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The role of glia in generating neuroplasticity

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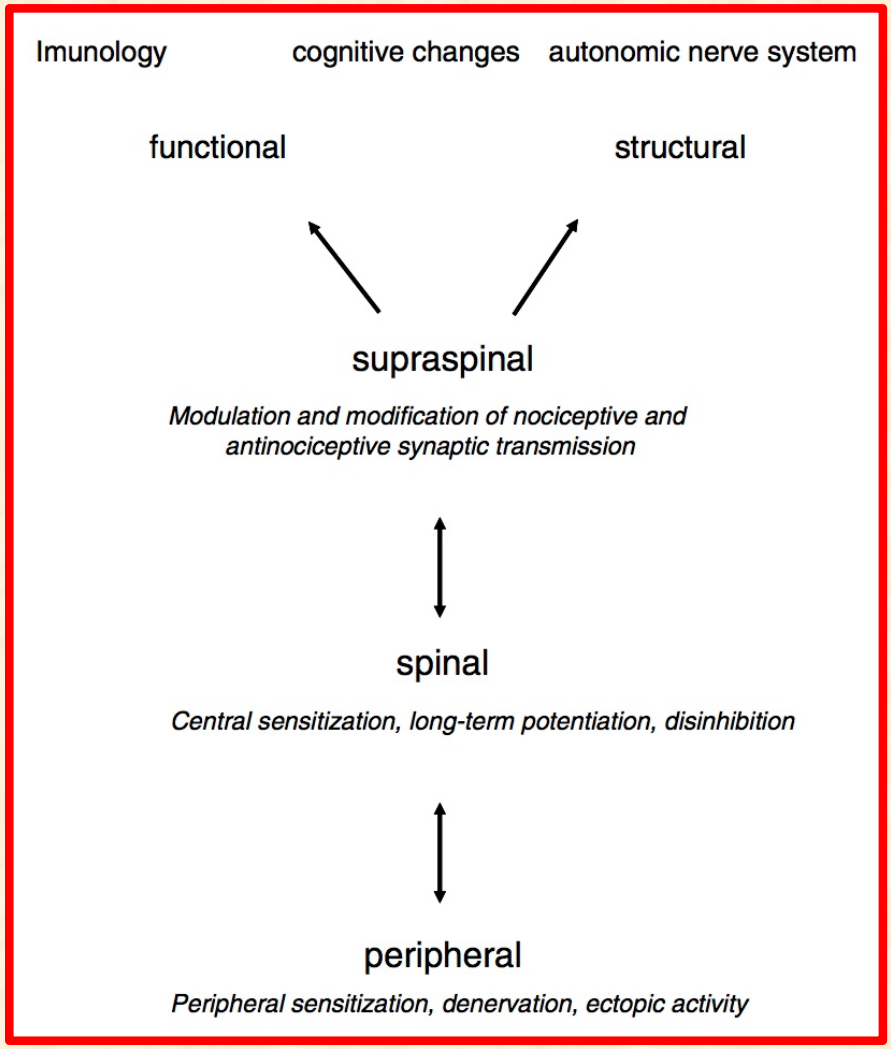
SASP ANNUAL MEETING 2017

Outline

- ☆ **The concept of brain plasticity under painful conditions**
- ☆ **The role of astrocytes and microglia in brain plasticity**
- ☆ **A possible role for satellite glial cells and for oligodendrocytes?**
- ☆ **Examples of the contribution of glial cells to post operative pain**

Plasticity in the brain under chronic pain conditions



May A., Pain 137:7-15 (2008)

Plasticity is a term used to refer to changes that occur in the established nervous system

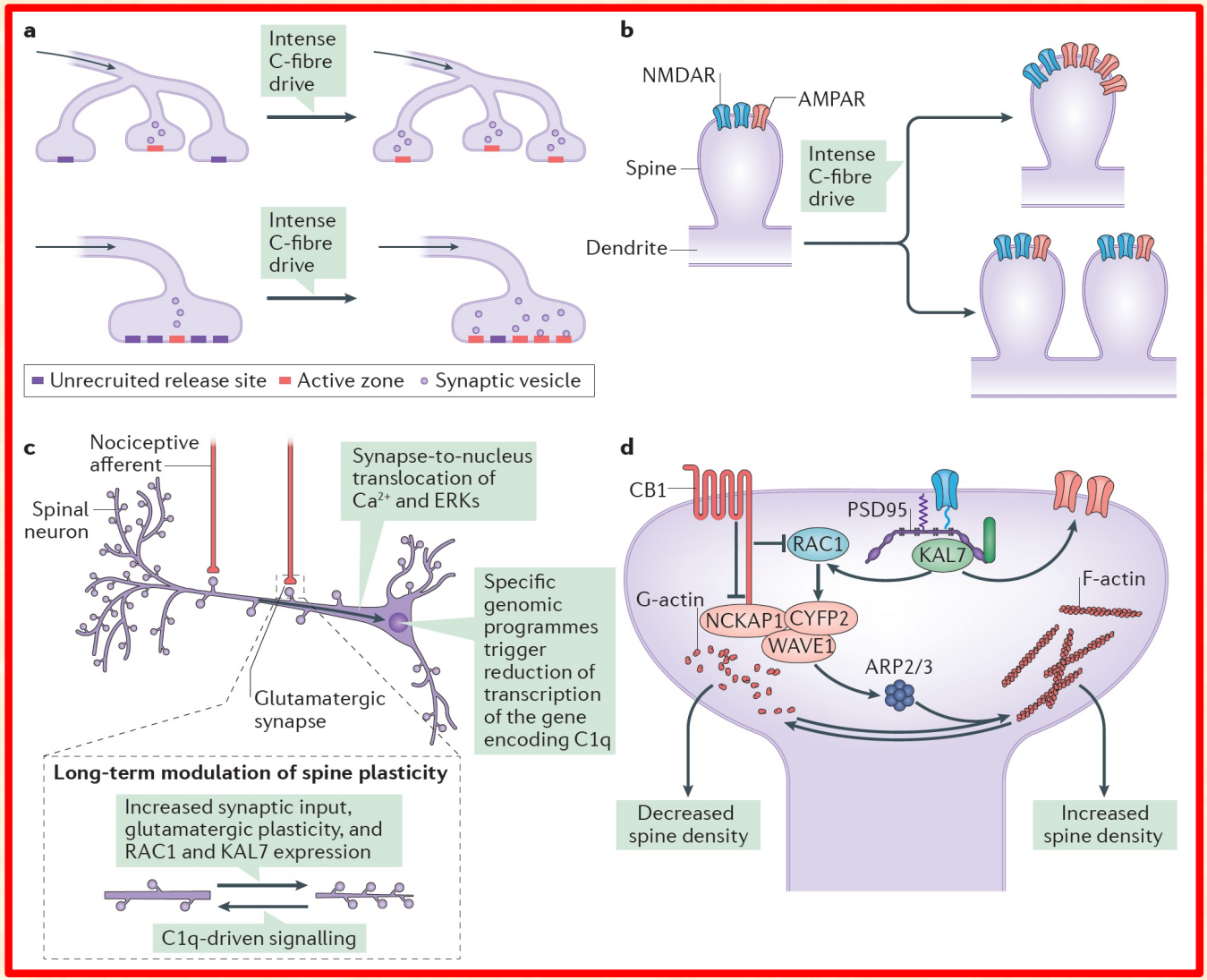
Plasticity is at the basis of learning and memory processes

MALADAPTIVE PLASTICITY → plasticity in the nervous system that leads to a disruption of the function and may be considered as a disease state

Mechanisms of synaptic plasticity in neuropathic pain

a. Intense afferent C-fiber stimulation results in an increase in active-zone recruitment

b. Persistent nociceptive activity (intense C-fibre drive) can result in increased spine size or density

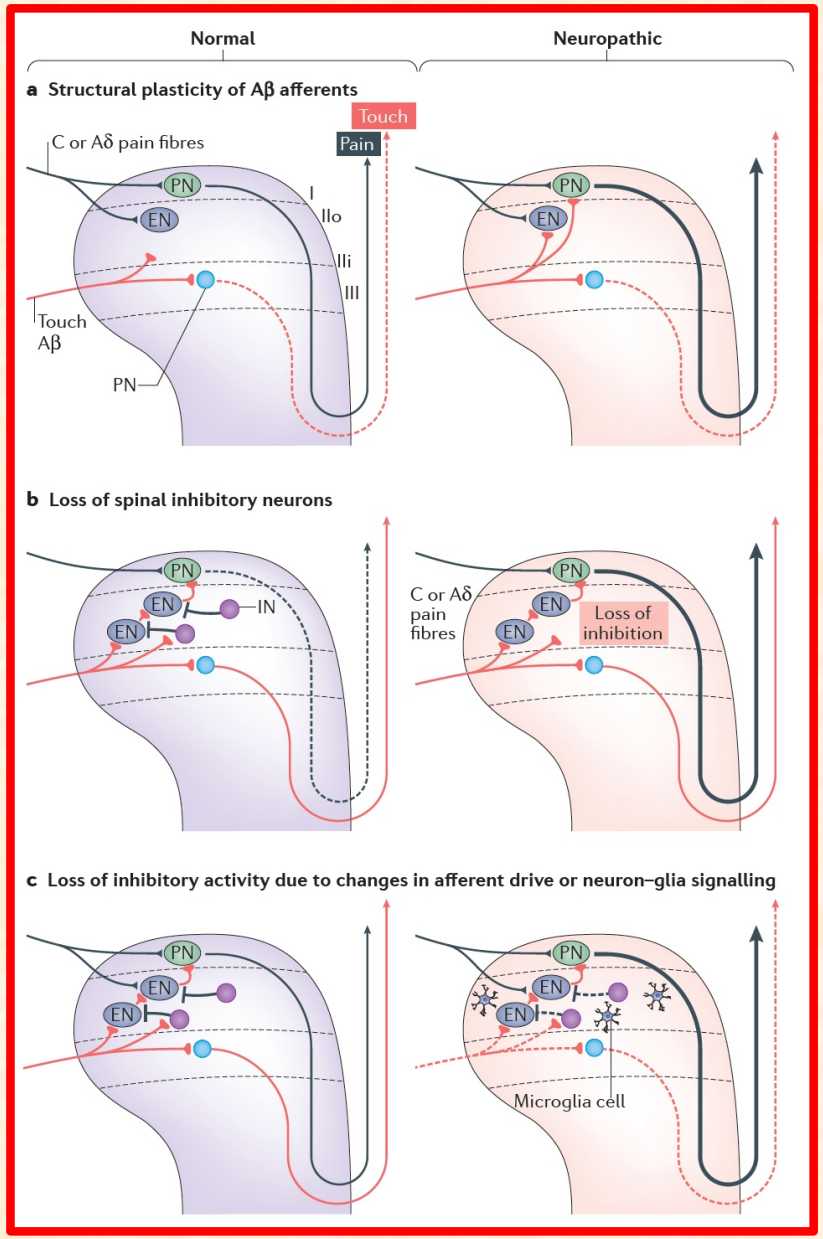


c. Postsynaptic messengers travelling from the synapse to the nucleus of the spinal neuron activate genomic programs, and thereby bring about long-term modulation of spine structure and density.

d. Persistent nociceptive activity-induced remodelling of the actin cytoskeleton involving spine stabilization are shown.

Kuner & Flor, Nat Rev Neurosci 18:20-30 (2017)

Mechanisms of plasticity in the spinal cord in neuropathic pain



EN, excitatory interneuron; IN, inhibitory interneuron; PN, projection neuron

a. Touch-sensitive low-threshold mechanoceptive fibres (Aβ fibres) sprout into superficial laminae that typically receive noxious inputs (C fibres and/or Aδ nociceptive fibres)

b. Disinhibition through a physical loss of spinal inhibitory neurons can activate crosstalk between touch and pain circuits

c. Alternative models involve:

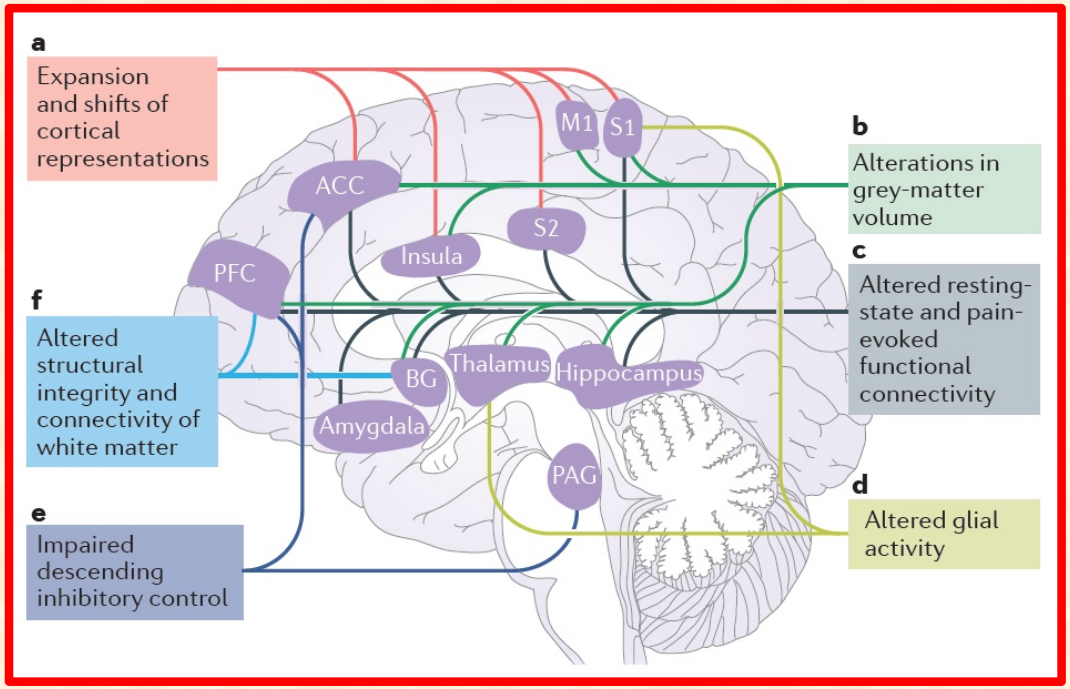
i. defects in the structure or activity of low-threshold mechanoreceptive fibres

ii. proliferation and activation of spinal glia, which modulate activity of spinal excitatory and inhibitory neurons via secreted mediators.

Whatever the mechanism involved, the final result is an *enhanced activity in spinal pain pathways* (*thicker black lines in the right panels*)

Kuner & Flor, Nat Rev Neurosci 18:20-30 (2017)

Brain plasticity in neuropathic pain



ACC, anterior cingulate cortex;
BG, basal ganglia;
M1, primary motor cortex;
PAG, periaqueductal grey;
PFC, prefrontal cortex;
S1, primary somatosensory cortex;
S2, secondary somatosensory cortex.

