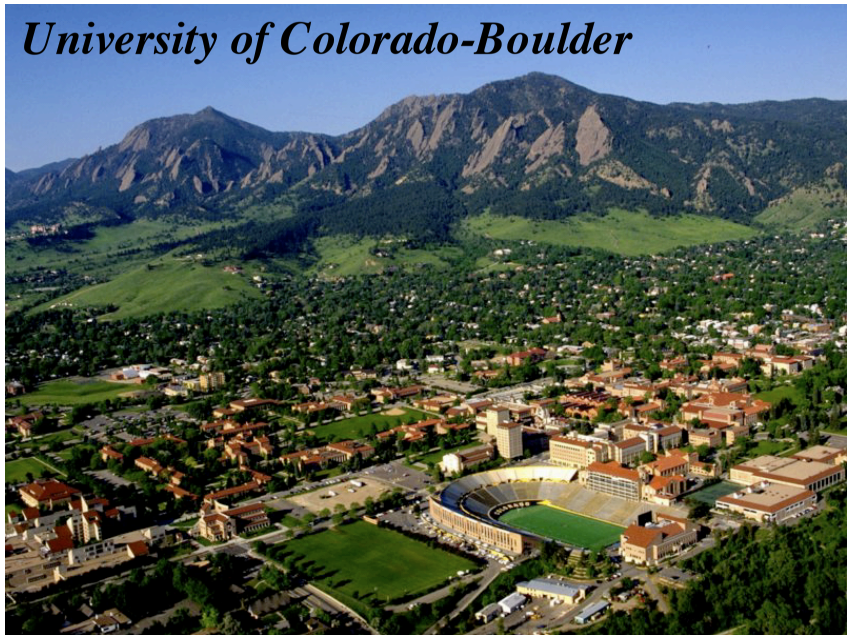
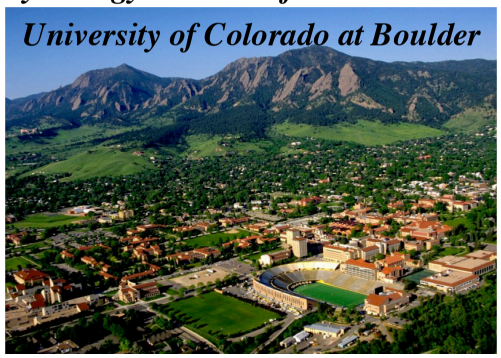



1

# *Glia as the Bad Guys:*
## *Implications of Glial Activation for Gulf War Illness*

**Linda R. Watkins**
***Psychology & Center for Neuroscience***
***University of Colorado at Boulder***



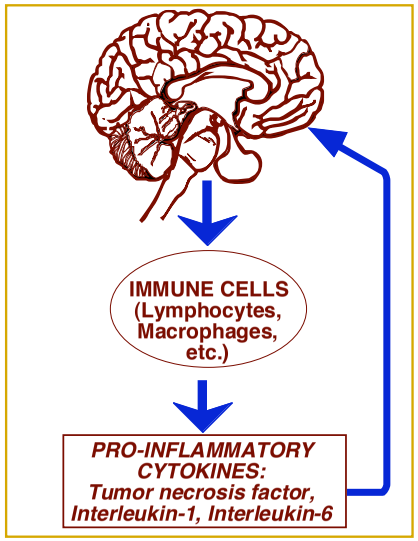
2

# *Global Concepts*

- **Views of pathological pain are changing**
- **New players in *pain*: Immune cells in & around peripheral nerves; Glia in spinal cord & brain**
- **New players in *opioid & alcohol actions*: Glia disrupt efficacy of opioids; enhance alcohol**
- **Glial "priming" by immune changes & CNS damage: setting the stage for Gulf War Illness?**
- **Drugs on the horizon?**
- **Is all of this a dysregulation of an ancient survival-oriented circuitry?**

3

## ***Bi-Directional* Immune/Brain Communication**




Maier & Watkins
*Psych. Review,*
1998

4

# *The Sickness Response*

Created By The

*Brain*

To Help you Survive
Viral & Bacterial Infections

5

# *The Sickness Response*

❖ ***Physiological Adjustments***

- ♦ **Fever**
- ♦ **Reduced carrier proteins**
- ♦ **Increased acute phase proteins**
- ♦ **Increased white blood cells; altered ions**
- ♦ **Increased sleep**
- ♦ **Etc.**

❖ ***Stress Response***

- ♦ **HPA activation**
- ♦ **Sympathetic nervous system activation**

6

# *The Sickness Response*

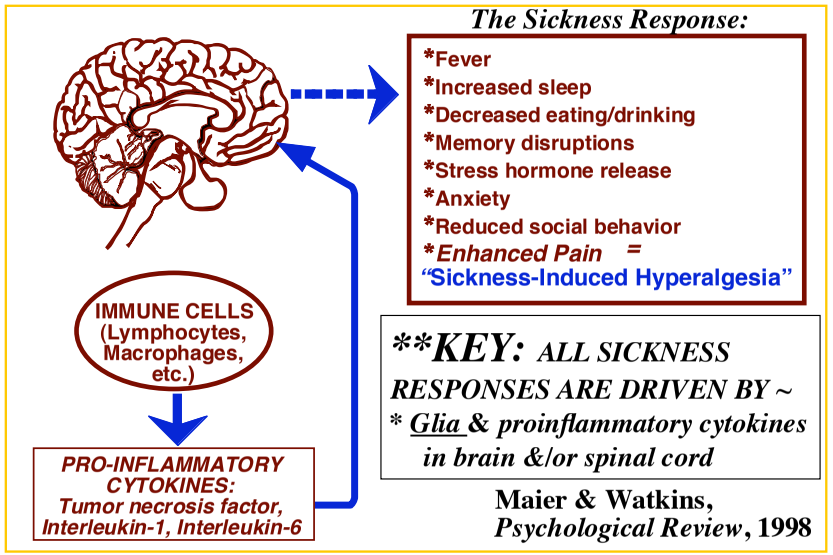
❖ ***Behavioral Adjustments***

- ♦ **Reduced food and water intake**
- ♦ **Reduced activity and exploration**
- ♦ **Reduced social behavior**
- ♦ **Reduced sexual behavior**
- ♦ **Disrupted learning & memory**
- ♦ **Anxiety**
- ♦ ***Enhanced pain responses***
- ♦ **Etc.**

