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# Chemical Mediators and Pain

Ellen Niederberger



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# PAIN

“An unpleasant, subjective, sensory and emotional experience associated with actual or potential tissue damage” (IASP, 1979)

- A universal experience that can span from mild discomfort to excruciating agony.
- Alters quality of life



## **Nociception**

measurable neurophysiological event of a type usually associated with pain; activity in nerve pathways, objective

## **Nociceptor**

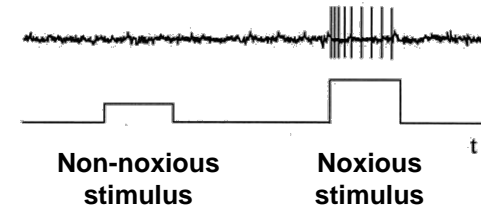
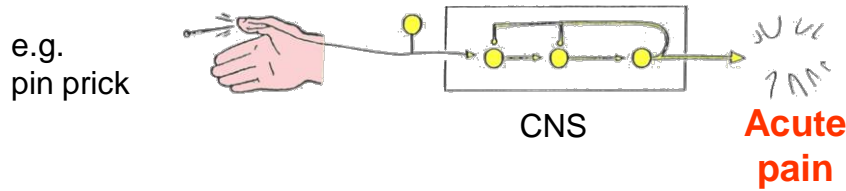
A receptor (free nerve ending) preferentially sensitive to a noxious stimulus or to a stimulus which would become noxious if prolonged.

## **Noxious Stimulus**

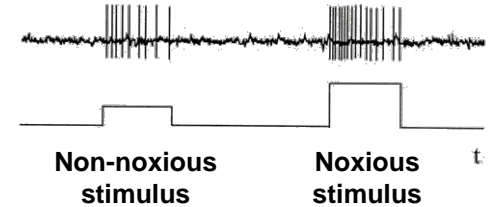
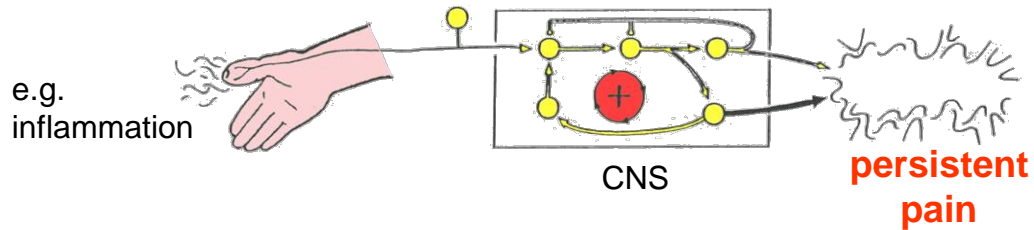
A stimulus which damages normal tissues.

# Pain types

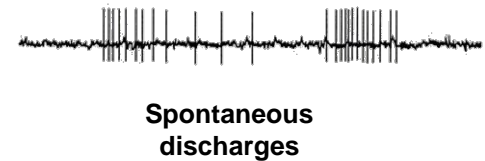
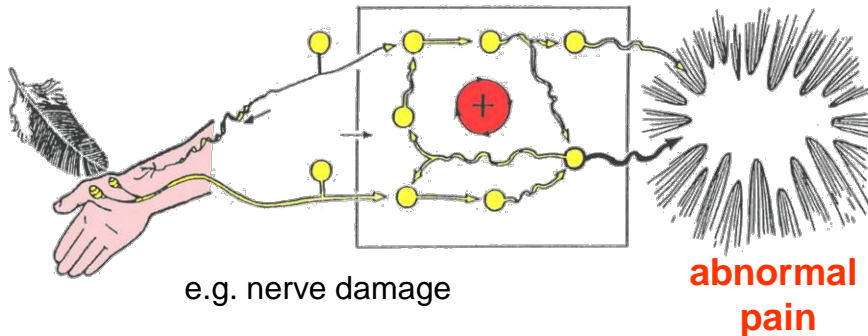
## Physiological nociceptive pain