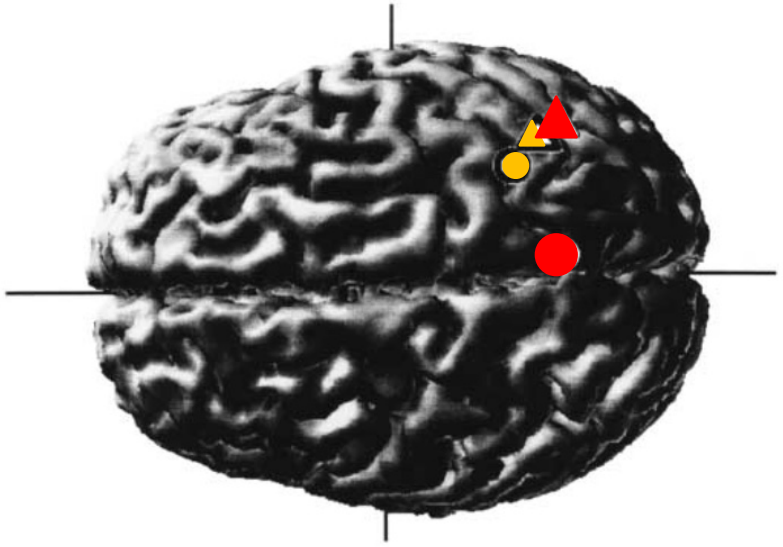
Kuner & Flor, Nat Rev Neurosci 18:20-30 (2017)

Chronic low back patients and control subjects were exposed to intracutaneous electric stimuli applied to the left back and index finger at a standard, a non-painful and a painful intensity.

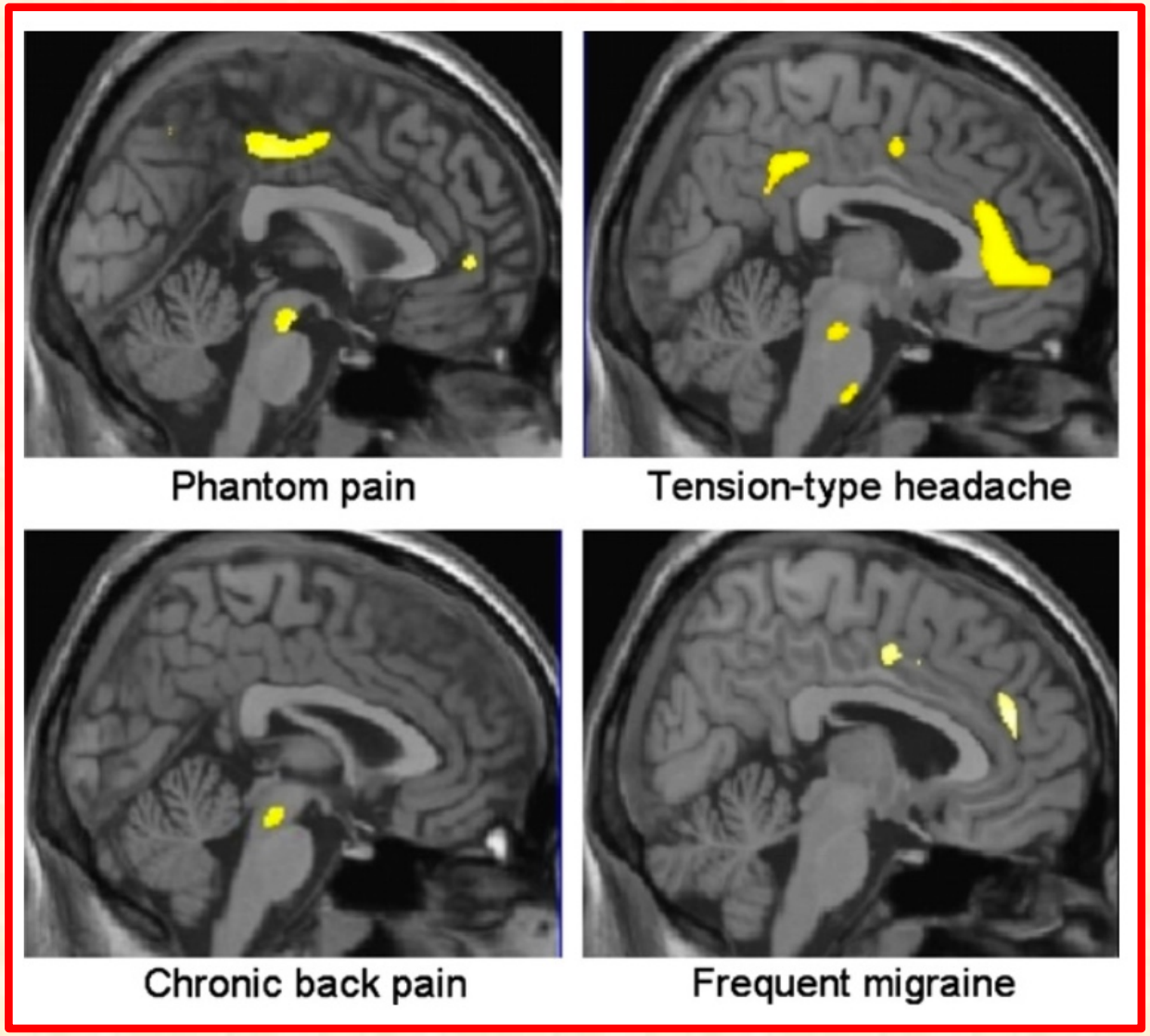
The location of the cortical activity to painful stimulation was determined in the 70–75 ms latency window by MRI.

In the medial-lateral direction, *the cortical representation of the back in the chronic back pain group had shifted more than 2.5 cm medially.*

Flor et al., Neurosci Lett 224:5-8 (1997)



Plasticity in the brain under chronic pain conditions



May A., Pain 137:7-15 (2008)

MRI images of the brain of patients suffering from different painful conditions have been superimposed with CTRL images.

Yellow areas indicate specific differences in gray matter structure, i.e. a decreased density possibly due to persistent chronic afferent inputs

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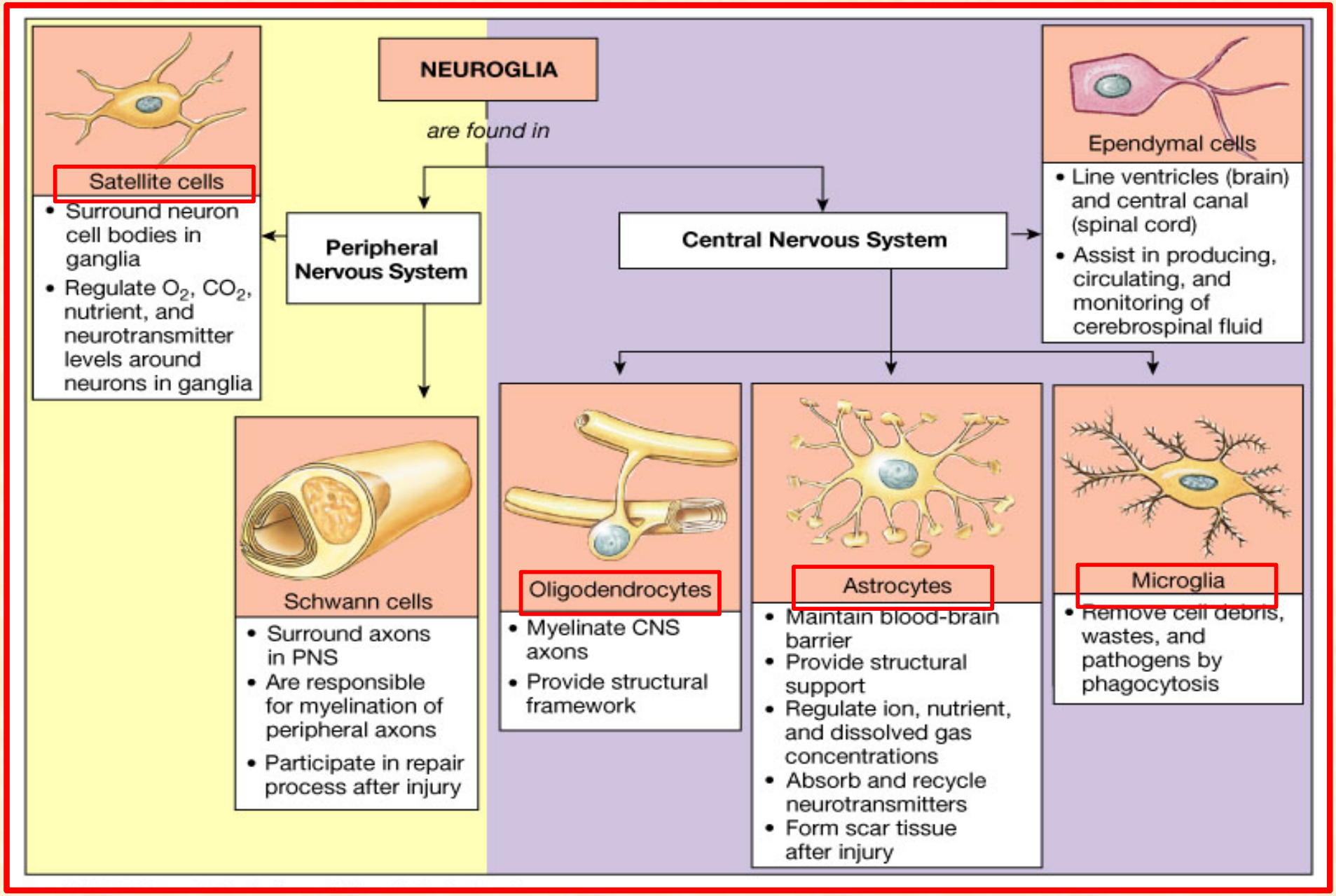
Types of glial cells in the CNS and PNS

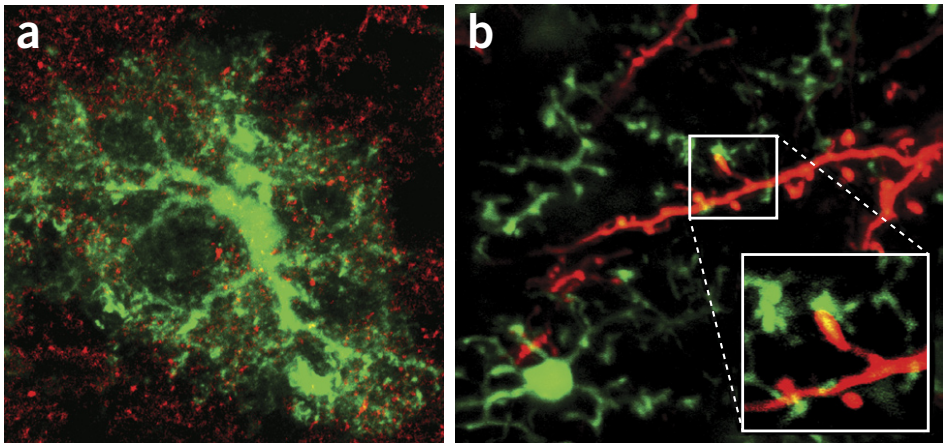


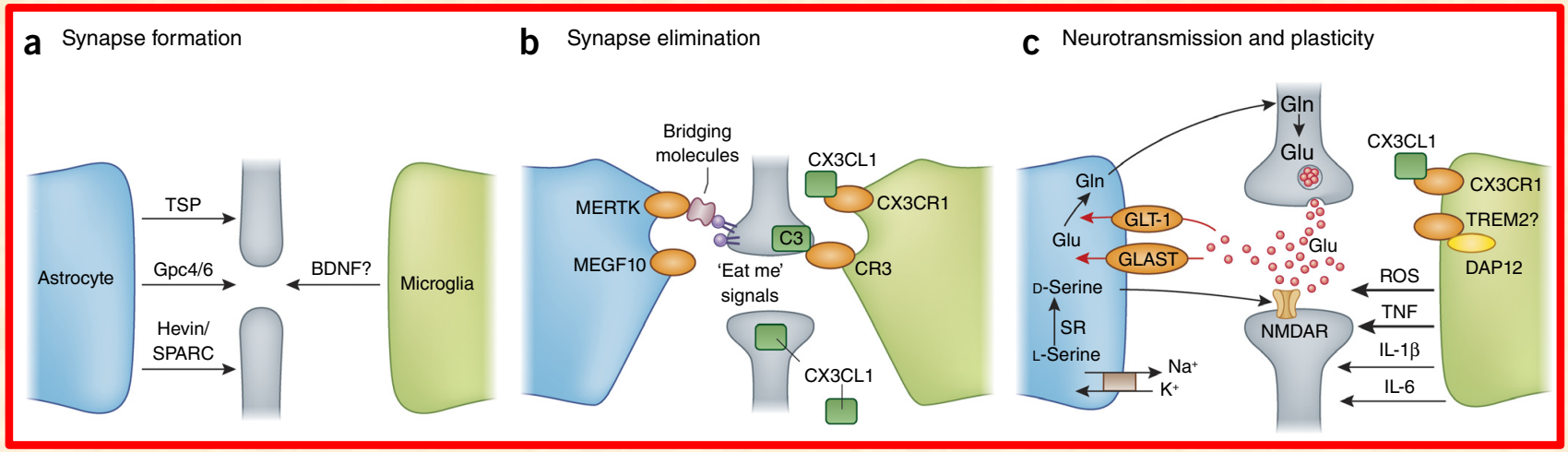
The close relationships between glial cells and neurons

Astrocytes and microglia interact with synapses

(a) Three- dimensional projection view of a GFP-expressing astrocyte associating with SV2-positive synaptic terminals.

(b) GFP-expressing microglia in close proximity to dendrites and spines.

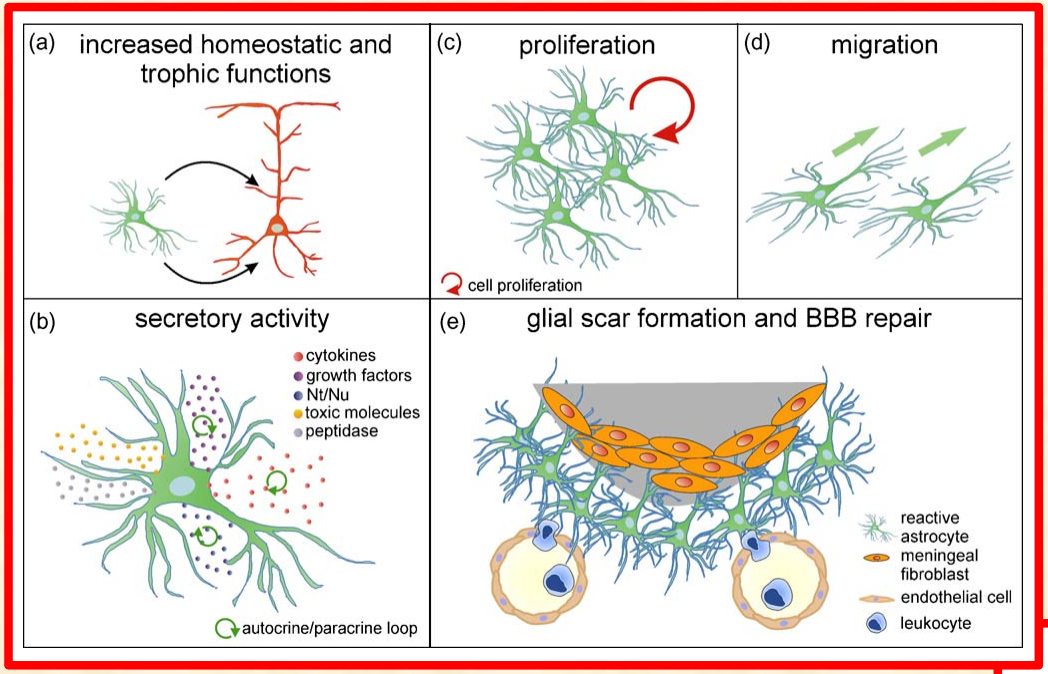




Astrocytes and microglia participate in synapse formation, elimination and plasticity

