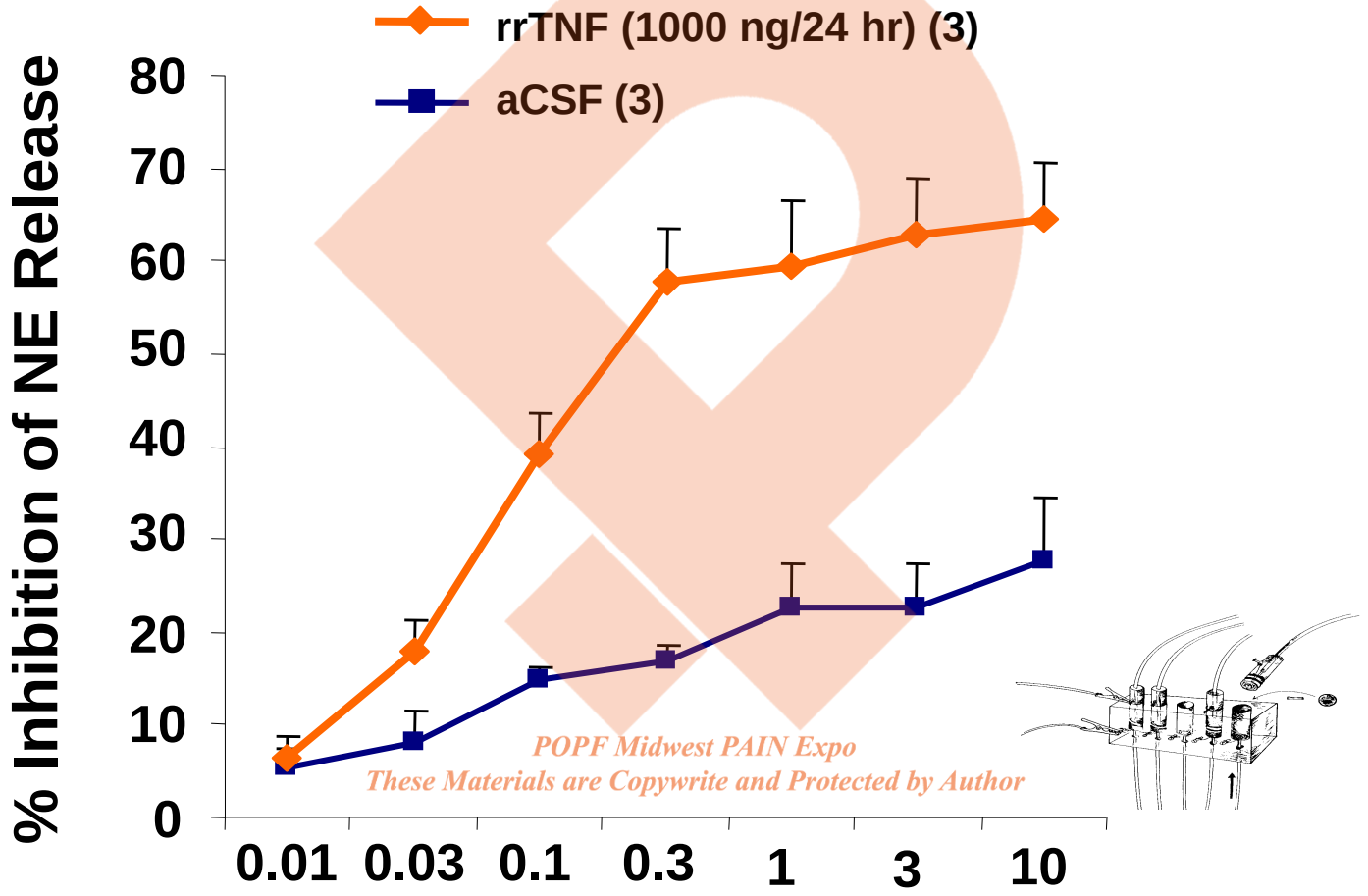
Intracerebroventricular Microinfusion



- Microinfusion of compound into right lateral cerebral ventricle for 14 days

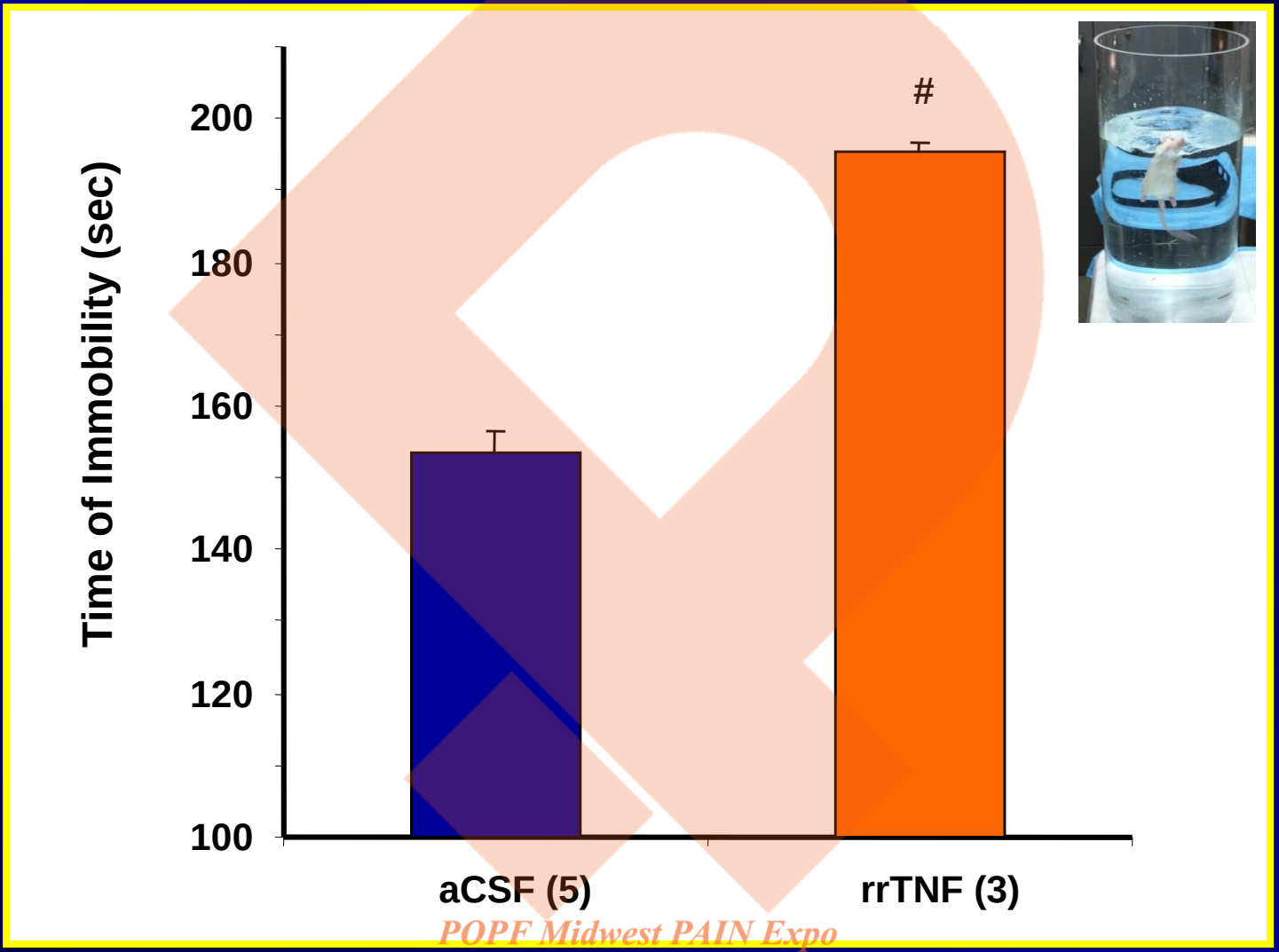
- Compounds Infused:
  - Artificial Cerebral Spinal Fluid (aCSF)
  - rrTNFα (1000 ng/24 hr, 14 days)

Field-Stimulated ^{3}H-NE Release



Reynolds JL, Ignatowski TA, Sud R, Spengler RN. Brain-derived tumor necrosis factor-α and its involvement in noradrenergic neuron functioning involved in the mechanism of action of an antidepressant. **J Pharmacol Exper Ther** 310:1216-1225, 2004.

FST-induced Immobility



Reynolds JL, Ignatowski TA, Sud R, Spengler RN. Brain-derived tumor necrosis factor-α and its involvement in noradrenergic neuron functioning involved in the mechanism of action of an antidepressant. **J Pharmacol Exper Ther** 310:1216-1225, 2004.

Conclusions

- Increase in central TNF induces depressive behavior.
- Blocking central TNF activity induces antidepressant-like behavior.
- Antidepressant drug administration reduces TNF expression.

Hypothesis

Alternative Agents – affect TNF systemically
Antidepressant Effects?

Drugs that decrease stress-induced TNF levels in the brain will prevent depressive-like behaviors.

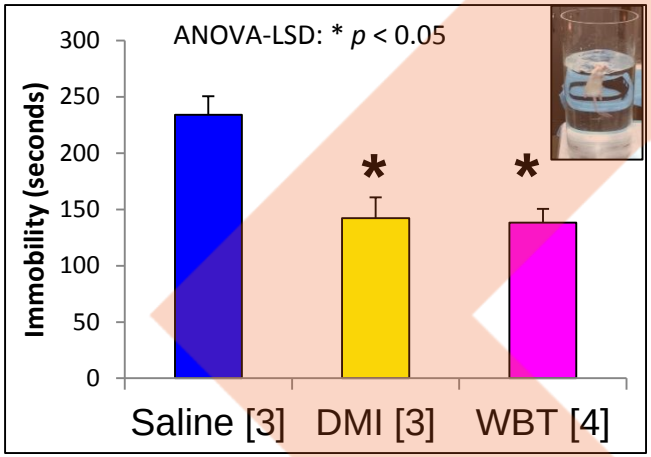
Methods

- Male Sprague-Dawley rats (150-175g)
  - Saline
  - Desipramine (10 mg/kg)
  - Zimelidine (3 mg/kg)
  - Wellbutrin (10 mg/kg)
  - Ketamine (5 mg/kg)
  - Ketamine (10 mg/kg)

