May 27, 2015
2015 NIH Pain Consortium Symposium

Panel on Neuro-glia Mechanisms of Chronic Pain

A Retrospective and the Role of Microglia & Astroctyes in Chronic Pain Mechanisms

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Glia and Pain: Pub Med Search

Microglia and pain: 1043

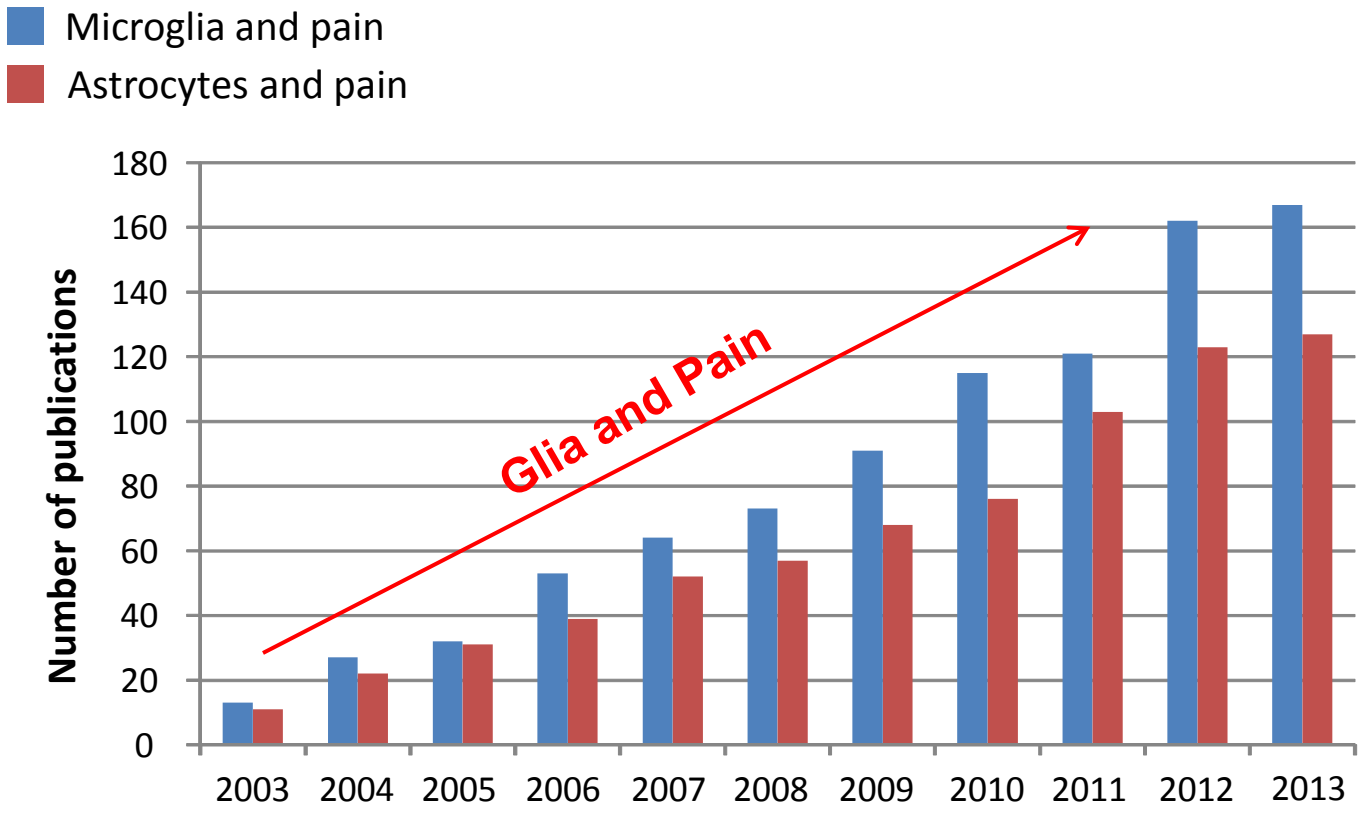
Astrocytes and pain: 769

Satellite glial cells and pain: 163

Oligodendrocytes: a few ?

Glia and pain: 1,717

Neuron and pain: 22,957



Glia have different activation states in chronic pain

1. **Gliosis: Changes of glial markers and morphology**

2. **UP-regulation of glial receptors and channels**

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3. **Intracelluar signaling: Phosphorylation of MAP kinases**

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4. **Production of glial mediators**

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Release of glial mediators (pain mediators)

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Interaction with neurons and central sensitization

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Chronic pain

Ji, Berta, and Nedergaard, *Pain*, 2013

I. Microglia and chronic pain

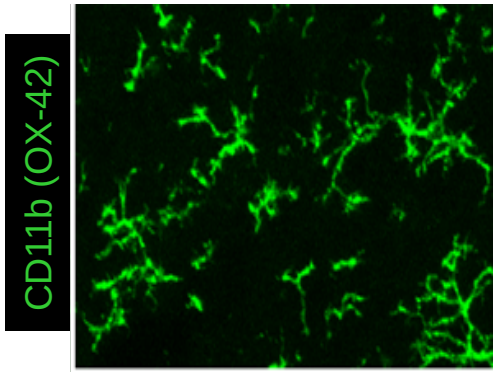
II. Astroglia and chronic pain

III. Gliopathy drives chronic pain

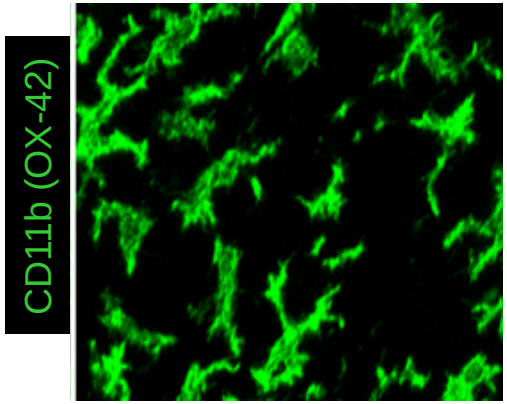
IV. Clinical significance & future directions