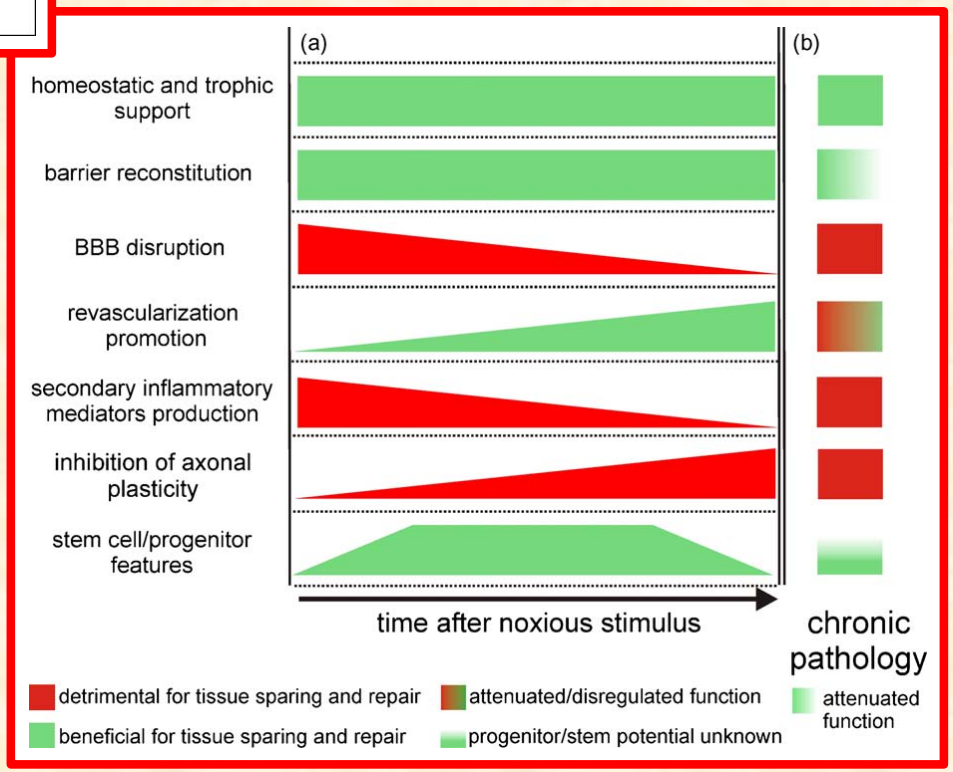
Modified from: Chiung et al., Nat Neurosci 18:1539-45 (2015)

Astrocyte reaction to injury



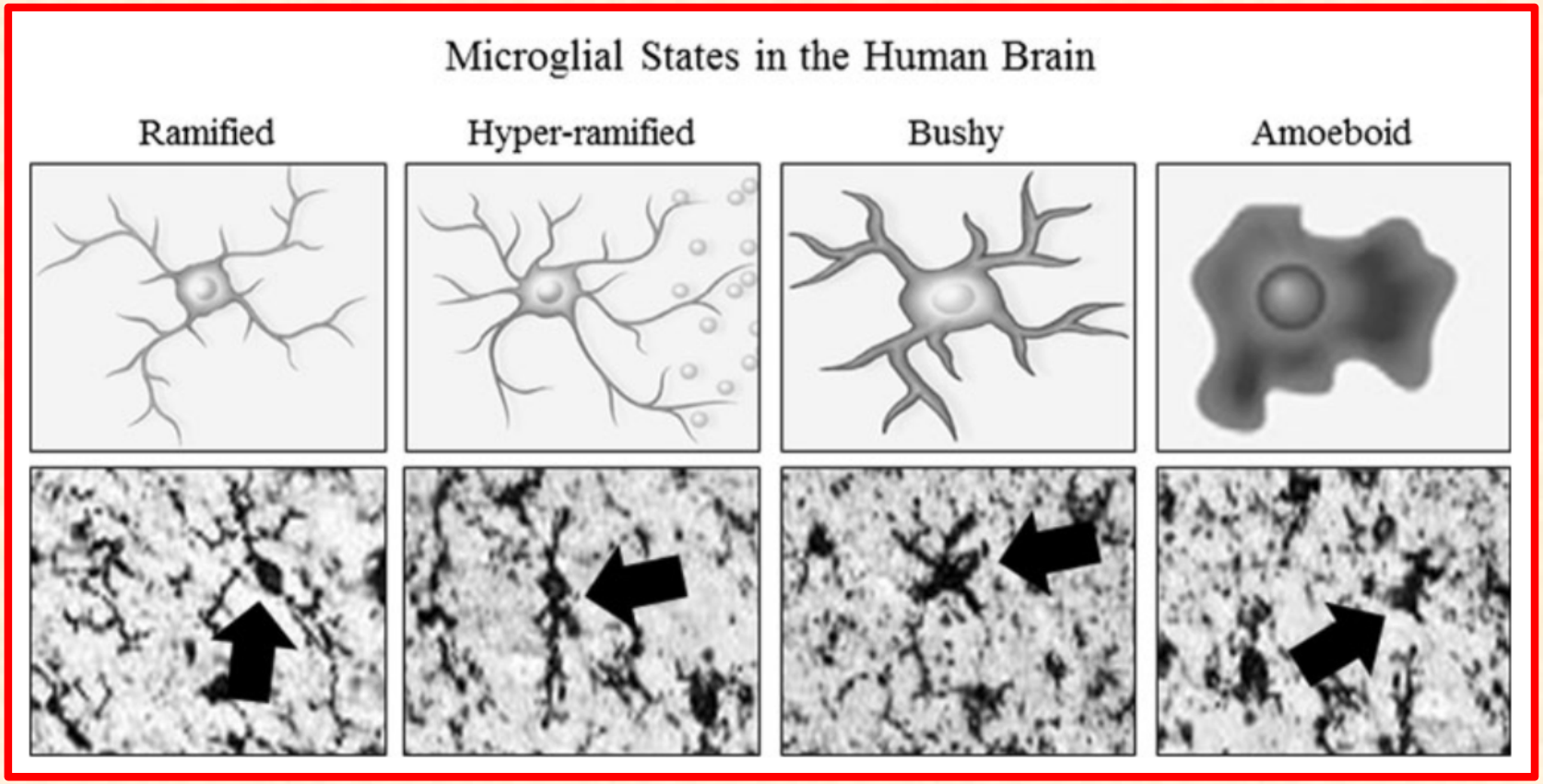
Step-by-step reactive astrogliosis

Double-edged sword effects of reactive astrocytes during acute and chronic brain pathologies



Buffo et al., Biochem Pharm 79:77-89 (2010)

MIcroglia reaction to injury



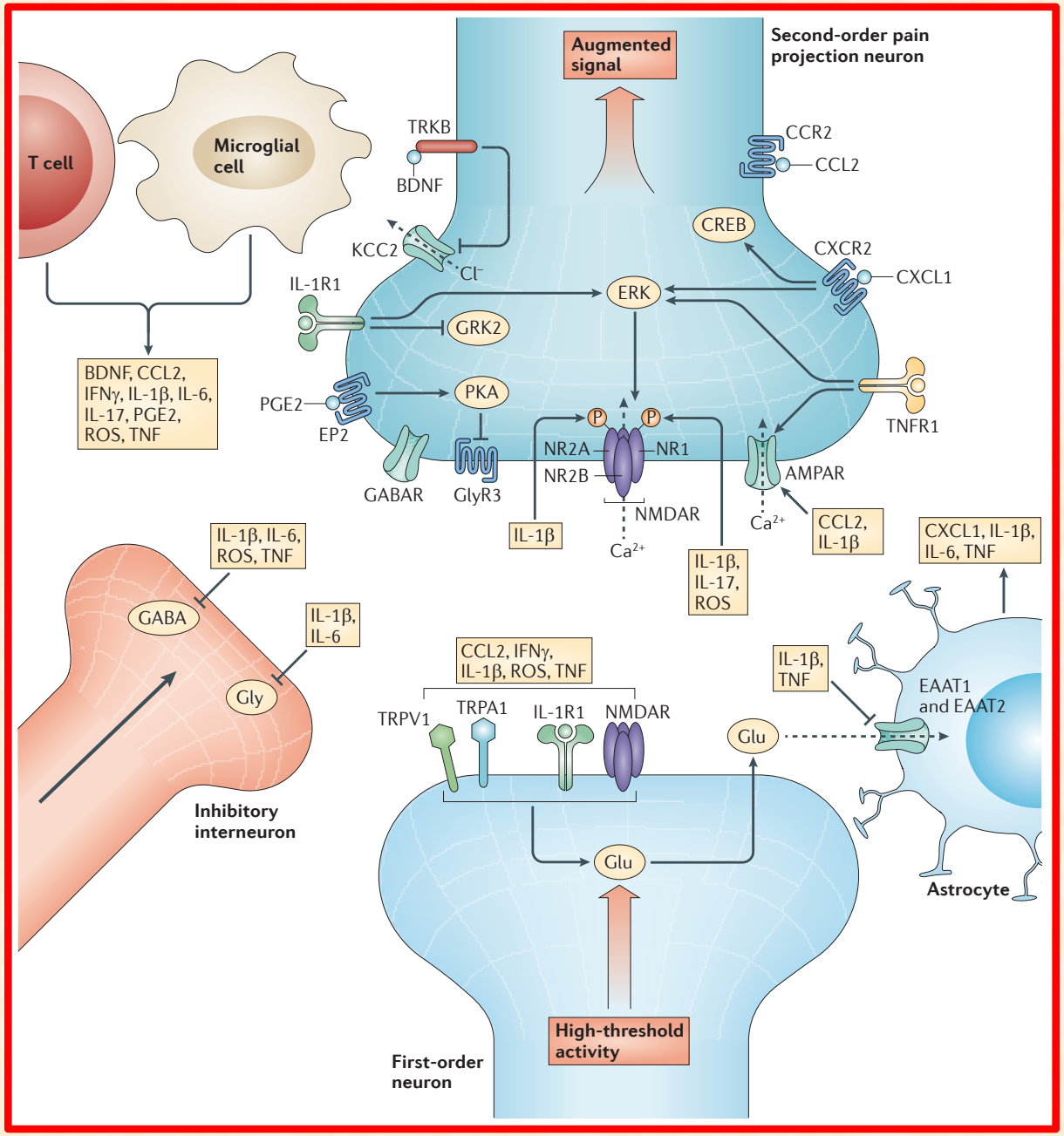
Crews & Vetreno, Psychopharmacology 233:1543-1557 (2016)

Upon activation, microglia cells modulates the expression of a plethora of receptors, enzymes, channels, and pro- or anti-inflammatory cytokines and chemokines

They can exibit either pro-inflammatory (*neurotoxic, M1*) and anti-inflammatory (*neuroprotective, M2*) phenotypes

Ji et al., PAIN 154:S10-S28 (2013)

Modulation of pain by the neuro-immune interface



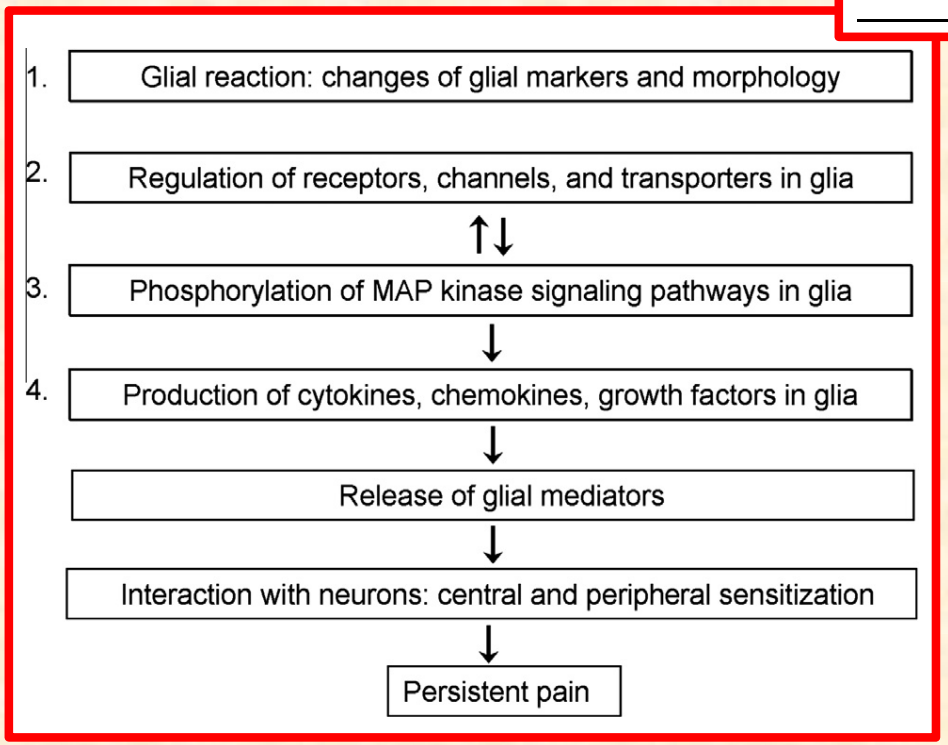
The complex interplay of different signalling pathways in the neuro-immune modulation of chronic pain in the spinal cord

Grace et al., Nat Rev Immunol 14:217-231 (2014)

Chronic pain as a "gliopathy"

Upregulation of the glial markers IBA1 and CD11b (*microglia*), and glial fibrillary acidic protein (GFAP; *astrocytes* and *satellite glial cells, SGCs*) in various pain conditions

