INFLAMMATION AND CANCER PAIN: BASIC MECHANISMS

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Cancer and Pain

- Pain: a common symptom
- Increases as cancer progresses to advanced stage
- Progression of tumor growth → chemotaxis of immune cells (macrophages, T cells, dendritic cells) coexist with cancer cells → production of mediators also found in inflamed tissue
- Related to inflammation
- Tissue & Nerve compression → injury

Background (1)

- Injury to tissues & nerves → inflammatory response
  - clear damaged cells, promote repair, contain pathogens
- 5 cardinal signs: pain ……
- Pain: nervous system process - tripartite system
  - Neurons-Immune Cells-Glia
  - Peripheral and Central mechanisms

Background (2): Tissue Injury-TLRs

- Inflammation triggered by innate immune system of pattern recognition receptors (Toll Like Receptors[TLRs])
- TLRs: bind endogenous molecules (eg. heat shock protein) from damaged tissue

What are these TLRs?

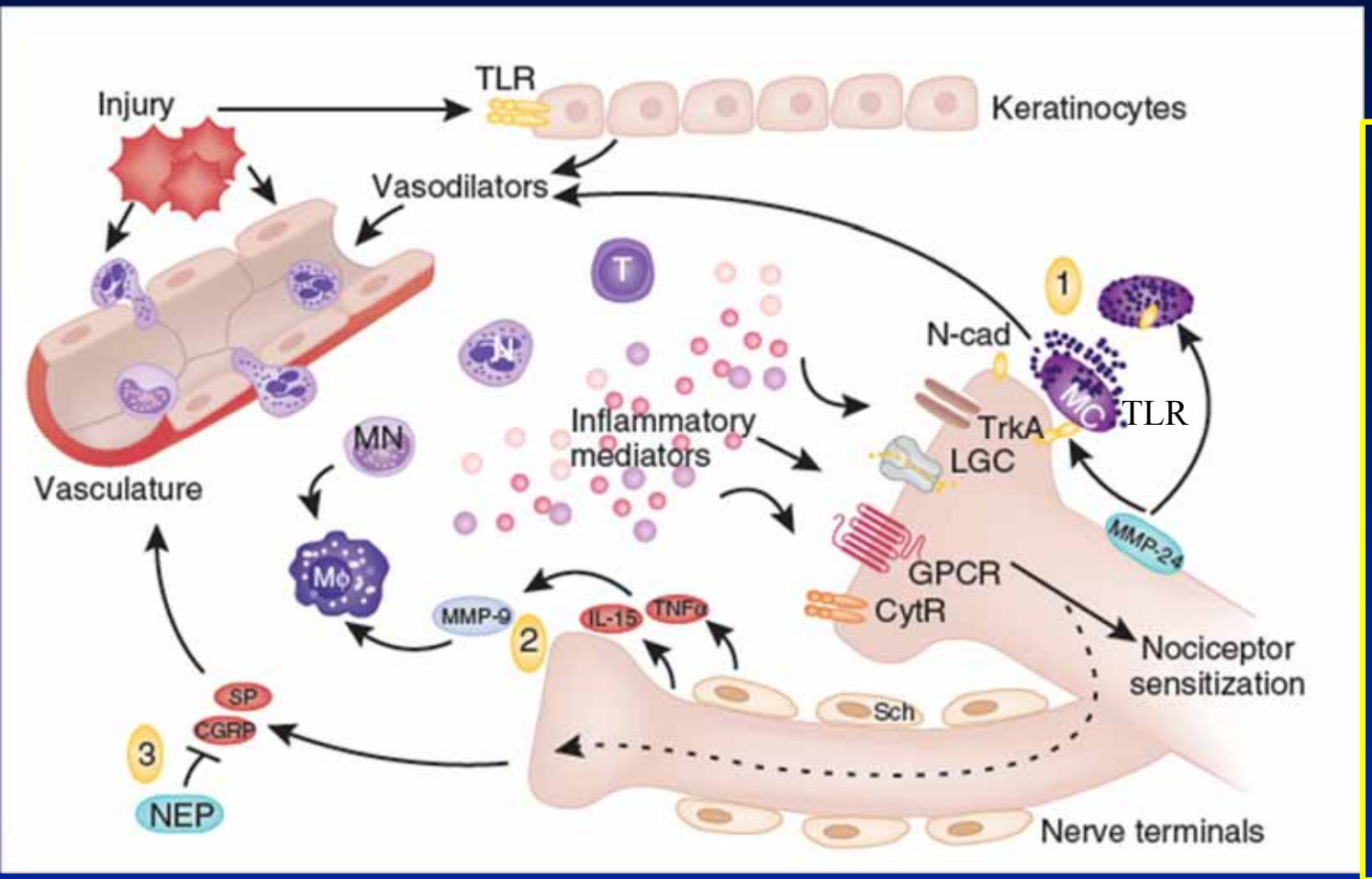
- Toll-Like Receptors: Family of pattern recognition receptors
- Monocytes, macrophages, dendritic cells, keratinocytes, mast cells, microglia, astrocytes, ….
- TLR4 (& TLR2) - recognises endogenous “danger signals”[HSP]
- TLR4
  - endotoxin (LPS) receptor;
  - Up regulated in neuropathic pain (animal models)
  - If TLR4 expression is blocked- pain does not develop
- Signalling: requires extracellular binding partner MD2 (myeloid differentiation factor) to associate before signalling commences

Background (3): TLR Signaling

- Binding to MD2-TLRs → NF-κB activation (intracellular) →
  - Pro-inflammatory cytokine release
    - IL-1β, TNFα, IL-6

- Tissue Injury: Resident immune cells, mast cells & macrophages also activated → proinflammatory cytokines, chemokines, vasodilators …