7

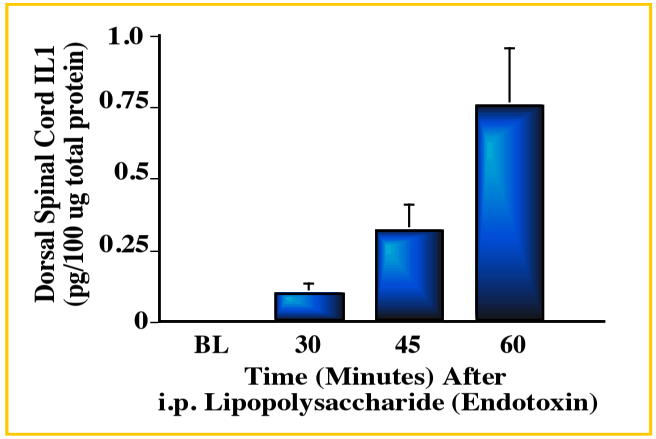


# *Immune-to-Brain Communication*



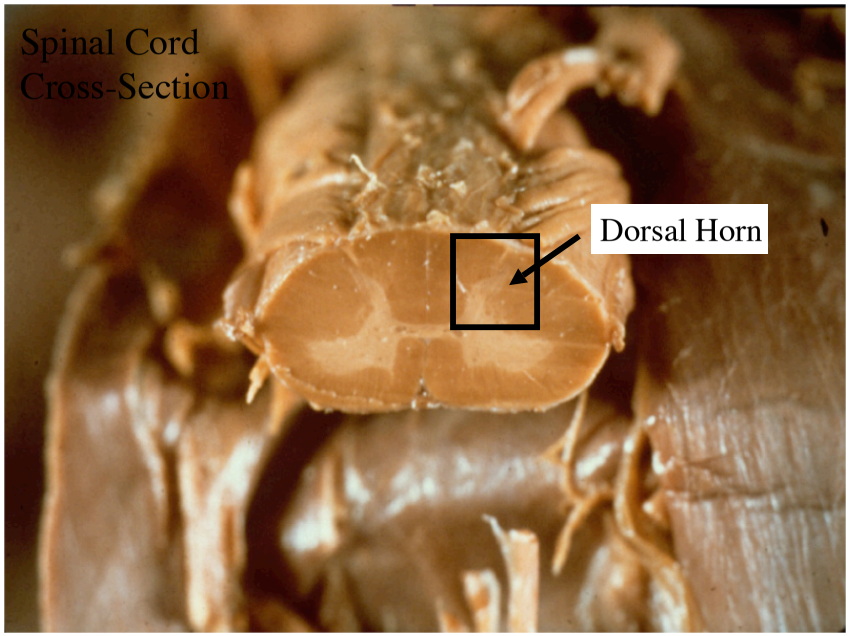

8

# *Abdominal Inflammation Rapidly Increases IL-1 in Dorsal Spinal Cord*



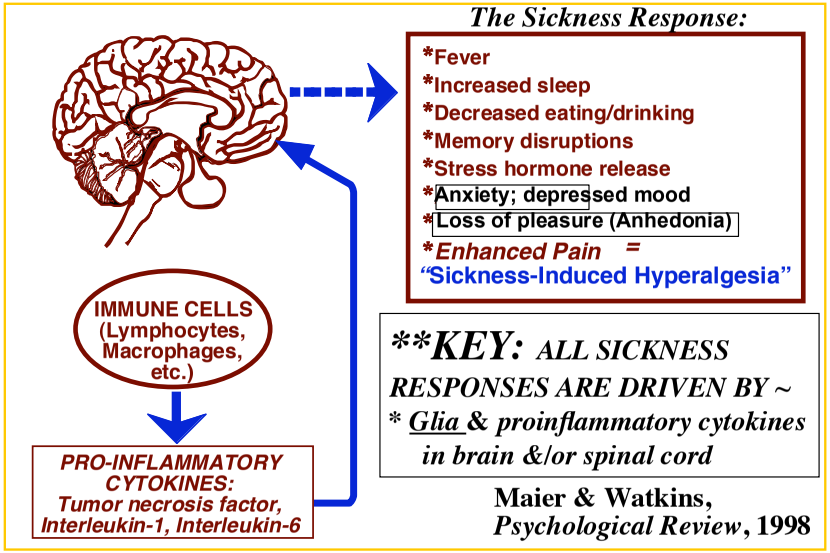

*Watkins, Milligan & Maier, In: Adv. Pain Res. Therapy, 2003*

9




10

# *Immune-to-Brain Communication*




11


12


13

## *Based on the Strength of the Glial Story for Pain, Across Labs & Across Diverse Animal Models: Translation to Clinical Trials for Neuropathic Pain*

*Avigen:*

**AV411 (oral Ibudilast; now MediciNova's MN-166)** - *dose ranging Phase II trial in Australia completed; approved for Phase II neuropathic pain trial in the US (Columbia Univ./ NIDA opioid trial ongoing )*

*Xalud Therapeutics:*

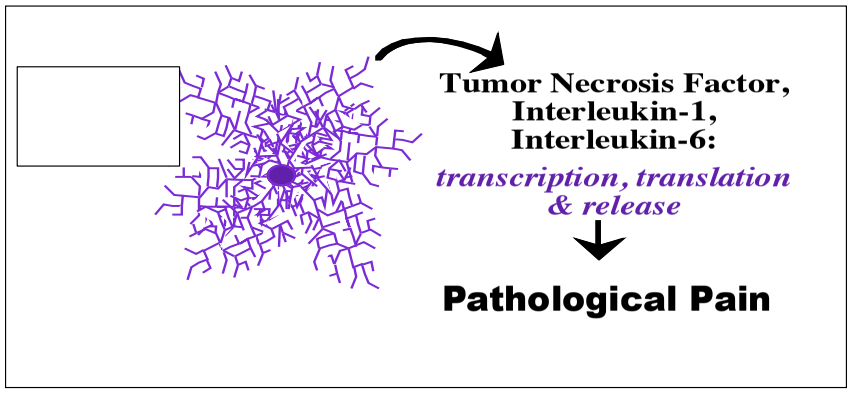
**XT101 (intrathecal Interleukin-10 DNA Therapy)**
*Investigational New Drug application upcoming*

*Adenosine Therapeutics/PGxHealth:*

**ATL313 (intrathecal drug that drives Interleukin-10)**
*In preclinical development*

14

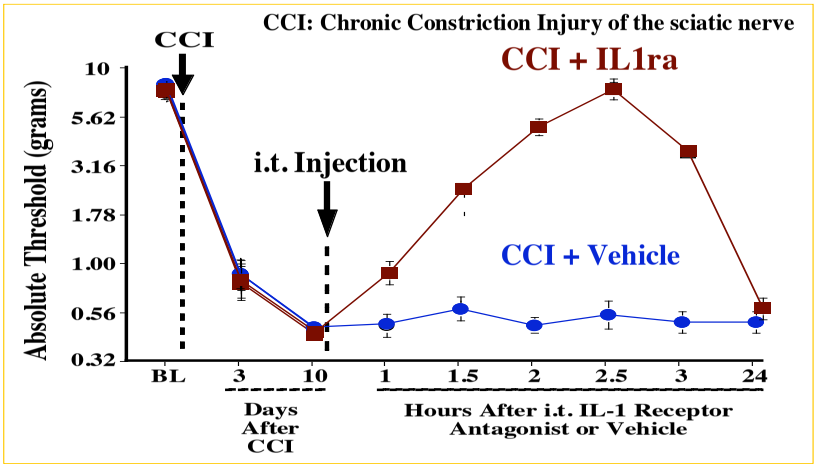
## *Glial Proinflammatory Cytokines: Major Players in Pathological Pain*




**Milligan et al., *Journal of Neurosci. '01;* Holguin et al. *Pain*, '04; Ledeboer et al. *Pain*, '05**

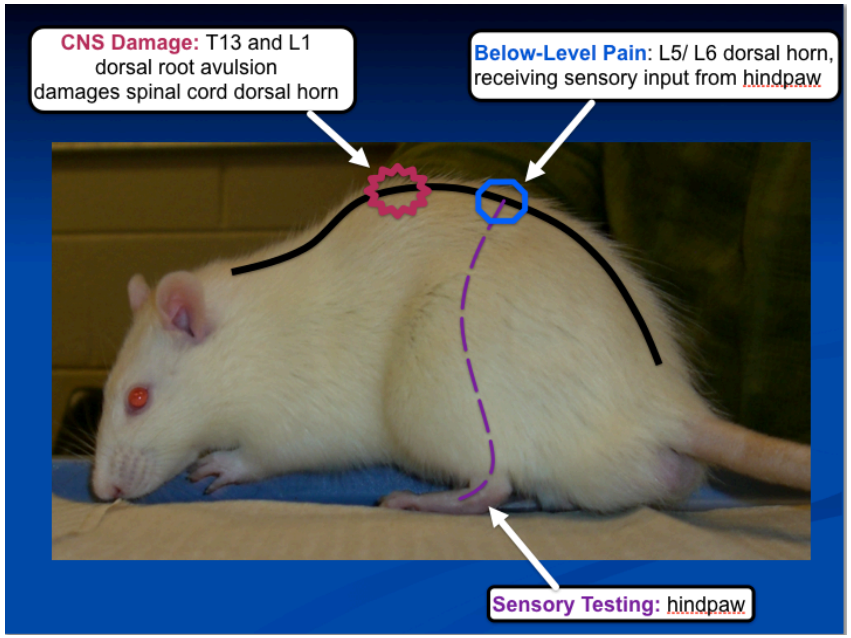
15

## *i.t. Interleukin-1 Antagonist Reverses Allodynia From CCI (even 2 mo.later!)*




**Milligan et al. *Eur. Journal of Neuroscience*, 2005**

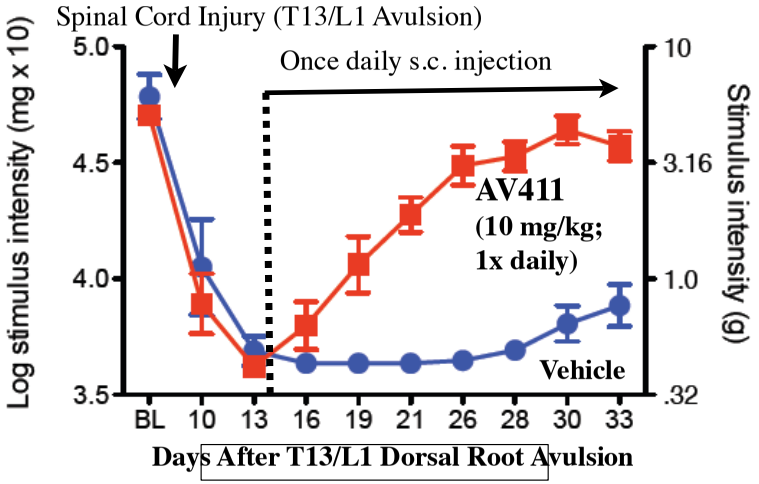
16




17

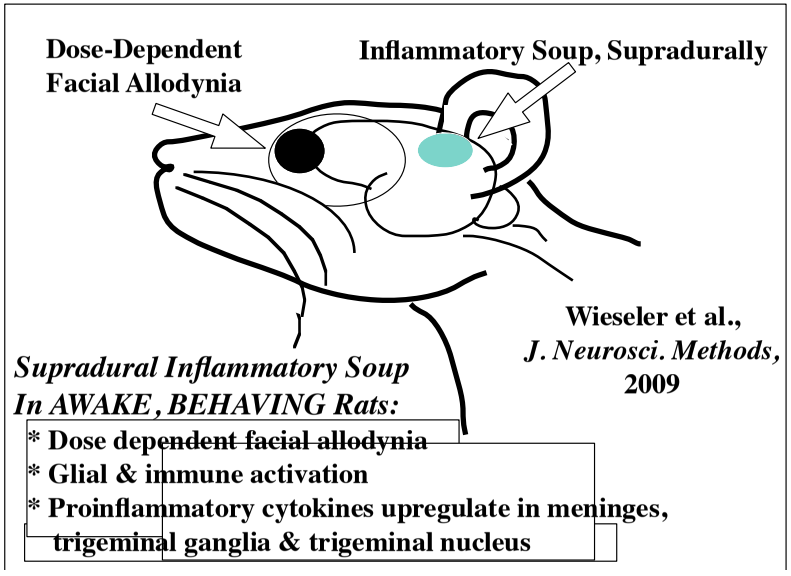
## *s.c. AV411, a Blood-Brain Permeable Glial Activation Inhibitor, Reverses Pain of Central Origin*