Microglia

Control

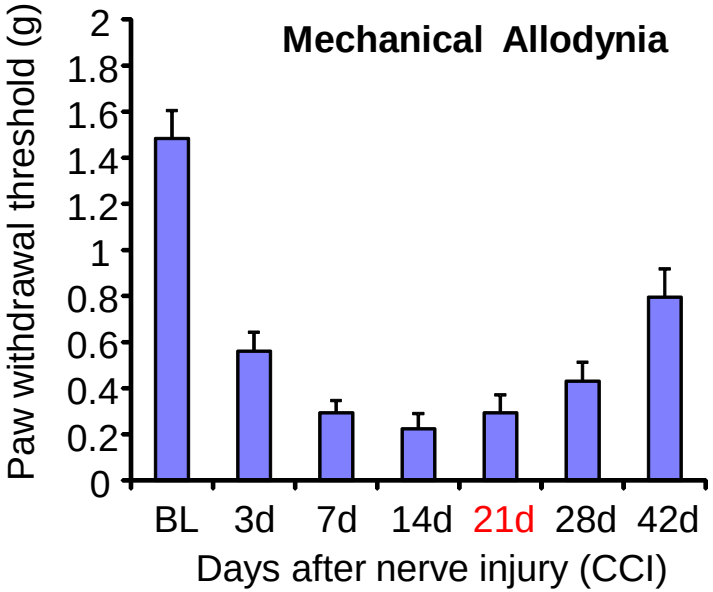


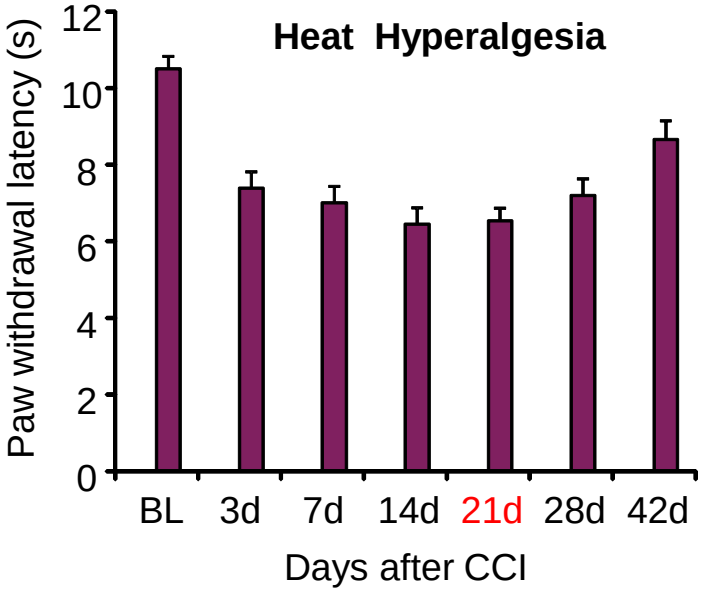
Nerve injury

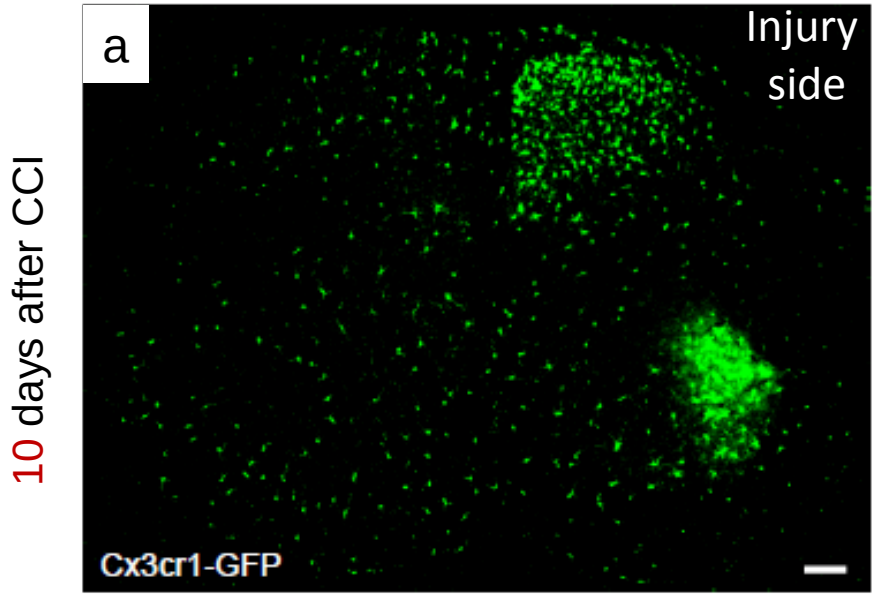


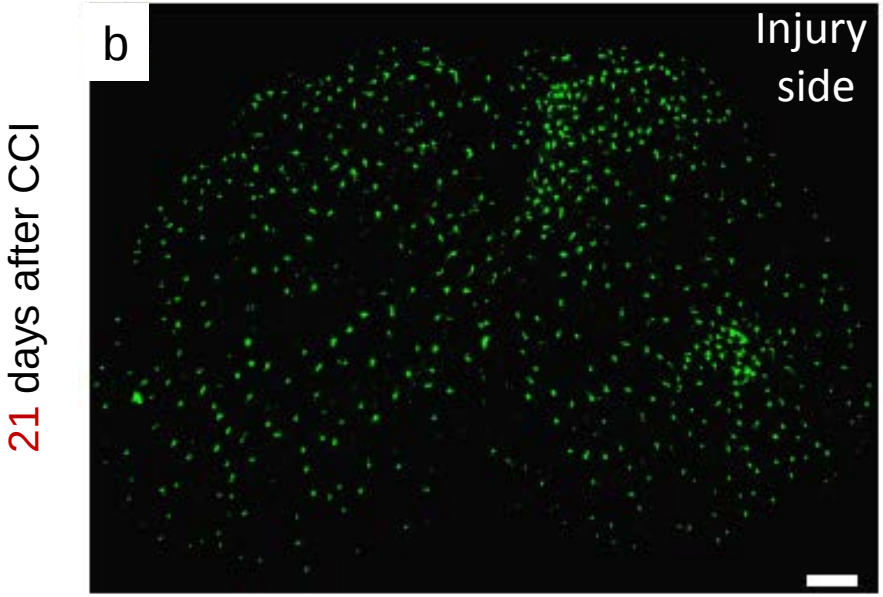
- Macrophages of the CNS
- Major effectors of the CNS innate response
- Express CD11b, IBA-1, CX3CR1
- Major source of cytokines in the CNS (TNF-α, IL-1β, IL-18)
- Produce growth factor BDNF
- Microgliosis (reactive) after nerve injury
- Pathogenesis of neuropathic pain
- Acute inflammatory pain?

Time-dependent microglia activation after CCI



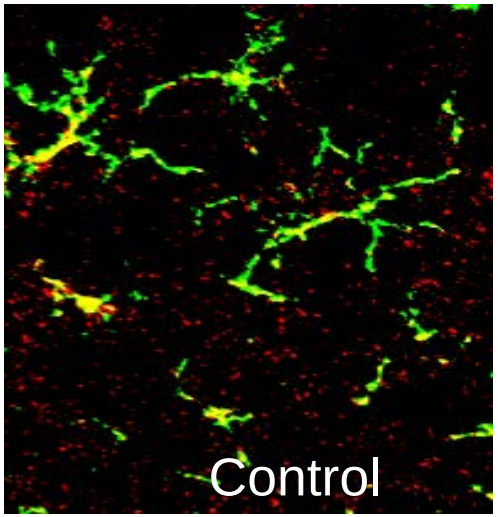


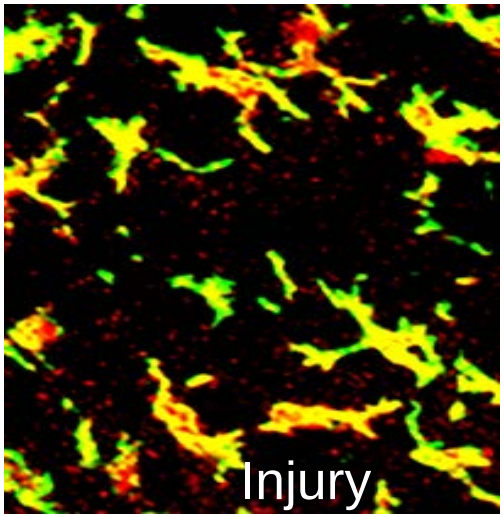




Phosphorylation of p38 MAPK after SNI

p-p38 / CD11b (OX-42)

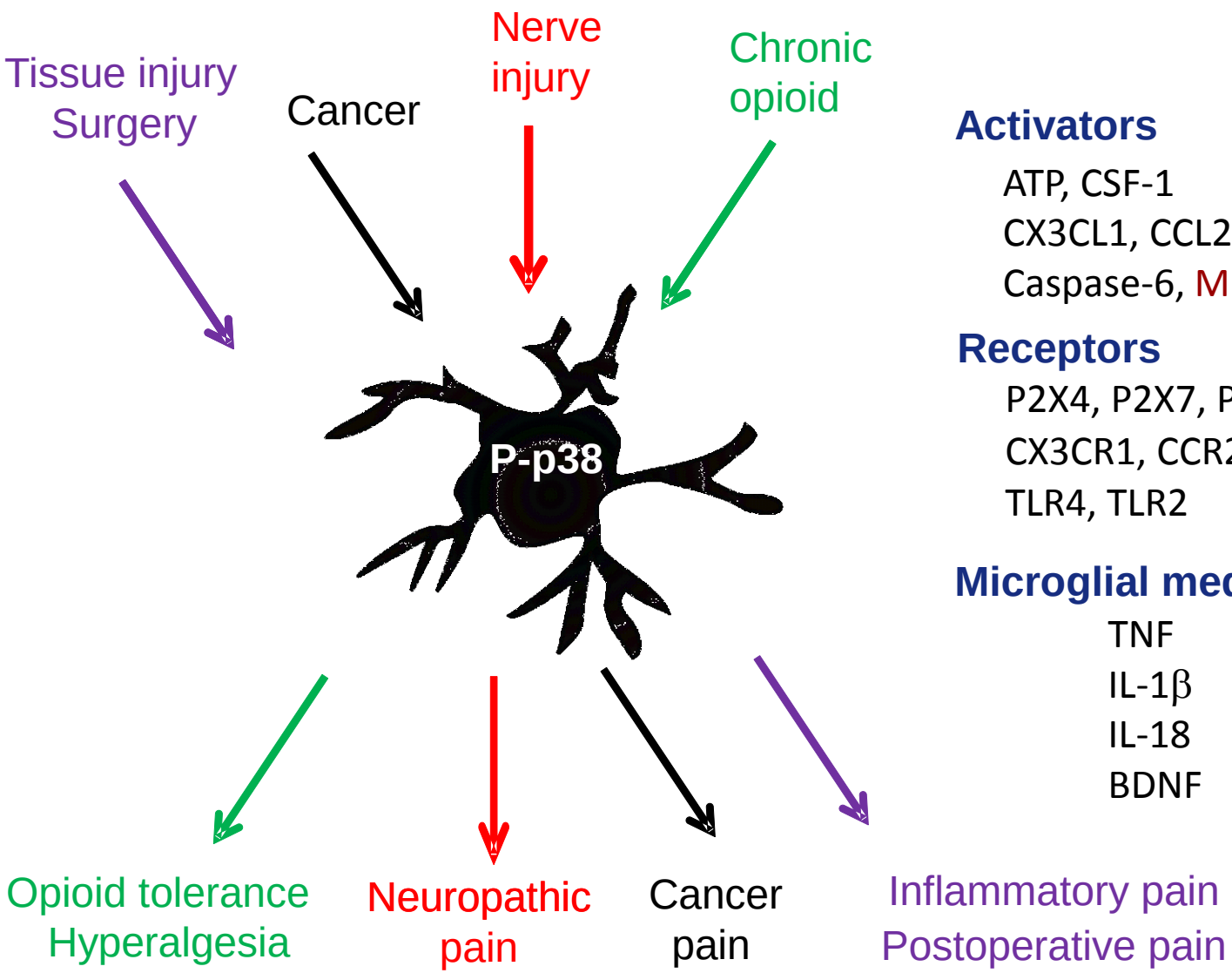




Numerous animal studies in different models (Intrathecal injection)

Some human studies (Systematic route) CNS effects?