Tissue Injury-Immune Activation-Nociceptor Sensitization

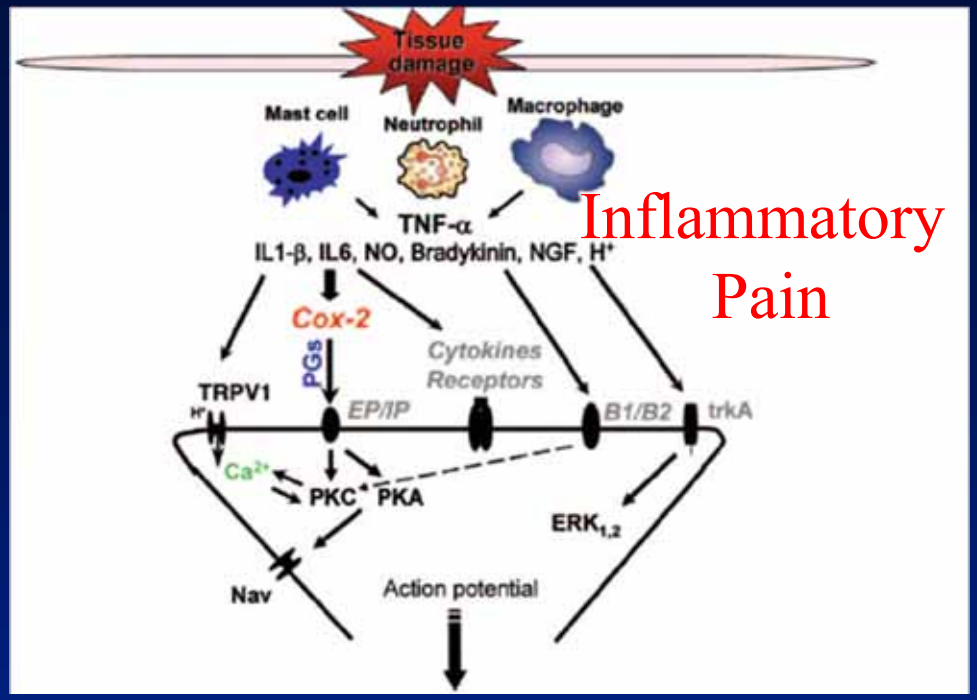


Injury activates TLRs.
Vasodilators released.
T cells, neutrophils, monocytes migrate.
Macrophages recruited.
Inflammatory mediators released.
Nerve terminals activated
Peripheral nociceptor sensitization

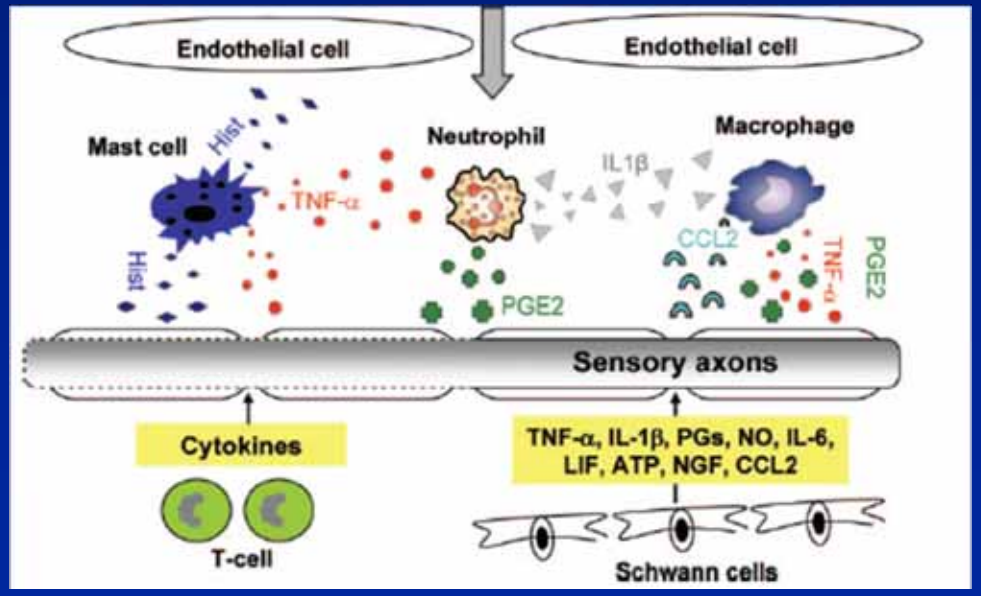
Immune cells contribute to peripheral sensitization – release soluble factors interacting with nociceptors. Note: immune system also release factors promoting tissue recovery and inflammation suppression