Results

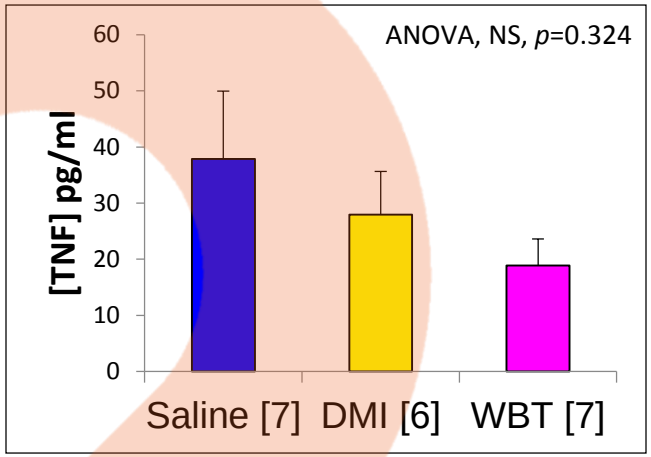
FST

5-min FST-Immobility

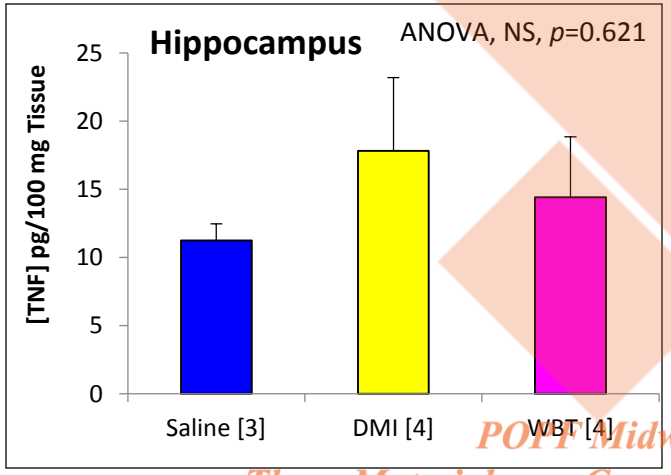


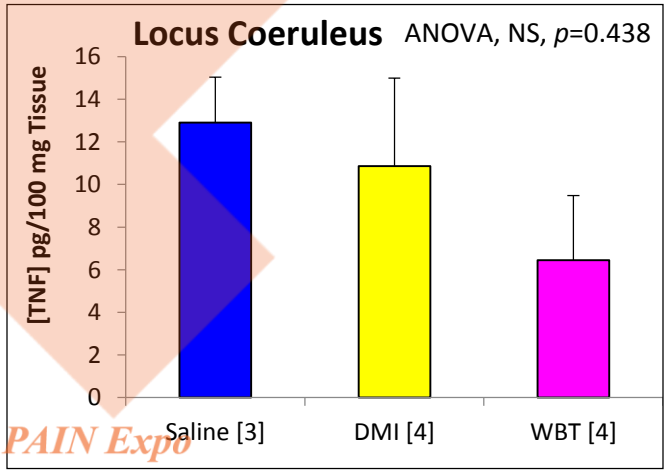
Bioactive TNF

[TNF] Serum



[TNF] Brain Tissue Homogenates

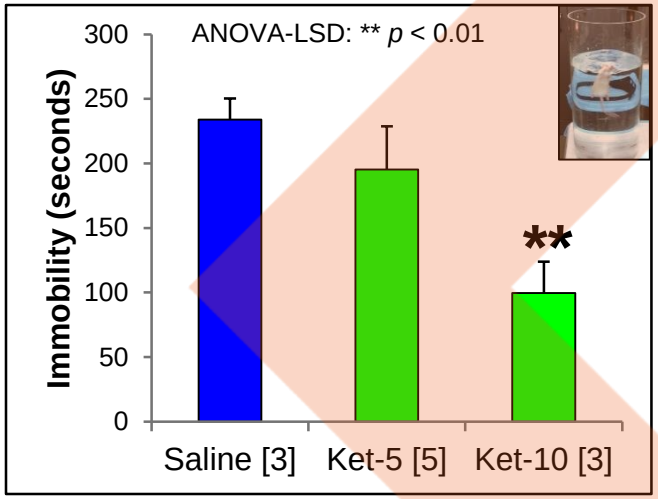


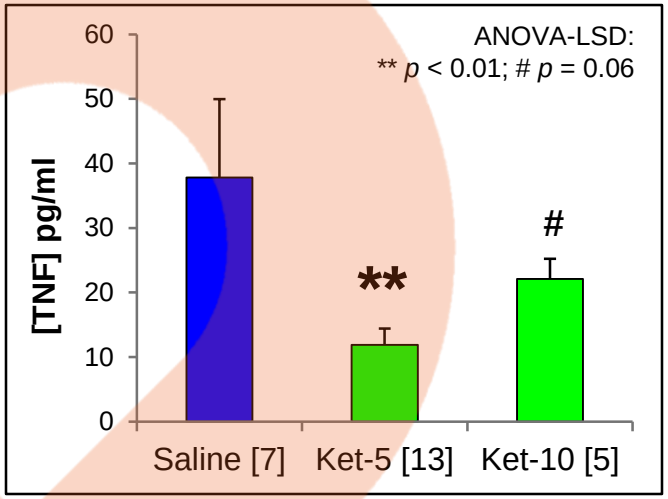


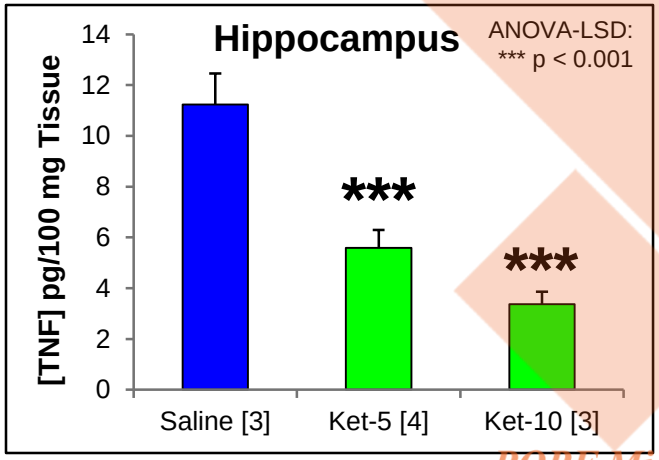
RESULTS

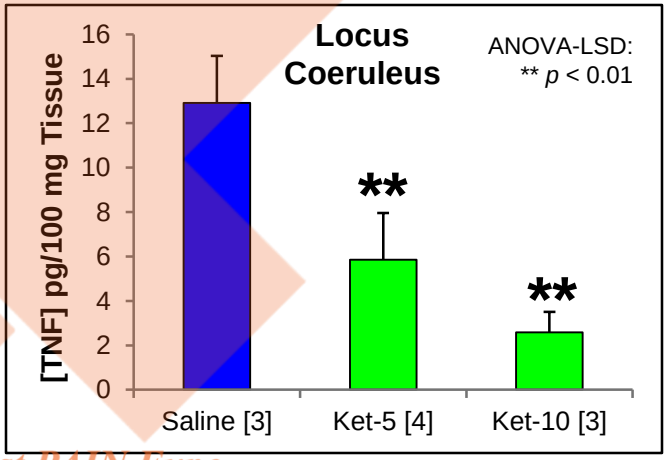
FST

Bioactive TNF









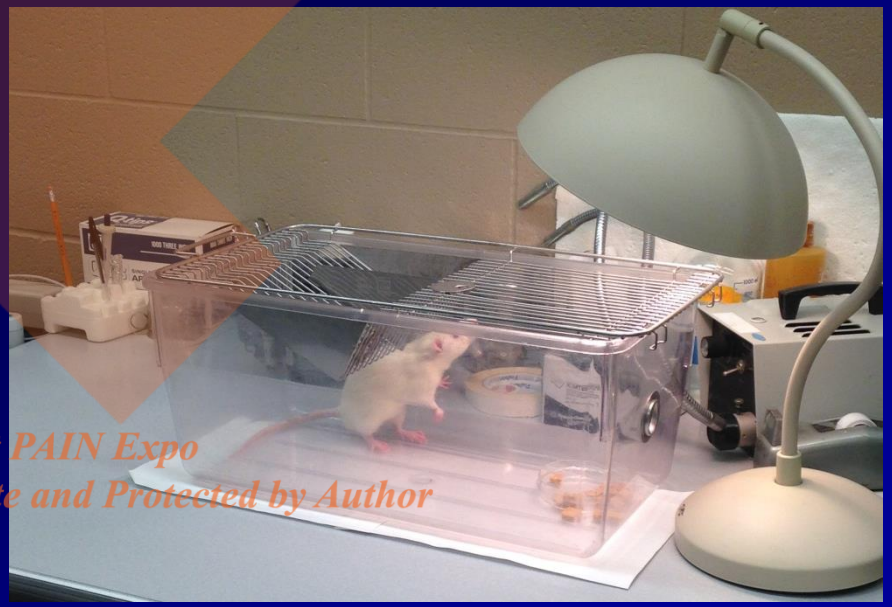
Conclusion: FST Studies

- Novel agent (ketamine)
  - Decrease immobility in FST
  - Decrease TNF levels in serum, hippocampus and locus coeruleus
- Ketamine = fast-acting

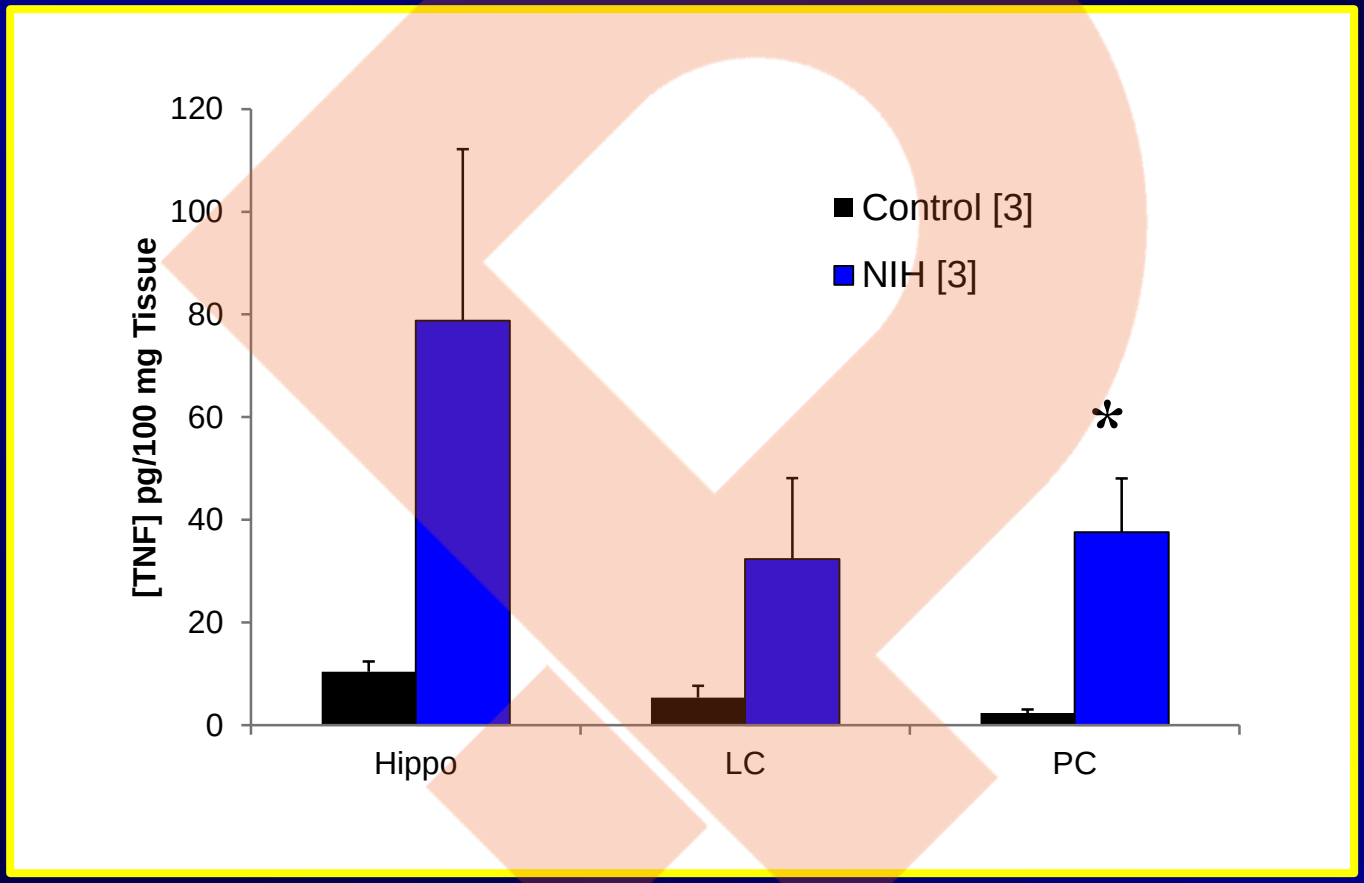
Suggests decreasing TNF levels mediates antidepressant activity