Intracellular signaling in microglia



Activators

ATP, CSF-1
CX3CL1, CCL21, CCL2
Caspase-6, MMP-9, cathepsin S

Receptors

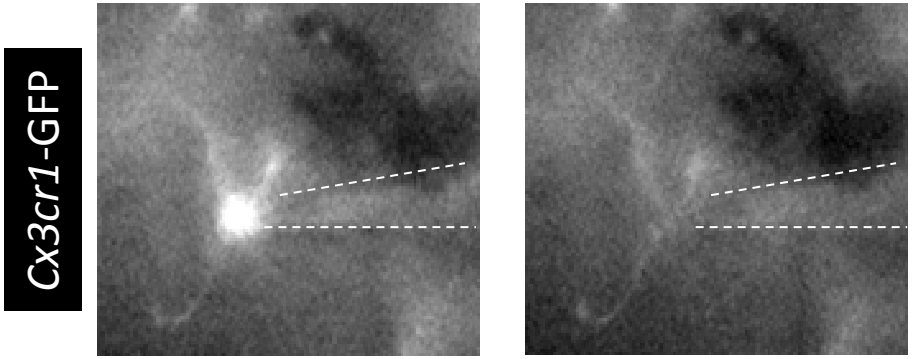
P2X4, P2X7, P2Y12, CSF1R
CX3CR1, CCR2
TLR4, TLR2

Microglial mediators

TNF
IL-1β
IL-18
BDNF

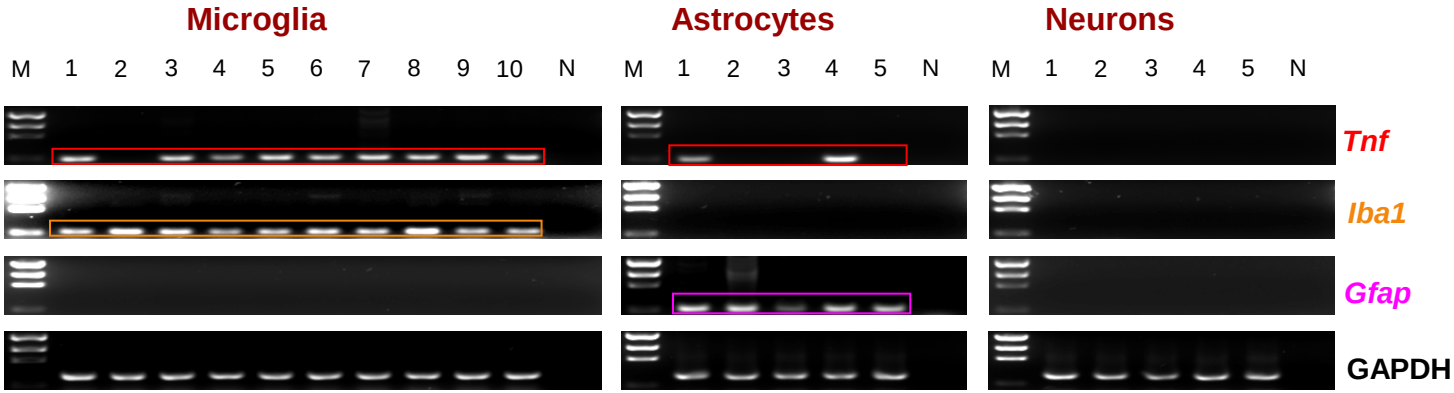
Single-cell analysis for microglial gene expression

Microglial cell collection from spinal cord slice of *Cx3cr1*-GFP mice



Before After

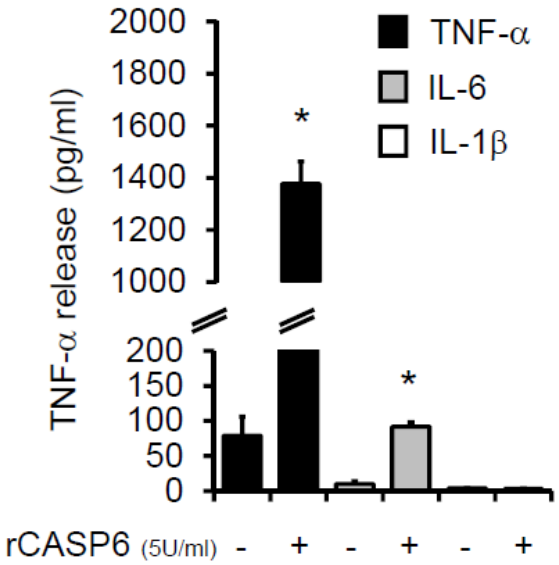
Single cell PCR

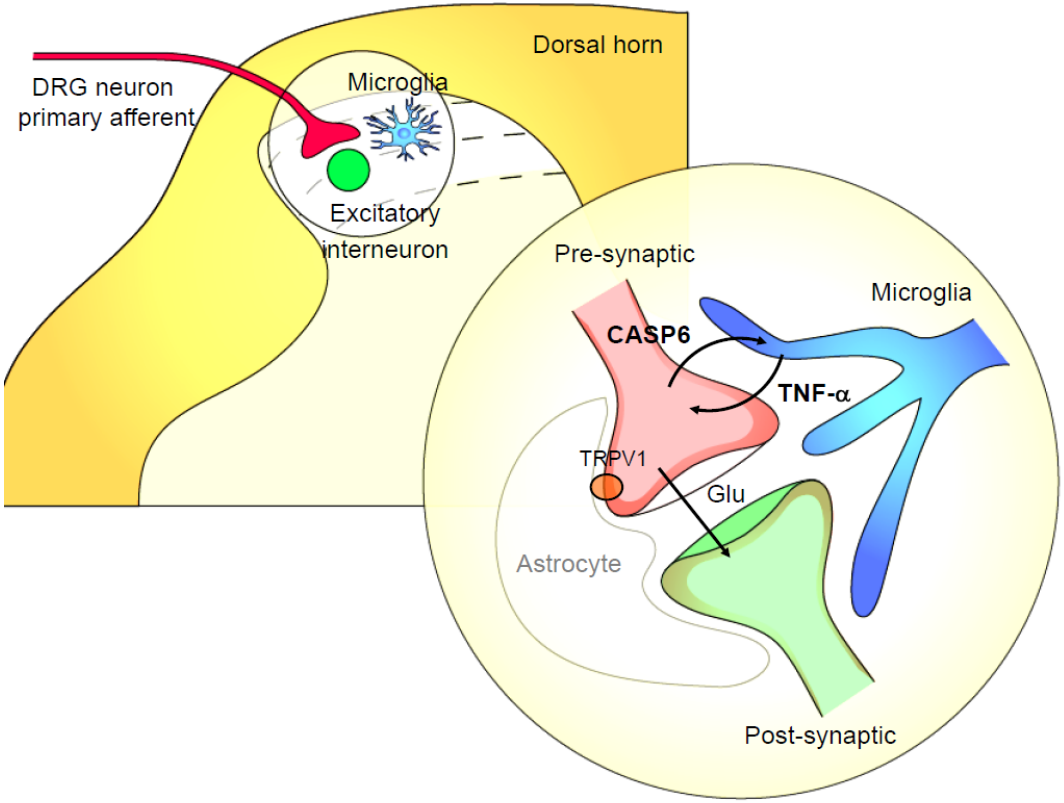


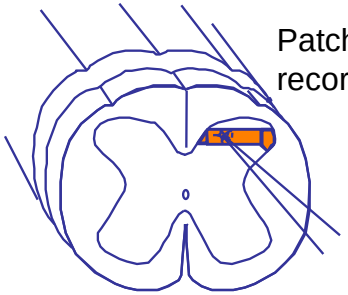
Microglia are primary source of TNF in the spinal cord.