**Wieseler et al., 2010 MS in review**



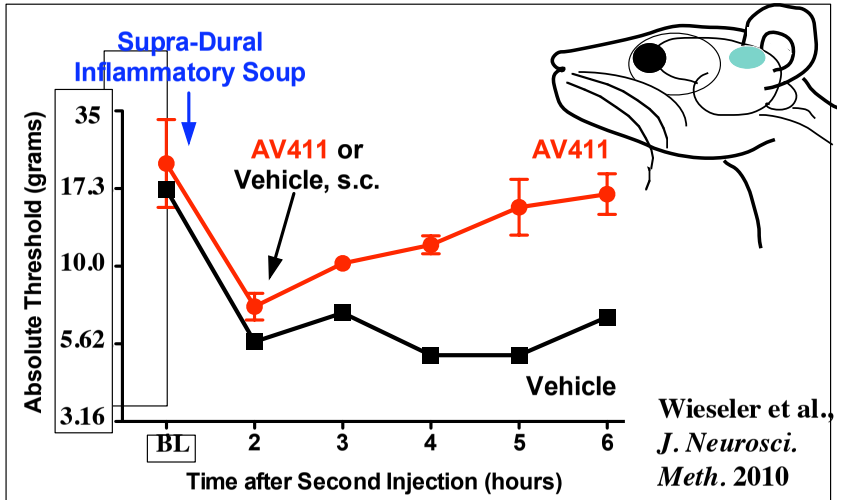

18

## *Relevance to Headache?*




19

## *Facial Allodynia following Supra-Dural Inflammatory Soup: Reversal by a Systemically Administered Immune/Glial Activation Inhibitor (AV411)*

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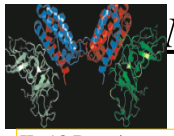
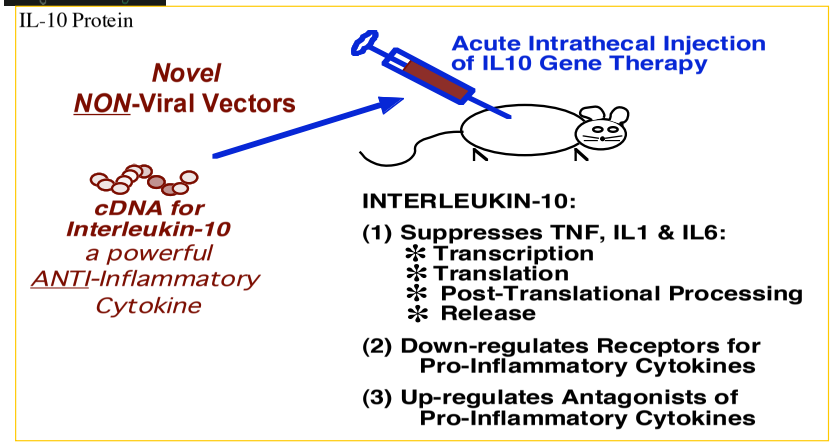


## *Non-Viral Gene Therapy to Induce the CNS Release of Interleukin-10*



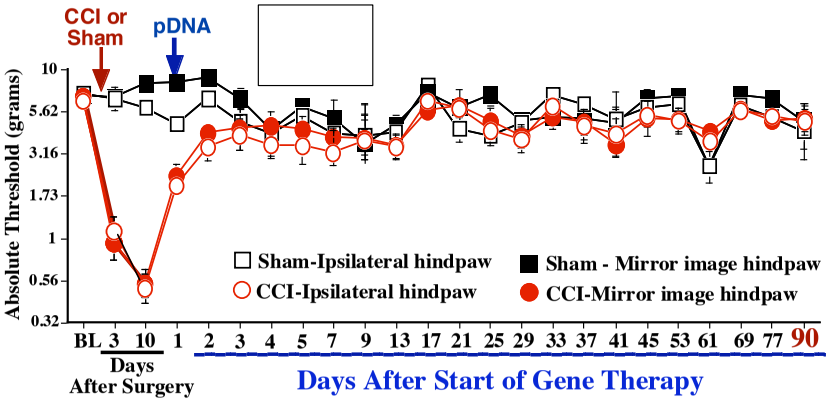

*And - Spinal cord neurons are "blind" to IL10 as they don't express receptors for it! (Ledeboer et al., J. Neuroimmunology '03)*

21

## *Intrathecal Non-Viral IL-10 Gene Therapy (XT101) Reverses CCI-Induced Neuropathic Pain For 3+ Months*

**Sloane et al., *Gene Therapy* 2009**

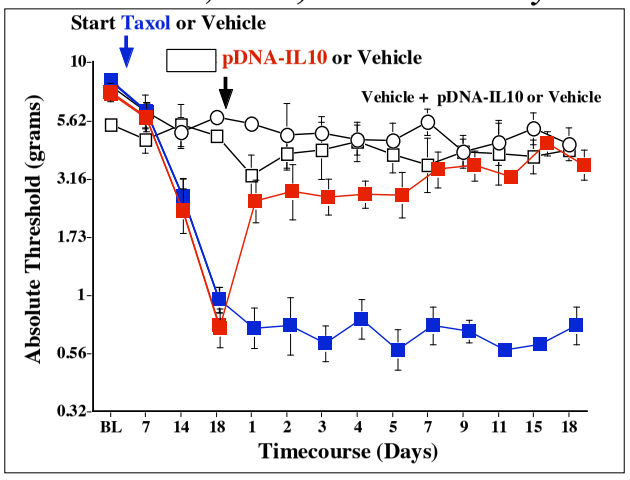
**Soderquist et al., *Pharmaceutical Research* 2010**




22

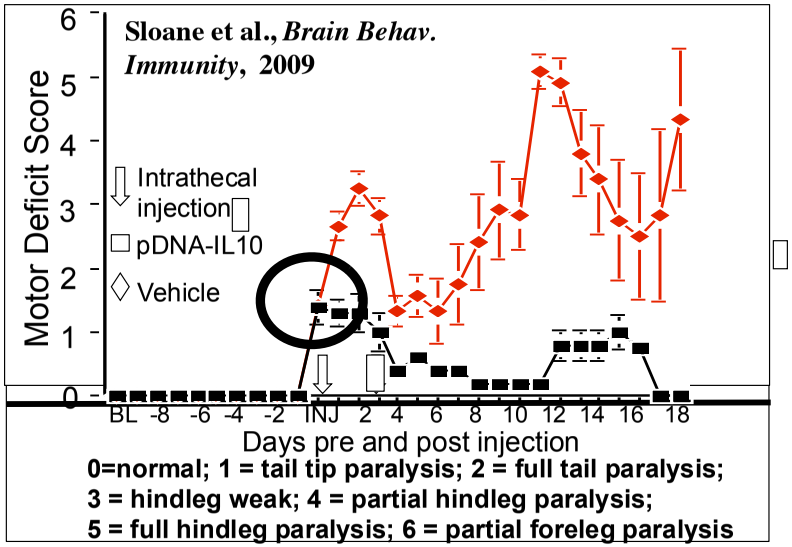
## *Reversal of Chemotherapy-Induced Neuropathic Pain by i.t. Non-Viral IL10 Gene Therapy (XT101)*