## Pathophysiological nociceptive pain



## Neuropathic pain



# Pain qualities

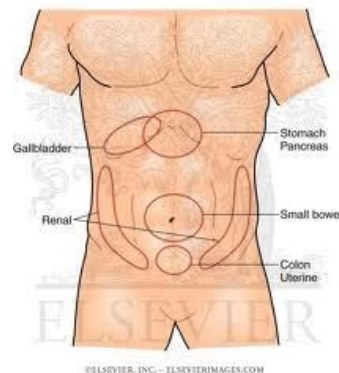
- **Somatic pain** (skin, muscles, joints, connective tissue)

- *surficial pain*, acute, easy to localize, short pain
- *deep pain* dull pain, e.g. headache



- **Visceral pain** (inner organs)

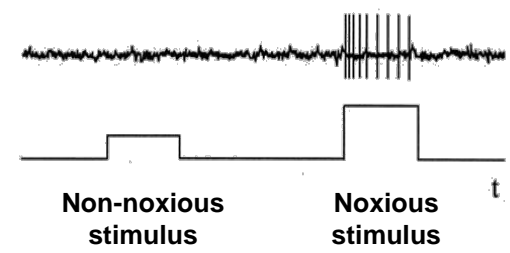
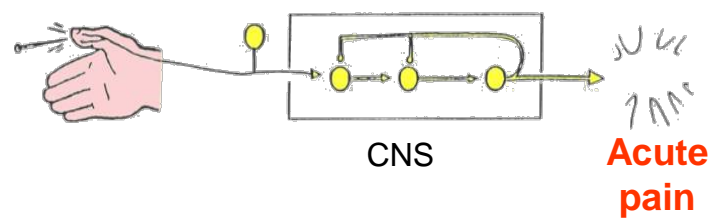
- dull pain



# Physiological pain

## Physiological nociceptive pain

e.g.  
pin prick



- high threshold
- short duration

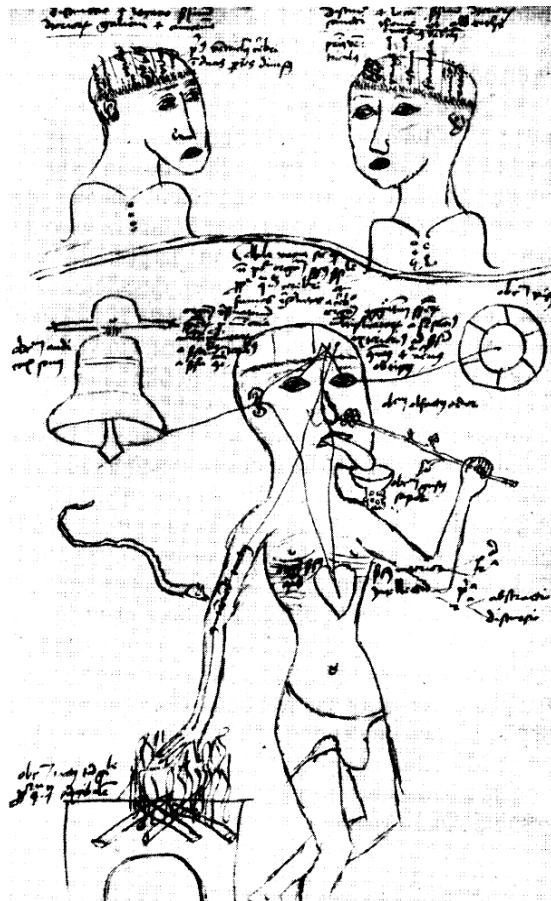
# Physiological pain

- body defence mechanism
- associated with an obvious cause
- rapid onset, acute pain
- protects us from external harm
- prevents further damage
- prevents activity while the body heals