Ren & Dubner. Nature (Medicine) 16, 1267, 2010

Inflammatory Immune Mediators in Peripheral Neuropathic/Cancer Pain



Inflammatory Pain



Peripheral Nerve Injury

- TNFα
- IL-1β
- IL-6
- NO
- PGs
- COX-2
- NGF

Thacker MA et al Anesth Analg 105, 838, 2007

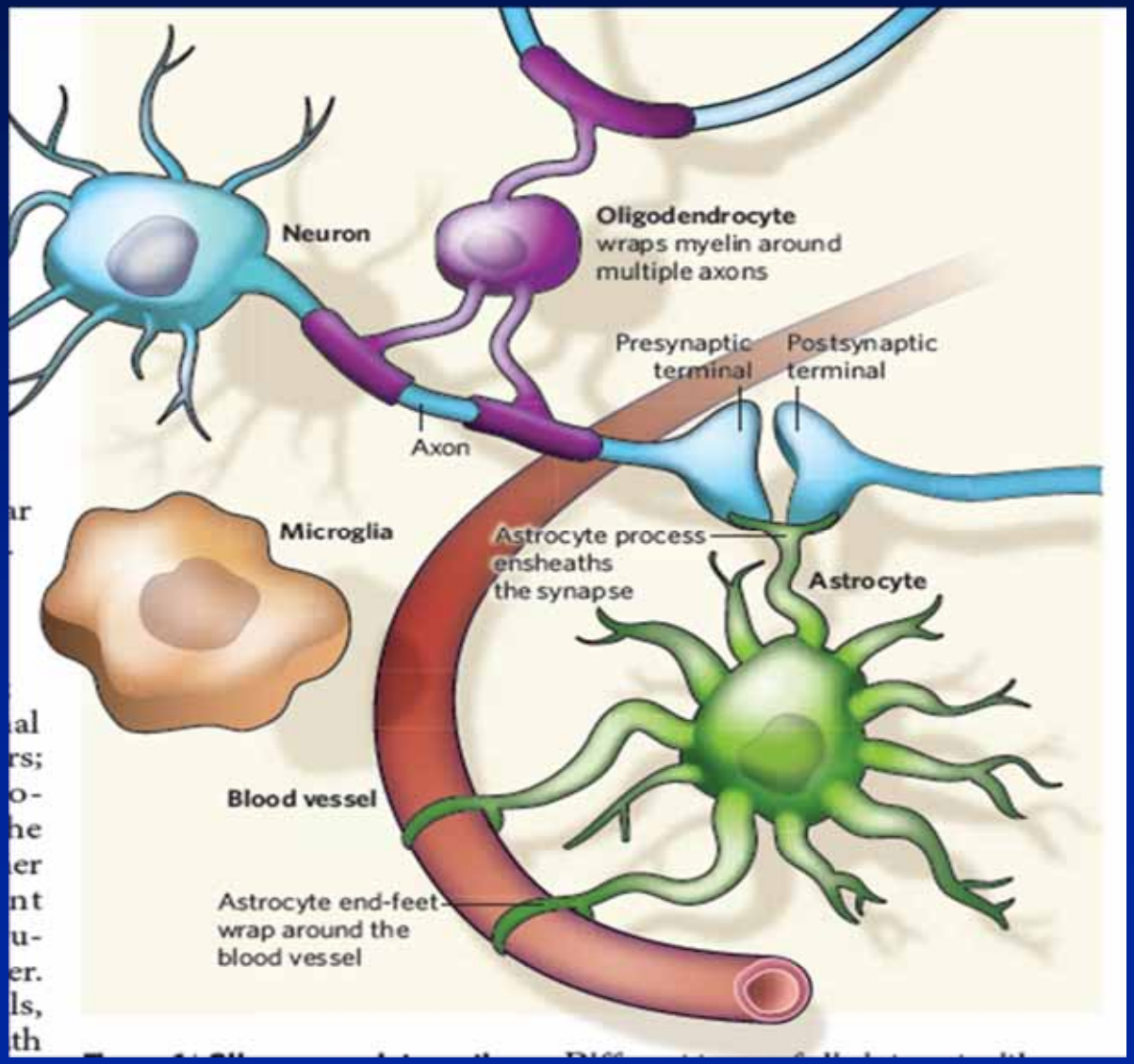
Background (5): Injury & **CNS Activation**

- Glia are activated- especially deep tissue injury
- Relayed to brain via peripheral immune activation, afferent nerve input, circulating cytokines, immune cell trafficking

Background (6): Peripheral Immune Signals

- IL-6: messenger from periphery to brain
- Fractalkine (chemokine): sustained input from peripheral tissue
  - Deep muscle, joint & visceral tissue injury
- Infiltration immune cells into CNS → chronic pain
- Blood:brain barrier permeability compromised (P-gp)