Novelty Induced Hypophagia (NIH)

- Trained 5 days
- Chronic administration (14 days; i.p.)
- Home cage test
- Novel cage test

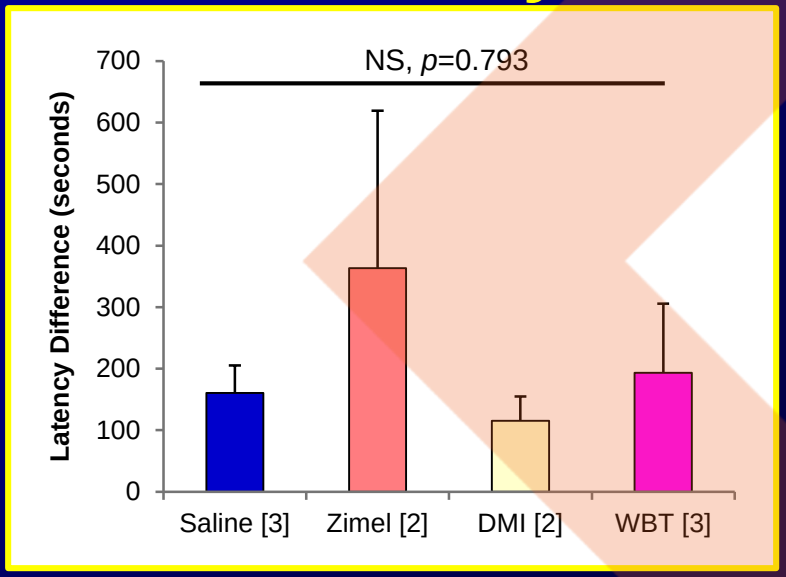


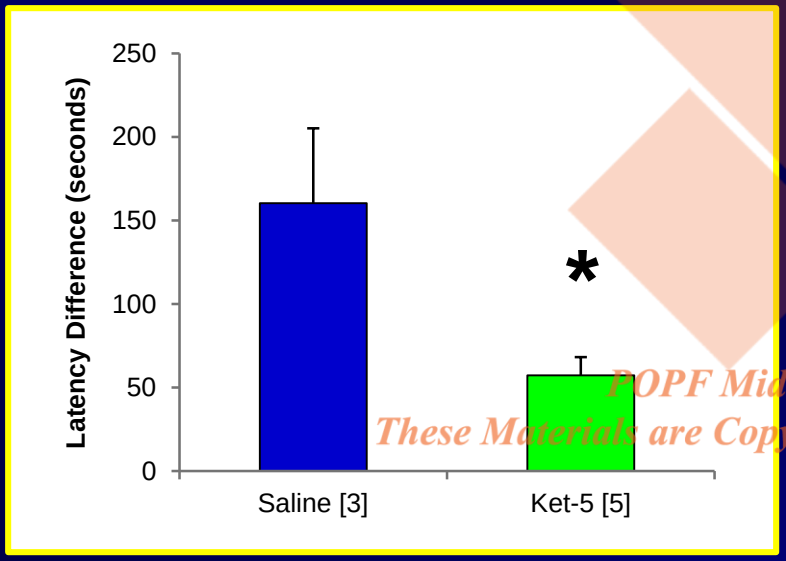
NIH-induced TNF Levels



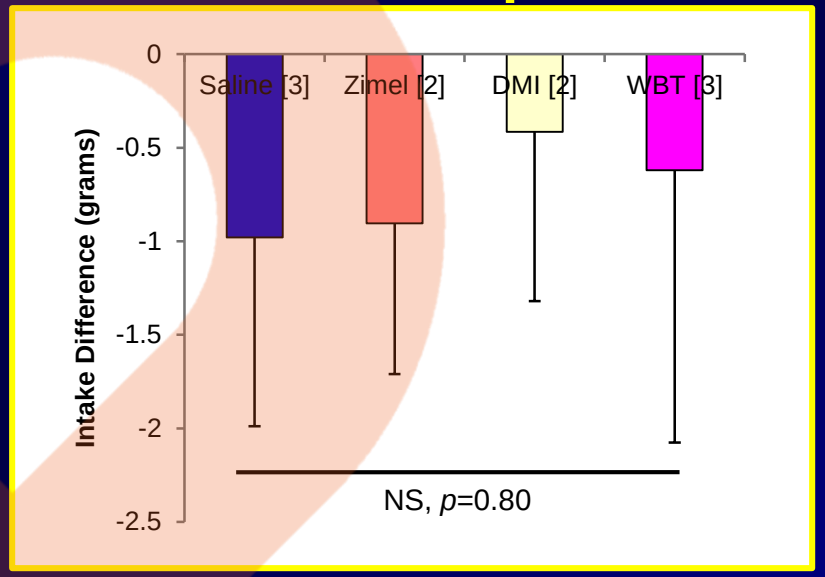
Results – NIH (acute)

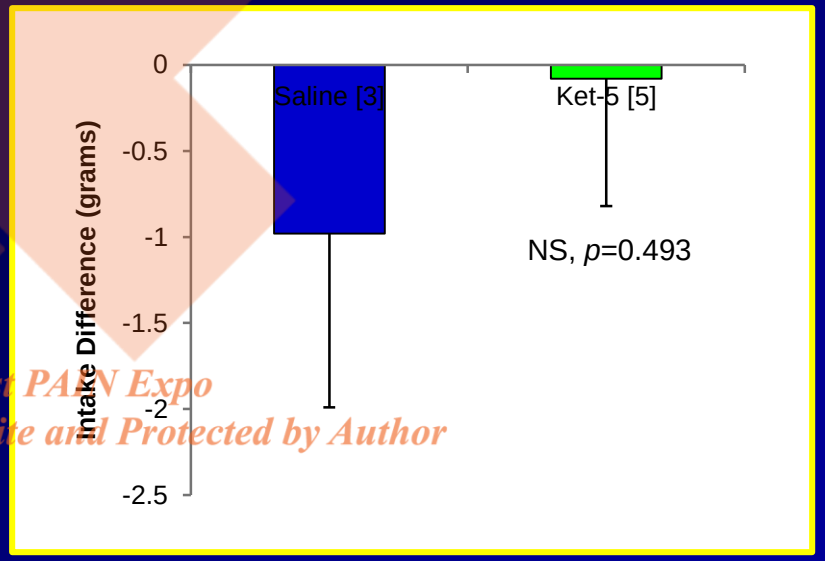
Latency





Consumption

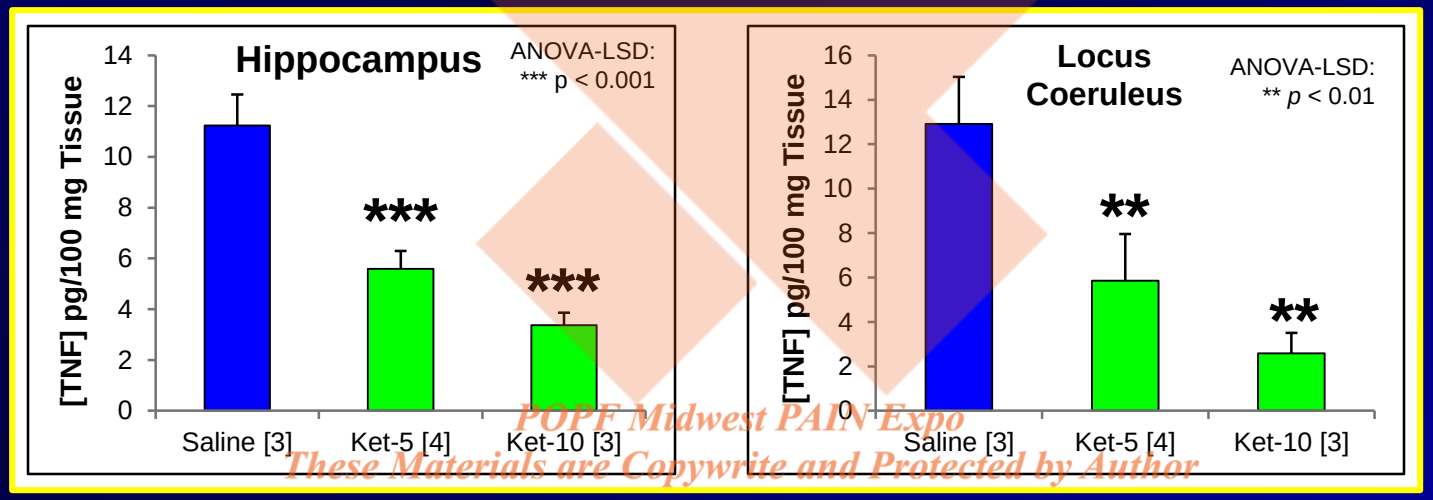




Conclusions: NIH Test (acute)

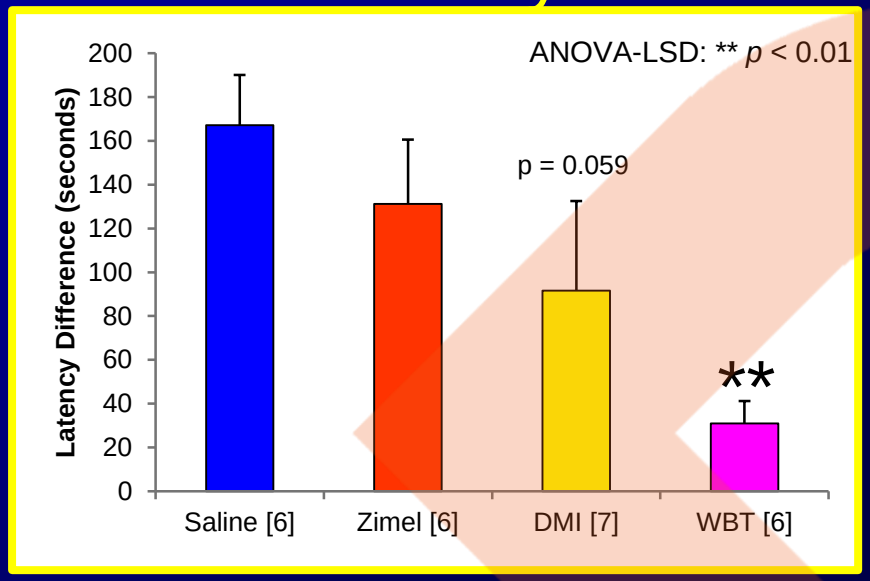
- Well-known antidepressants (desipramine, wellbutrin, zimelidine) do not work following acute administration.
- Ketamine shows decrease in latency.