- pain indifference
- pain insensitivity

# Pain transmission (historical view)

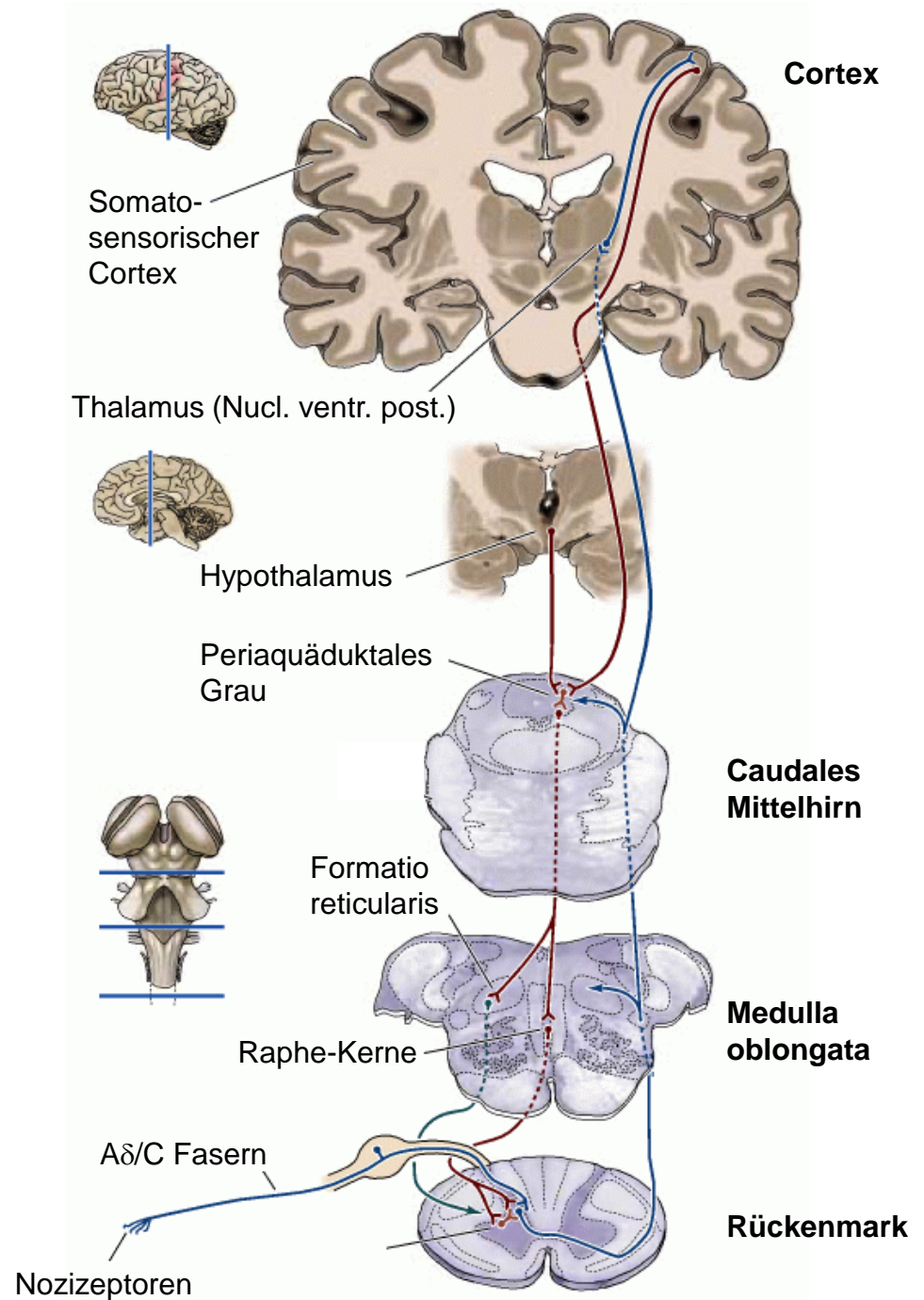


Gerard de Harderwyck 1496