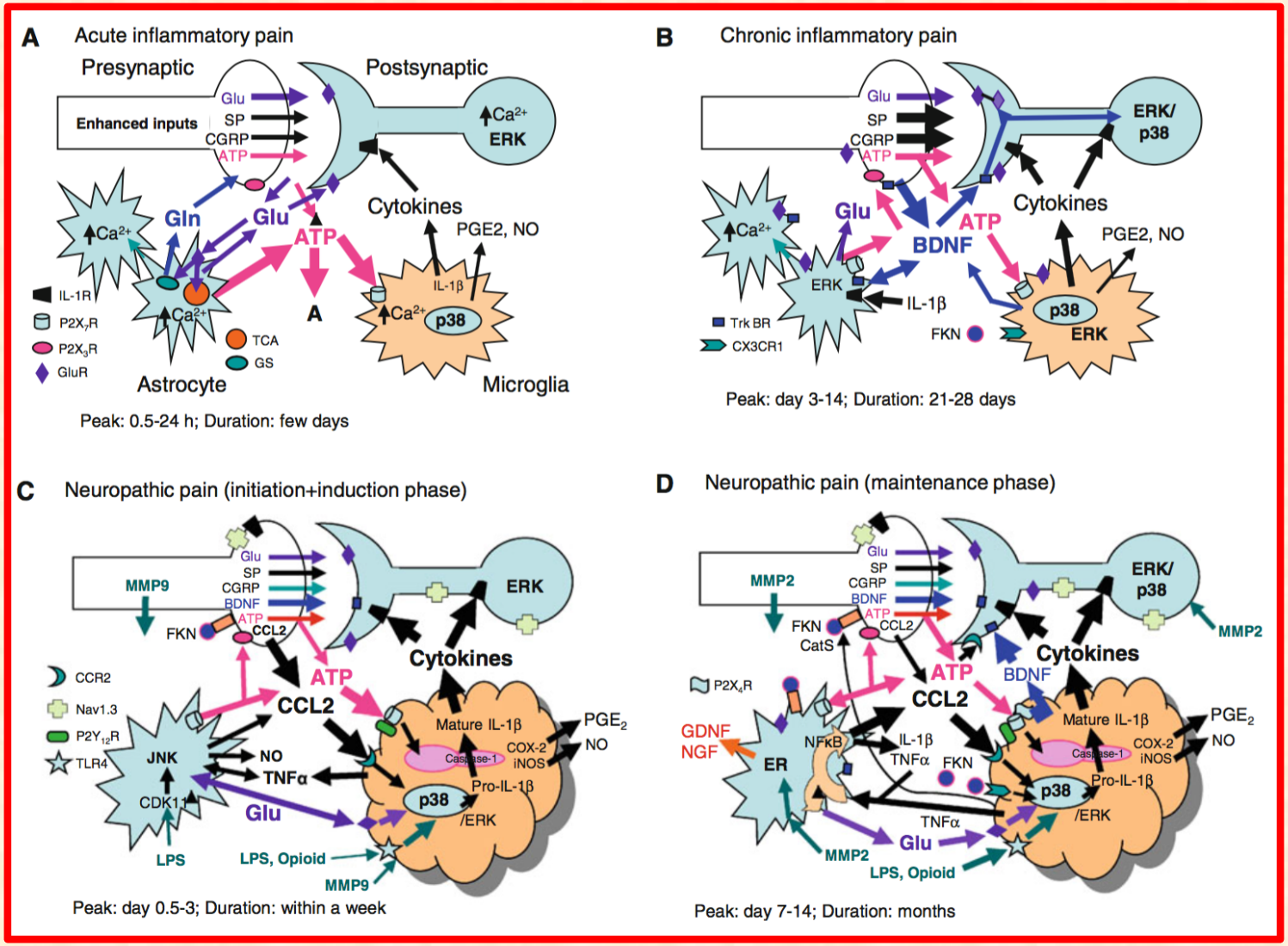
Pain conditions	Microglia	Astrocytes	SGCs
Nerve injury	↗	↗	↗
Spinal cord injury	↗	↗	
Paw incision	↗	↗	
Inflammation	↔/↗	↗	↗
Joint arthritis	↗	↗	↗
Bone cancer	↔/↗	↗	↗
Skin cancer	↔	↗	
Chemotherapy	↔/↗	↗	↗
Diabetes	↗	↗	
HIV neuropathy	↔	↗	
Chronic opioid	↗	↗	
Acute opioid	↔	↔	↗



Four steps of glial cell reaction to painful stimuli

Ji et al., PAIN 154:S10-S28 (2013)

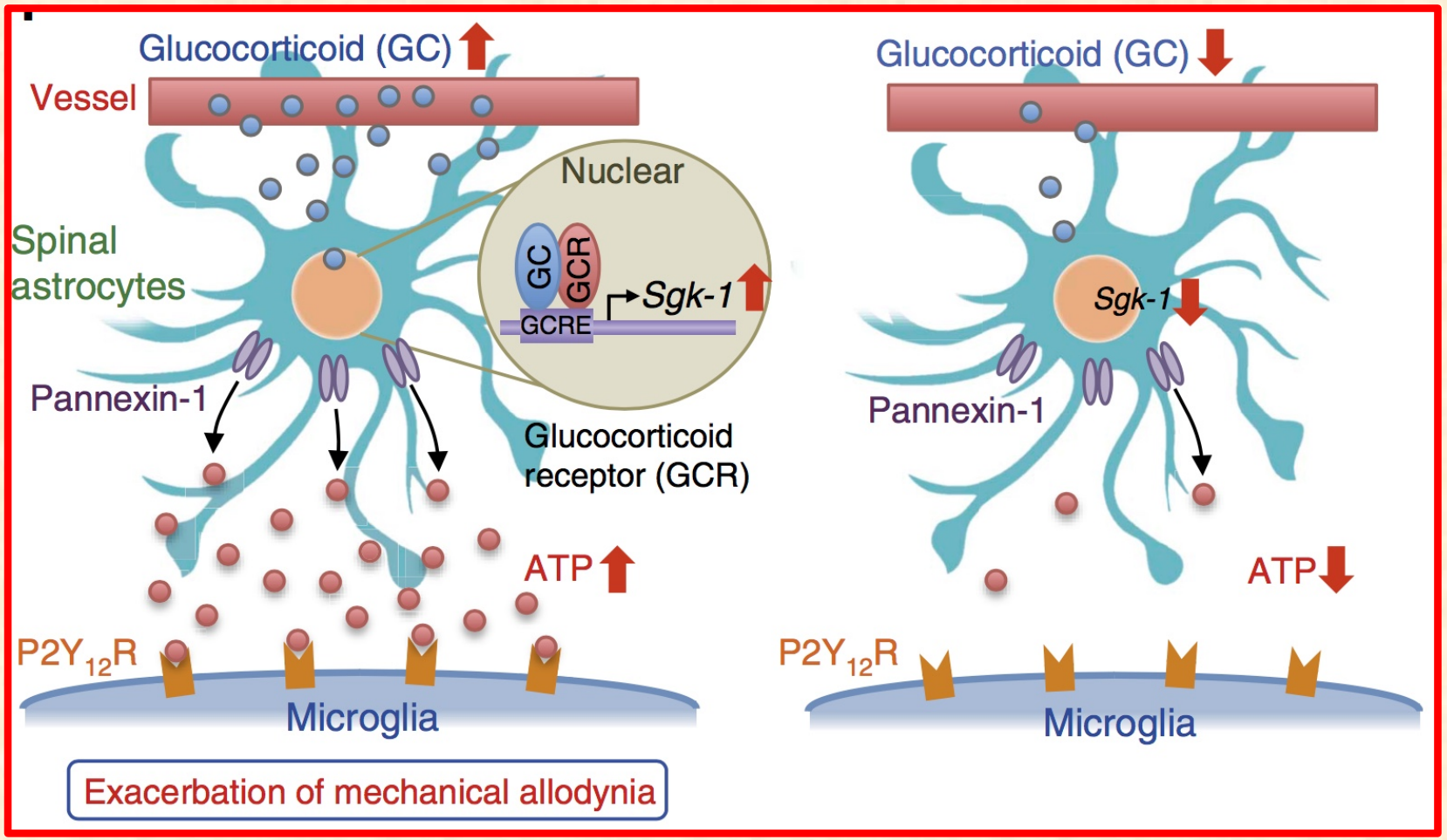
Altered neuron-glia signaling pathways in the transition from acute to chronic pain



Some of the signalling pathways that modulate the astrocyte-neuron-microglia connection and are involved in transition from acute to chronic inflammatory (A→B) and neuropathic (C→D) pain

Chiang et al., Neurochem Res 37:2419-2431 (2012)

Modulation of astrocytic ATP release by glucorticoids underlies diurnal exacerbation of mechanical allodynia

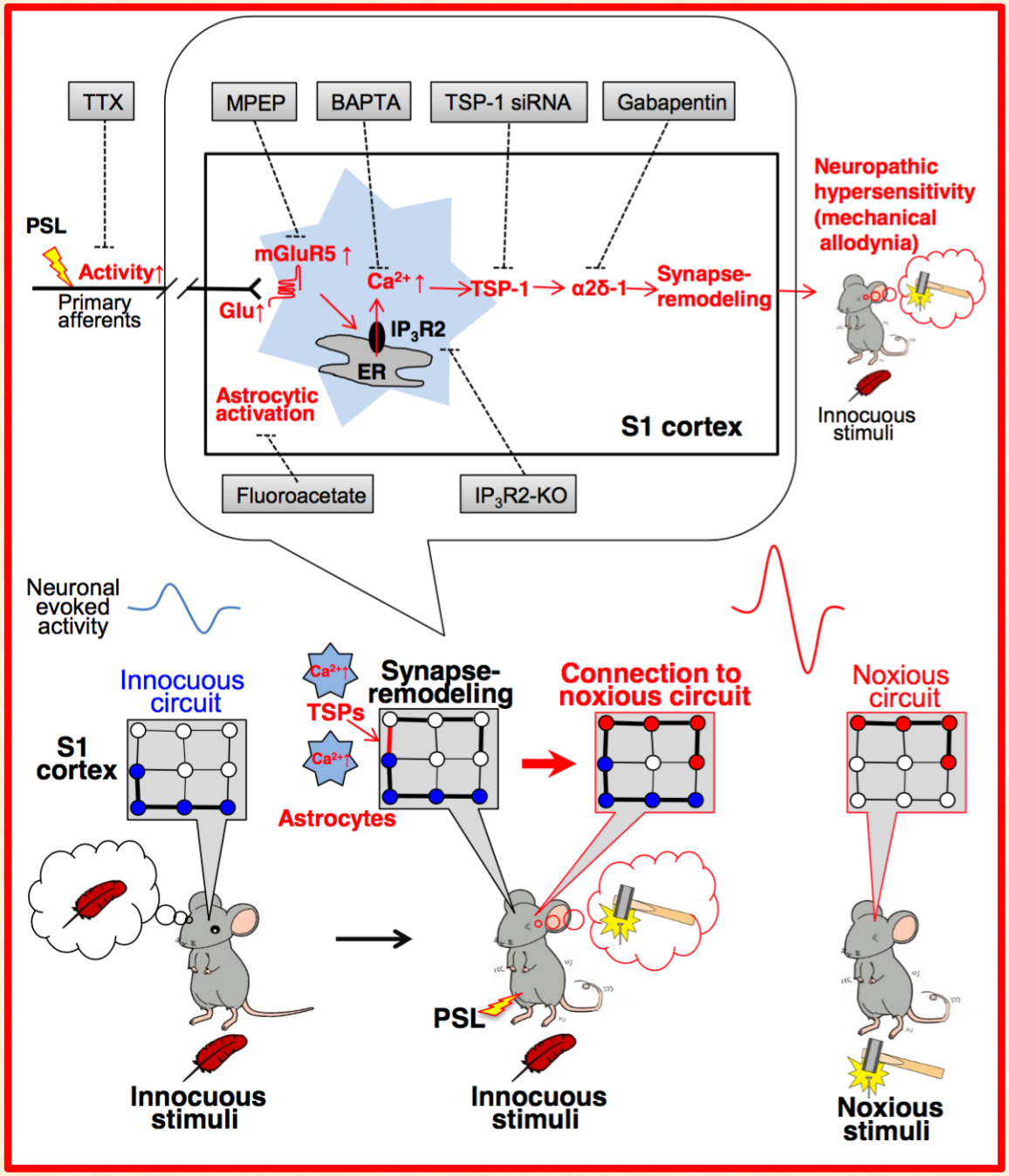


Koyanagi et al., Nat Commun 7:13102 (2016)

Temporal elevations in glucocorticoid levels induce the expression of SGK-1 in spinal astrocytes, thereby enhancing extracellular ATP release through pannexin-1 hemichannels.

ATP released from astrocytes binds to $P2Y_{12}$ receptors on activated microglia. Stimulated $P2Y_{12}$ receptors induce downstream events, which result in a decrease in the threshold of mechanical allodynia (*partial sciatic nerve ligation in rats*).

Reactive astrocytes mediate synaptic rewiring in the S1 cortex



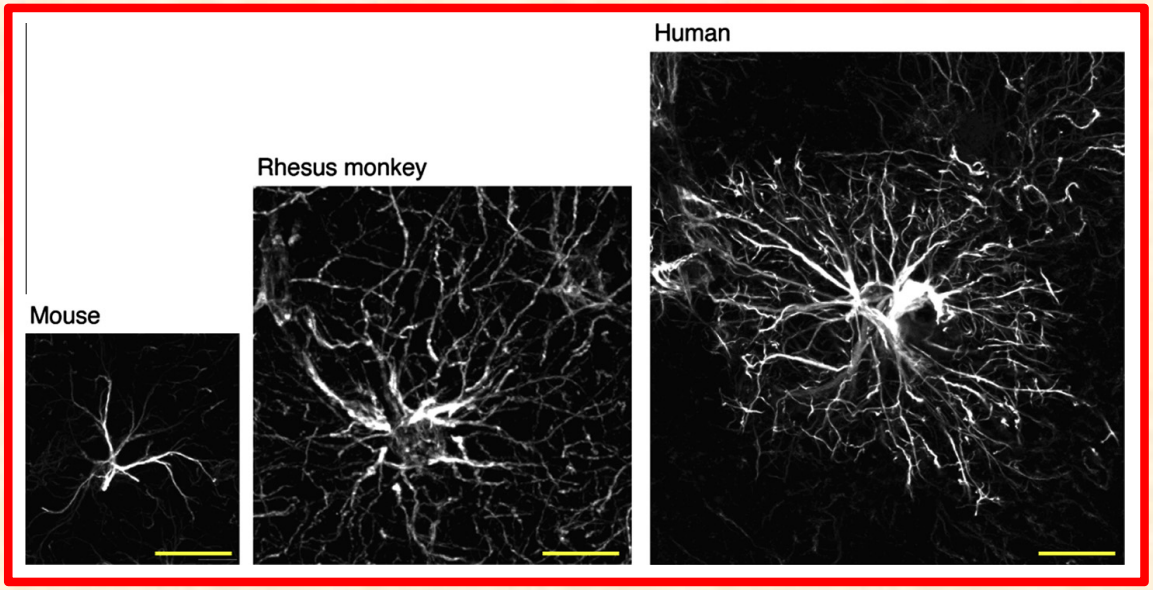
Immature-type astrocyte signaling within the S1 cortex is activated by peripheral injury during the first week post-injury time.

Elevation of astrocytic Ca^{2+} activity releases TSP-1 that binds to the neuronal α2δ-1 receptors to initiate increased synapse remodeling.