Ketamine is effective at decreasing depressive behavior following acute administration.

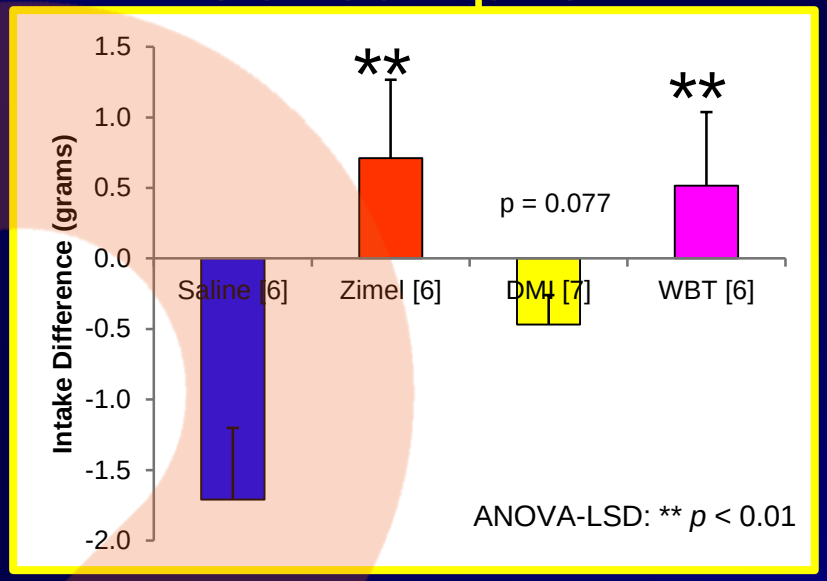


RESULTS – NIH (CHRONIC)

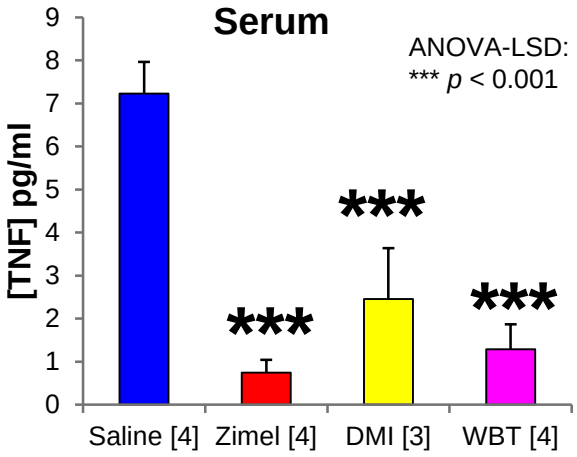
Latency

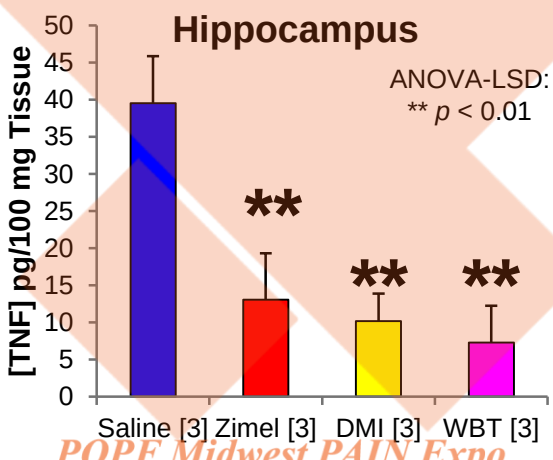


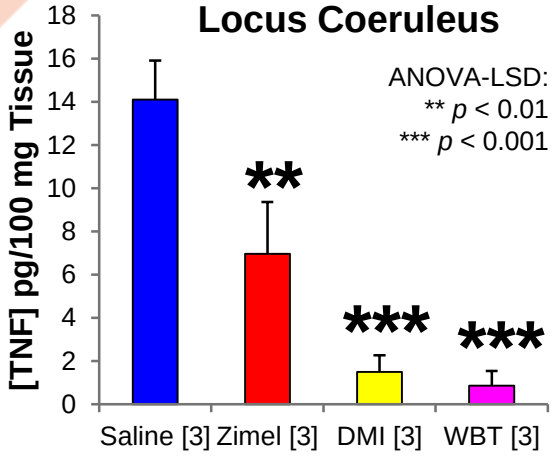
Consumption



Bioassay: Levels of TNF







Conclusions: NIH Test (chronic)

- Zimelidine, desipramine, and wellbutrin
  - Decreased latency (not zimelidine)
  - Increased consumption (not desipramine)
  - Decreased TNF in serum, hippocampus and locus coeruleus

Decrease in TNF is associated with antidepressant activity

TNF

Neuromodulator

Role in Depressive Behavior

Role in Neuropathic Pain

POPF Midwest PAIN Expo

Neuropathic Pain

- Pain resulting from injury to the nervous system
- Central component
- Characteristic features: Hyperalgesia

  Allodynia

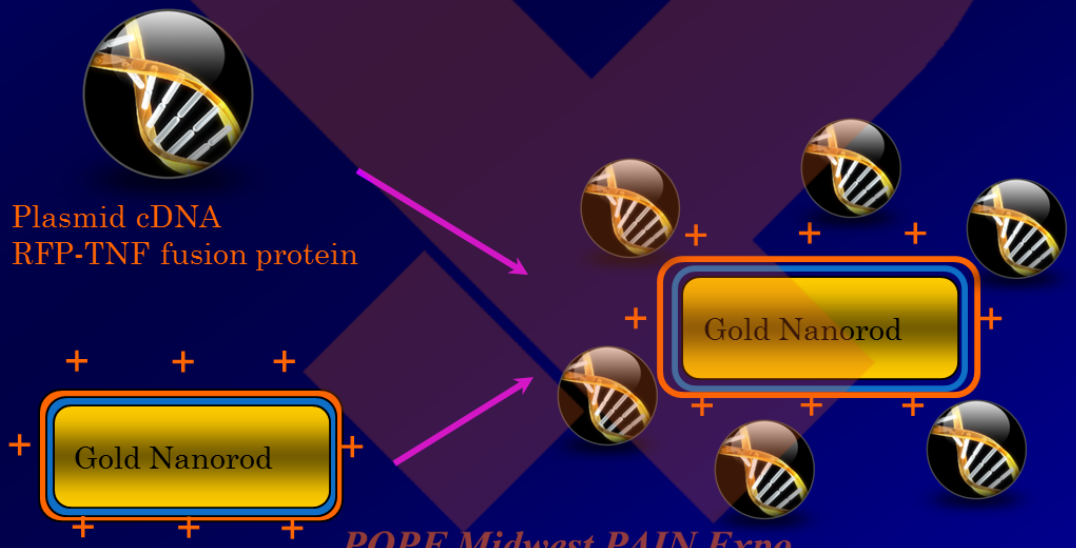
Model	ICV	Pain
CCI	---	++
---	TNF	++
CCI	TNF	++++
CCI	TNF-Ab	---

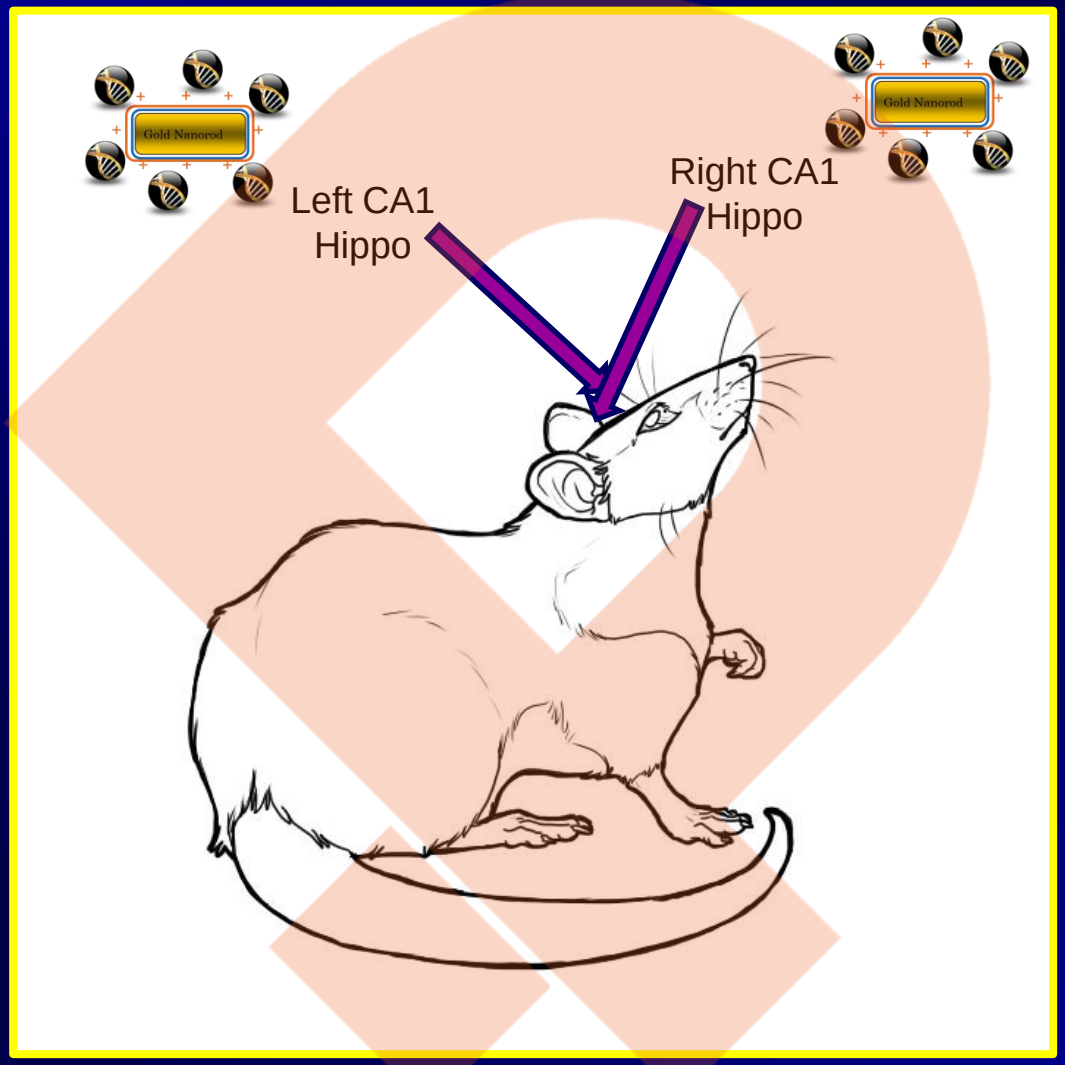
Ignatowski TA, Covey WC, Knight PR, Severin CM, Nickola TJ, Spengler RN. Brain-derived TNFα mediates neuropathic pain. **Brain Res** 841:70-77, 1999.
Covey WC, Ignatowski TA, Knight PR, Spengler RN. Brain-derived TNFα: Involvement in neuroplastic changes implicated in the conscious perception of persistent pain. **Brain Res** 859:113-122, 2000.