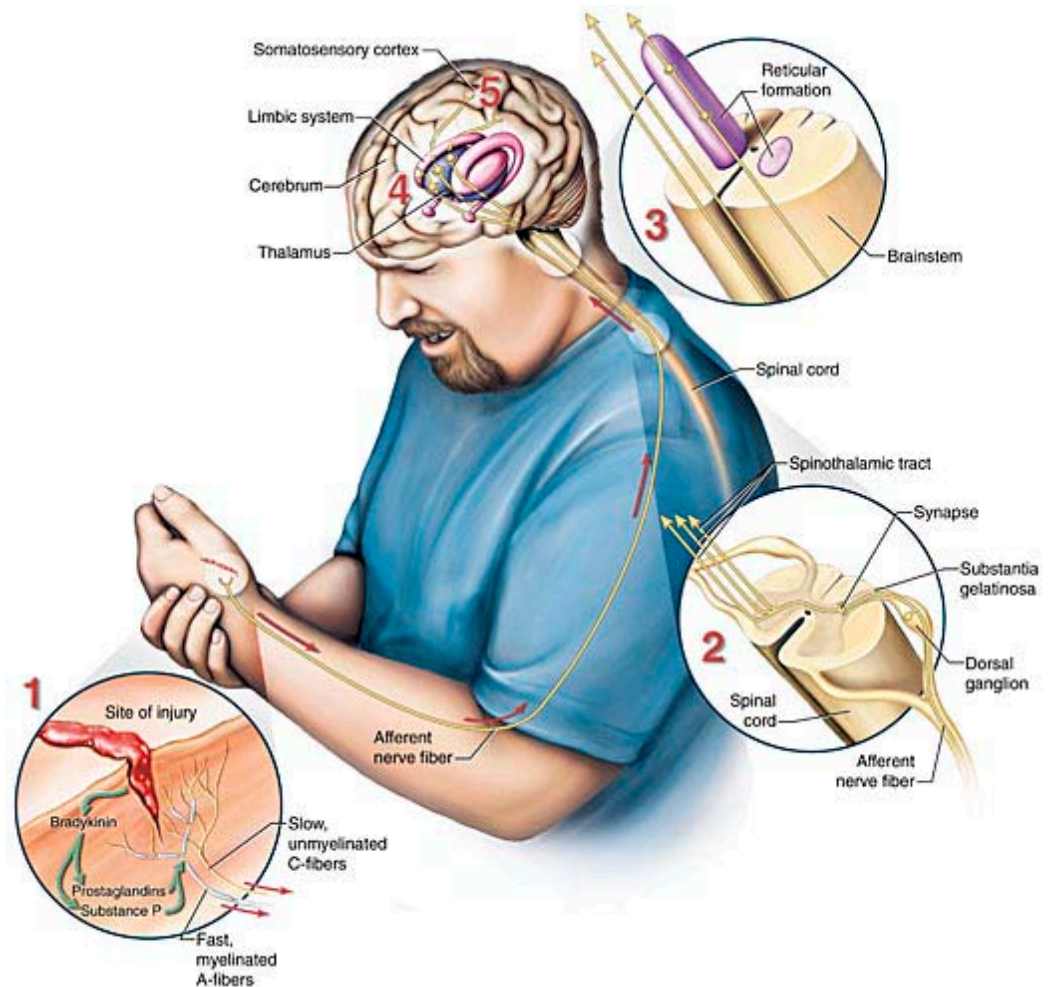


René Descartes 1664

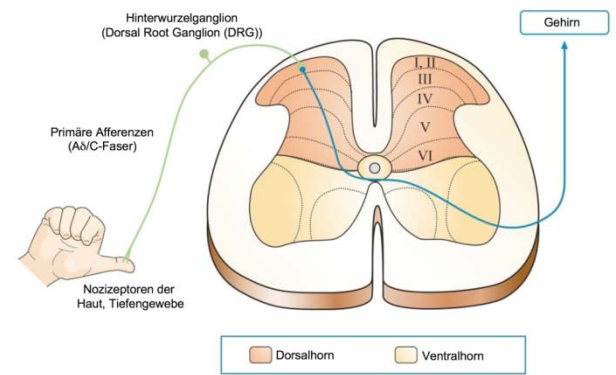
**Ascending nociceptive  
and  
descending  
antinociceptive system**