**Ledeboer et al., *Brain, Behav. Immunity* 2007**




23

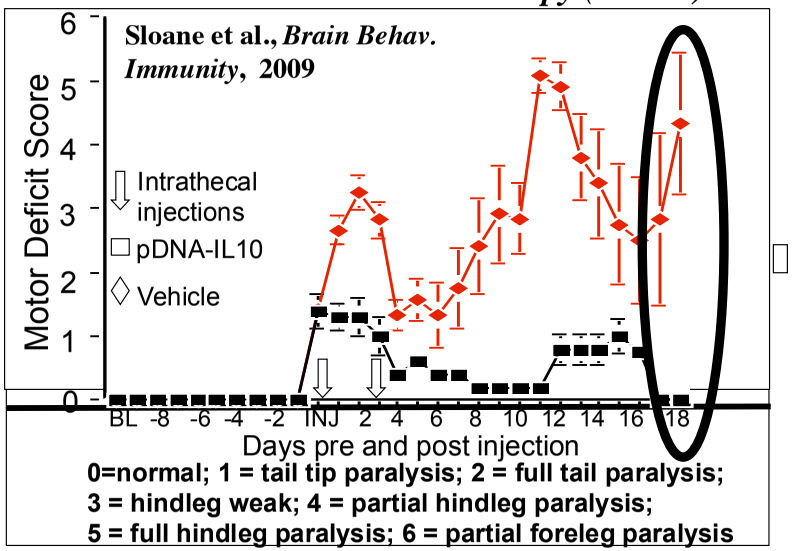
## *Blockade of Rat Multiple Sclerosis (EAE) Paralysis by i.t. Non-Viral IL-10 Gene Therapy (XT101)*




24


25

## *Blockade of Rat Multiple Sclerosis (EAE) Paralysis by i.t. Non-Viral IL-10 Gene Therapy (XT101)*




26

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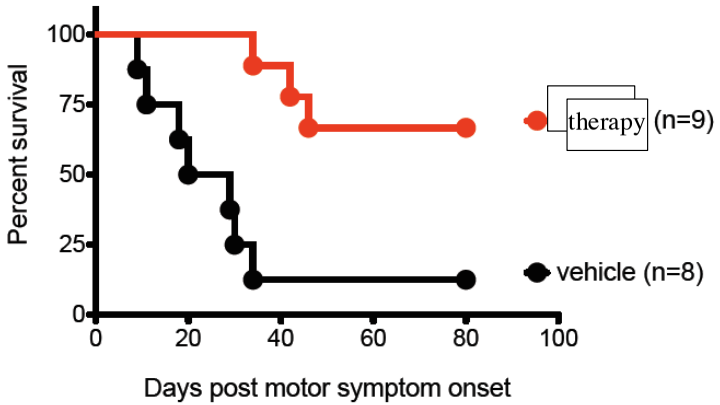
## *Intrathecal IL-10 is Good for You:*

***Single* intrathecal dose of another IL-10 perseverantly inducing drug suppresses paralysis and vastly enhances survival**

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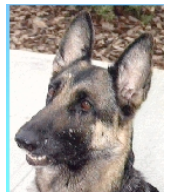
## *Degenerative Myelopathy*



*Chronic, progressive neurodegenerative disease
* Initial signs: thoracolumbar spinal cord disease

## *Similar Conditions in Humans*

- ***Multiple Sclerosis***
  - **Immune-related demyelinating disorder**
- ***Amyotrophic Lateral Sclerosis***
  - **Axonal loss disease**
    - *** Genetic**
    - *** Proinflammatory cytokines**
    - *** Free radicals**

We can access a population of these dogs for testing pDNA-IL10

30



## *Degenerative Myelopathy*

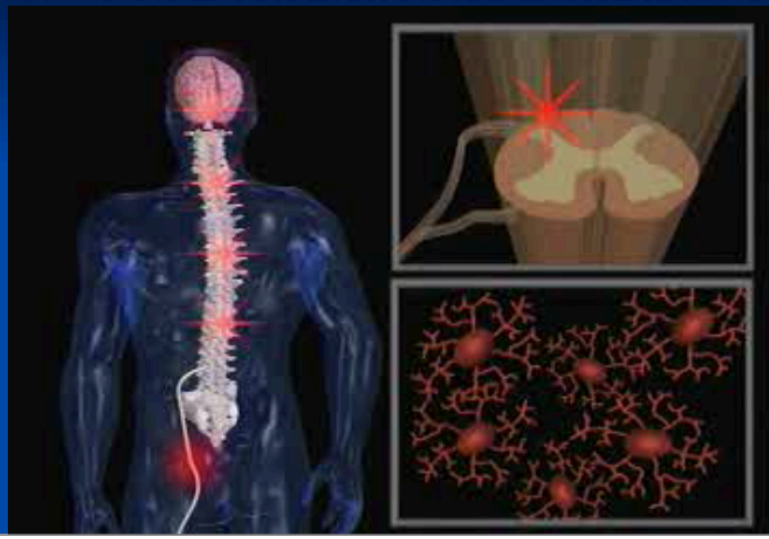
ASYMMETRIC SPASTIC PARAPARESIS AND GENERAL PROPRIOCEPTIVE ATAXIA

from Awano et al., *PNAS* 2000

31

## *How Does i.t. IL-10 Gene Therapy Work?*

***Xalud Therapeutics now developing for clinical trials (XT-101)***



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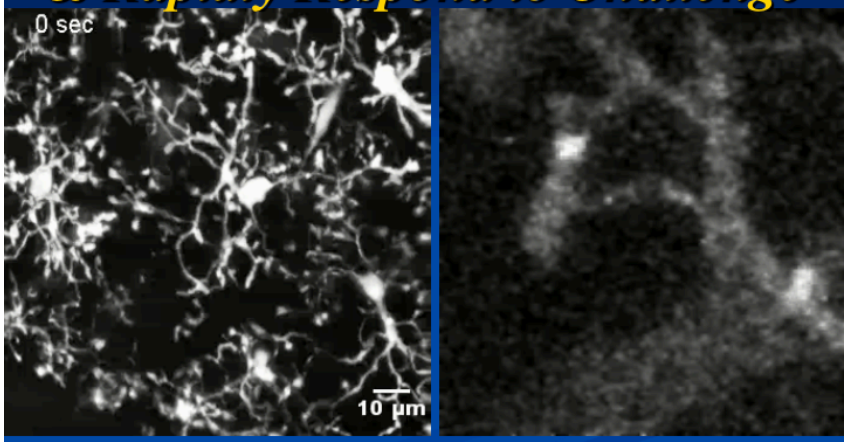
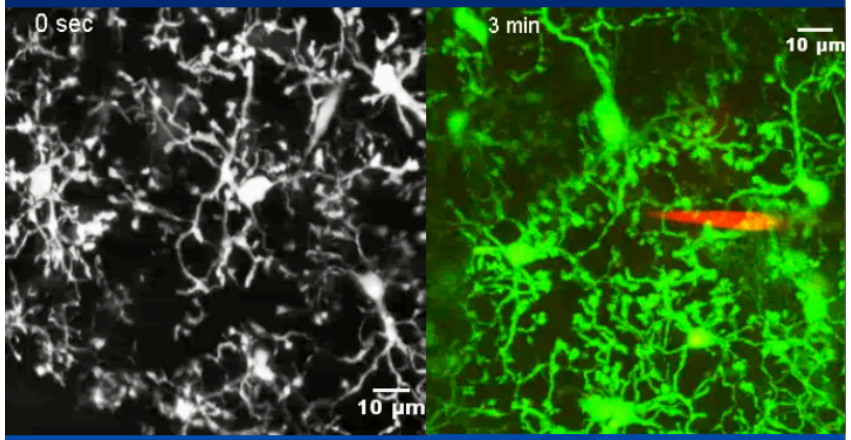
## Microglia Actively Survey the CNS & Rapidly Respond to Challenge




Videos from: Davalos et al., *Nature Neuroscience* supplements, 8 (2005) 752-758; & Nimmerjahn et al., *Science* supplements, 308 (2005) 1314-1318

33

## Microglia Actively Survey the CNS & Rapidly Respond to Challenge




Videos from: Davalos et al., *Nature Neuroscience* supplements, 8 (2005) 752-758

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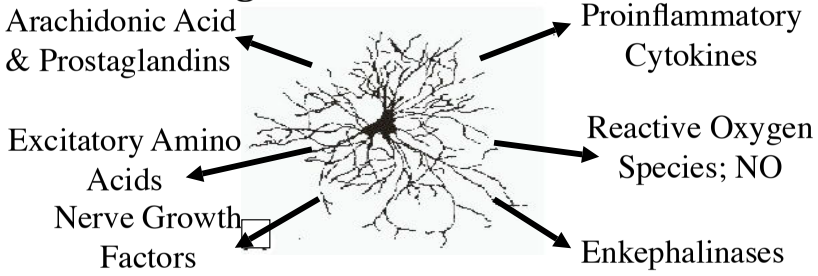
## Glia Release Classic Pain Modulatory Substances