This probably connects the S1 innocuous circuits to noxious circuits, and finally mediates the long-lasting mechanical allodynia

Kim et al., JCI 126:1983-1997 (2016)

Striking differences among astrocytes from rodents, monkeys, and humans



In human cortex, astrocytes are more than 2-fold larger in diameter and extend 10-fold more GFAP-positive primary processes than their rodent counterparts

The domain of a single human astrocyte has been estimated to contact up to 2 million synapses

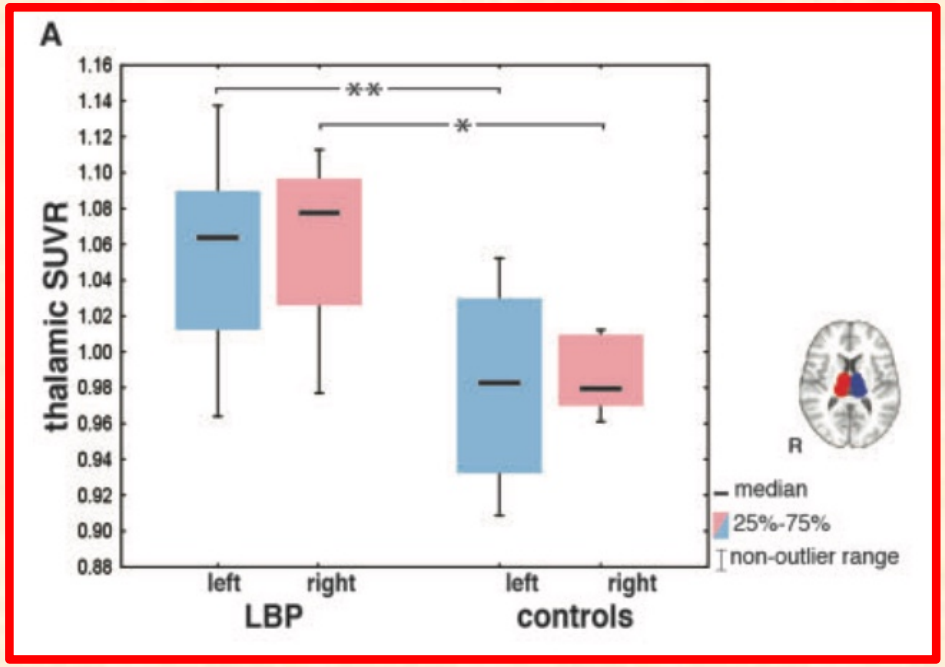
Human astrocytes could play a more sophisticated role in chronic pain than rodent astrocytes. Importantly, astrocyte reaction, but not microglial reaction, is associated with chronic pain in HIV-infected patients

Ji et al., PAIN 154:S10-S28 (2013)

Activation of CNS glia in chronic low back pain patients

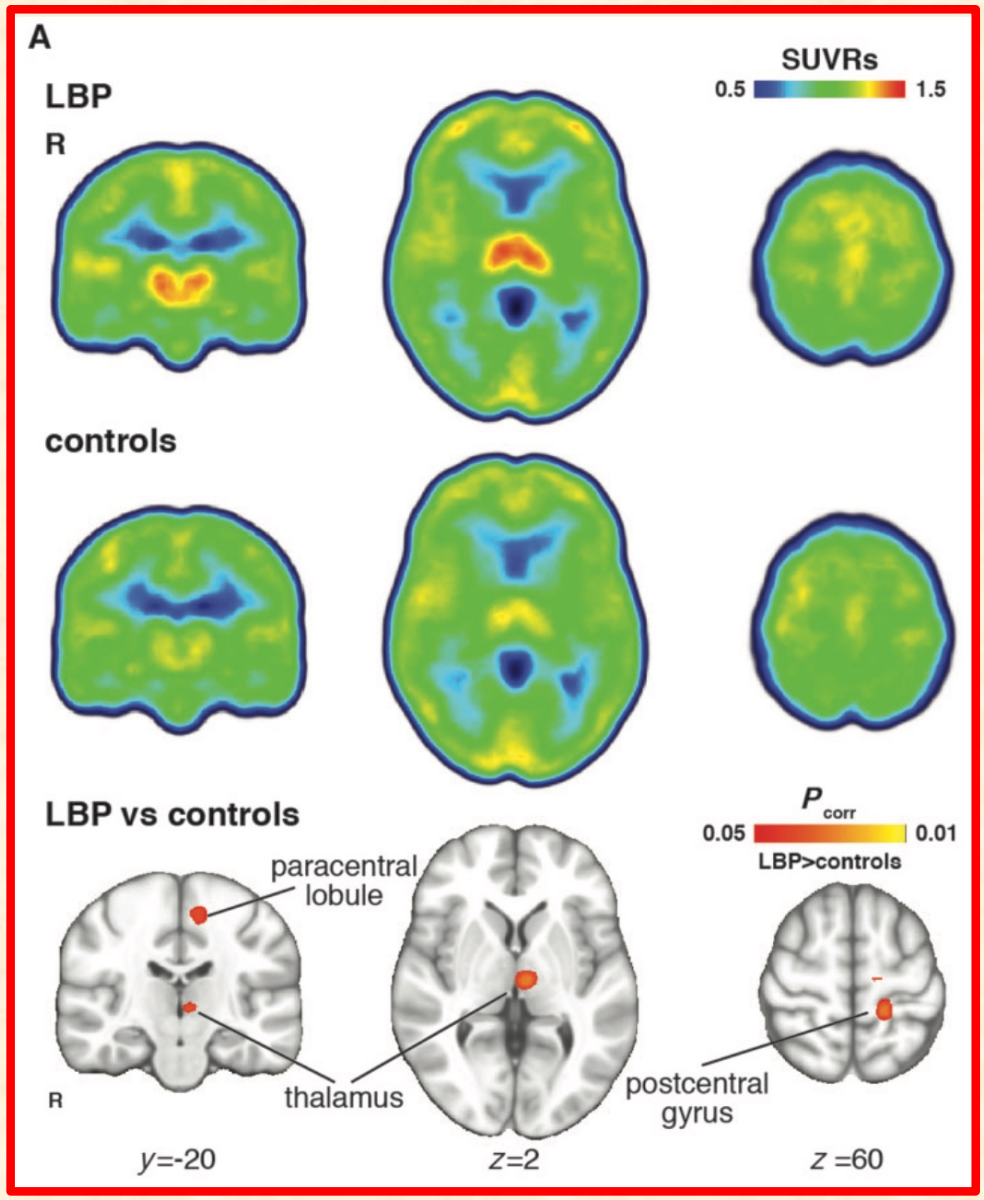
Standardized Uptake Values (SUV):

mean radioactivity/injected dose/weight)



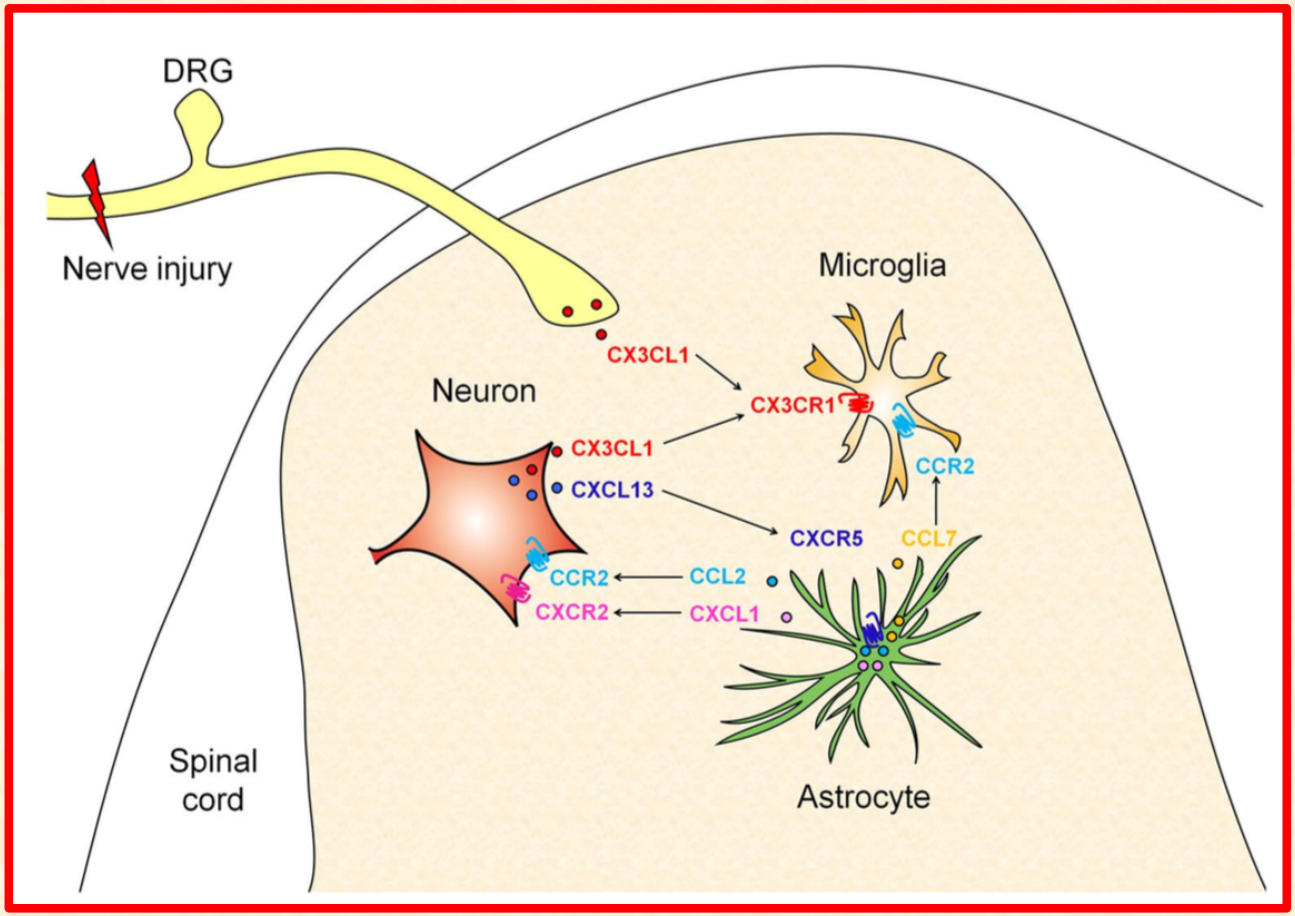
The PET radioligand ^{11}C-PBR28 binds to the translocator protein (18kDa) (TSPO), a protein upregulated in activated microglia and reactive astrocytes in animal models of pain.

Data show a significantly increased uptake in the thalamus of patients and a positive correlation with the plasma concentrations of IL-6.



Loggia et al., BRAIN 138:604-615 (2015)

Neuroinflammation in neuropathic pain: the neuron-glia-chemokine connection



CX3CL1 (fraktalkine) is physiologically expressed by neurons but can be induced in astrocytes by peripheral nerve injury. Acts on its receptor CX3CR1 to activate microglia

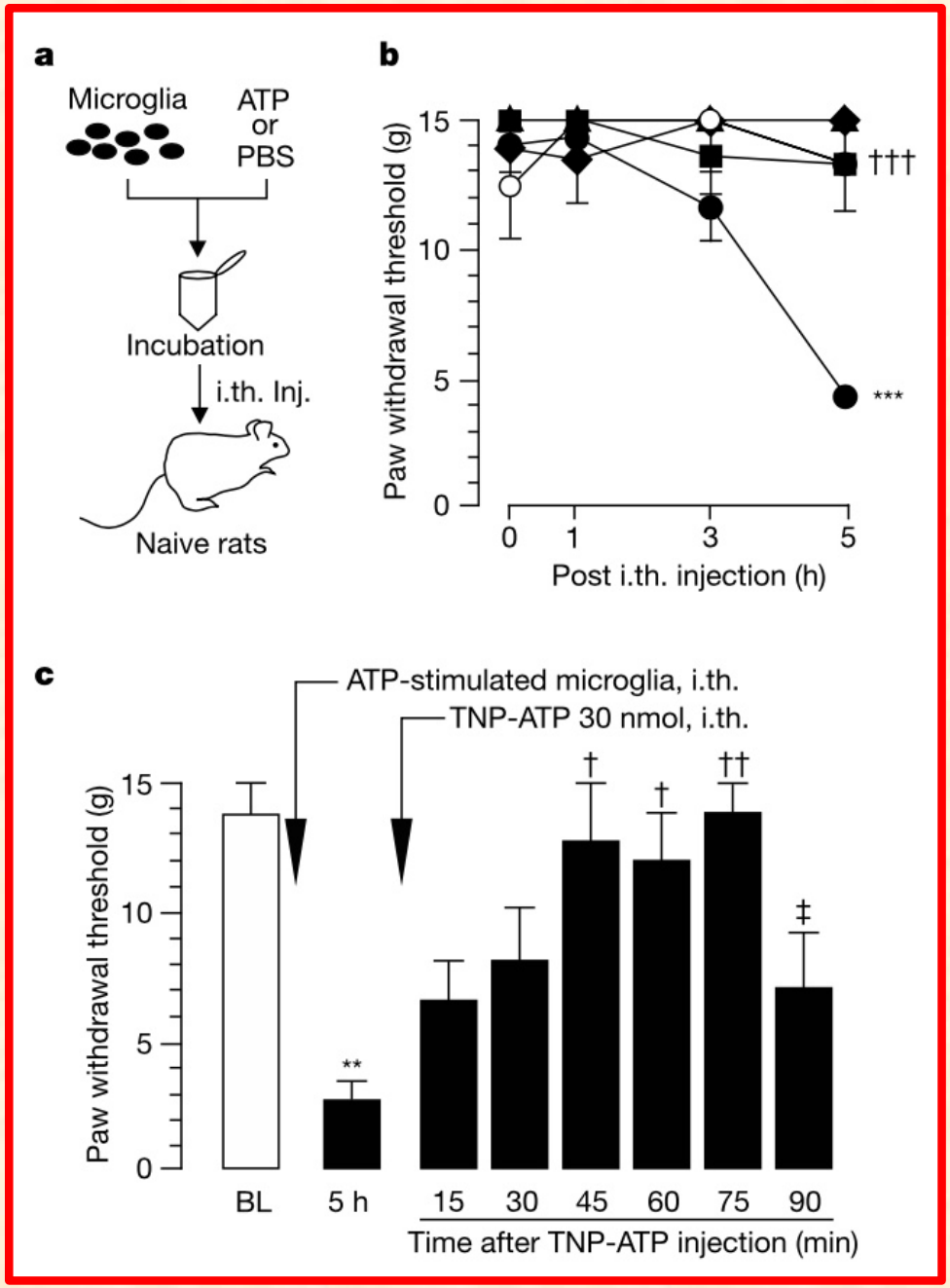
CXCL13 releases from spinal neurons and acts on CXCR5 to induce astrocyte activation

The activated astrocytes release CCL2 and CXCL1, which act on their major receptors CCR2 and CXCR2 on spinal neurons to enhance excitatory synaptic transmission

CCL7 may be released from astrocytes and acts on CCR2 to activate microglia cells

Zhang et al., Cell Mol Life Sci in press 2017

The central role of activated microglia in neuropathic pain



The intraspinal injection of activated glia produces tactile allodynia, a hallmark of neuropathic pain, in naïve rats

Allodynia is reverted by the purinergic antagonist TNP-ATP, acting on the P2X4 receptor channel

Tsuda et al., Nature 424:778-783 (2003)

The P2X4 receptor subtype in neuropathic pain

In spinal cord microglia, P2X4-evoked Ca^{2+} response is increased after peripheral nerve injury, leading to activation of p38 and release of BDNF

Ulmann et al., J Neurosci. 28:11263-11268 (2008); Trang et al., J Neurosci. 29:3518-3528 (2009)

The CCL21 chemokine (only expressed in damaged neurons) rapidly induces P2X4 receptor expression in spinal cord microglia, and is necessary for the development of tactile allodynia

Biber et al., The EMBO J. 30:1864-1873 (2011)