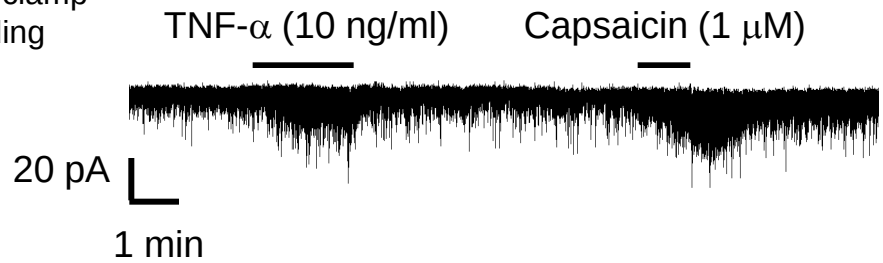
Berta et al., *JCI*, 2014

Caspase-6 releases TNF-α from microglia to modulate synaptic transmission







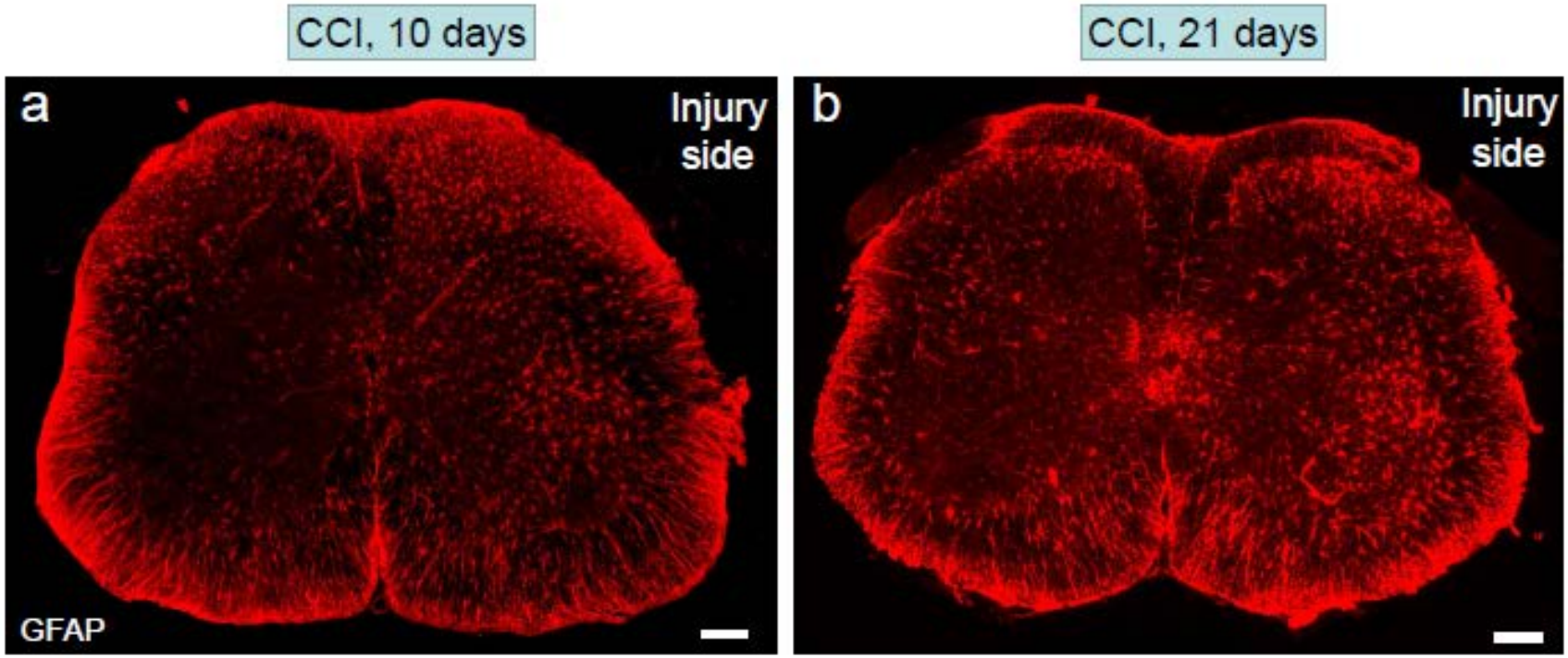


Berta et al., *JCI*, 2014

Astrocytes

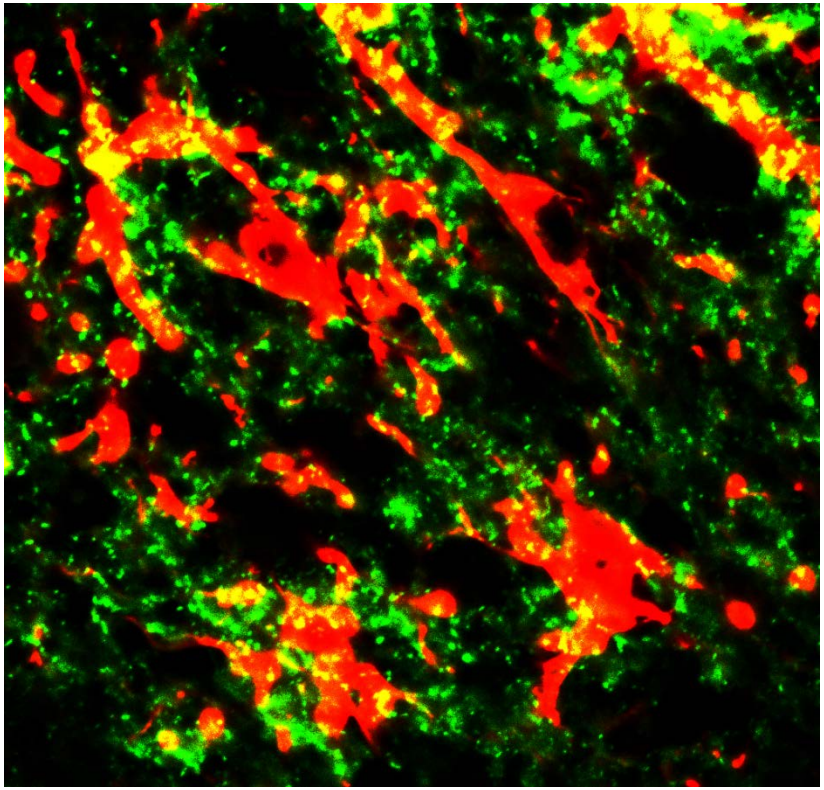
- Most abundant cells in the CNS
- Express GFAP, GLT1, Cx43, ALDH1-L1
- Provide structural and tropical support for neurons
- Control synapse formation & insulate synapses
- Interact with blood vessels
- Form networks via gap junctions
- Form glymphatic system to clear toxins
- Maintain glutamate and K^+ homeostasis
- Release gliotransmitters such as ATP, glutamate, cytokines, chemokines

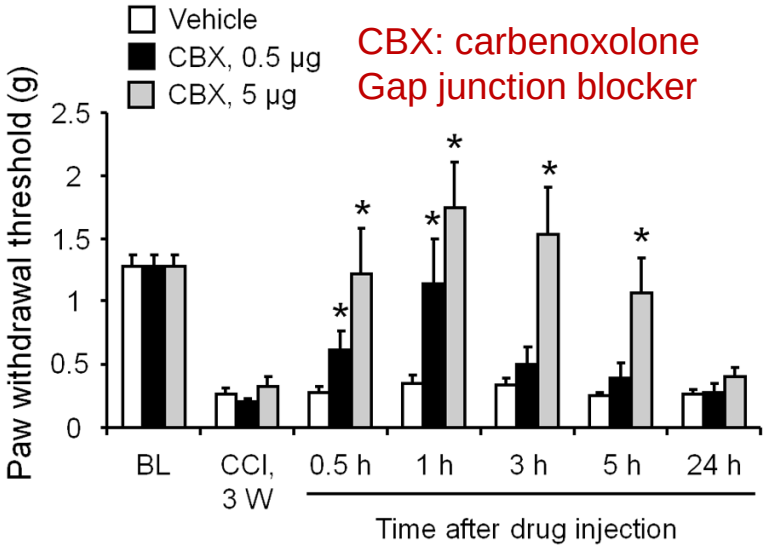
Persistent activation of astrocytes in neuropathic pain