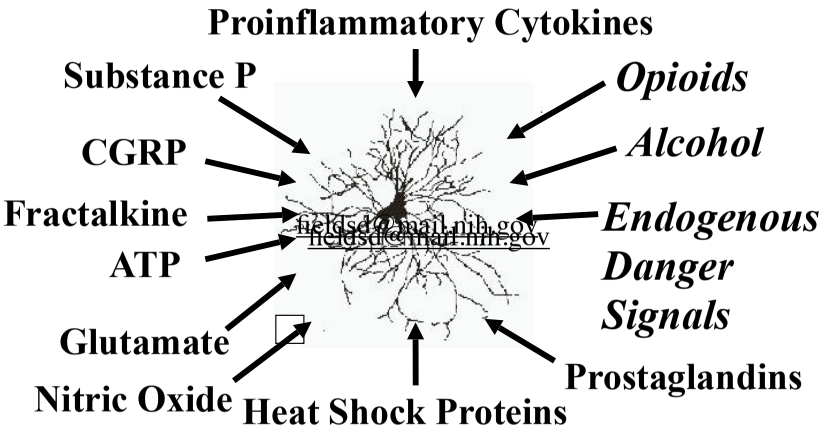
### Activated glia release:




- ***Amplify pain signaling to spinal cord***
- ***Amplify pain transmission to brain:***
  - ***upregulate AMPA & NMDA number/function***
  - ***downregulate GABA & outward K+ currents***

35

## What Activates Glia?




**Watkins & Maier**, *Nature Rev. Drug Disc. '03*

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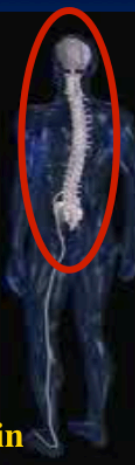
## Glial Dysregulation of Opioids

**Activation of:**
- *Microglia
- *Astrocytes

**Release of:**
- *Interleukin-1
- *Interleukin-6
- *Tumor Necrosis factor
- •Each Enhance Pain
- •Effects Synergize



**Results in:**
- * ↓Analgesia
- * Naïve tolerance
- * ↑Tolerance
- * ↑Dependence
- * ↑Reward
- * ↑Side Effects

Watkins et al., *Trends in Pharmacological Sciences* 2009

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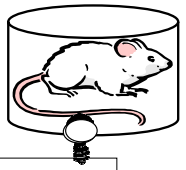
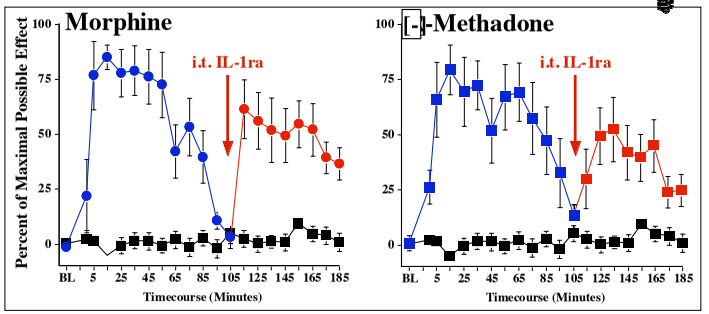
## Glial Activation Opposes Both i.t. Morphine & i.t. Methadone Analgesia






Hutchinson et al., *Brain Behavior & Immunity*, '08

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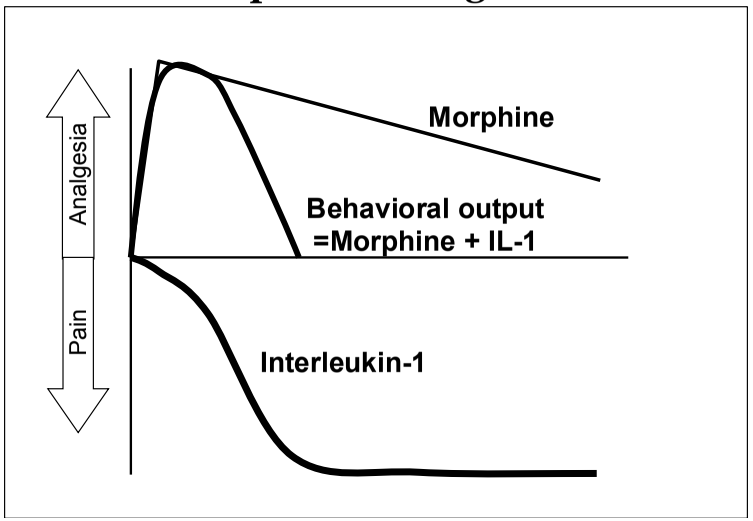
## Intrathecal IL-1ra Unmasks Morphine Analgesia

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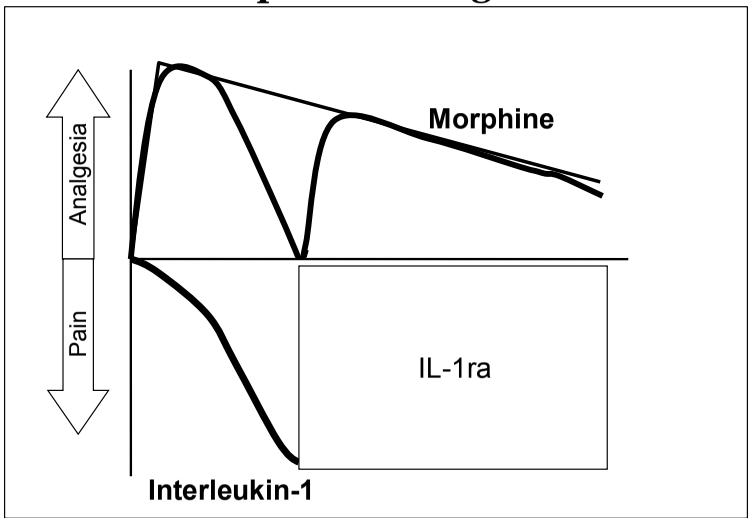
## Intrathecal IL-1ra Unmasks Morphine Analgesia




40

***For Opioids, like Morphine:***
***Current Data Strongly Predict That***
***Blocking Glial/Immune Activation Will:***

- ❖Improve opioid analgesia
- ❖Suppress opioid tolerance
- ❖Suppress opioid dependence
- ❖Suppress opioid reward linked to drug craving/drug seeking
- ❖Suppress respiratory depression

*..... and it won't just be for opioids (e.g. alcohol)*
Watkins et al., *Trends in Pharmacological Sciences* 2009

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# *So...what is this Mystery Receptor?*

***To target it, one must know what it is***

**Toll-Like Receptor-4 (TLR4):**
***Classically....***
**"not me, not right, not OK" receptors**
**TLR4 detects:**
***bacteria (lipopolysaccharide; LPS)**
***endogenous danger signals (damage/death)**
***Relevant to Gulf War Illness?***

**** All classes of opioids used clinically***
Hutchinson et al., *TSWJ* 2007; *Br Behav Immun* 2008

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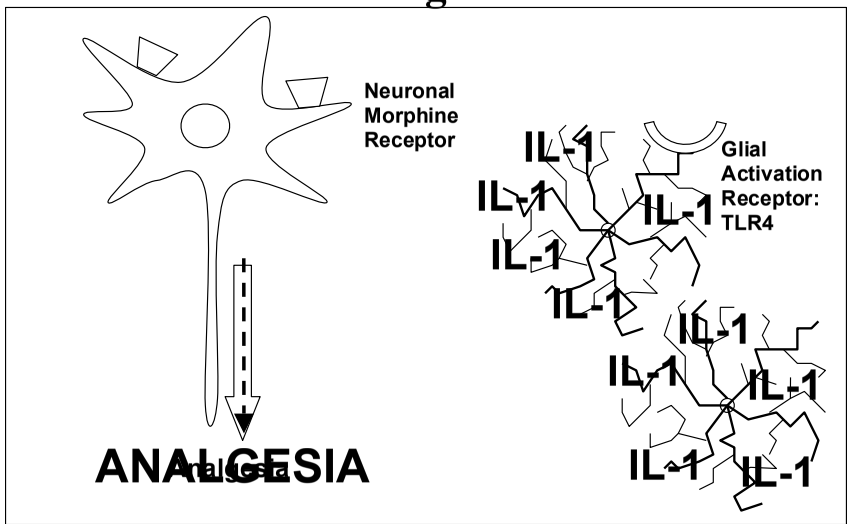
## Opioid Activation of Glia Suppresses Analgesia

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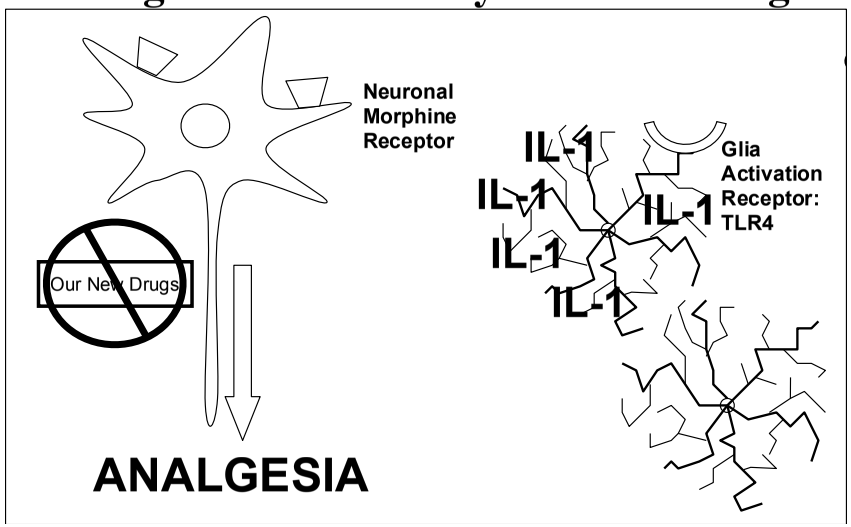
## Opioid Activation of Glia Suppresses Analgesia: Blockade by Our New Drugs

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## *Glial TLR4*

***~ the "not me, not right, not okay" receptor ~***

***is also activated by Endogenous Danger Signals that drive Neuropathic Pain***

If that's True, then ....