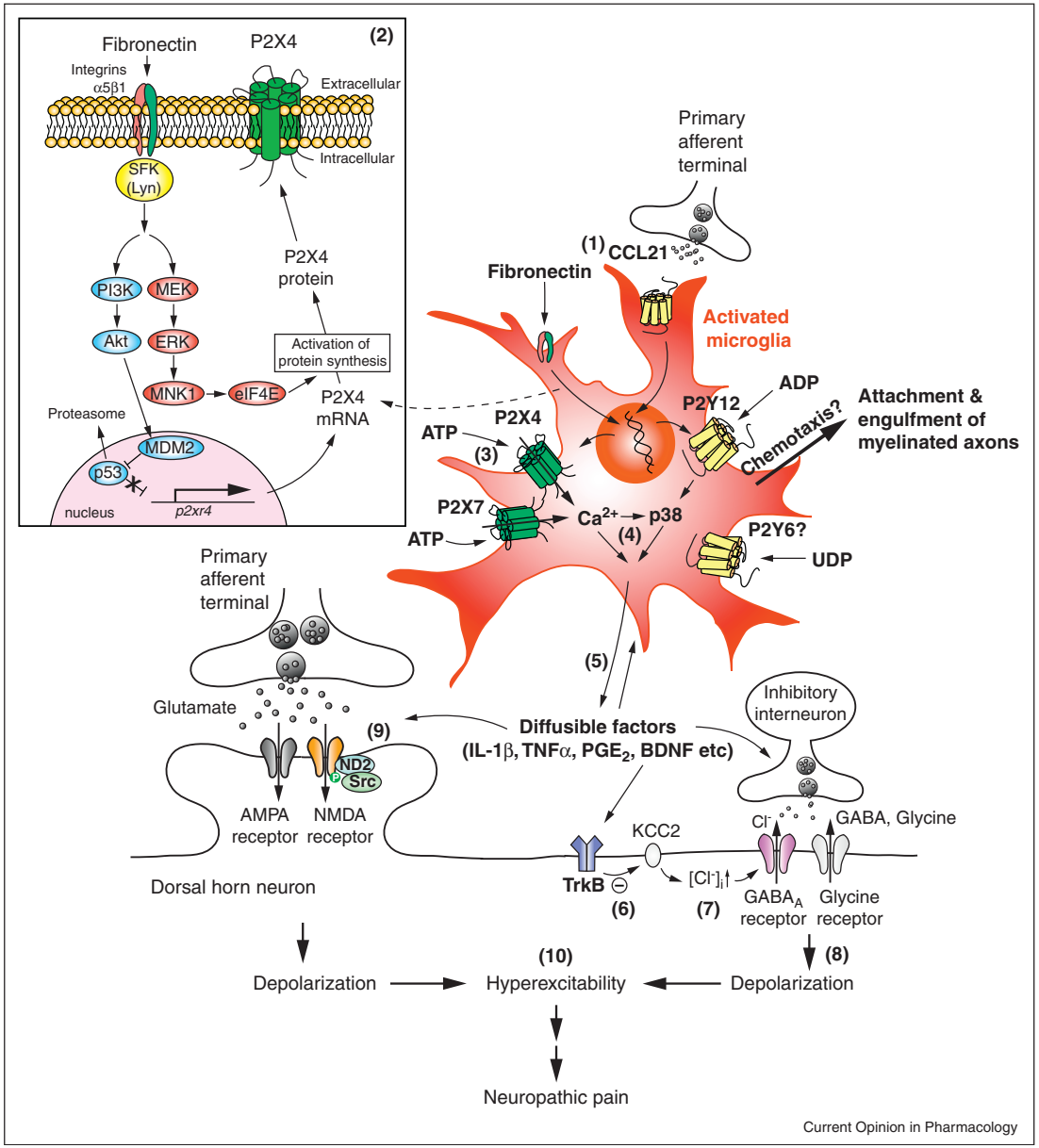
The P2X4 receptor subtype is necessary to the development of nerve injury-induced tactile allodynia and, to a lesser extent, of peripheral inflammation

Tsuda et al., Mol Pain 5:28 (2009)

Spinal cord neurons express the P2X4 receptor subtype as well, which modulates inflammasome activation and IL1beta release after spinal cord injury

de Rivero Vaccari et al., J Neurosci. 32:3058-3066 (2012)

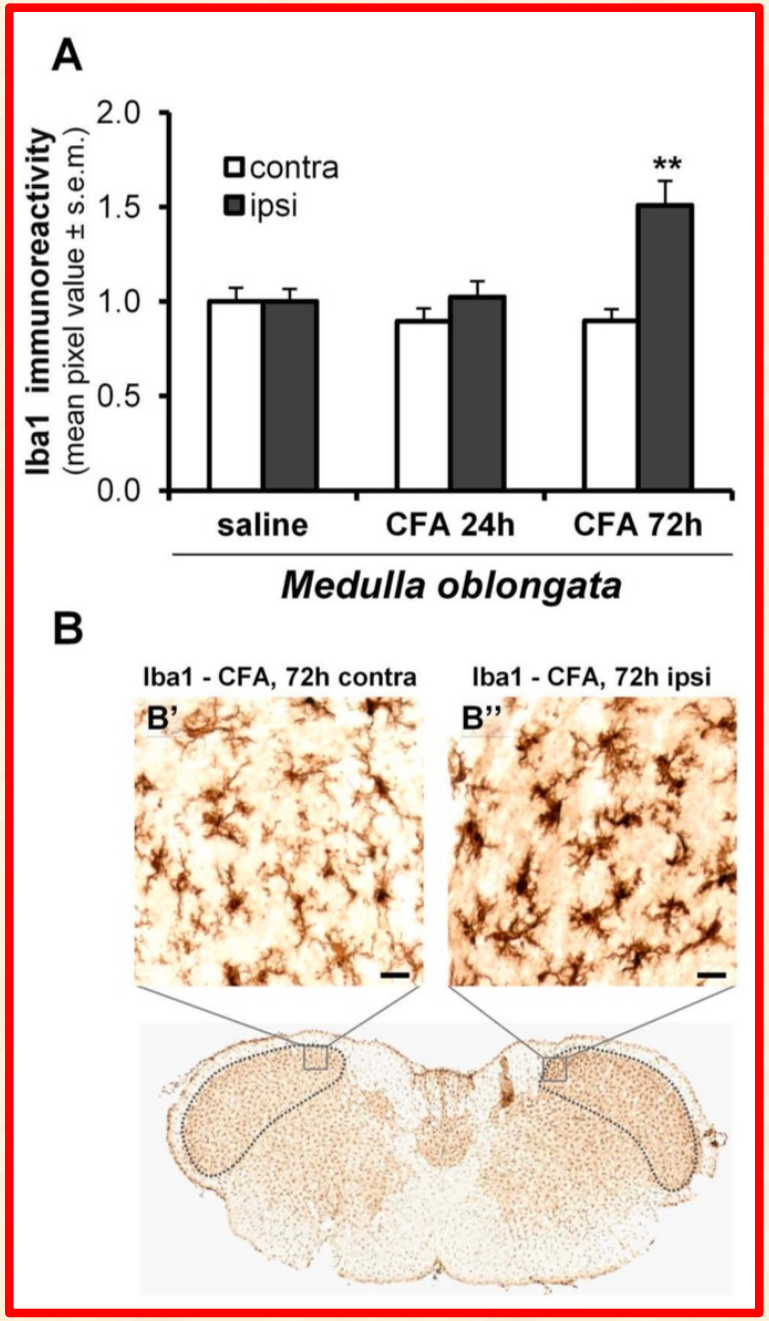
The central role of spinal microglia in neuropathic pain



The purinergic system is critically involved in the modulation of functions of activated microglial cells and in the development of nerve injury-associated pain.

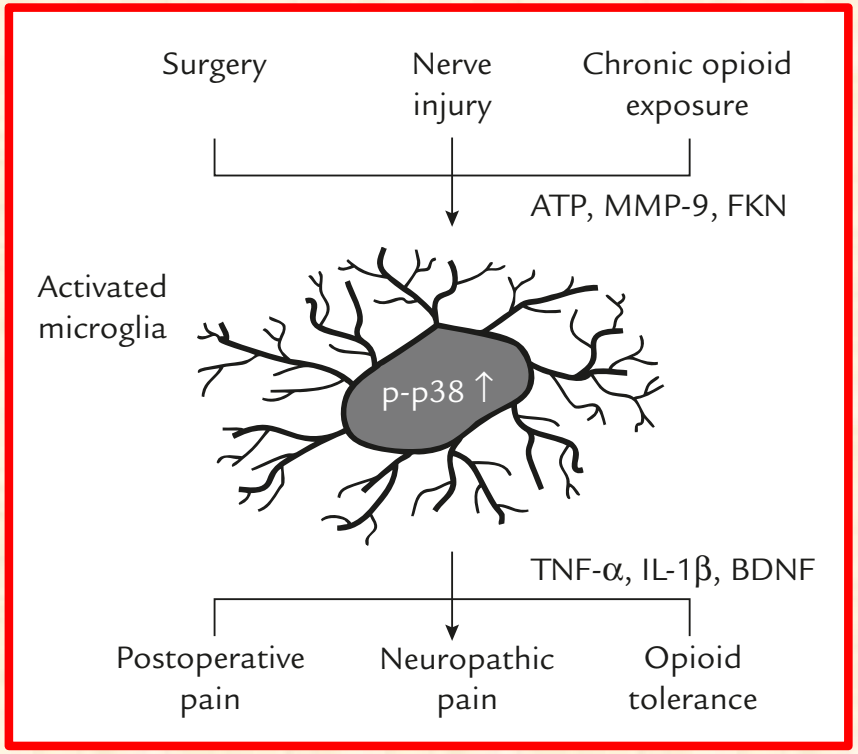
Tsuda et al., Curr Opin Pharmacol. 12:74-79 (2012)

Activation of spinal microglia in inflammatory trigeminal pain

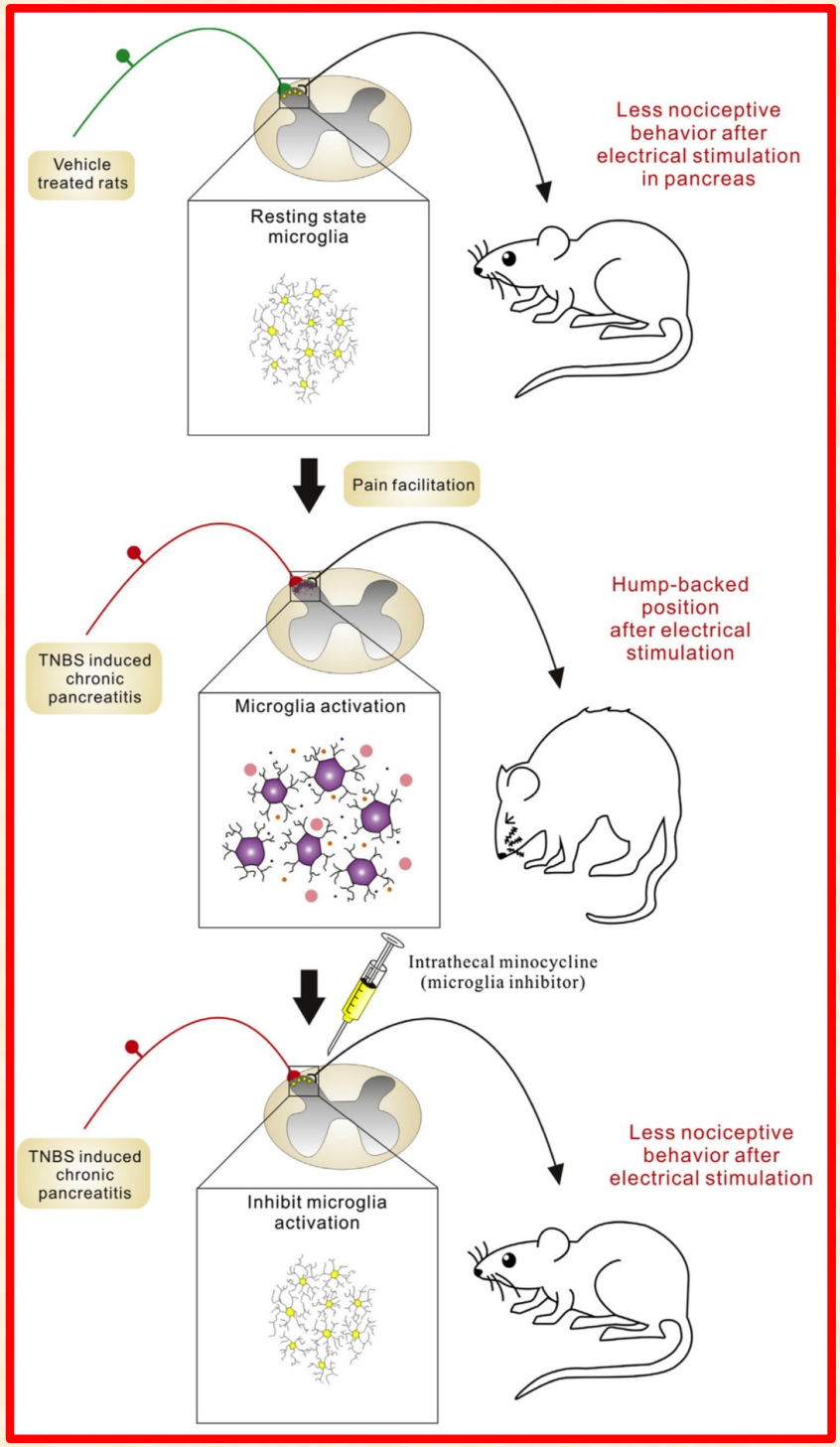


Villa et al., Mol Pain 6:89 (2010)

Glia-produced pain mediators can initiate and maintain postoperative pain, neuropathic pain, and antinociceptive tolerance of opioids, via the induction of hyperexcitability of nociceptive neurons in the spinal cord dorsal horn



Wen et al., J Formos Med Assoc 110:487-494 (2011)



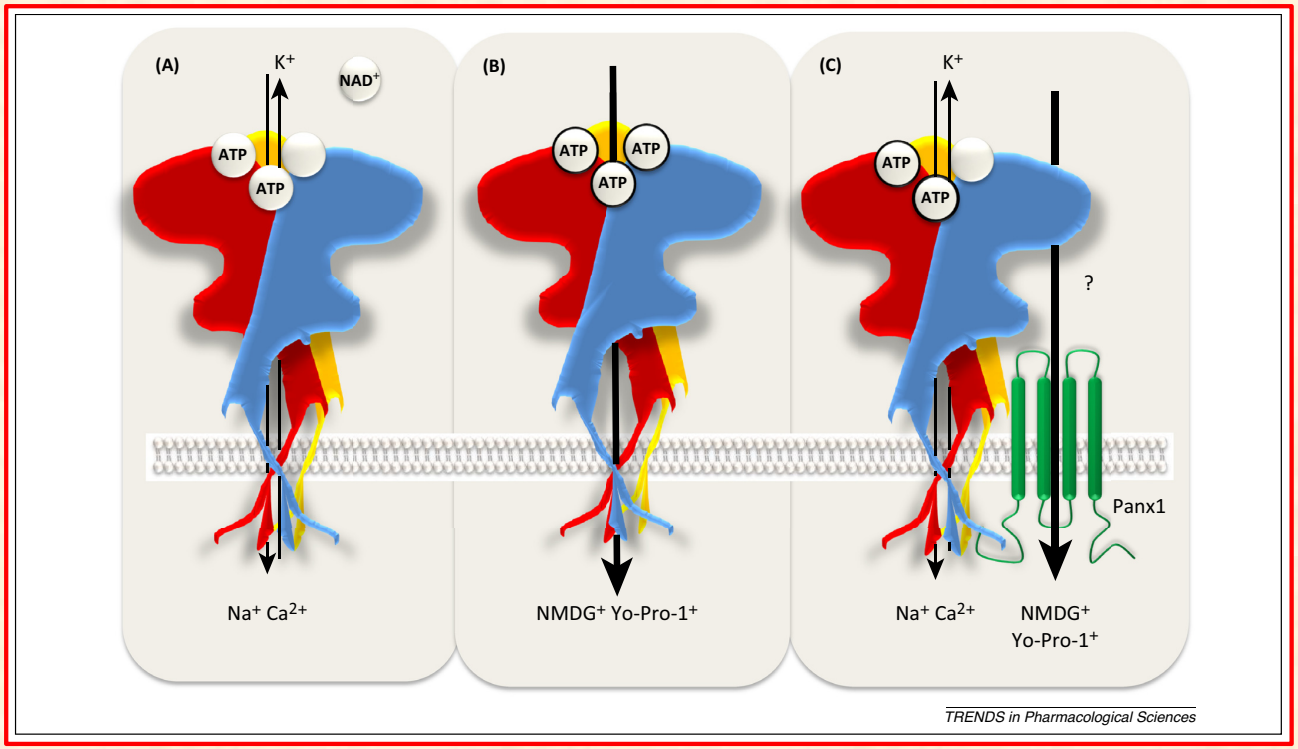
Therapeutic approaches targeting activated microglia