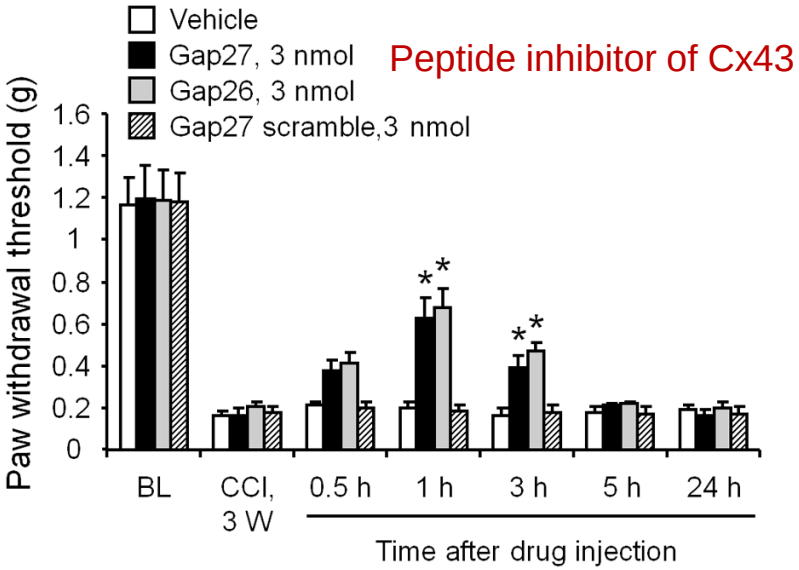


Astrocytic Cx43 maintains late-phase neuropathic pain

Cx43 / GFAP

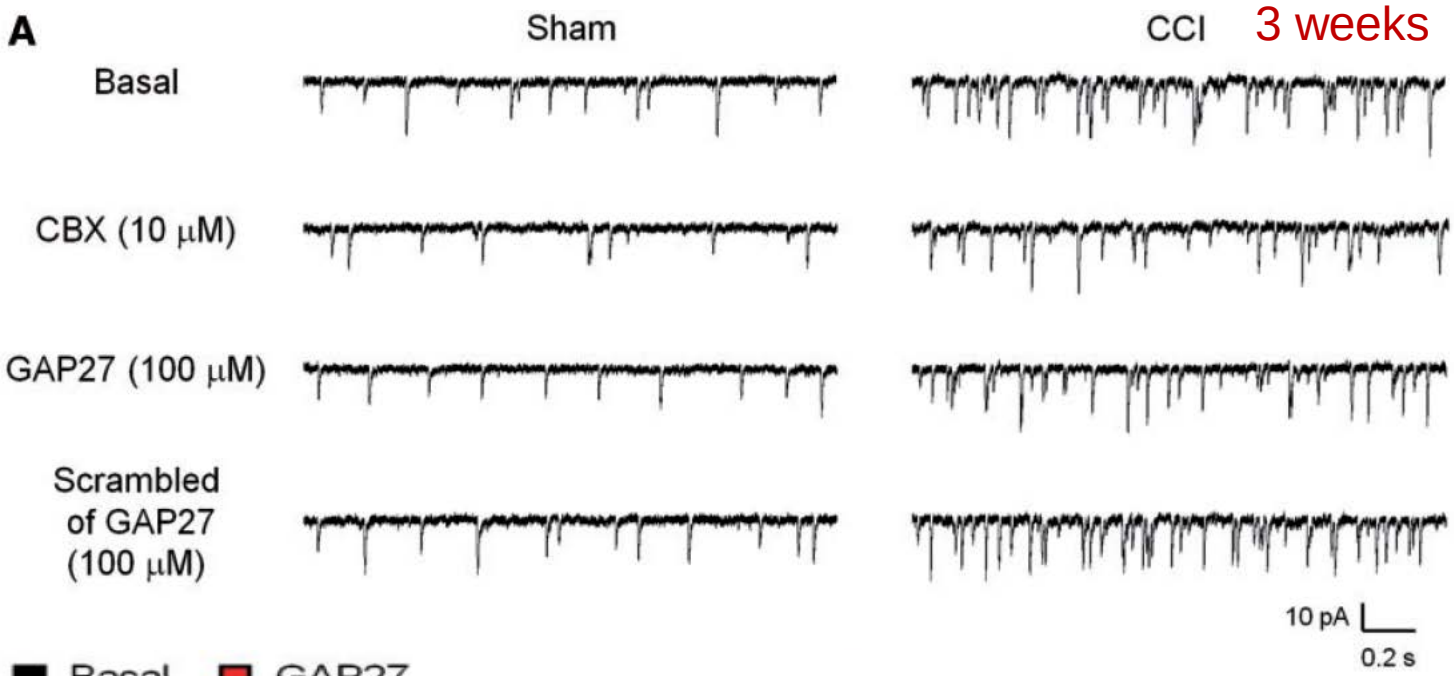


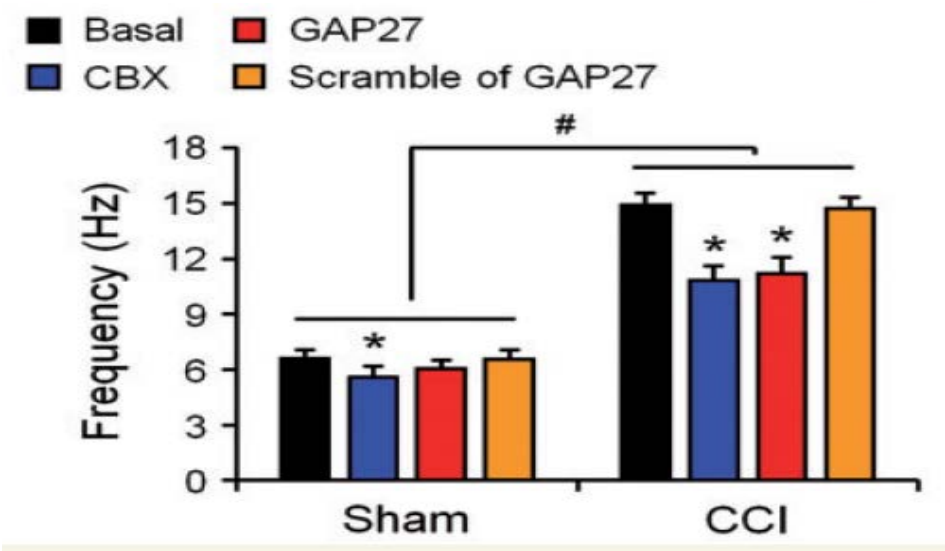


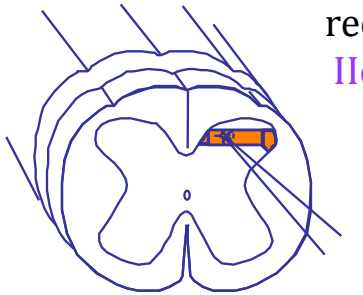


Chen et al., Brain, 2014

Cx43 modulates synaptic transmission after CCI



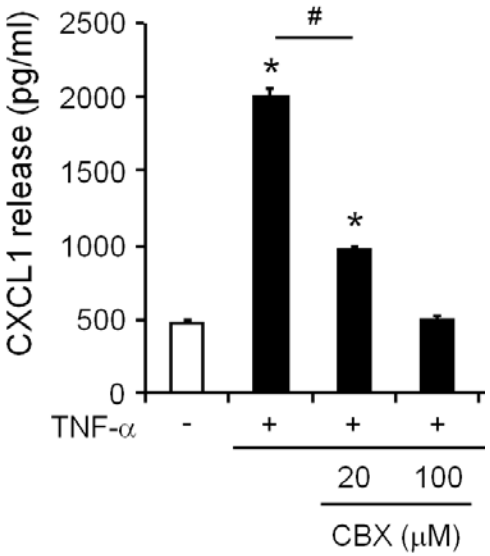


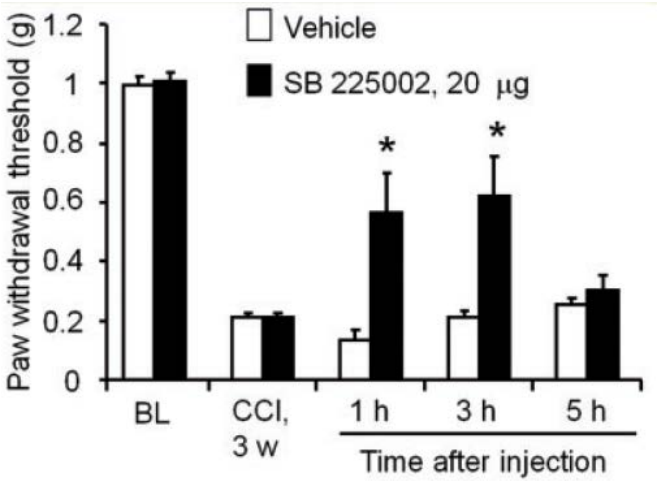


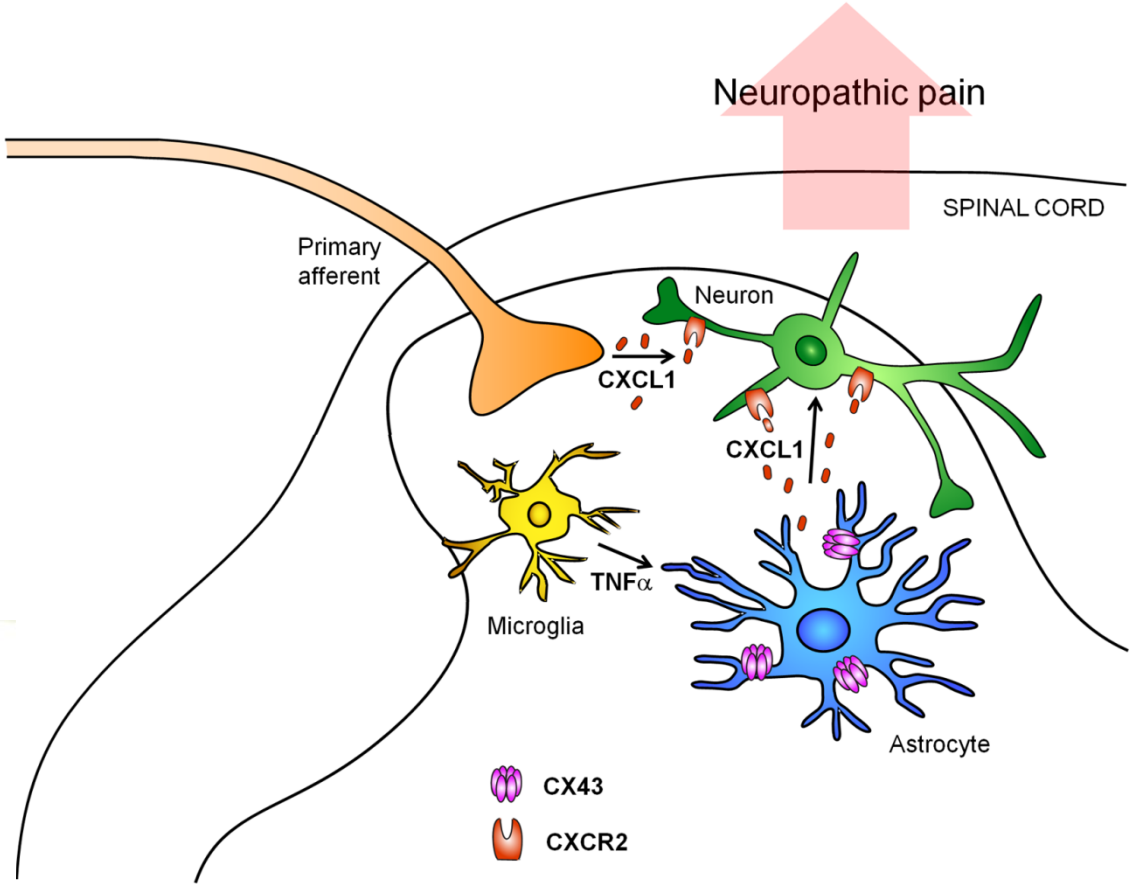
Patch clamp recording in IIo neurons

Cx43 maintains neuropathic pain

via CXCL1 release from astrocytes

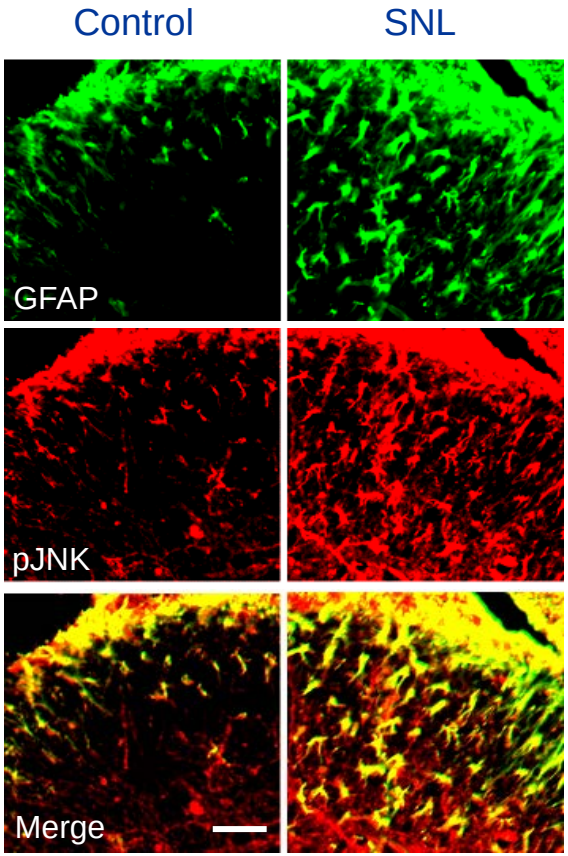