* Might that suggest that blocking TLR4 can do more than just potentiate opioid analgesia?

* Might our novel blood-brain barrier permeable TLR4 antagonists also be stand-alone treatments for pathological pain?

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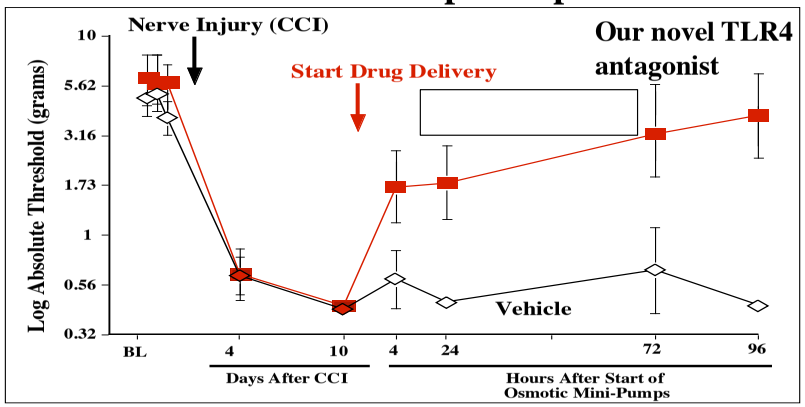
## *Toll Like Receptor-4 (TLR4):*

**Our novel orally available, blood brain barrier permeable TLR4 antagonist, reverses neuropathic pain**




Hutchinson et al., *Eur. J. Neurosci.* 2008

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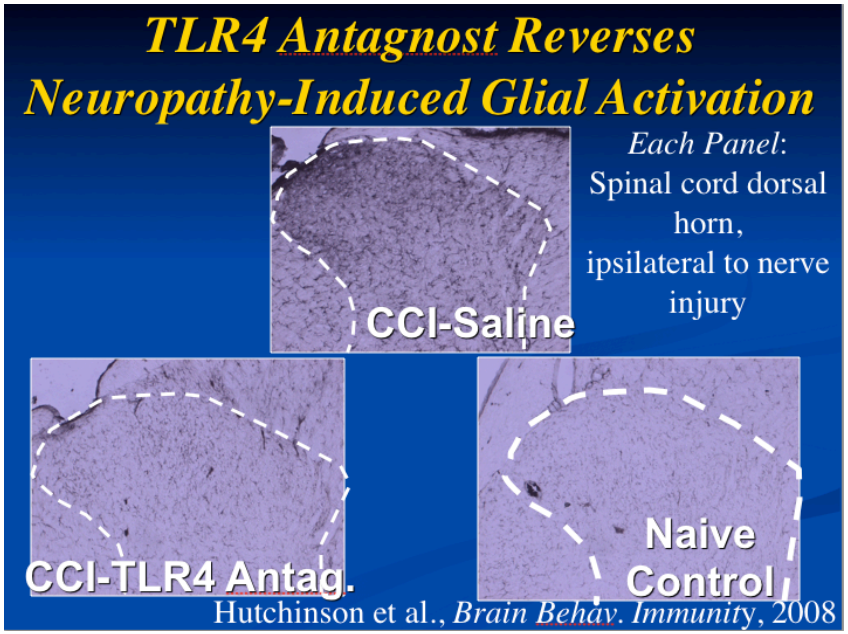
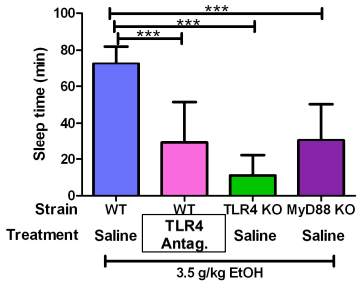


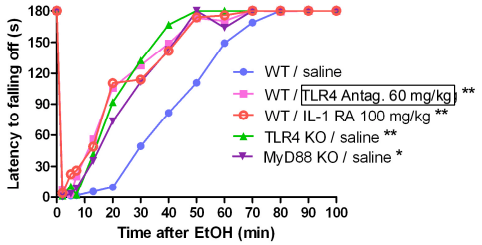

47

## *Glial TLR4 & Alcohol*

**TLR4 & MyD88 KO mice & TLR4 antagonist treated mice have shorter EtOH sleep time.**

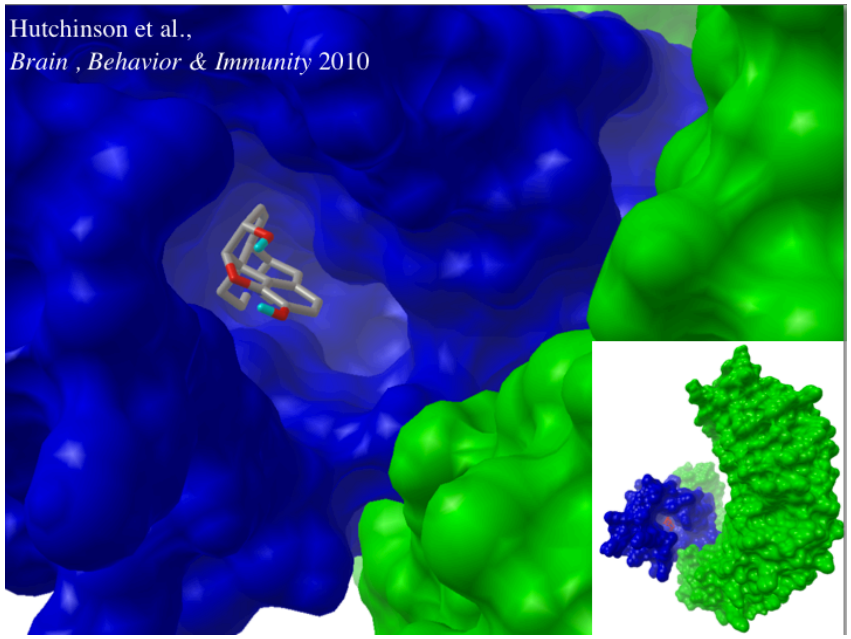



**TLR4 & IL-1 Antagonists, TLR4 KO, & MyD88 KO attenuate EtOH-induced rotarod motor impairments.**




Hutchinson et al., 2010

48

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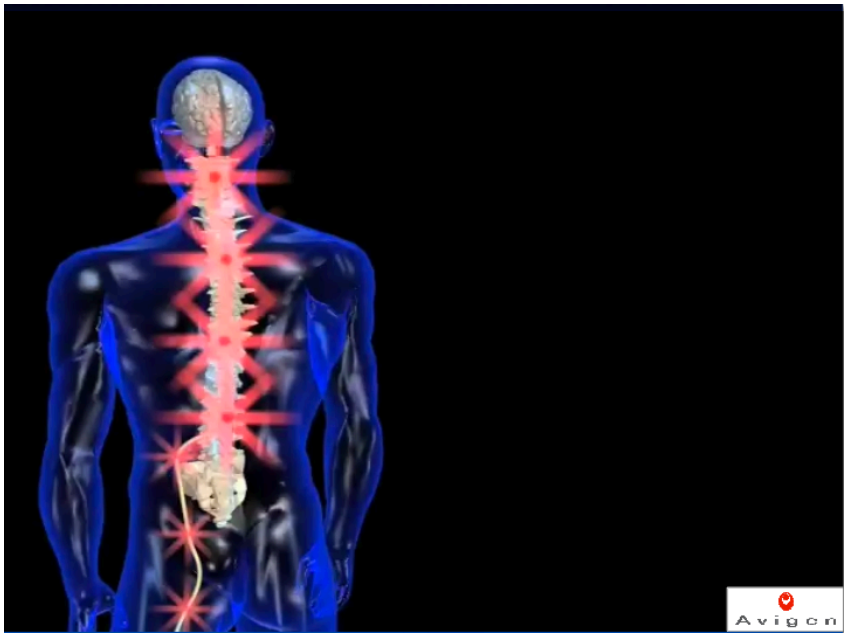