# Nociceptive Transmission

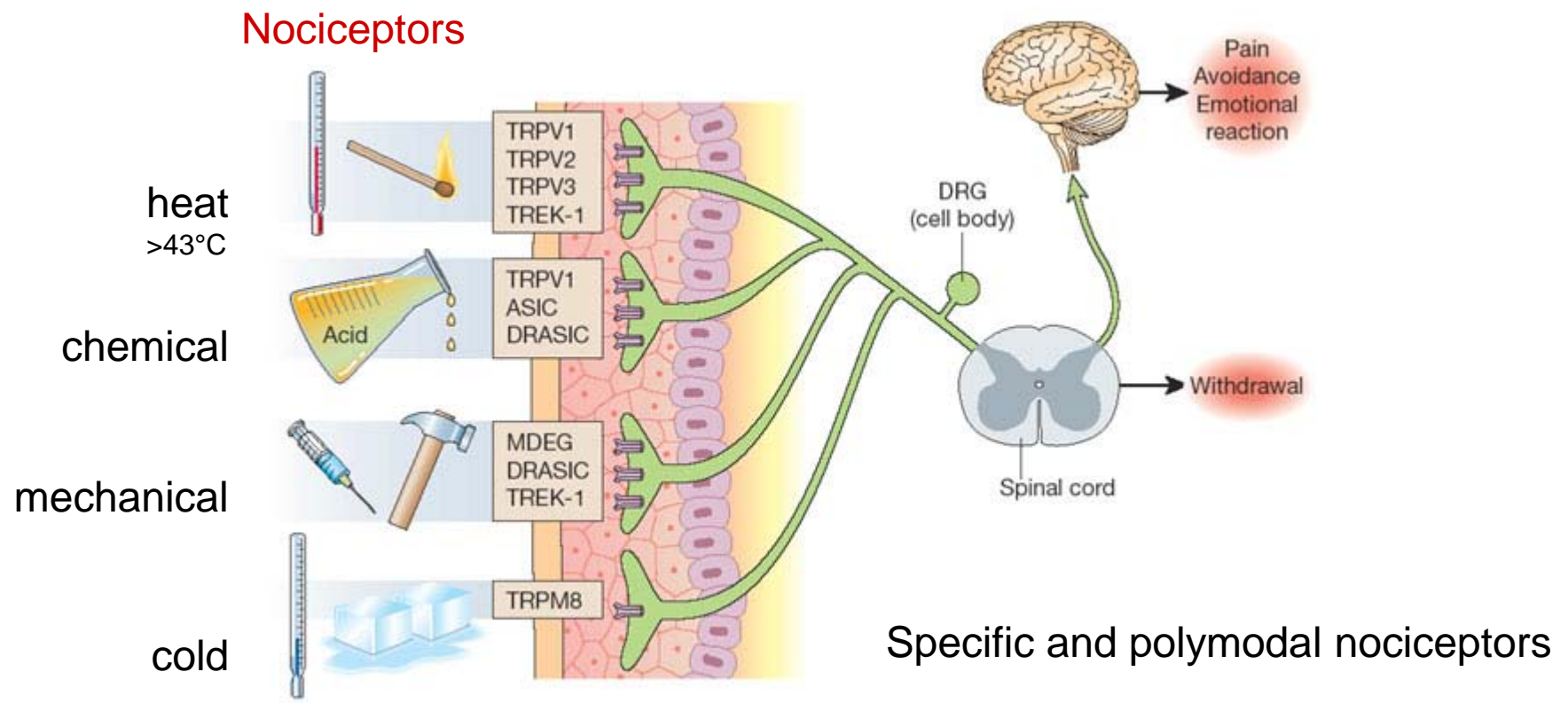


- 1 Periphery
- 2 Spinal Cord
- 3 Brain



(Schmidtko et al. 2009, modified)