CNS: Glia- More than Just Brain



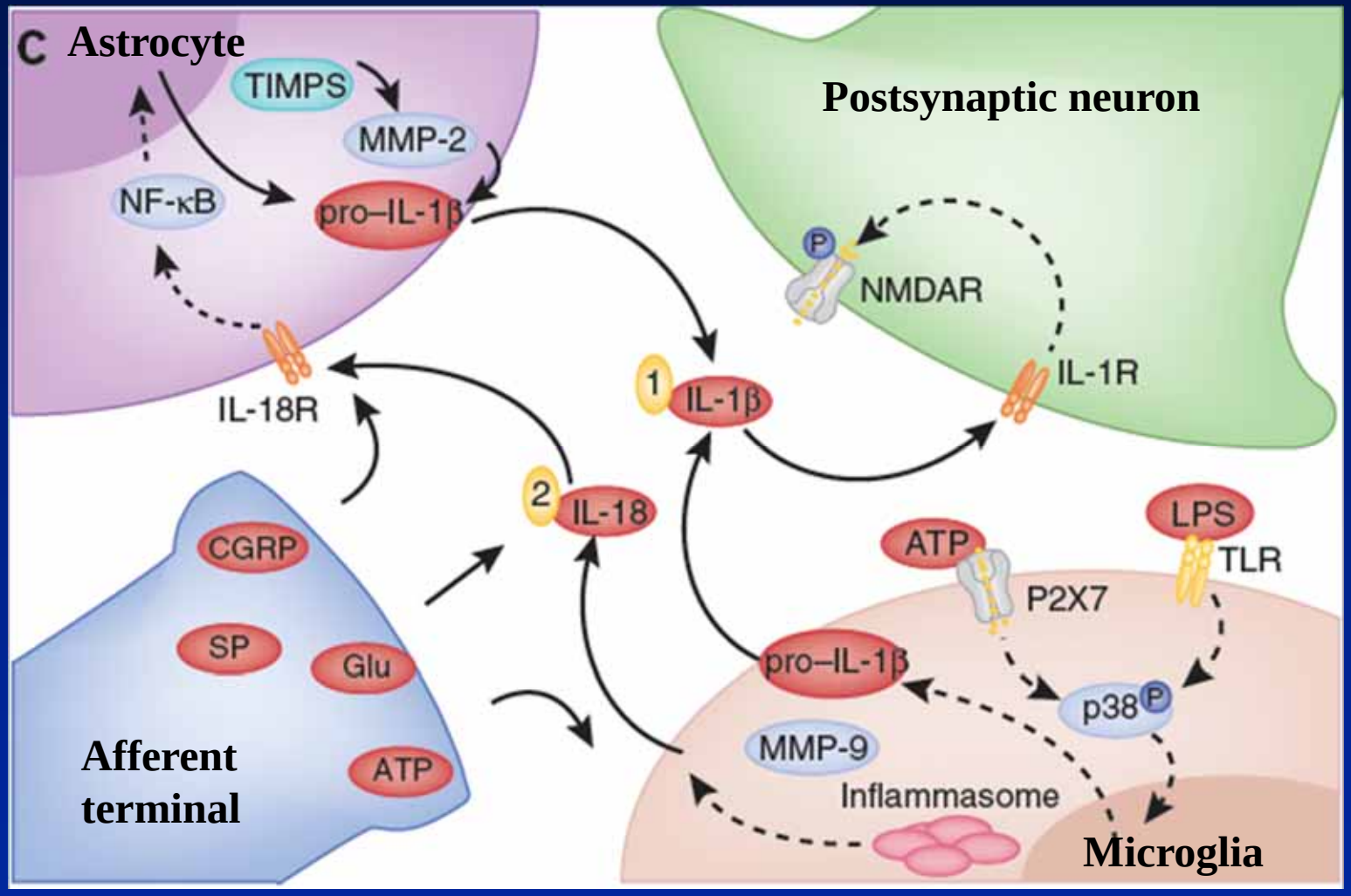
Astrocytes: ensheath neuronal synapses

Microglia: surveillance monitors for danger signals: stress, pain, infections

Endogenous signal: Heat shock protein, LPS

TLR activation → IL-1β, IL-6, TNFα

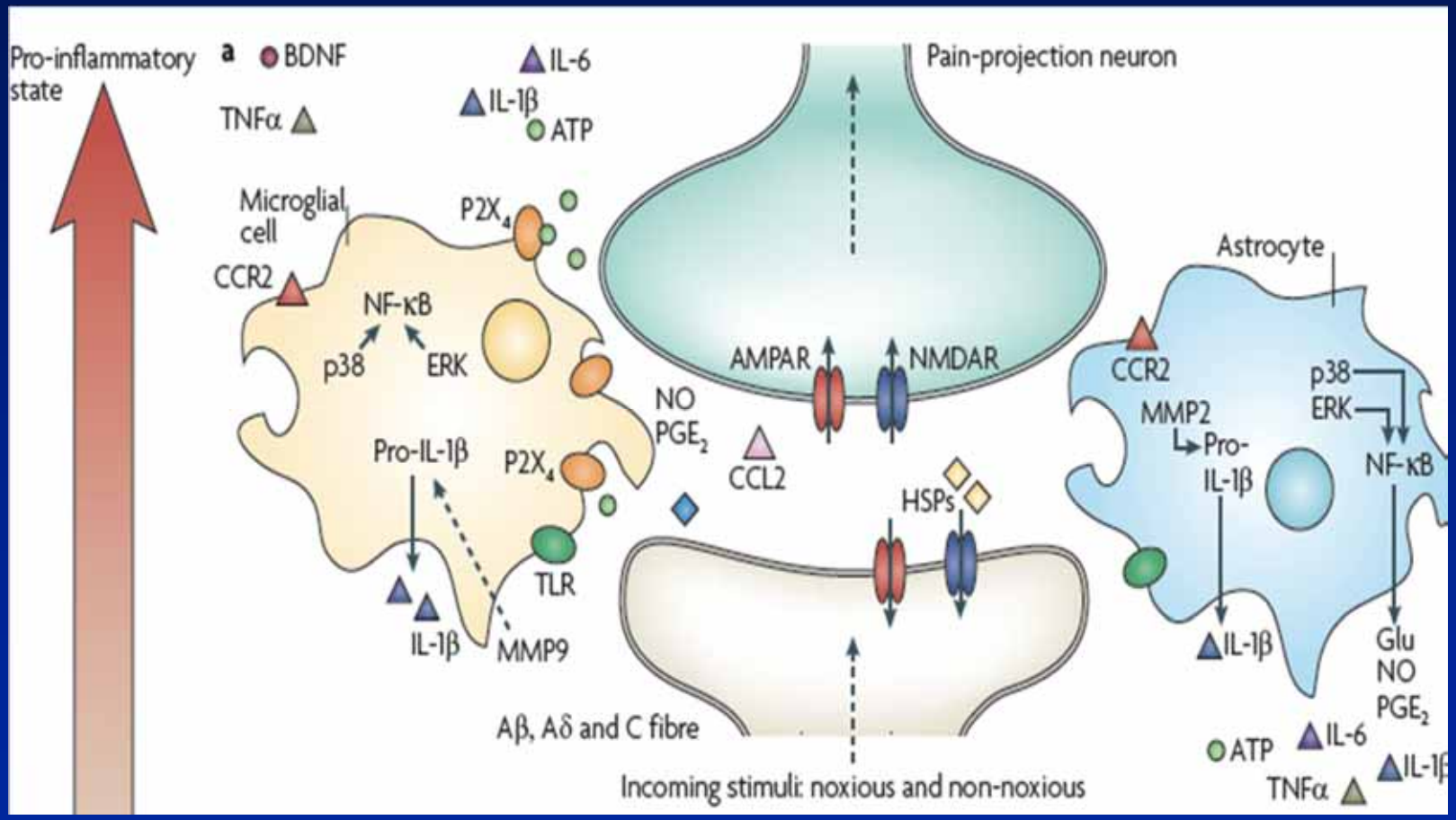
Peripheral Injury Activation of Glia & Neurons: Dorsal Horn Spinal Cord



TLR activation by LPS, HSP of glia → IL-1β → modulation of NMDA on post synaptic neurons → Central Sensitization

Ren & Dubner. Nature (Medicine) 16, 1267, 2010

Nerve damage affects dorsal horn neurones: activation of glia: mediators



IL-1β
TNFα
IL-6
NO
PG
NGF

Milligan ED & Watkins LR. Nat Rev Neurosci 10, 23, 2009

Key Role of IL-1β

- Key cytokine modulating microglia, astrocytes, neurons
- Selectively upregulated in astrocytes - spinal cord & other sites in animal models of cancer pain

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J.-T. QIAO,[c] B. M. BERMAN[a] AND L. LAO[a]

INTERLEUKIN 1β facilitates bone cancer pain in rats by enhancing NMDA receptor NR-1 subunit phosphorylation Neurosci 154, 1533, 2008