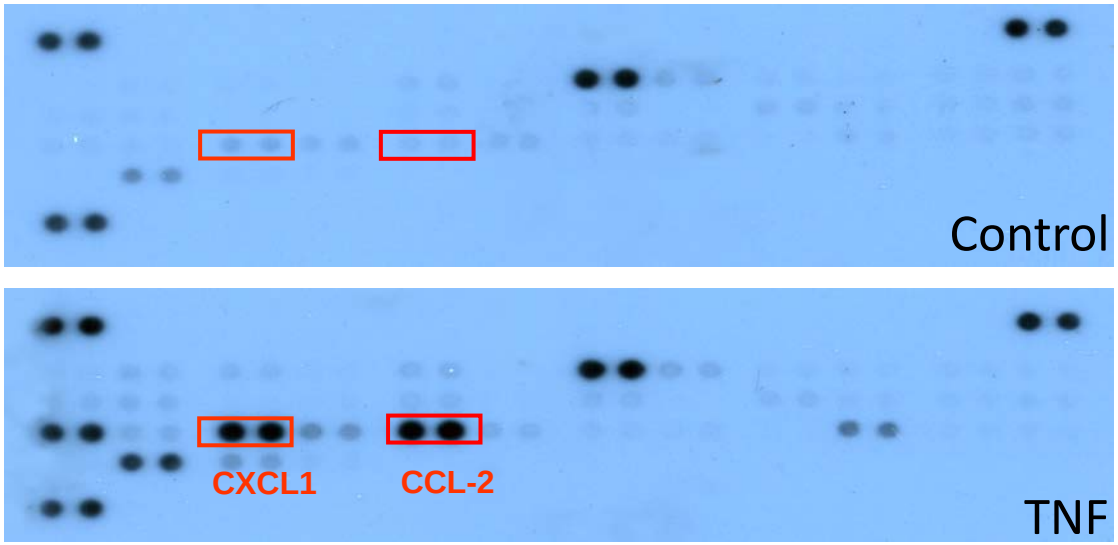


Chen et al., Brain, 2014

Astrocytes produce chemokines via JNK



Gao and Ji, Neurotherapeutics, 2010



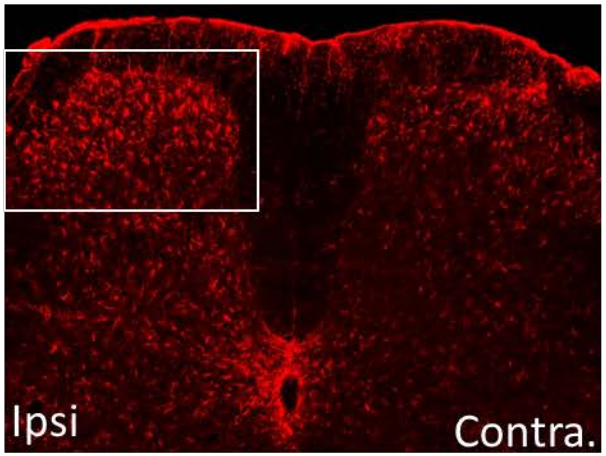
Astrocyte-neuron interactions

Chemokines (Astrocytes)	Chemokine receptors (Neurons)
CCL2/MCP-1	CCR2
CXCL1/KC	CXCR2

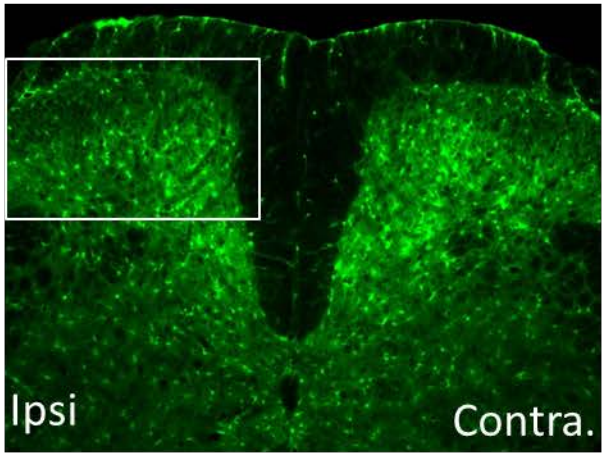
Different types of spinal cord astrocytes

CCI injury

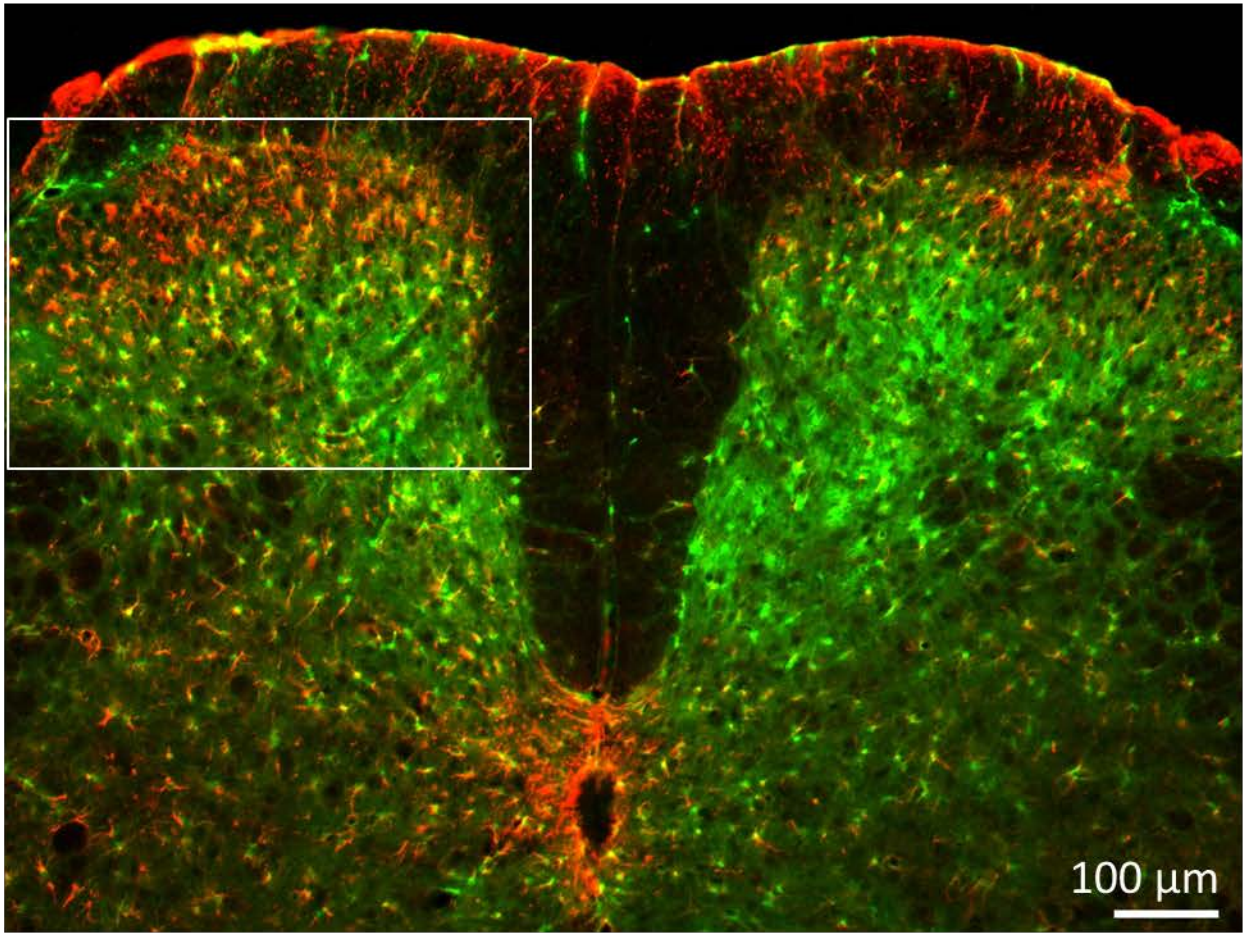
GFAP



Aldh1-EGFP



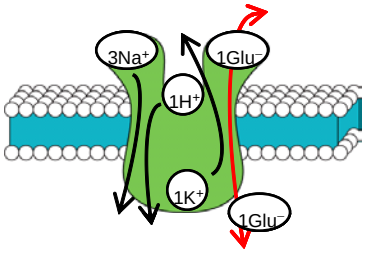
Merge GFAP / Aldh1



ALDH1-L1 (Aldehyde dehydrogenase 1, L1)