50

## *Endogenous Danger Signals: Might they explain symptoms years after Gulf War damage to the CNS?*

1. Gulf war veterans show loss of gray & white matter
2. Clearance of myelin debris from the CNS takes years; how many years is unknown
3. Exposure to such endogenous danger signals cause glial activation & "priming"
4. The symptoms observed should reflect where the CNS degeneration occurs

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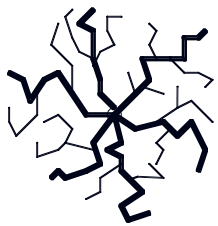
## *States of Glial Activation: Not Just "Off" or "On" Anymore!*

Basal State: Boring but Vigilant



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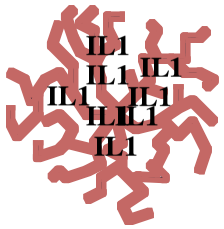
# *States of Glial Activation: Not Just "Off" or "On" Anymore!*

Activated State: Proinflammatory

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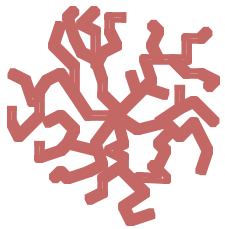
# *States of Glial Activation: Not Just "Off" or "On" Anymore!*

***"Primed"* State:**

* Can occur for a period of time after prior activation
* No longer producing proinflammatory products...

but....Ready for Action!



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# *States of Glial Activation: Not Just "Off" or "On" Anymore!*

**Reactivation from the"Primed" State:** ***Explodes*** into Action in Response to a New Challenge!


**Aging**
**Stress**
**Trauma**
**Opioids**

***Sets the Stage For Chronic Pain??Gulf War Illness?***

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# *So...what do aging, stress, trauma/inflammation, & opioids all have in common?*

**Up-regulation of Toll-Like Receptor-4 (TLR4):**
***Classically....***
**"not me, not right, not OK" receptors**

Hutchinson et al., *TSWJ* 2007; *Br Behav Immun* 2008

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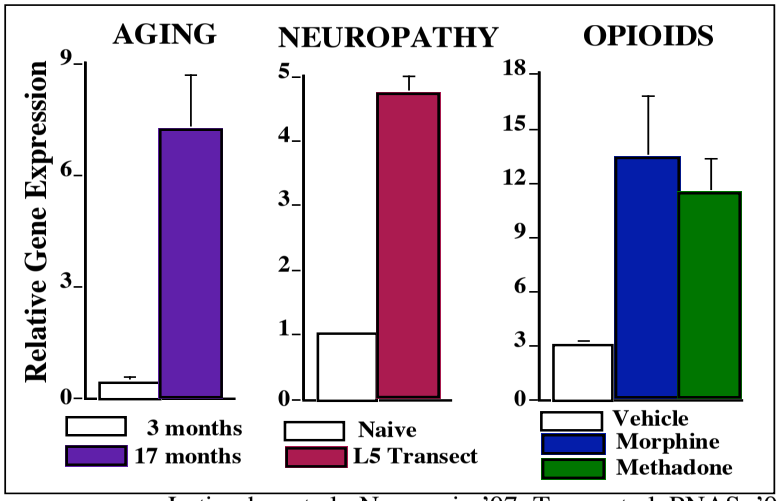
# *Upregulation of TLR4 mRNA*

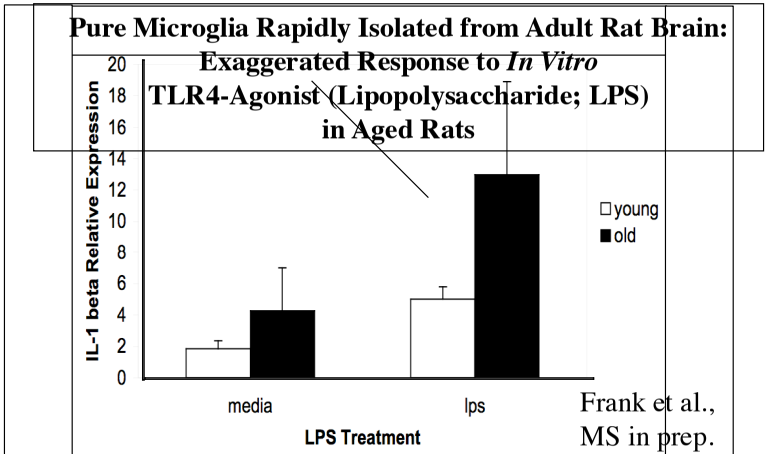



Letiembre et al., Neurosci., '07; Tanga et al. PNAS, '05;
Hutchinson et al., TSWJ, '07



# *States of Glial Activation: Not Just "Off" or "On" Anymore!*

## *"Priming" of Glia by Aging*

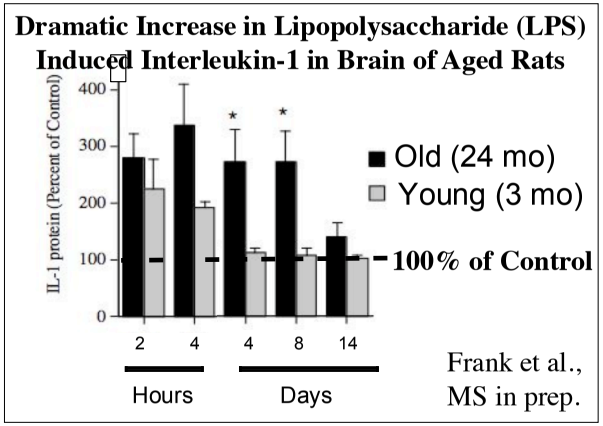



Frank et al.,
MS in prep.



# *States of Glial Activation: Not Just "Off" or "On" Anymore!*

## *"Priming" of Glia by Aging*

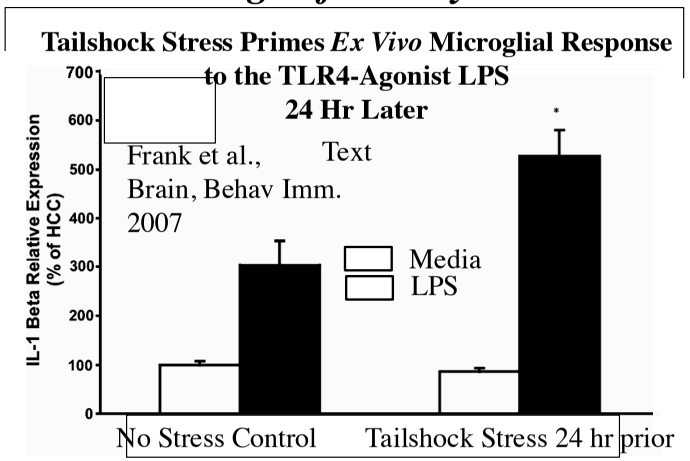



Frank et al.,
MS in prep.

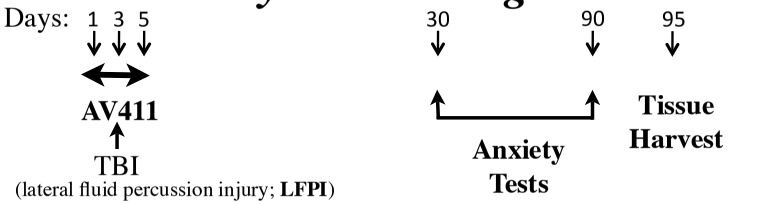


# *States of Glial Activation: Not Just "Off" or "On" Anymore!*

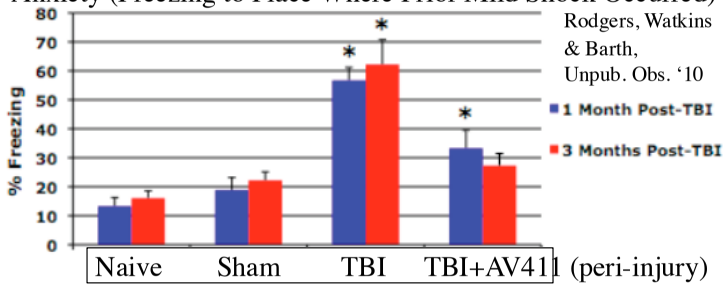
## *"Priming" of Glia by Stress*



# *Traumatic Brain Injury: Anxiety & Neurodegeneration*




Anxiety (Freezing to Place Where Prior Mild Shock Occurred)




Rodgers, Watkins & Barth, Unpub. Obs. '10

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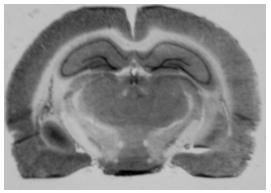
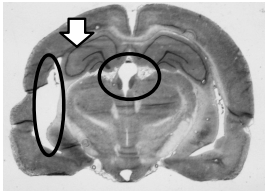
# *Traumatic Brain Injury: Anxiety & Neurodegeneration*