The injection of drugs blocking glial cells activation can inhibit, delay or reverse pain

(e.g., visceral pain; chronic pancreatitis induced by trinitro-benzene sulfonic acid, TNBS)

Lu, J Chin Med Assoc 77:3-9 (2014)

The peculiar features of the P2X7 receptor subtype



Permeable to molecules with a molecular weight up to 900 Da and to fluorescent dyes

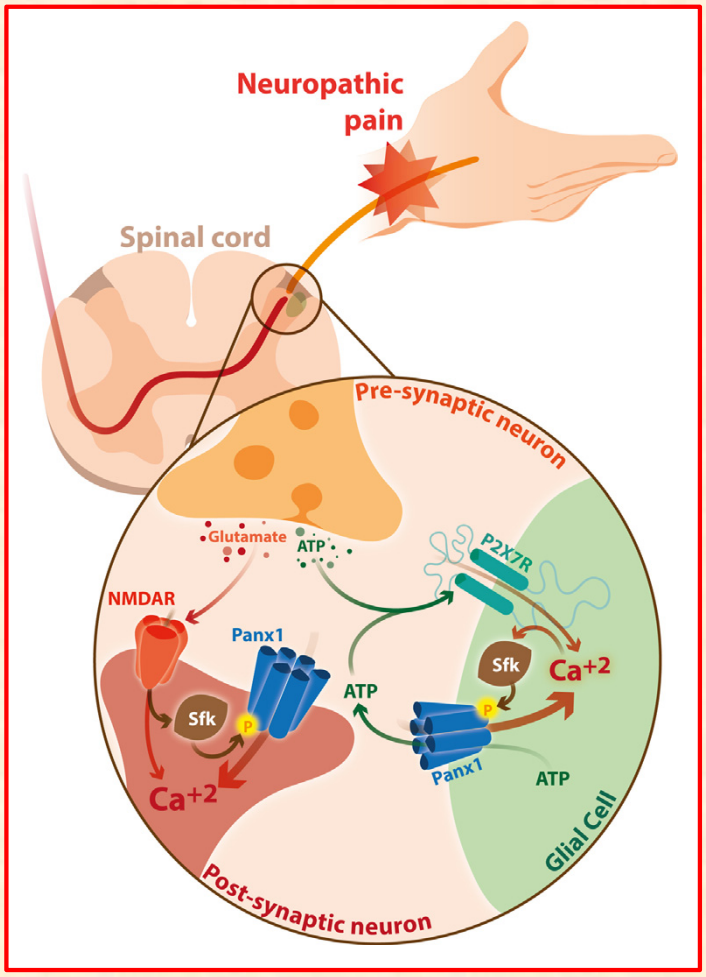
Open state **Membrane PORE**

Sperlágh & Illes., TiPS 35:537-547 (2014)

Effects of P2X7 receptor activation

- *Plasma membrane "blebbing"*
- *Release of IL1β, TNFα and PGE_2 from inflammatory cells*
- *Fusion of macrophages*

The P2X7 receptor subtype in neuropathic pain



Bravo et al., Pharm Res 101:86-93 (2015)

After intense and continuous discharge of the damaged peripheral neuron there is ATP release, which in turn activates glial P2X7R among other purinergic receptors.

When activated, P2X7R raises intracellular calcium that activates MAPK and p38, and release pro-inflammatory cytokines, which sensitize neurons.

Furthermore, P2X7R may be opening Panx1 via Src kinases, and this channel could enhance the nociceptive activity of P2X7R, by secreting ATP.

A possible role at the level of satellite glial cells in dorsal root ganglia has been also hypothesized

Zhang et al., JBC 290:14647-14655 (2015)

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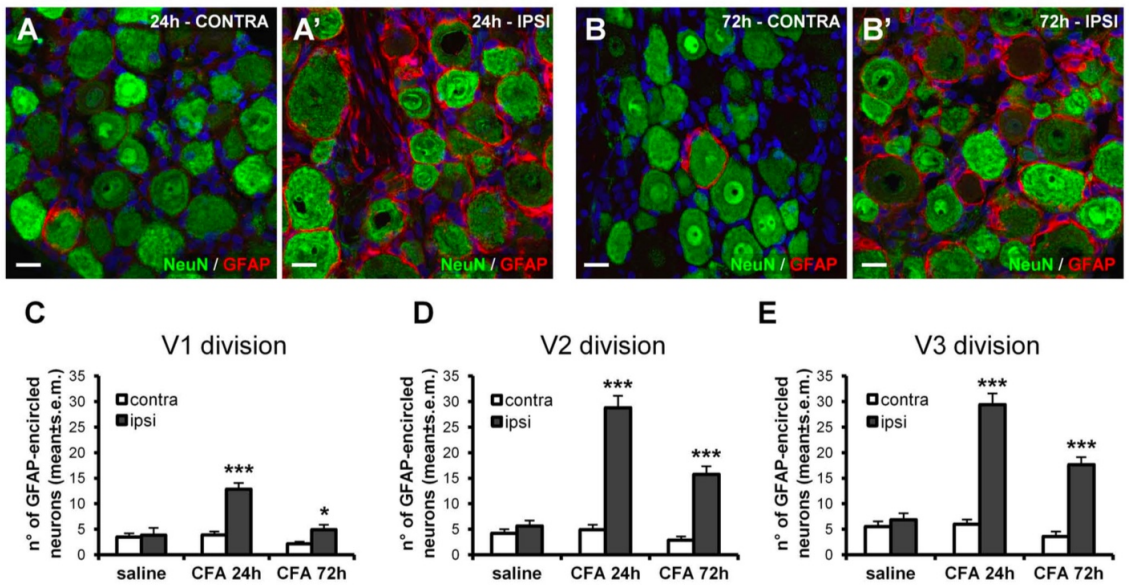
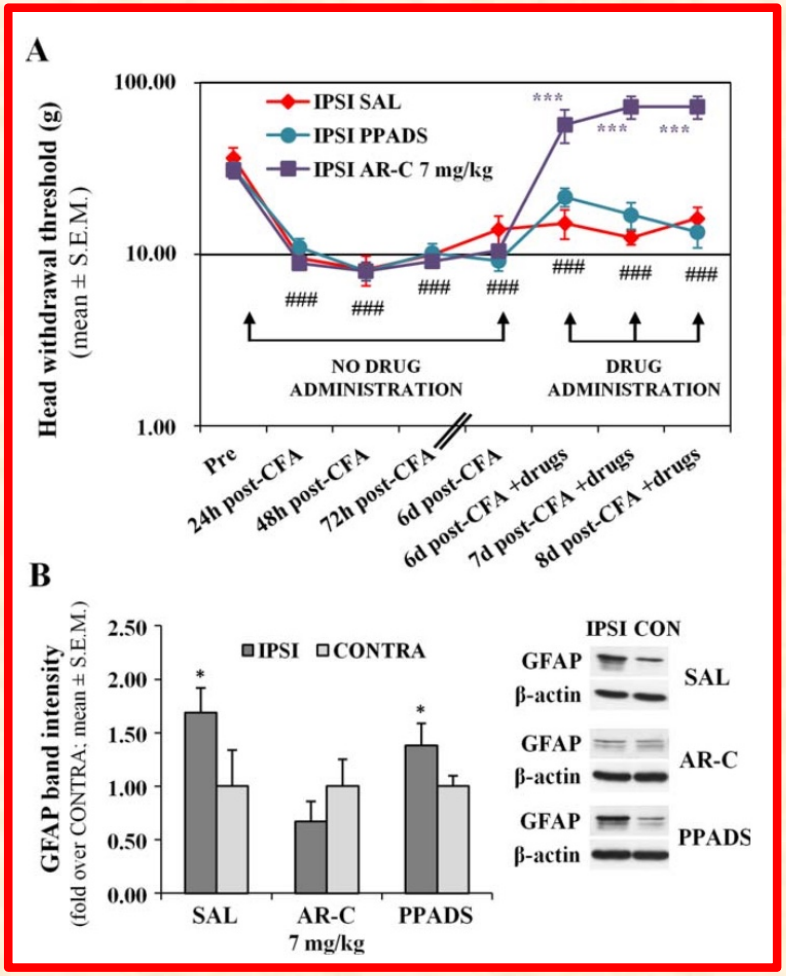
Inhibition of activated SGCs protects from mechanical allodynia induced by inflammatory trigeminal pain



Villa et al., Mol Pain 6:89 (2010)

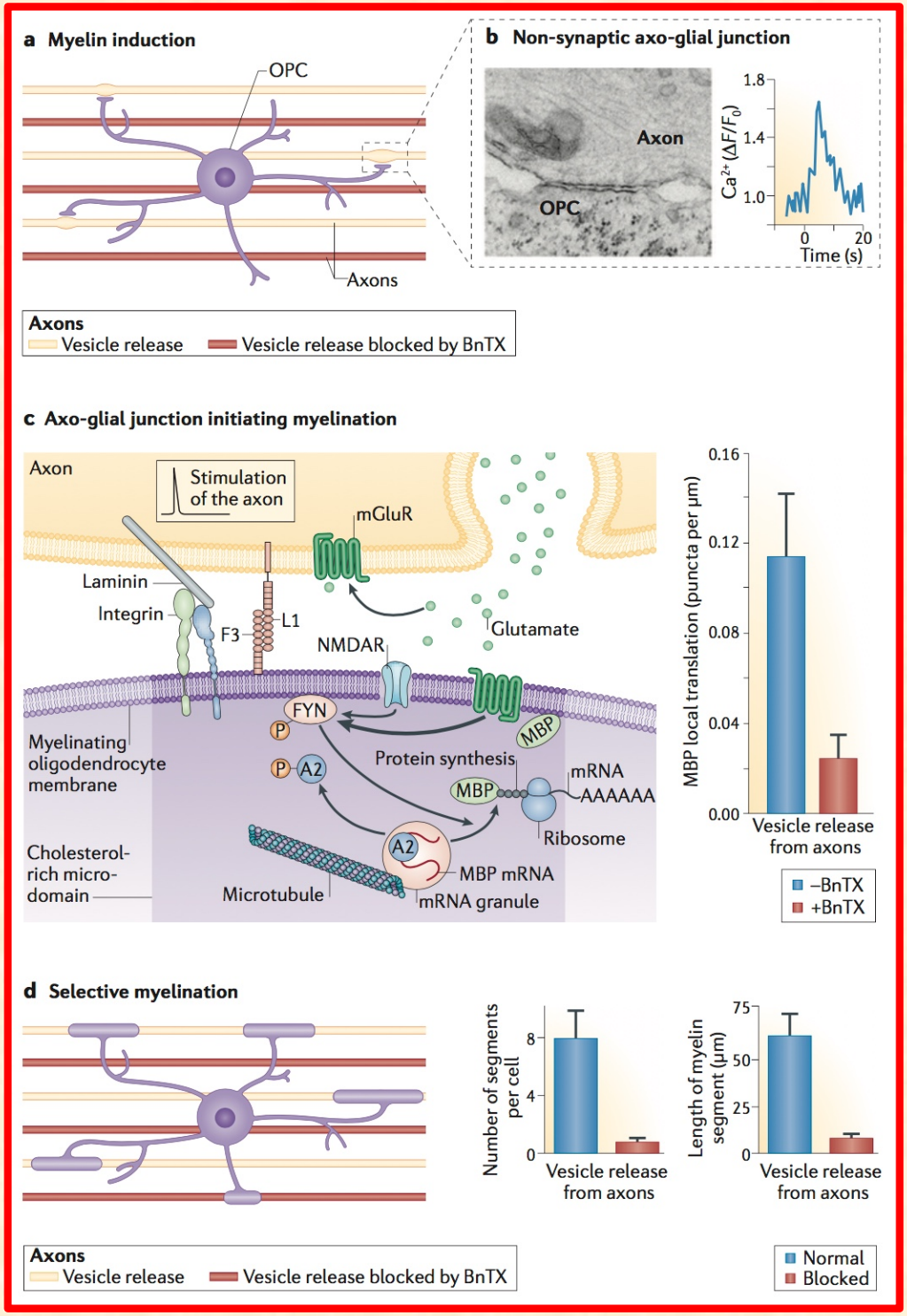
SGCs are activated and upregulate GFAP in the trigeminal ganglion after induction of inflammatory trigeminal pain (*CFA injection in the TMJ*)



Administration of the AR-C118925 compound, acting on glial purinergic $P2Y_2$ receptors, leads to inhibition of SGCs and reverses mechanical allodynia under sub-chronic pain conditions

Magni et al., Glia 63:1256-1269 (2015)

A new mechanism for neuroplasticity: activity-dependent myelination



Non-synaptic junctions on myelinating glia promote preferential myelination of electrically active axons

Formation of the axo-glial signalling complex and local synthesis of MBP are inhibited by axonal firing when NMDAR and mGluR activation are blocked by BnTX

Three weeks after stimulating action potentials in axons, the number and length of myelin segments formed on axons releasing synaptic vesicles (yellow axons in diagram on the left and blue bars in graphs on the right) was much higher than on axons in which vesicle release was blocked by BnTX

Fields D., Nat Rev Neurosci 16:756-767 (2015)