Is a molecular imbalance in TNF levels sufficient and necessary to produce neuropathic pain in the CCI model?

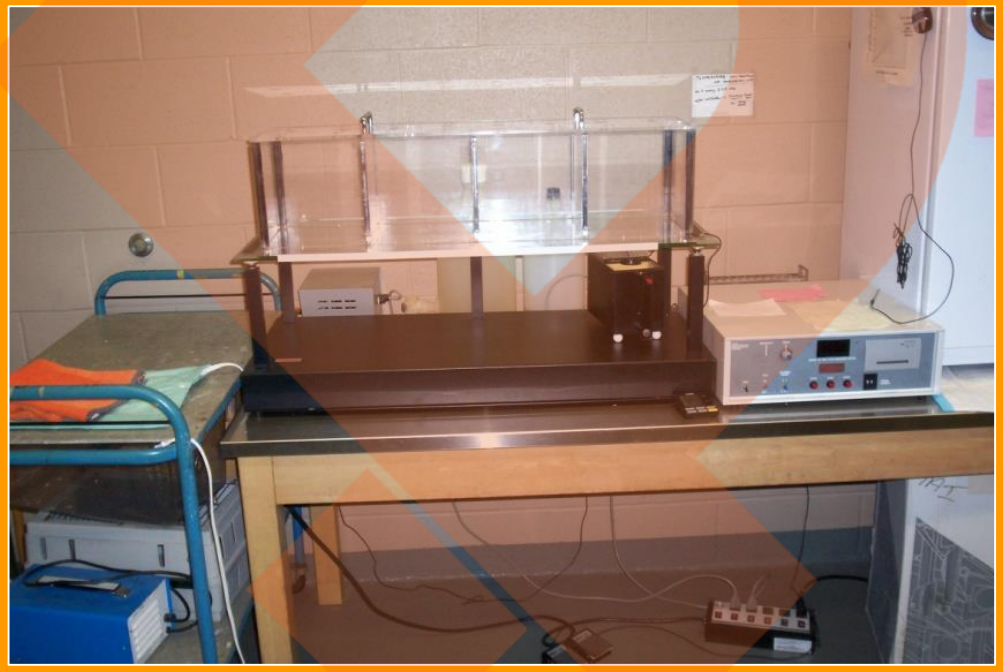
Is a molecular imbalance in TNF levels sufficient to produce neuropathic pain in the CCI model?

GNR-pDNA(TNF)-RFP





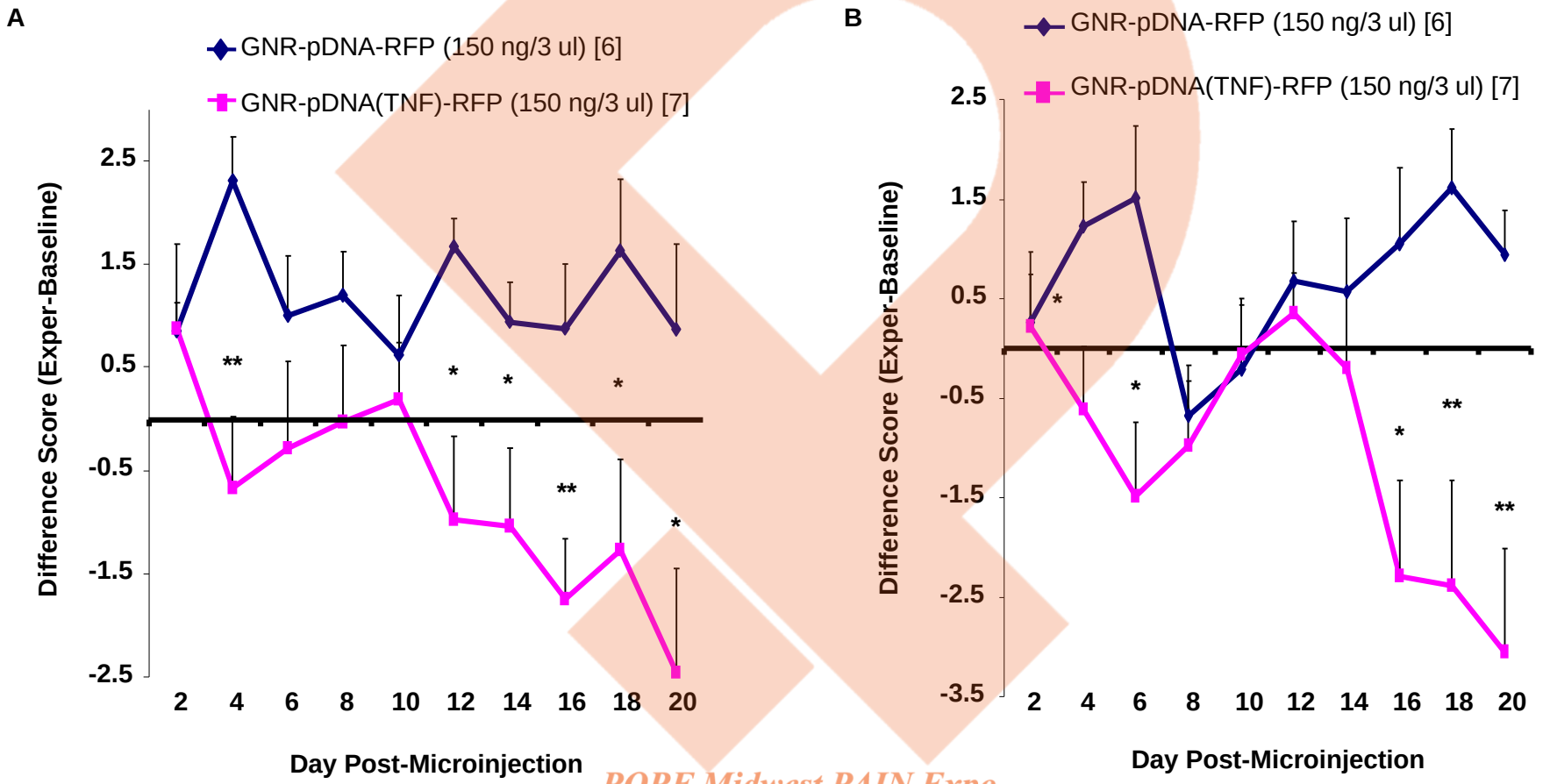
Paw Algesia Apparatus



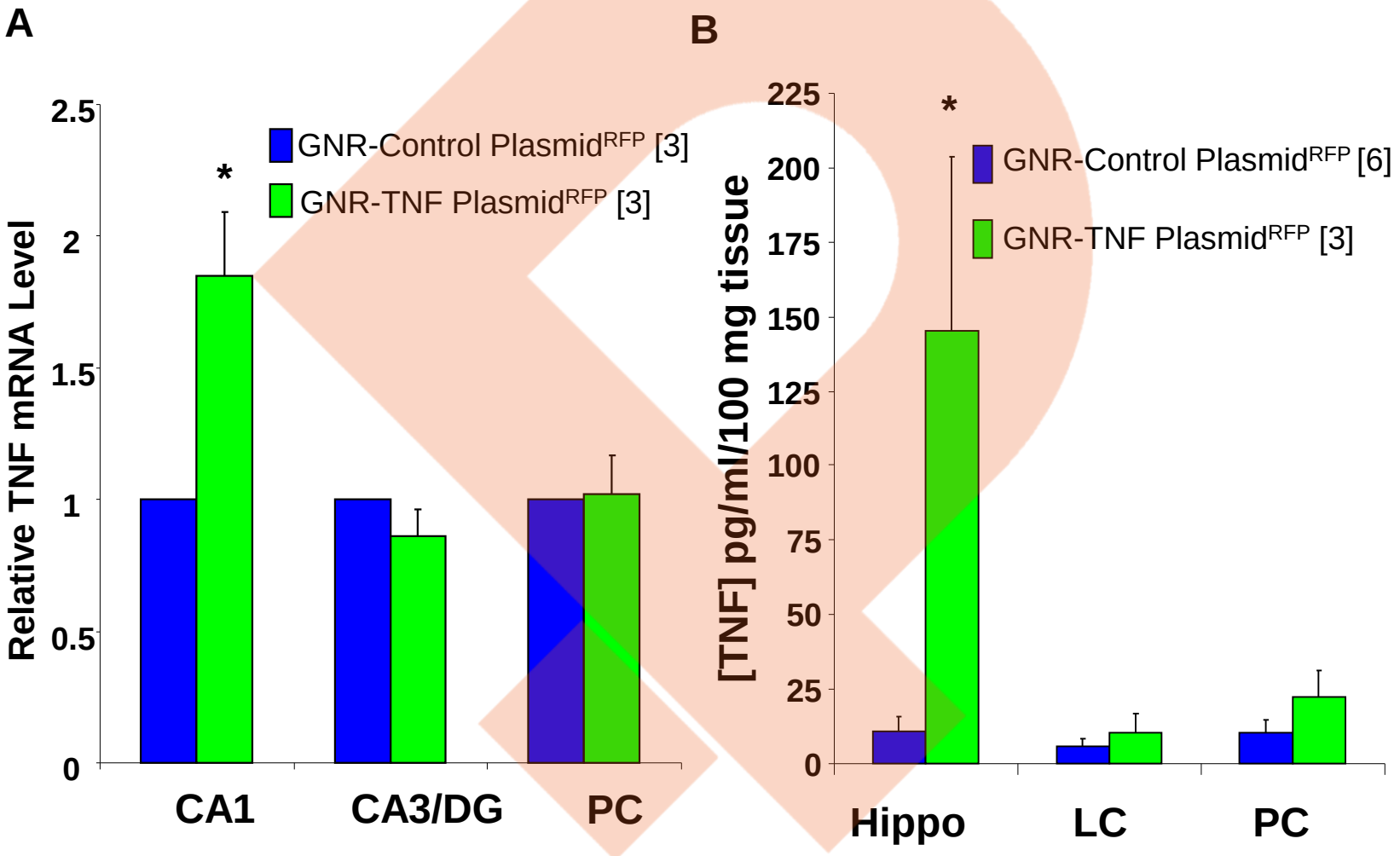
Paw Withdrawal Latency

Right Hind Paw

Left Hind Paw



Martuscello RT, Spengler RN, Bonoiu AC, Davidson B, Helinski J, Ding H, Mahajan S, Kumar R, Bergey EJ, Knight PR, Prasad PN, Ignatowski TA. Increasing TNF levels solely in the rat hippocampus produces persistent pain-like symptoms. PAIN 153:1871-1882, 2012