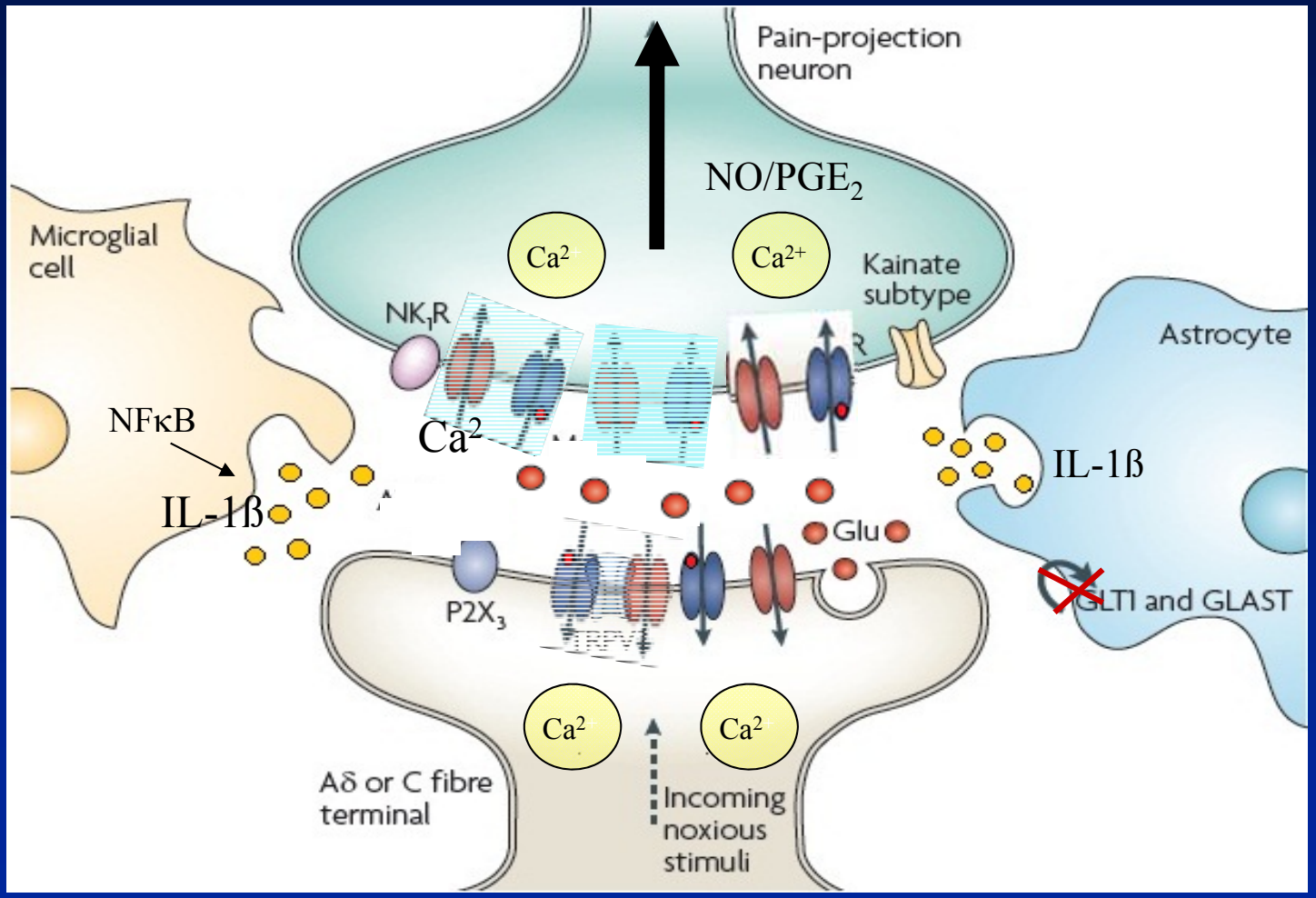
- spinal IL-1β upregulated in this model

How do Cytokines Cause Pain? IL-1ß

- phosphorylates NMDA receptor -> channel opening-> ↑ Ca^{2+} influx→ ↑ NO & ↑ PGE2 → amplify excitability pain projection neurons

- Inhibits Glu reuptake

- Upregulates NMDA & AMPA receptors expression



Inflammatory Biomarkers & Pain

IASP

PAIN® 152 (2011) 460–463

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www.elsevier.com/locate/pain

Clinical note

Cancer pain and its relationship to systemic inflammation: An exploratory study

Barry J.A. Laird[a,*], Angela C. Scott[a], Lesley A. Colvin[b], Amy-Louise McKeon[a], Gordon D. Murray[a], Kenneth C.H. Fearon[a], Marie T. Fallon[a]

[a] University of Edinburgh, UK
[b] Western General Hospital, Edinburgh, UK

- CRP a valid serum biomarker of IL-6
- Positive correlation between log serum CRP and EORTC Pain Symptom Scale

$r = 0.126$ ($P = 0.036$); $r = 0.163$ ($P = 0.032$)

Cancer Pain Genetics: Cytokines (1)