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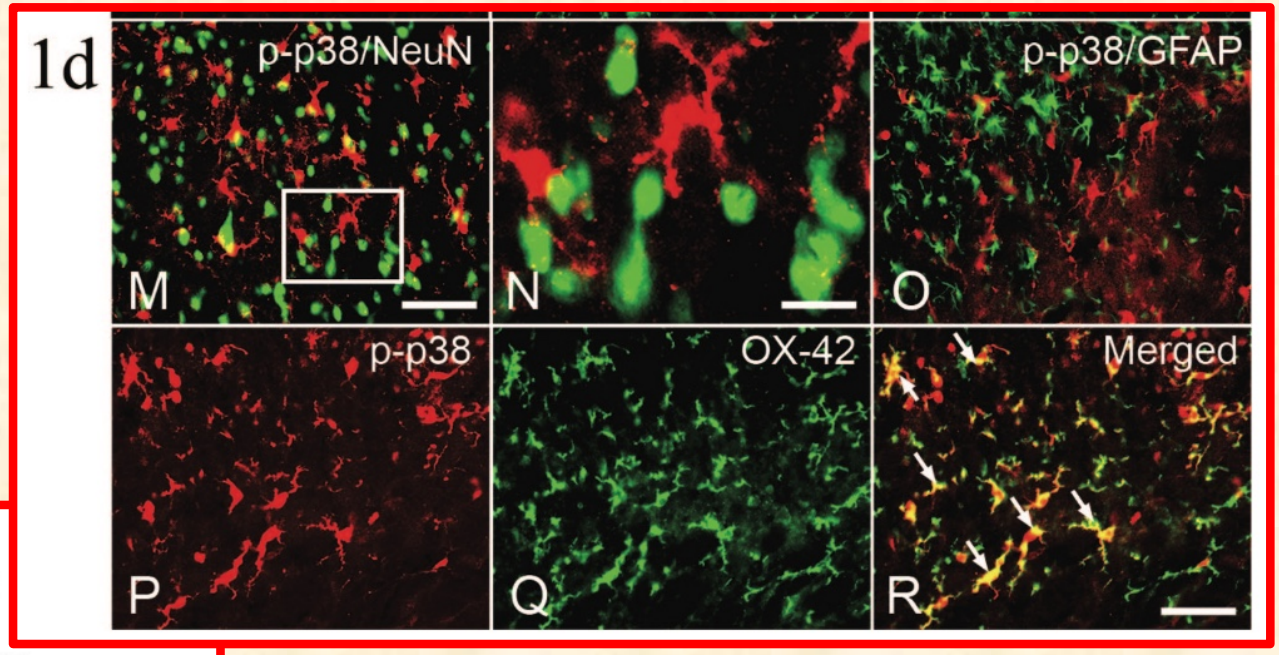
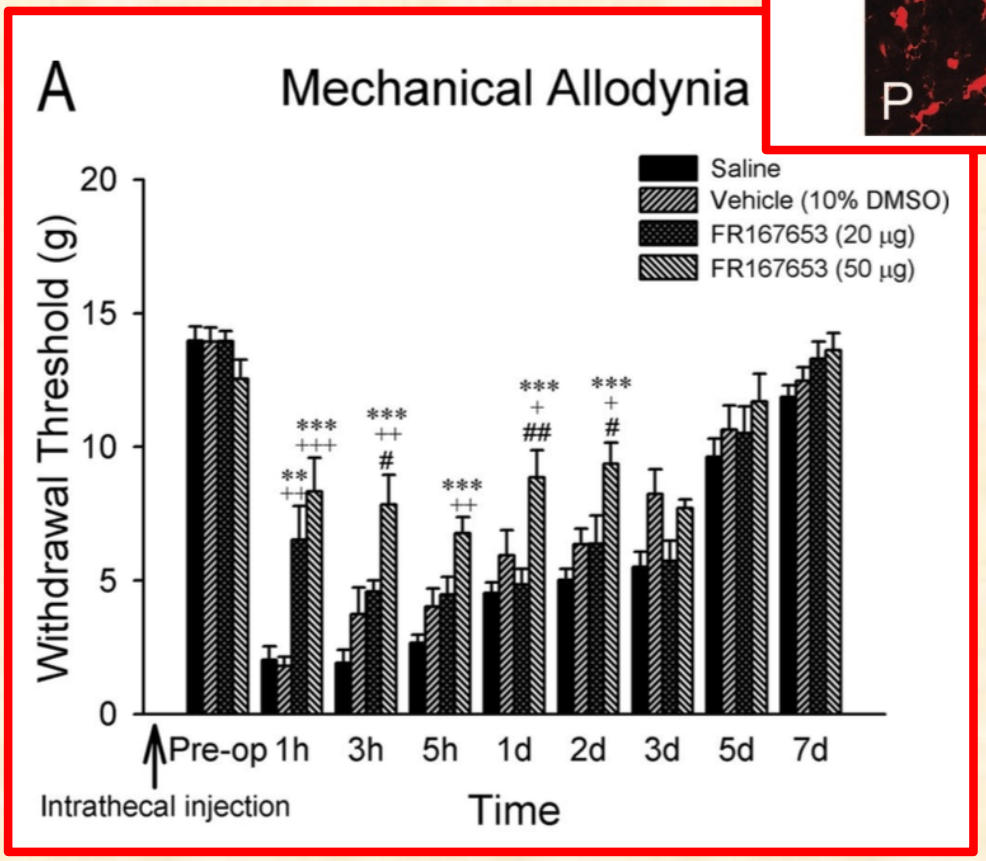
Blockade of microglia activation reliefs from surgery-induce allodynia

p38 MAP kinase is activated in spinal microglia after surgery (*plantar incision in rats*)

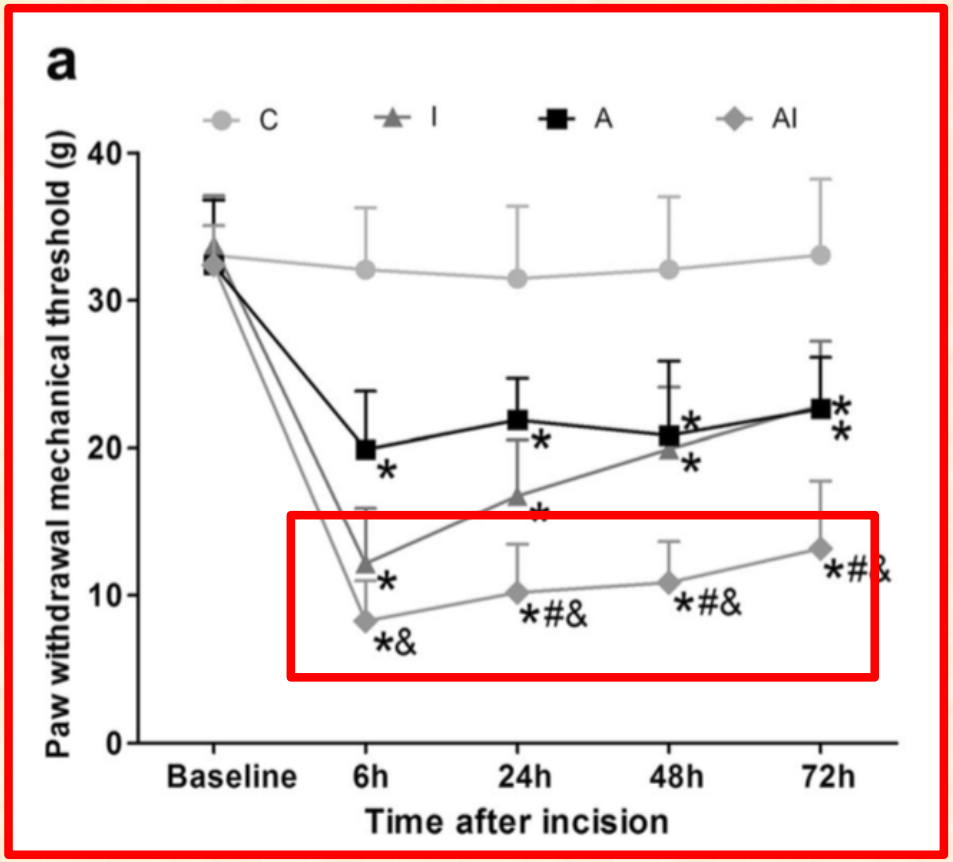




The p38 inhibitor FR167653 attenuates plantar incision-induced p38 activation in the ipsilateral spinal dorsal horn and reverses mechanical allodynia up to 2 days post operation

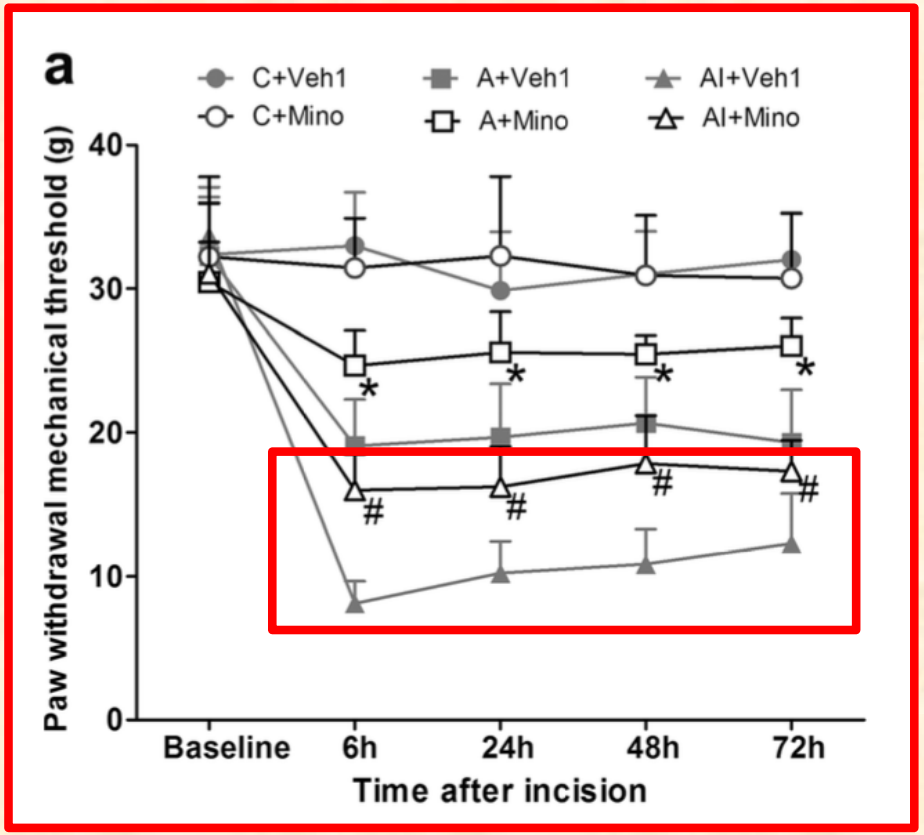
Wen et al., Anesthesiology 110:155-165 (2009)

Stress-induced microglia contributes to preoperative anxiety-induced postoperative hyperalgesia



A single prolonged stress (SPS) induces postoperative hyperalgesia and activation of spinal microglia (*plantar incision*)

Control, group C; Incision, group I; SPS, group A; SPS + incision, group AI



The microglia inhibitior minocycline reduces postoperative hyperalgesia

Sun et al., Mol Neurobiol DOI 10.1007/s12035-016-9976-1 (2016)

Neuropharmacology. 2016 Jun;105:607-17. doi: 10.1016/j.neuropharm.2016.02.024. Epub 2016 Feb 23.

Activation of glial glutamate transporter via MAPK p38 prevents enhanced and long-lasting non-evoked resting pain after surgical incision in rats.

Reichl S[1], Segelcke D[2], Keller V[2], Jonas R[3], Boecker A[2], Wenk M[2], Evers D[2], Zahn PK[4], Pogatzki-Zahn EM[5].

Author information

Experimental Neurology 261 (2014) 836–843

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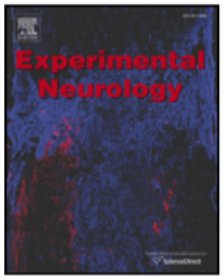
journal homepage: www.elsevier.com/locate/yexnr



Regular Article

Over-expression of P2X7 receptors in spinal glial cells contributes to the development of chronic postsurgical pain induced by skin/muscle incision and retraction (SMIR) in rats

Yan-Lu Ying [a,1], Xu-Hong Wei [b,1], Xue-Bing Xu [c,*], Shou-Zhang She [a], Li-Jun Zhou [b], Jing Lv [a], Dai Li [b], Bin Zheng [a], Xian-Guo Liu [b]

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Can modulators of the neuro-immune interface be effective in pain?

Table 1 | **Modulators of the neuroimmune interface**

Drug name	Cellular targets	Mechanism of action	Clinical use
Amitriptyline	Microglia	Disrupts TLR4 signalling by binding to MD2	Depression
ATL313 (Santen Pharmaceutical)	Microglia and astrocytes	• Adenosine A_{2A} receptor agonist • PKA and PKC activation	NA
BAY 60–6583 (Bayer HealthCare)	Microglia and astrocytes	Adenosine A_{2B} receptor agonist	NA
Ceftriaxone	Astrocytes	Increases EAAT2 expression by inhibiting NF-κB activity	• Bacterial meningitis • Lyme disease
Dilmapimod	Microglia	Selective p38 MAPK inhibition	NA
FP-1	Microglia	TLR4 antagonist	NA
Glatiramer acetate	T cells	Promotes generation of anti-inflammatory T cell phenotypes	Multiple sclerosis
Ibudilast (MN-166; MediciNova)	Microglia, astrocytes and T cells	Non-selective phosphodiesterase inhibitor	• Asthma • Post-stroke dizziness
Methotrexate	T cells	Suppresses expression of cell adhesion molecules	• Breast cancer • Rheumatoid arthritis
Minocycline	Microglia, T cells and neurons	• Inhibits NF-κB translocation to the nucleus • Inhibits NFAT1	Acne vulgaris
Paroxetine	Microglia	$P2X_4R$ antagonist	Depression
Propentofylline (Aventis Pharma)	Microglia, astrocytes and neurons	• Inhibits cAMP and cGMP phosphodiesterases • Adenosine reuptake inhibitor	Ischaemic stroke
Resolvin D1 (Resolvyx Pharmaceuticals)	Microglia and neurons	• Attenuates pro-inflammatory cytokine release • Inhibits TRPV1 • Reverses NMDAR subunit phosphorylation	NA
Resolvin E1 (Resolvyx Pharmaceuticals)	Microglia and neurons	• Attenuates pro-inflammatory cytokine release • Inhibits TRPV1 • Attenuates glutamate release • Reverses NMDAR subunit phosphorylation	NA
Rifampin	Microglia	Disrupts TLR4 signalling by binding to MD2	Tuberculosis
(+)-naloxone	Microglia	Disrupts TLR4 signalling by binding to MD2	NA
(+)-naltrexone	Microglia	Disrupts TLR4 signalling by binding to MD2	NA

A_{2A}, adenosine receptor 2A; A_{2B}, adenosine receptor 2B; cAMP, cyclic AMP; cGMP, cyclic GMP; EAAT2, excitatory amino acid transporter 2; MAPK, mitogen-activated protein kinase; MD2, myeloid differentiation protein 2; NA, not applicable (no current clinical application); NFAT1, nuclear factor of activated T cells 1; NF-κB, nuclear factor-κB; NMDAR, ionotropic glutamate receptor; $P2X_4R$, P2X purinoceptor 4; PKA, protein kinase A; PKC, protein kinase C; TLR4, Toll-like receptor 4; TRPV1, transient receptor potential cation channel subfamily V member 1

Grace et al., Nat Rev Immunol 14:217-231 (2014)

LABORATORY OF MOLECULAR AND CELLULAR PHARMACOLOGY OF PURINERGIC TRANSMISSION

MARIA P. ABBRACCHIO

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The Journal of Neuroscience, May 7, 2008 • 28(19):4949–4956 • 4949

Neurobiology of Disease

$P2Y_{12}$ Receptors in Spinal Microglia Are Required for Neuropathic Pain after Peripheral Nerve Injury

Hidetoshi Tozaki-Saitoh,[1] Makoto Tsuda,[1] Hiroyuki Miyata,[1] Kazuaki Ueda,[1] Shinichi Kohsaka,[2] and Kazuhide Inoue[1]
[1]Department of Molecular and System Pharmacology, Graduate School of Pharmaceutical Sciences, Kyushu University, Higashi, Fukuoka 812-8582, Japan, and [2]Department of Neurochemistry, National Institute of Neuroscience, Kodaira, Tokyo 187-8502, Japan

Time-dependent upregulation of microglial $P2Y_{12}$ ipsilateral to nerve injury

Administration of selective $P2Y_{12}$ antagonists (intrathecal AR-C69931MX or oral clopidogrel) prevented the development of tactile allodynia

Mice lacking $P2Y_{12}$ displayed impaired tactile allodynia after nerve injury

$P2Y_{12}$ receptors regulates microglial process extension and chemotaxis, through integrin β1

Ohsawa et al., Glia 58:790-801 (2010)

The $P2Y_{12}$ receptor subtype is expressed by TG satellite glial cells only after lingual neuropathic pain induction, and its antagonism relieve pain

Katagiri et al., Mol Pain 8:23 (2012)