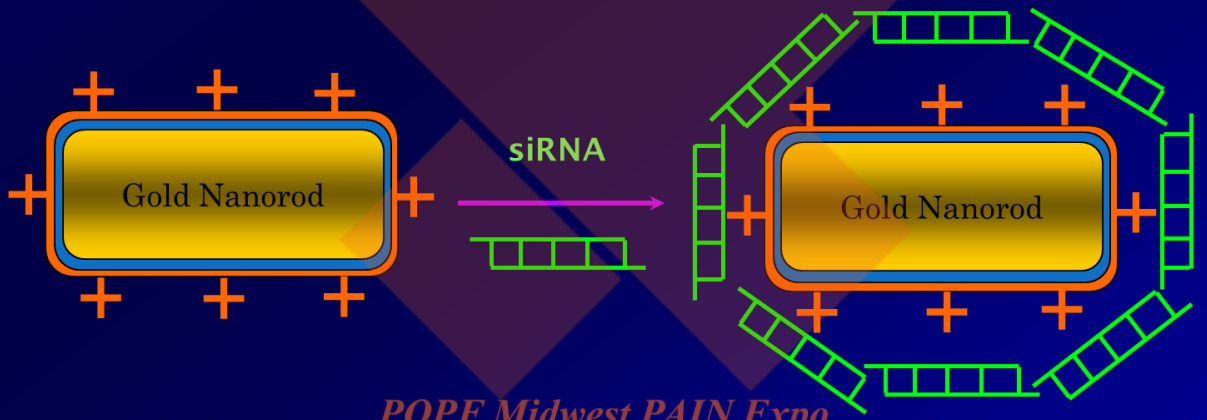
Martuscello RT, Spengler RN, Bonoiu AC, Davidson B, Helinski J, Ding H, Mahajan S, Kumar R, Bergey EJ, Knight PR, Prasad PN, Ignatowski TA. Increasing TNF levels solely in the rat hippocampus produces persistent pain-like symptoms. PAIN 153:1871-1882, 2012.

Conclusions

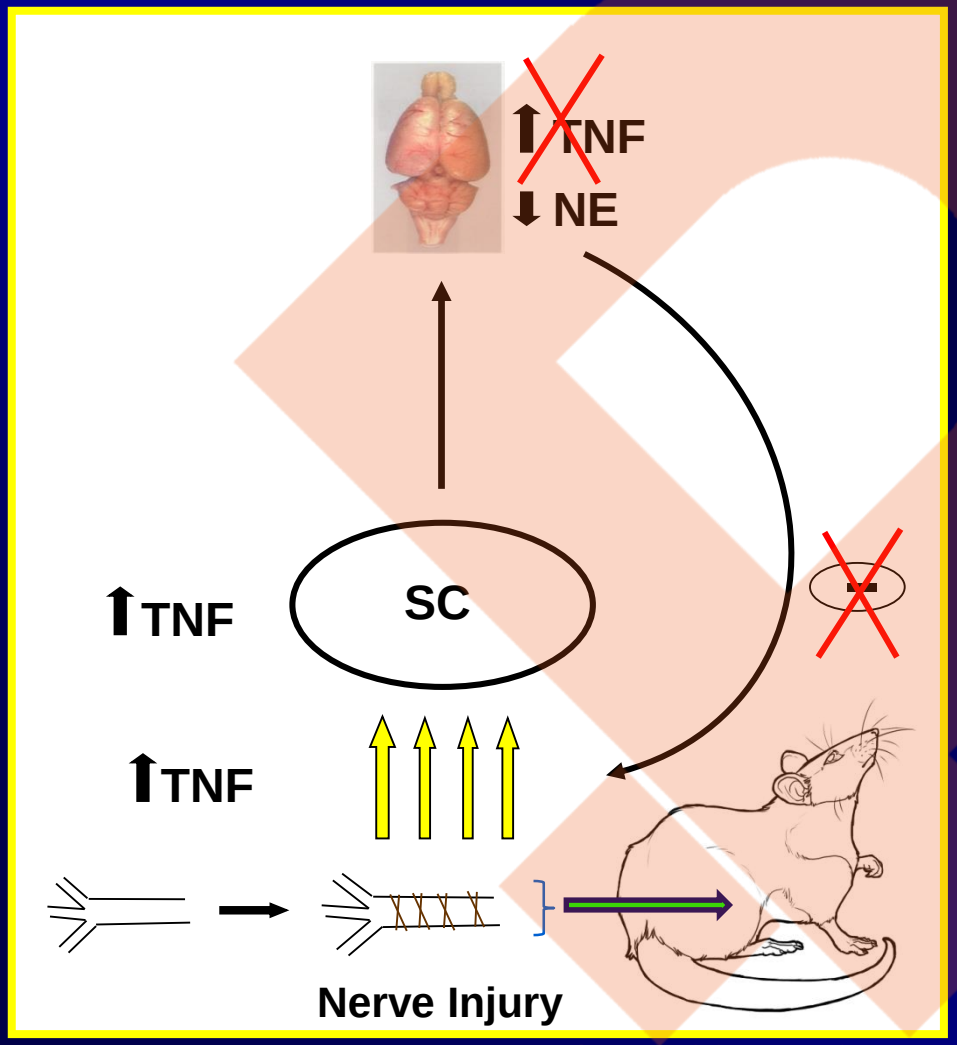
Sustained increases in the levels of TNF in the hippocampus induce chronic pain

Is a molecular imbalance in TNF levels <u>necessary</u> to produce neuropathic pain in the CCI model?

TNF siRNA^{Cy3}



CCI Neuropathic Pain Model



Ignatowski TA, Covey WC, Knight PR, Severin CM, Nickola TJ, Spengler RN. Brain-derived TNFα mediates neuropathic pain. **Brain Res** 841:70-77, 1999.

Covey WC, Ignatowski TA, Knight PR, Spengler RN. Brain-derived TNFα: Involvement in neuroplastic changes implicated in the conscious perception of persistent pain. **Brain Res** 859:113-122, 2000.

Covey WC, Ignatowski TA, Renauld AE, Knight PR, Nader ND, Spengler RN. Expression of neuron-associated TNFα in the brain is increased during persistent pain. **Reg Anesth Pain Med** 27:357-366, 2002.

Ignatowski TA, Reynolds JL, Sud R, Knight PR, Spengler RN. The dissipation of neuropathic pain paradoxically involves the presence of tumor necrosis factor-α (TNF). **Neuropharmacology** 48:448-460, 2005.

Sham (8-21)

CCI (6-21)