Gliopathy in chronic pain: Astrocyte dysregulation

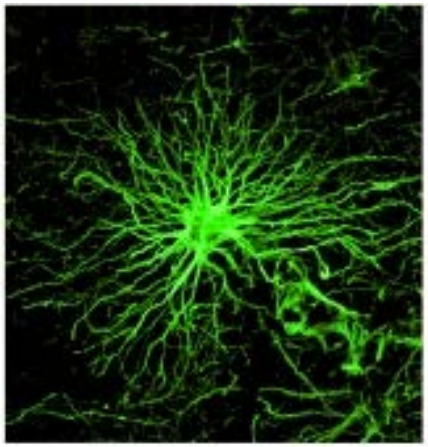
Glutamate homeostasis



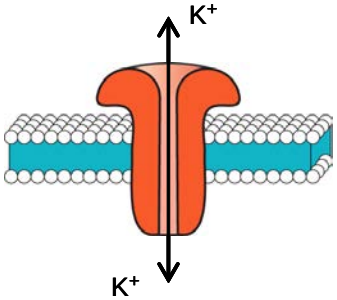
GLT1, GLAST

Hyper-excitability

Astrocyte



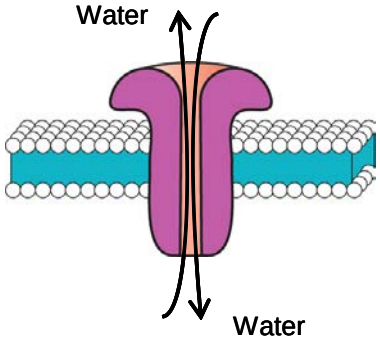
Potassium homeostasis



Kv4.1, Kv5.1

Hyper-excitability

Water homeostasis

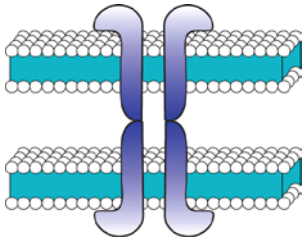


AQP4 dysfunction

Edema

Chemokines (CXCL1, CCL2)

Long-range signaling



Cx43 dysfunction

Paracrine signaling

ATP, glutamate

Modified from Verkhratsky et al., *ASN Neuro*, 2012

Ji et al., Pain, 2013

Targeting glial cells for chronic pain

- Chronic pain is a result of "**gliopathy**".
- Microglia and astrocytes are important players in chronic pain development and maintenance.
- Glia modulate pain via "neuro-glia interactions
- Glia-produced mediators (cytokines and chemokines) are **neuromodulators** and can powerfully modulate synaptic transmission.

Strategies

- Cytokine inhibitors (TNF, IL-1b, IL-6)
- Anti-inflammatory cytokines IL-10, TGF-β
- Chemokine inhibitors (CXCL1/CCR2, CCL2/CXCR2)
- MAPK inhibitors (p38, JNK, ERK)
- Proteases inhibitors (MMP-9, MMP-2, CASP6, Cathepsin S)
- Cx43, P2X7, TLR4 inhibitors
- **Cell therapy (bone marrow stem cells)**

What can we learn from cancer therapy?

Cancer

- **Chemotherapy**
- **Immune therapy**
- **Combination therapy**

Chronic Pain

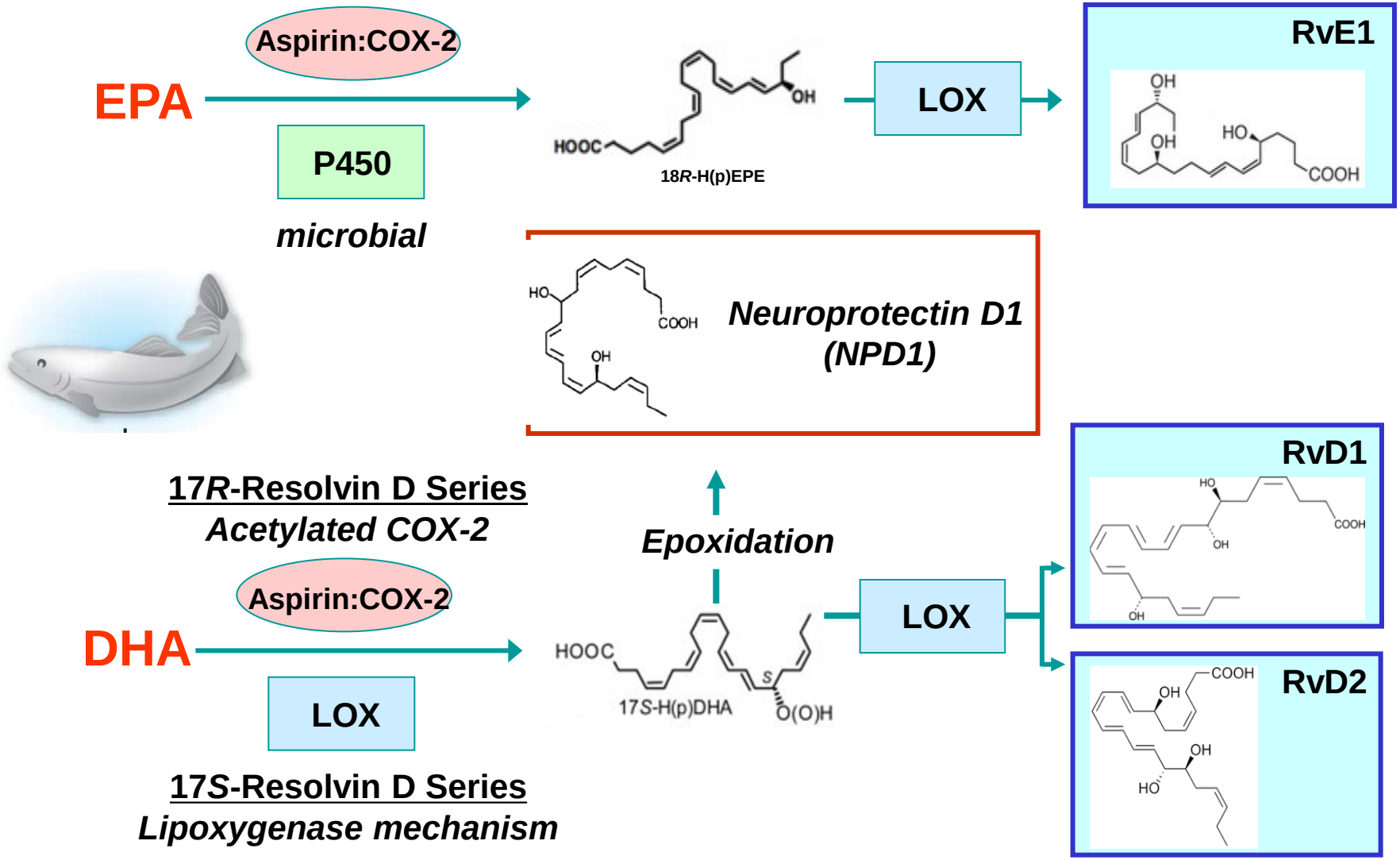
- **Neuron-targeting therapy**
- **Glia-targeting therapy**
- **Combination therapy**

Strategies

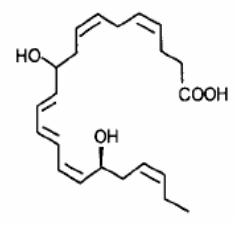
- Multiple drugs to target neurons and glia separately
- One drug that can target both neurons and glia

Biosynthesis of resolvins and neuroprotectin

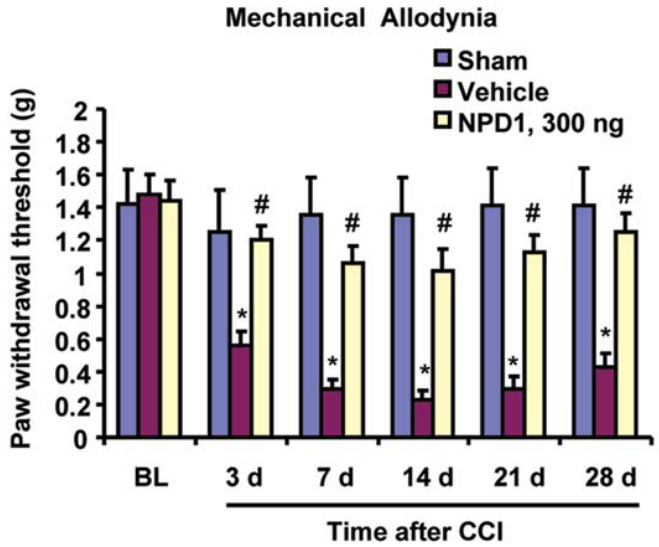
(Anti-inflammatory and pro-resolution mediators)