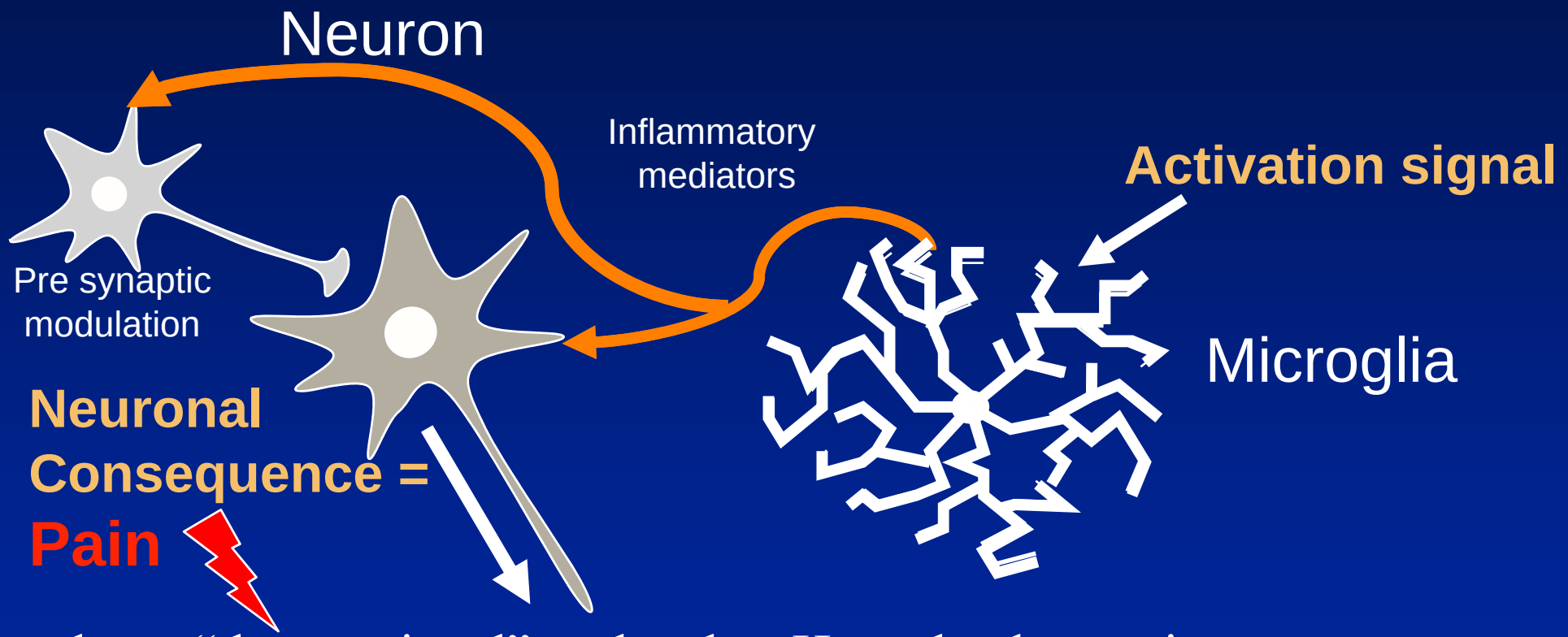
- Reyes-Gibby et al (Cancer Epidemiol Biomarkers Prev 16, 2745, 2007)
  - Newly diagnosed NSCLC patients
  - *IL8* (-251T/A): associated with severe pain
    - OR=2.35 (1.10-5.03) TA/AA vs TT
  - No association: TNF-a (-308G/A), *IL6* (-174G/C)
  - IL-8 function: chemokine (CXCL8)
    - Mediator inflammatory response, promotes immune cell migration, induces astrocyte migration, microglia proliferation, induces TNFα, IL-6

Cancer Pain Genetics: Cytokines (2)

- Reyes-Gibby et al (Cancer Epidemiol Biomarkers Prev 17, 3262, 2008)
  - Follow up previous study: 140 patients for pain treatment
  - TNF-a (-308G/A): associated with pain severity (P=0.03)
  - *IL6* (-174G/C): CC carriers associated with higher morphine equivalent dosing requirements
    - OR = 4.7 (1.2-12.01)
    - Mechanism(s) unclear- IL-6: Periphery pronociceptive; Central- pro – or antinociceptive; ↑ MOP, ↓ P-glycoprotein;

Role of Glia in Pain Neuropathies

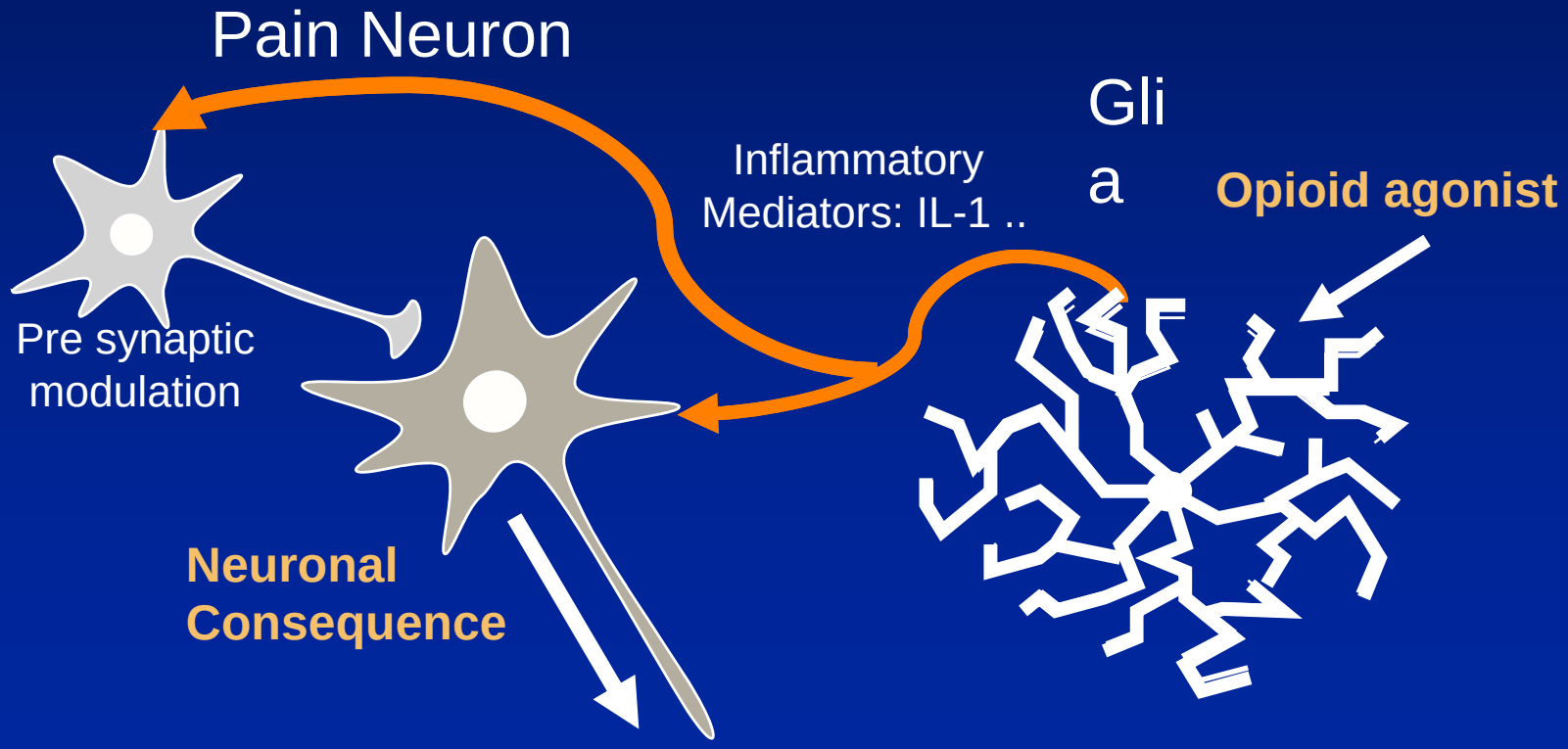
- Damaged Neuron, Virus, Diabetes ….