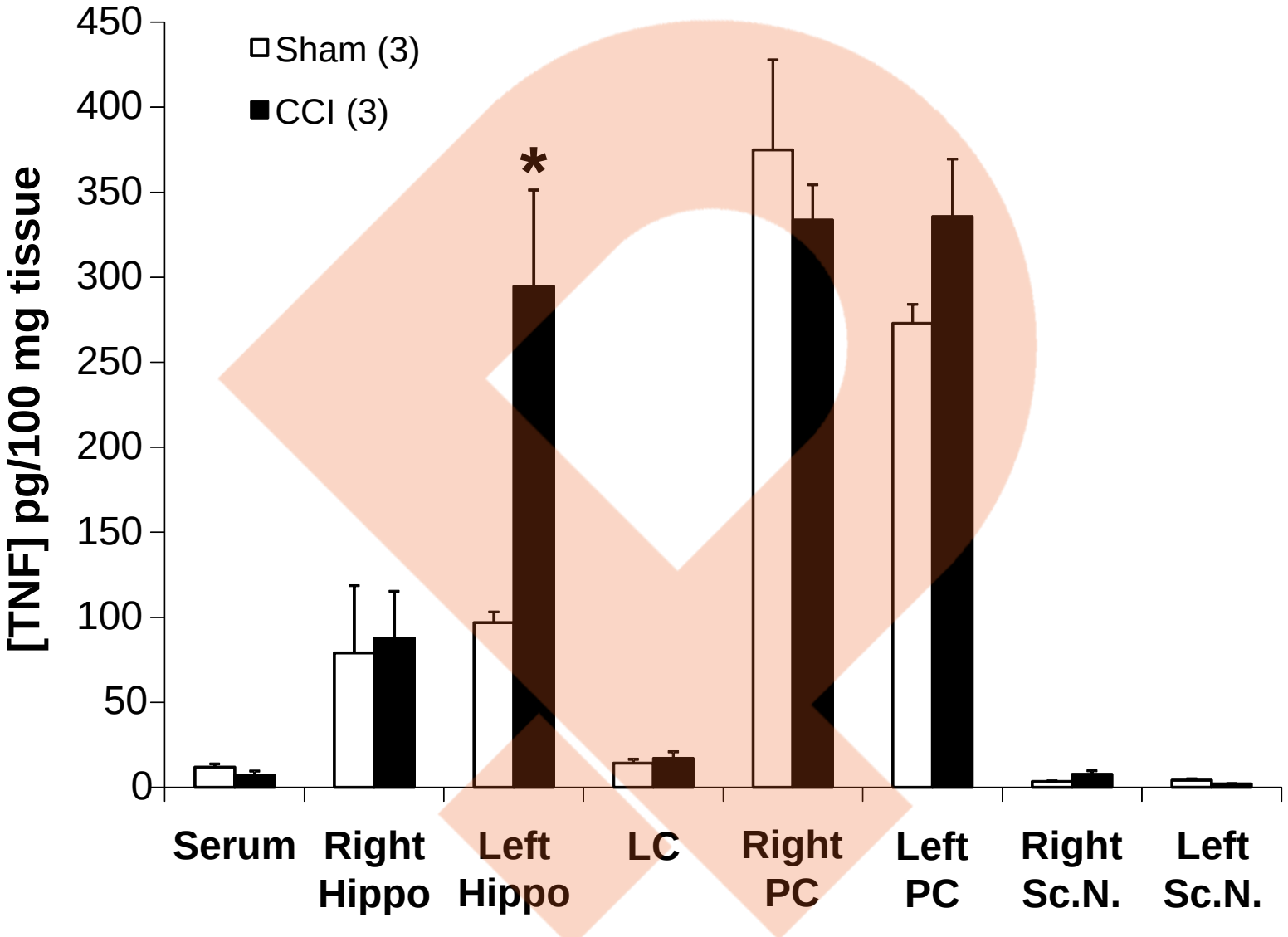
Difference Score

2
1
0
-1
-2
-3
-4
-5
-6
-7

***
***
***
***
***

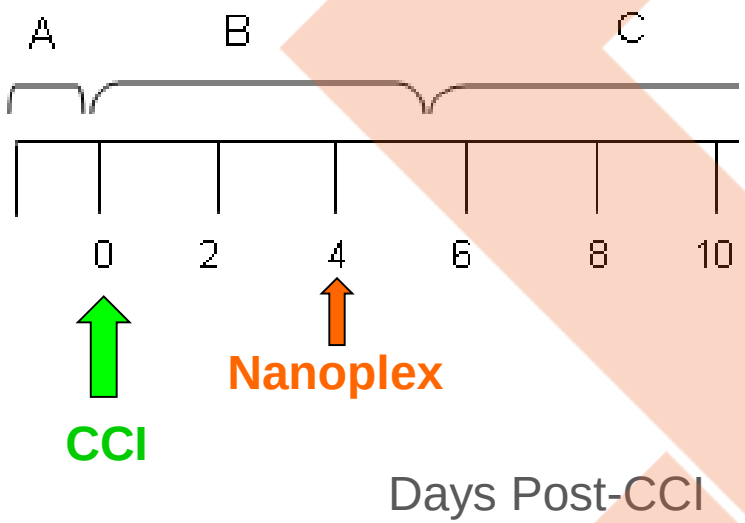
0
2
4
6
8
10

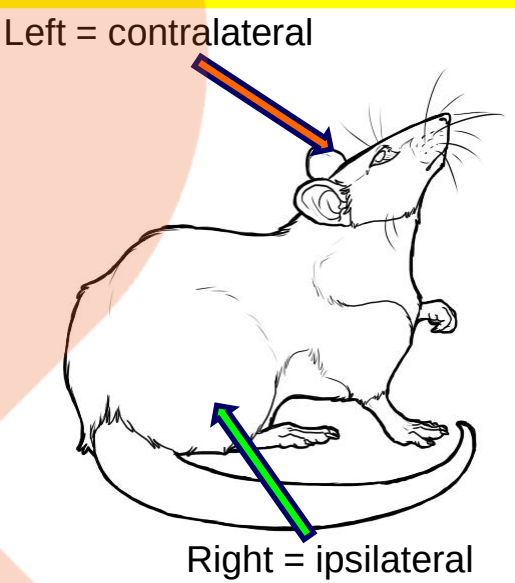
Day Post-Surgery



Gerard E, Spengler RN, Bonoiu AC, Davidson BA, Mahajan SD, Ding H, Kumar R, Prasad PN, Knight PR, Ignatowski TA. Chronic pain is relieved by nanomedicine-mediated decrease of hippocampal TNF. **PAIN** 156:1320-1333, 2015.

Nanoplex Treatment Paradigm





Sham (8-21)
CCI (6-21)
Sham + control nanoplex (7)
CCI + control nanoplex (6)

Microinjection

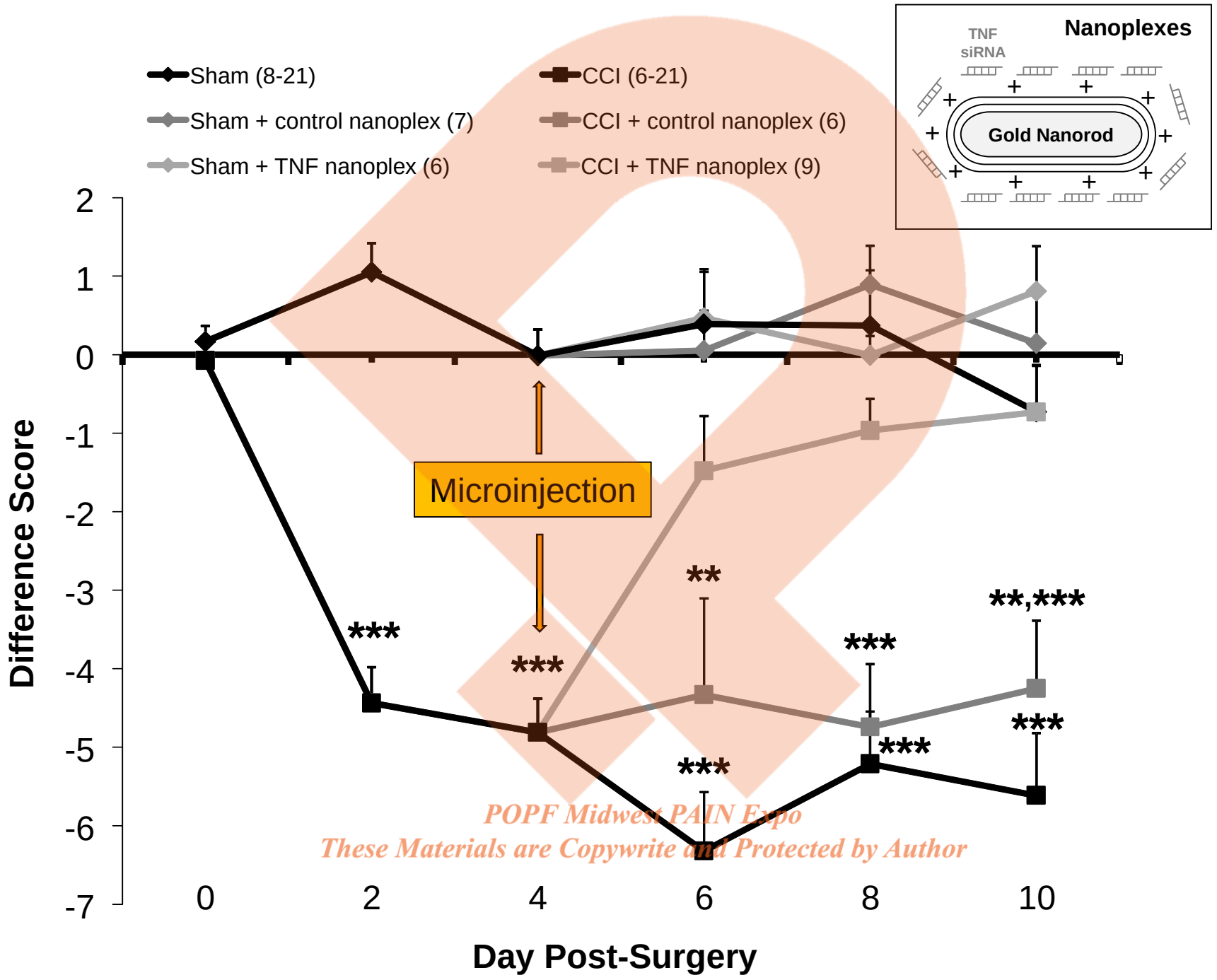
***
***
**
***
***
,*
***

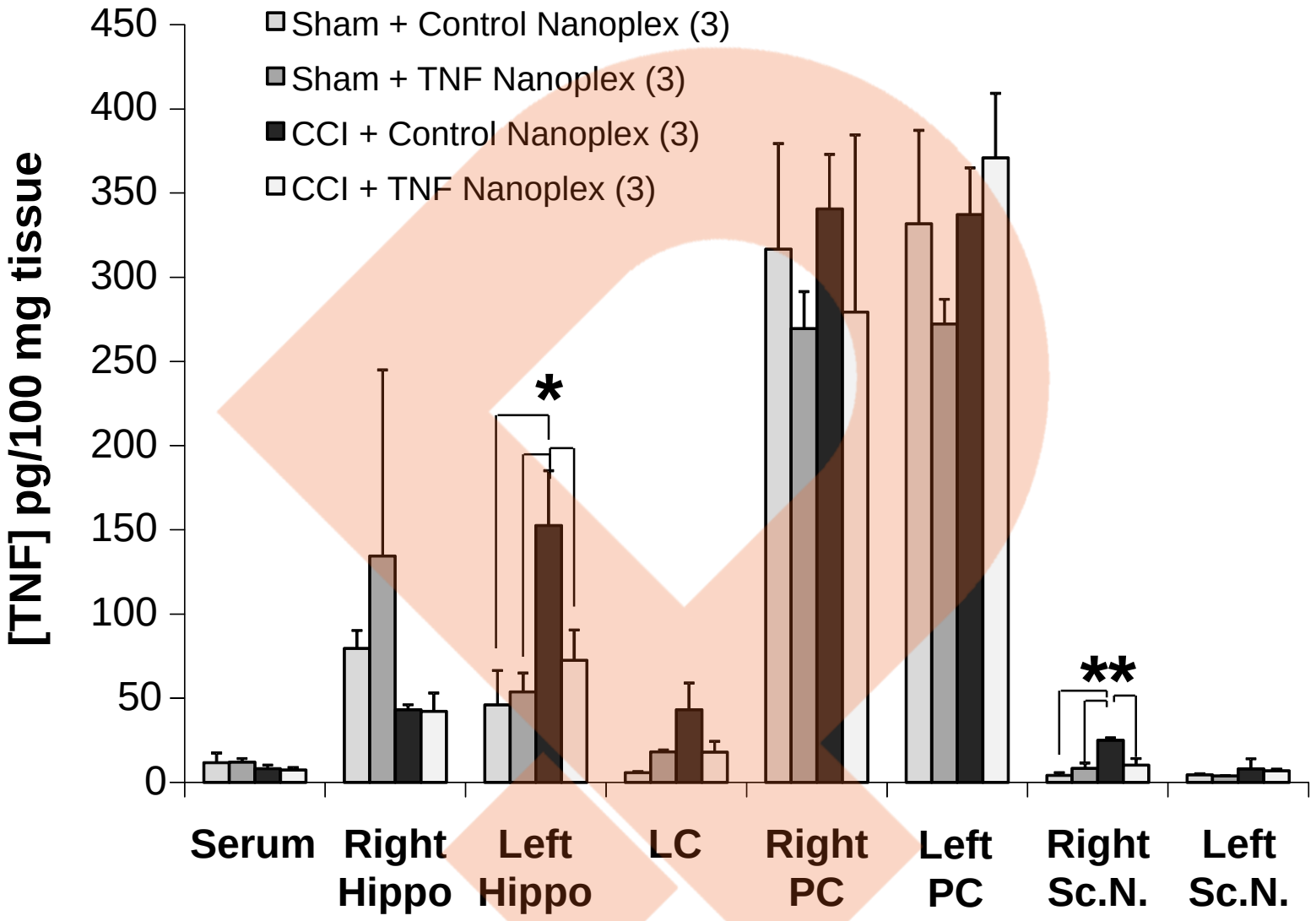
Difference Score

2
1
0
-1
-2
-3
-4
-5
-6
-7

0
2
4
6
8
10

Day Post-Surgery


Sham (8-21)
CCI (6-21)
Sham + control nanoplex (7)
CCI + control nanoplex (6)
Sham + TNF nanoplex (6)
CCI + TNF nanoplex (9)
Nanoplexes
TNF siRNA
Gold Nanorod
Microinjection
Difference Score
Day Post-Surgery
2
1
0
-1
-2
-3
-4
-5
-6
-7
0
2
4
6
8
10
***
***
**
***
***
,*
***
POPF Midwest PAIN Expo
These Materials are Copywrite and Protected by Author