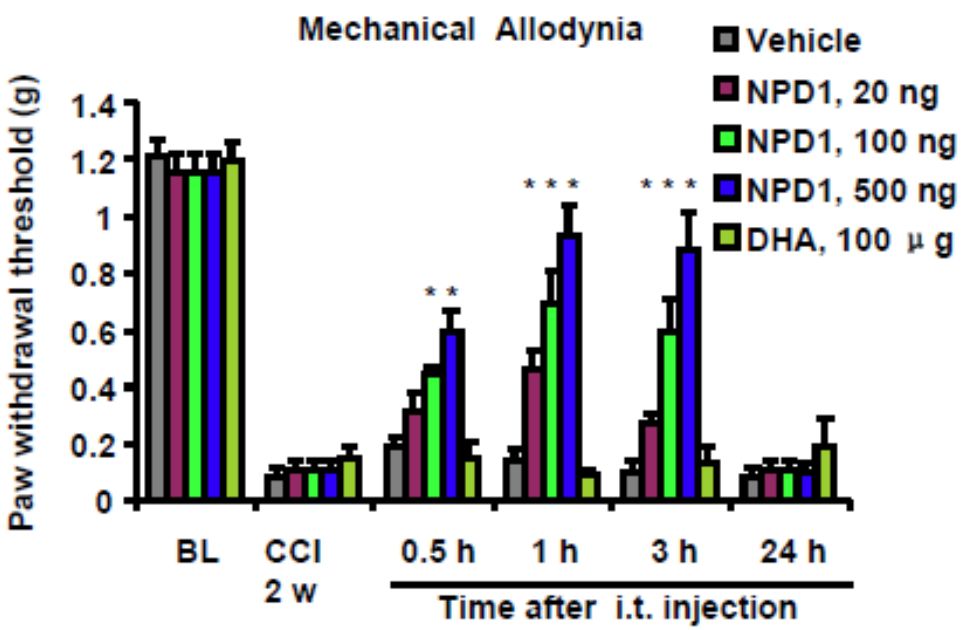


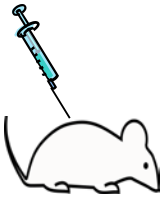
Neuropathic pain relief by neuroprotectin D1 (NPD1)

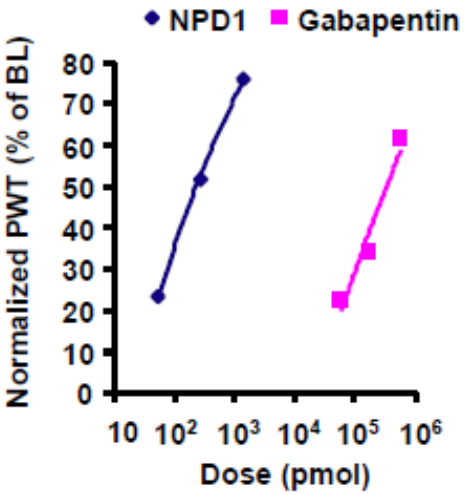


NPD1





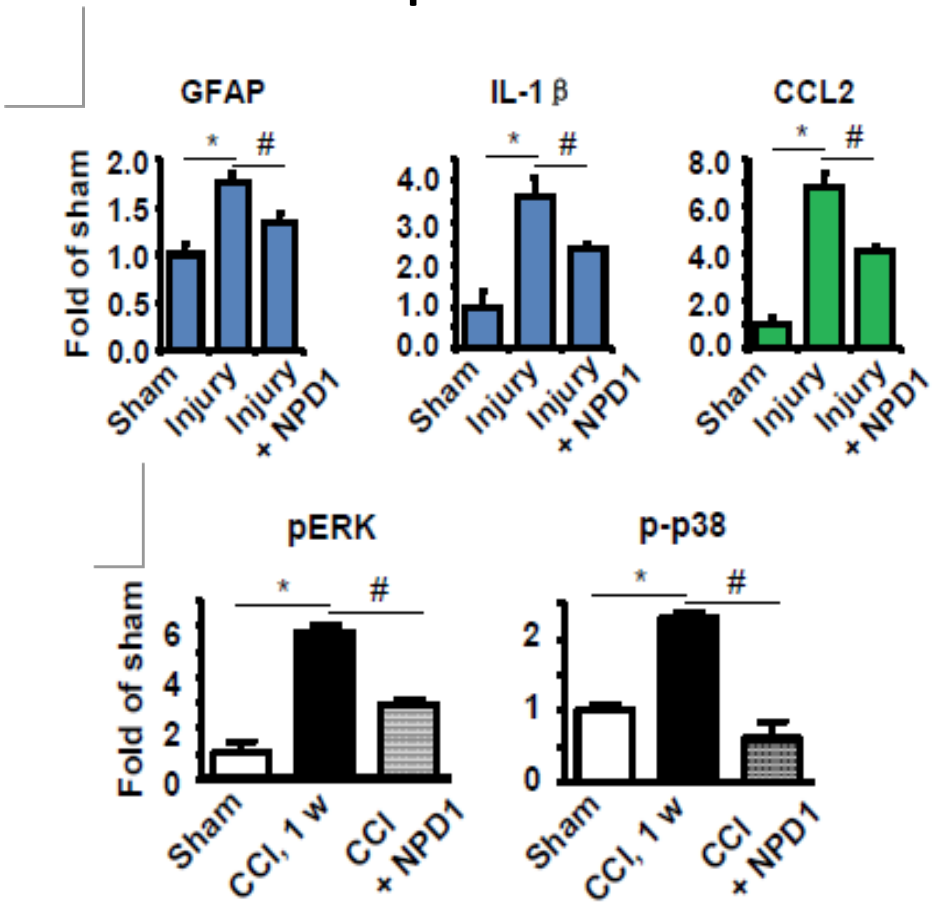




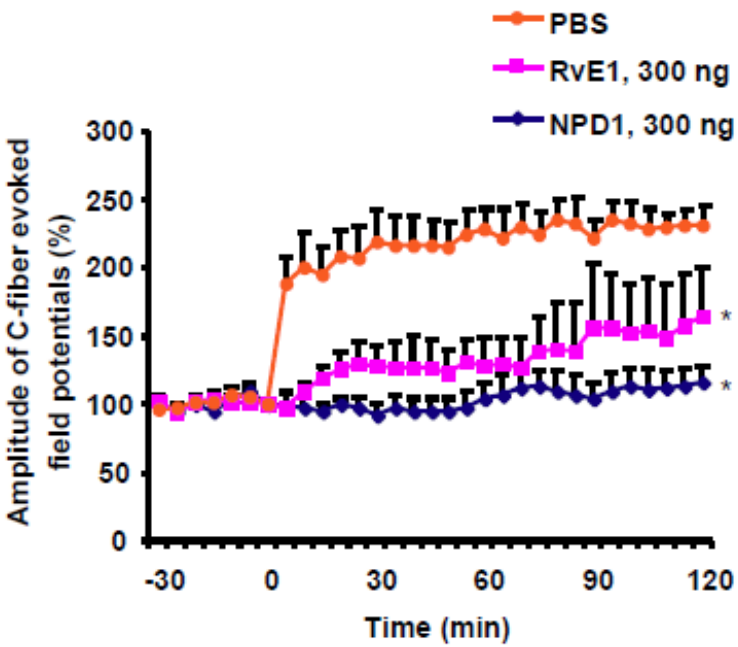
Xu et al., *Annals of Neurology*, 2013

Neuroprotectin D1 (NPD1) modulates glial and neuronal activities after nerve injury

Neuroinflammation in the spinal cord after CCI



Long-term potentiation in the spinal cord



Xu et al., *Annals of Neurology*, 2013

Neuroprotectin D1 (NPD1) based therapies

Glia activation and Neuroinflammation

TNF-α
IL-1β
CCL2
CXCL1
BDNF

↓

Maladaptive synaptic plasticity

↓

Chronic pain

NPD1

- Anti-inflammation
- Pro-resolution
- Very safe
- Inhibit TRPV1 function
- Inhibit neural plasticity
- Inhibit glial activation
- Promote regeneration
- Protect neurons
- Limitation: unstable

- **NPD1: Prevention of nerve trauma and chemotherapy induced neuropathic pain**
- **Small molecule receptor agonist of NPD1R: Treatment of established chronic pain**

Future directions

- Identify new markers for microglia and astrocytes
- Unbiased approaches to screen mediators produced by glial cells
- Further investigations into neuro-glia interactions
- Other glial types (satellite glia, oligodendrocytes)
- Selective tools to target glia (chemical genetics, DREADD)
- Sex-dependent glial signaling
- Age-dependent glial signaling
- Glia activation in human brain
- Glymphatic system in chronic pain

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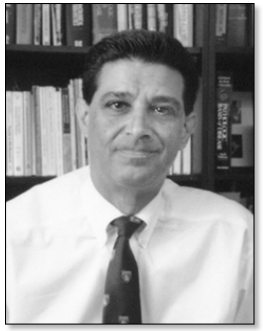
(Resolvins and protectin)

Maiken Nedergaard

Charles Serhan



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