- Neurons release "danger signal" molecules: Heat-shock proteins
- Microglia mop-up molecules via toll-like receptors (TLR2, 4)- signalling
- Constant TLR signalling → ↓ (-ve) feedback → proinflammatory cytokines (IL-1)
- Prolonged proinflammatory pain response "allergy in dorsal horn spinal cord"

Opioids also Dysregulate Glia

- Glia are activated
- Proinflammatory response
- Enhance Pain - decrease analgesia
- Increased tolerance, dependence, reward, etc



Dorsal horn glial activation from any source → enhanced pain

Morphine activates neuroinflammation in a manner parallel to endotoxin

PNAS 109, 6325-6330, 2012

Xiaohui Wang[a], Lisa C. Loram[b,c], Khara Ramos[b,c], Armando J. de Jesus[a], Jacob Thomas[d], Kui Cheng[a], Anireddy Reddy[b,c], Andrew A. Somogyi[d], Mark R. Hutchinson[e], Linda R. Watkins[b,c], and Hang Yin[a,c,f,1]

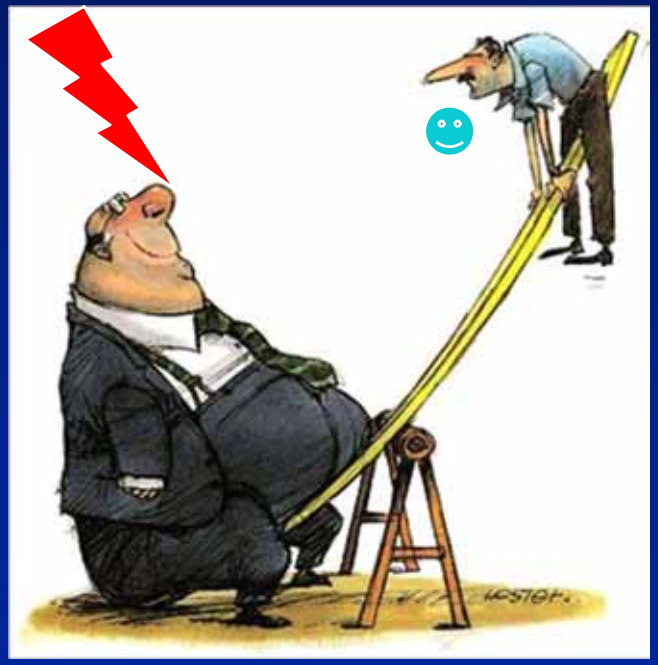
- Morphine binds to MD2 (an accessory protein of TLR4)
- MD2-TLR4 oligomerization
- TLR4 signaling activation
- Release of proinflammatory/neuroexcitatory cytokines (IL-1β)
- Similar mechanism to LPS (endotoxin)

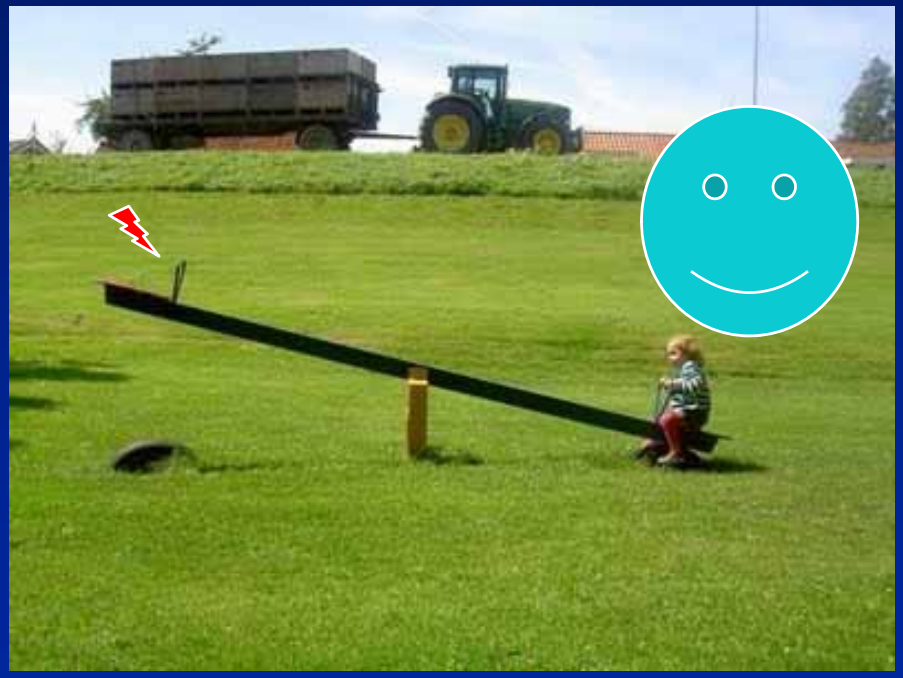
OPIOIDS: Mu Receptor Binding & TLR4-Signal Activation: Different **Rank Order**

Mu Receptor	⬆ TLR4 Signal
Buprenorphine	M3G
M6G	Oxycodone
Morphine, methadone	Fentanyl
Fentanyl	Pethidine
Oxycodone	methadone, morphine
Pethidine	Buprenorphine
M3G	M6G
(+)-naloxone/ (+)-naltrexone)	(+)-naloxone/ (+)-naltrexone)