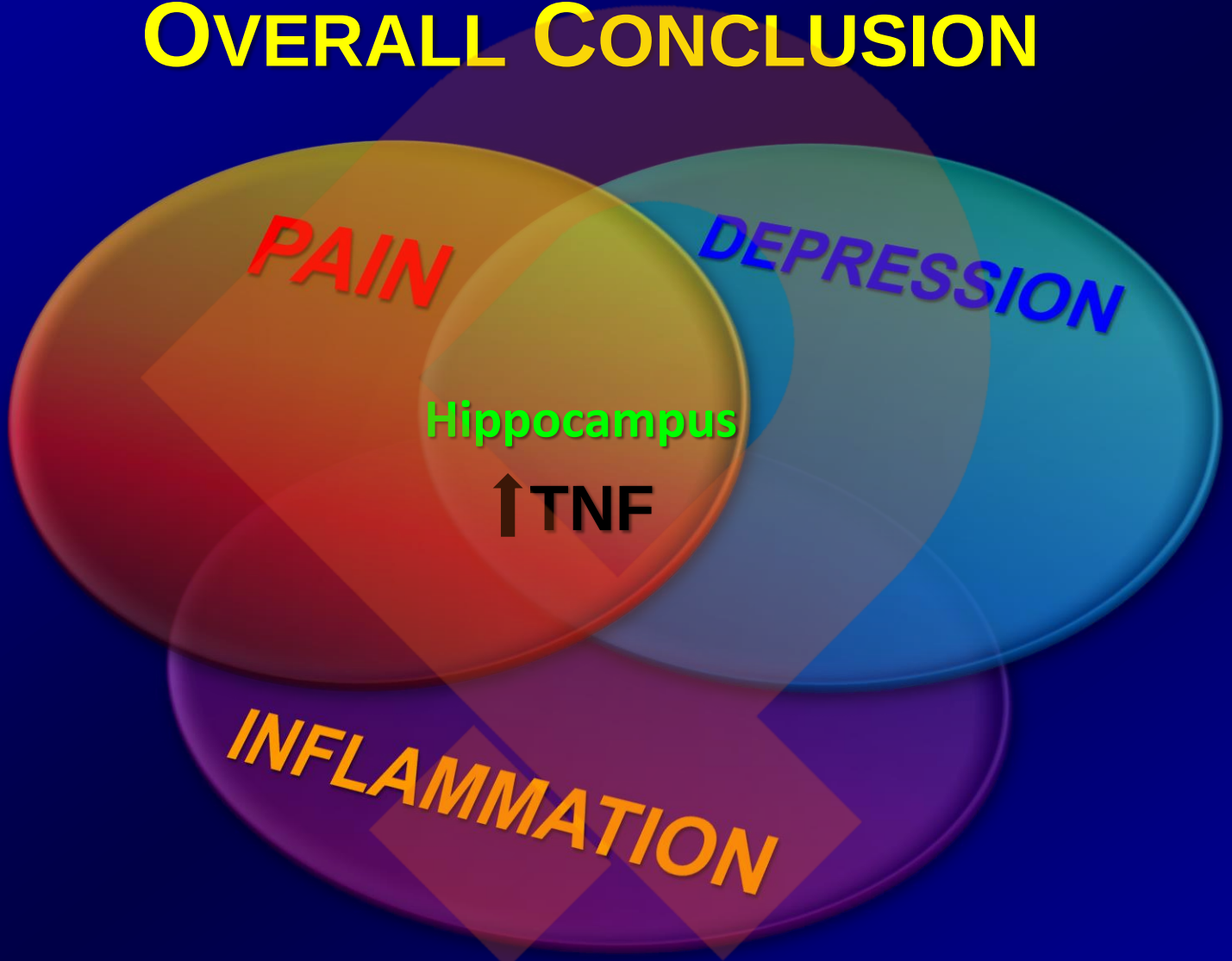
Conclusions

- Rats undergoing neuropathic pain and displaying characteristic chronic pain symptoms that received a single hippocampal microinjection of TNF nanoplexes showed:
  - Complete alleviation of pain (thermal hyperalgesia).
  - Transient alleviation of pain (mechanical allodynia).

- CCI-induced enhanced levels of TNF (bioactivity) within the contralateral hippocampus were reduced to control levels following TNF nanoplex microinjection.

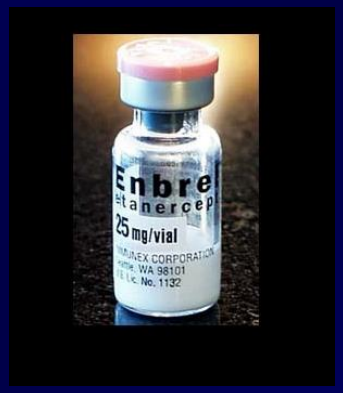
Decreasing elevated levels of TNF specifically in the brain may provide effective relief of chronic pain.

Overall Conclusion



Fasick V, *et al*., Neurosci Biobehav Rev 53: 139-159, 2015

Neuropathic Pain: CRPS



Current Treatments

NSAIDS
Morphine derivatives
Tricyclic Antidepressants
Anti-convulsants

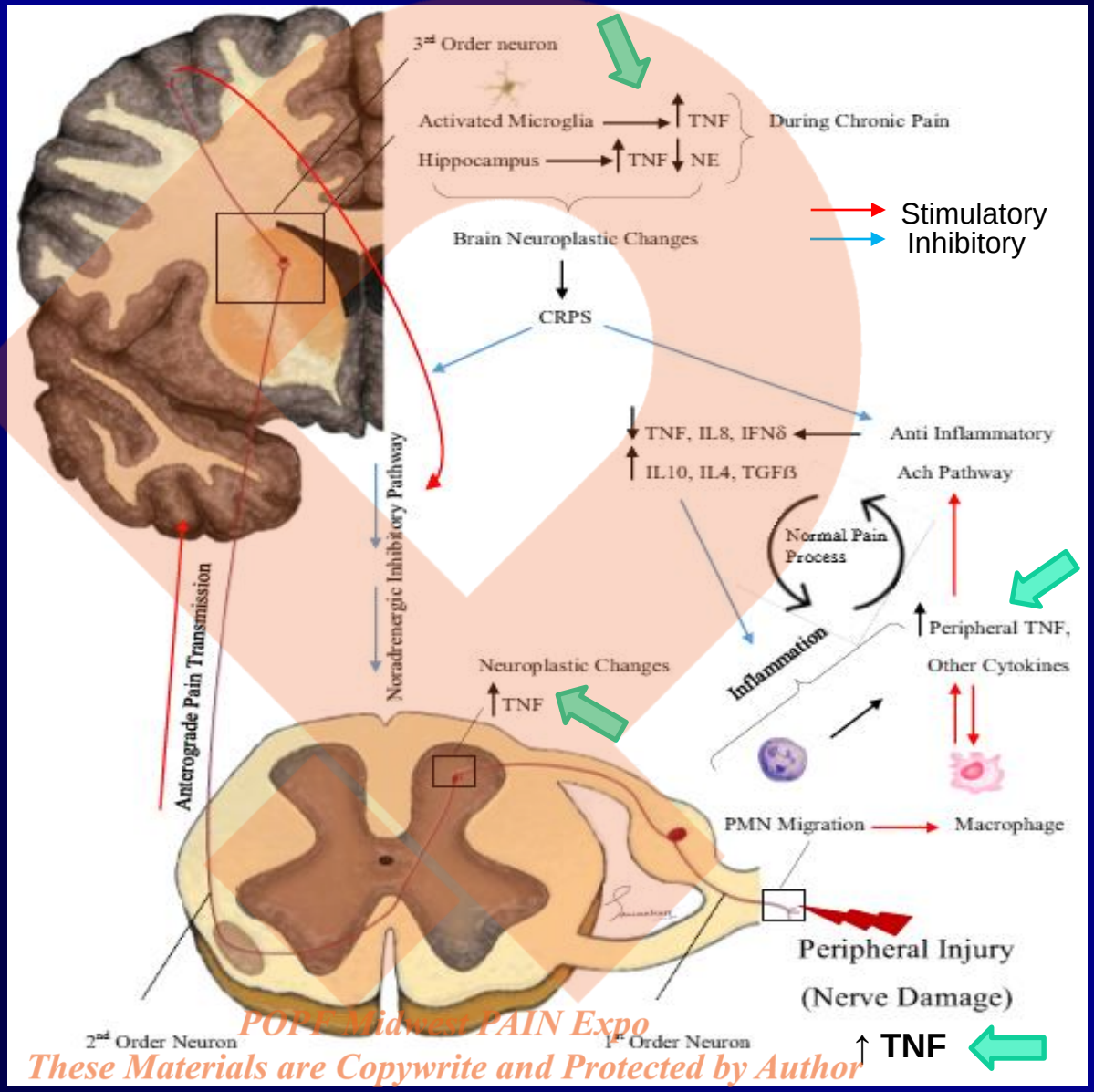
Alternative Therapies

NMDA Antagonists
Electrical Stimulation
HBOT
Anti-TNF Molecules

Ongoing Studies & Future Directions

- TNFR; Transcription factors involved in TNF signaling pathway
- Antidepressant/antinociceptive mechanism of action
  - Atypical agents/alternative therapies
  - Affective component of analgesics
    - CPP
- Systemic nanoplex administration
  - intravenous; intrathecal; perispinal

TNF in CRPS



Ignatowski TA, Samankan S, and Spengler RN. Molecular Pathophysiology and the Role of TNF in the Neuro-inflammatory Reflex. In: CRPS: Past, Present & Future; Nader, N.D. and Visnjevac, O. (Eds). NOVA Science Publishers, Inc., Hauppauge, New York 2015. *In press*.

Acknowledgements

Collaborators
Robert N. Spengler, PhD
Paul R. Knight, MD, PhD
Bruce A. Davidson, PhD
Paras N. Prasad, PhD
Adela Bonoiu, PhD
Supriya D. Mahajan, PhD

Students
Reeteka Sud, PhD
Victoria Fasick, MA, DO
Regina Martuscello, MA, PhD
Elizabeth Gerard, MA
Ashley Re, MA
Ellyce Clonan, BS, MA
Abdel Rahman Alnaji, BS

Technical Assistance
Annette Featherstone, HT ASCP
Shabnam Samankan, MD
Wade Sigurdson, PhD

Funding Support
NIH 5R01NS41352 (RNS, PRK, TAI)
NARSAD (TAI)
United Spinal Assoc (TAI)
UB-MDRF (A. Re; E. Clonan)
UB-CURCA (A. Alnaji)
Dept. PAS