Days: < 95 >

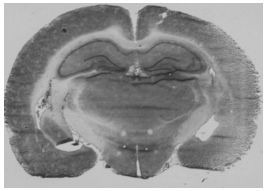
Tissue Harvest



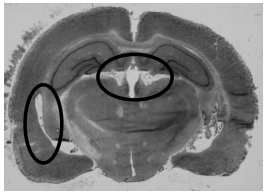
Naïve



TBI



Sham



TBI + AV411 (glial inhibitor, just peri-injury)

Rodgers, Watkins & Barth, Unpub. Obs. 2010

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# *"Two-Hit" Hypothesis: A 2nd "Hit" Can Create a Faster, Stronger, Longer Response by Glia*

FIRST "HIT"

* **Aging**
* **Stress**
* **Trauma/ Inflammation**
* **Opioids**

**Critical Window of Time**

SECOND "HIT"

**Activation of TLR4 by Bacterial LPS, etc.**

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# *So..... Does PRIOR glial activation alter the pain response to a NEW challenge?*

FIRST "HIT"

2 weeks

SECOND "HIT"

BL

test

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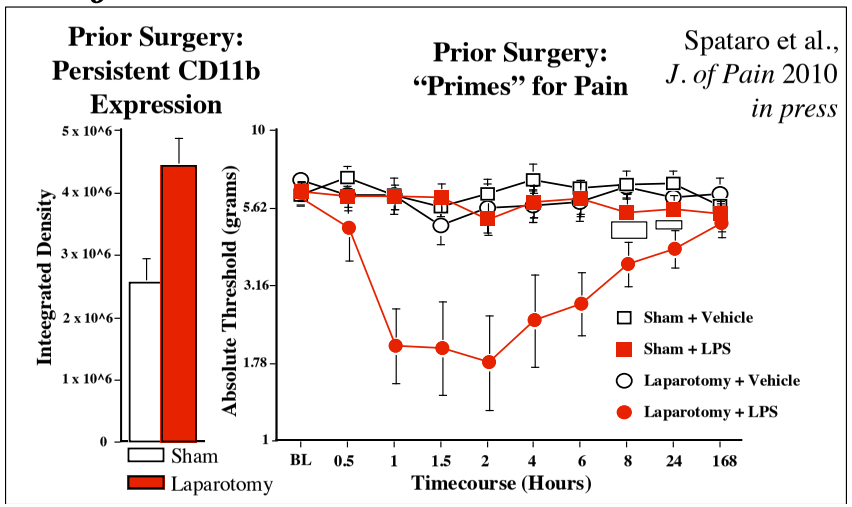
# *Prior Surgery (Laparotomy): Weak Inflammation Now Induces Pain*






# *So..... Does PRIOR glial activation alter the pain response to a NEW challenge?*

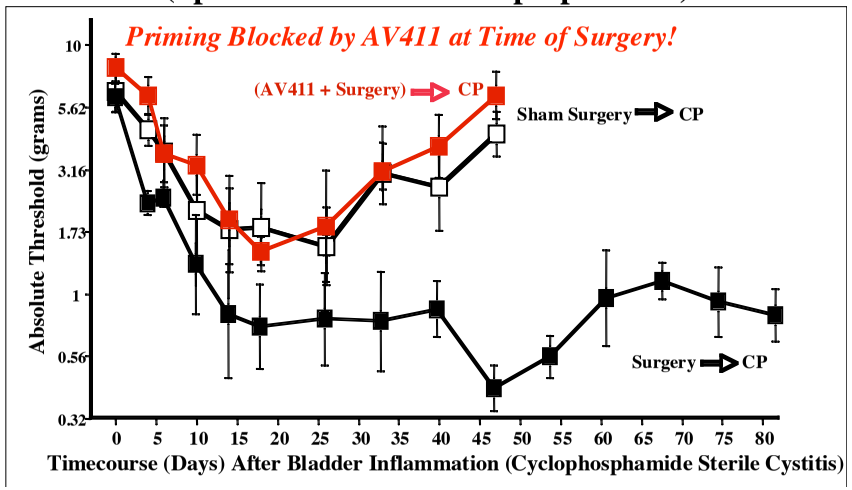

(+/- Glial Inhibitor)

BL test



# *Prior Laparotomy Amplifies Later Visceral Pain (Cystitis): Blocked by AV411*

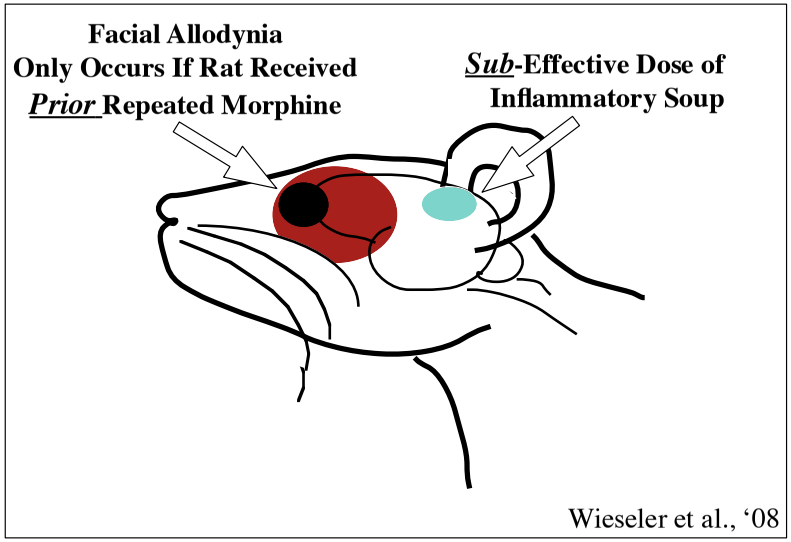
**(Spataro et al. '10 MS in preparation)**







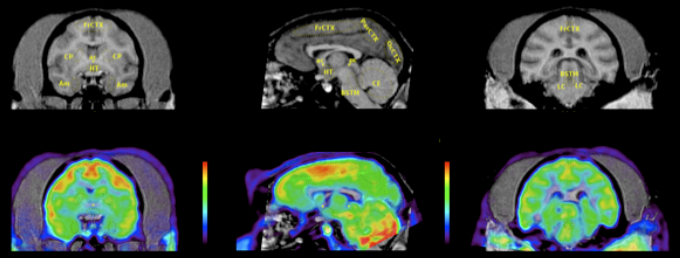

# Opioid "Priming" of Headache



Rio Pharmaceuticals

## Evaluation of norepinephrine CNS pathway integrities with

Alzheimer's, Parkinson's & PTSD



RP-105: $^{18}$F-Tracer NET PET Biomarker

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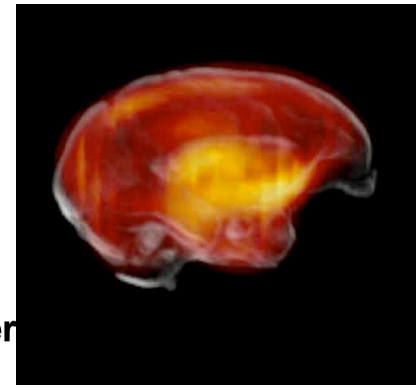
Rio Pharmaceuticals

## Evaluation of serotonin CNS pathway integrities with PET

Depression
Inflammation
Neurodegeneration

RP-110: $^{18}$F-Tracer
SERT PET Biomarker



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## *So, While Speculative…*
## *the Data to Date on Priming Suggest:*
## Conclusions-1

- Glial responses change, dependent upon history & time after prior activating event
  *Aging ~ Stress ~ Trauma ~ Opioids…*
  *Gulf War-Associated Neurodegeneration?*
- Primed glia now over-respond to new challenges: faster, stronger, longer
- Primed glia can change:
  * "no pain" to "pain"
  * "pain" to "enduring pain"

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## Conclusions - 2
## *Toll-Like Receptor 4 (TLR4) as an Intriguing Glial Activation Receptor*

- Glial TLR4: "not me, not right, not OK" receptor - bacteria, danger signals, opioids
- Activation of TLR4 produces proinflammatory responses: cytokines & other neuroexcitatory substances
- Upregulated by aging, stress, trauma & inflammation & opioids
  *Gulf War-Associated Neurodegeneration?*

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# General Conclusions

- **Glial responses can create & maintain pain facilitation**
  - * ***Physiologically*** **as a Sickness Response**
  - * ***Pathologically*** **when triggered by neuropathy, neurotropic viruses, etc**
  - * ***Pharmacologically*** **by clinically relevant opioids**
- **Glial activation now also linked to opioid tolerance, opioid dependence/withdrawal, opioid reward**
- **Proinflammatory cytokines are key**
- **Glial priming can profoundly amplify glially driven effects**
- **Targeting glia & glial products may provide a novel approach to pain control, increasing opioid efficacy, dereasing alcohol's effects, treating Gulf War Illness**

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