Opioid Activation

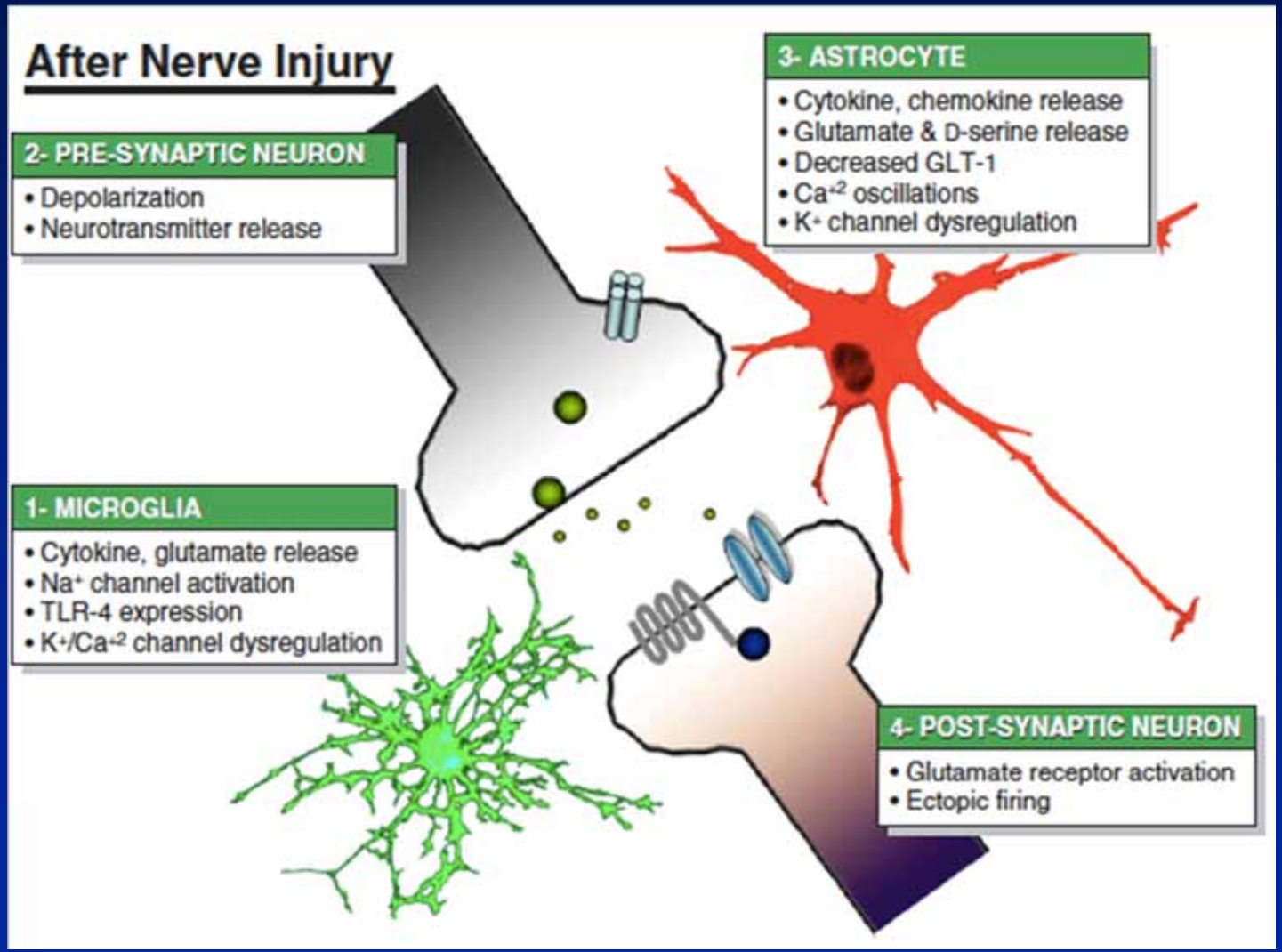
- Mu receptor: analgesia
- TLR4/MD2 Signalling: pain





Opioids have two targets: efficacy & adverse effects in a particular patient may be dependent on which target is predominant

Tetrapartite Synapse:
Microglia, Astrocyte, Pre-synaptic Neuron, Post-Synaptic Neuron

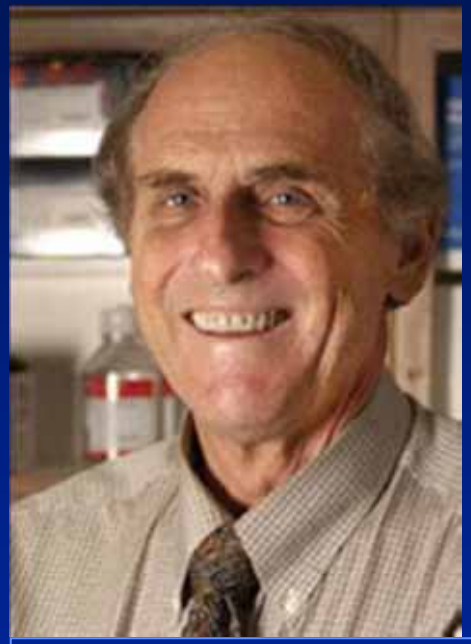


De Leo et al.
Pain 122, 17, 2006

The Nobel Prize in Physiology or Medicine: 2011

Bruce Beutler

Jules Hoffmann



Ralph Steinman

"for their discovery concerning activation of innate immunity"

"for his discovery of the dendritic cell and its role in adaptive immunity"

Summary

- Cancer pain → Tissue & Nerve Injury → inflammation
- Tri- (tetra-)partite system: neurons (pre- & post synaptic)-immune cells-glia
- Direct Peripheral and Central mechanisms
- Pro-inflammatory cytokine activation from TLR4/MD2 signalling is pivotal: NF-κB → IL-1β
- Perhaps a bell-shaped dose-response curve for opioids
- Targeting glial IL-1β release – a potential therapeutic strategy for cancer pain

Acknowledgements

- Dr Mark Hutchinson, Dr Janet Coller (Adelaide)
- Prof Linda Watkins (Colorado)