# Nociceptive Transmission (Periphery)

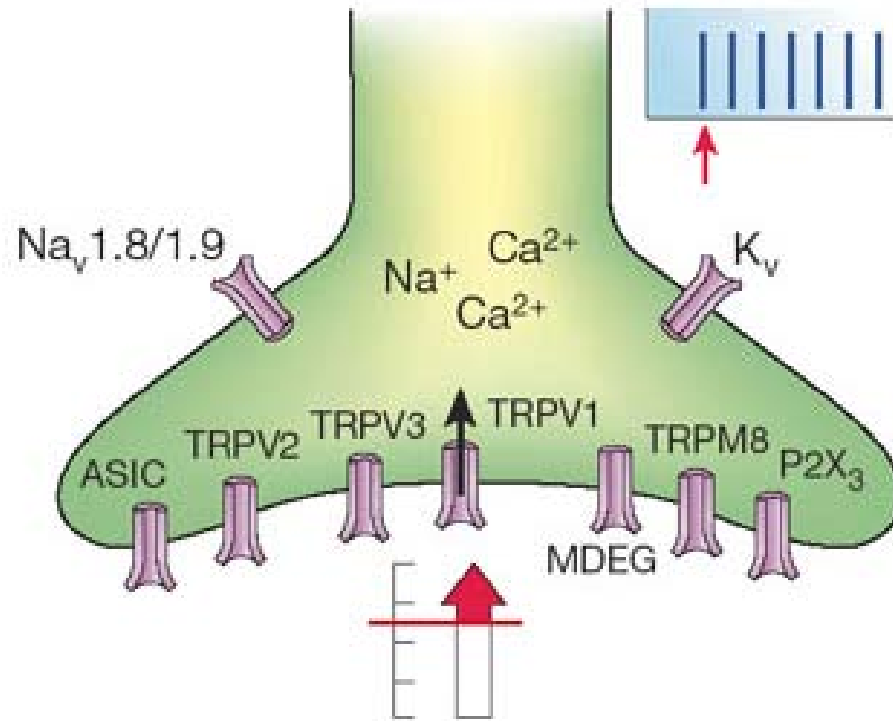




# Tissue-specific nociceptors

- **Skin nociceptors**
- **nociceptor in the musculoskeletal system**
- **Visceral nociceptors**
- **Trigeminal nociceptors**
- **(Irritation sensors e.g. reflexes, itching)**

# Chemical mediators in the primary afferent



# Functional properties of primary afferents

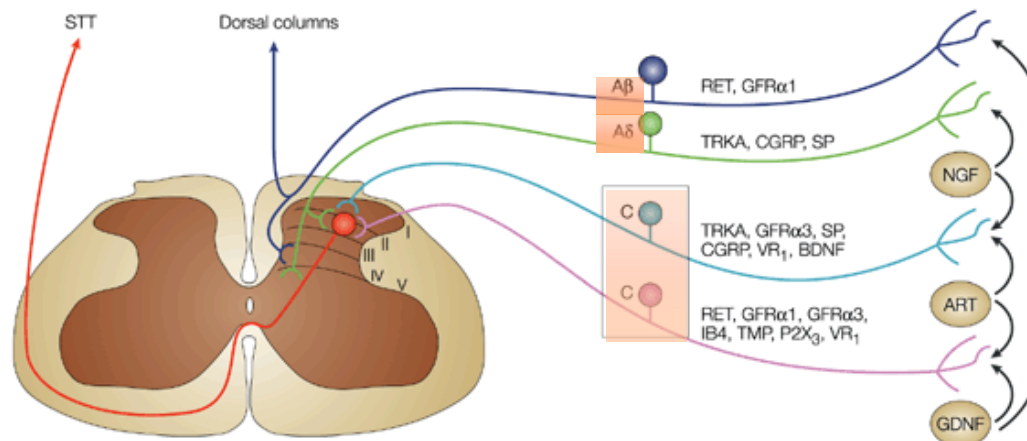
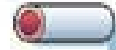
- **A $\alpha$ - and A $\beta$ -fibres** (  $\varnothing$  15  $\mu$ m, myelinated, conduction velocity 30-120m/sec) **non-nociceptive**




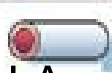
- **A $\delta$ -fibres** (  $\varnothing$  2-3  $\mu$ m, thinly myelinated, conduction velocity 2-40m/sec) **nociceptive**

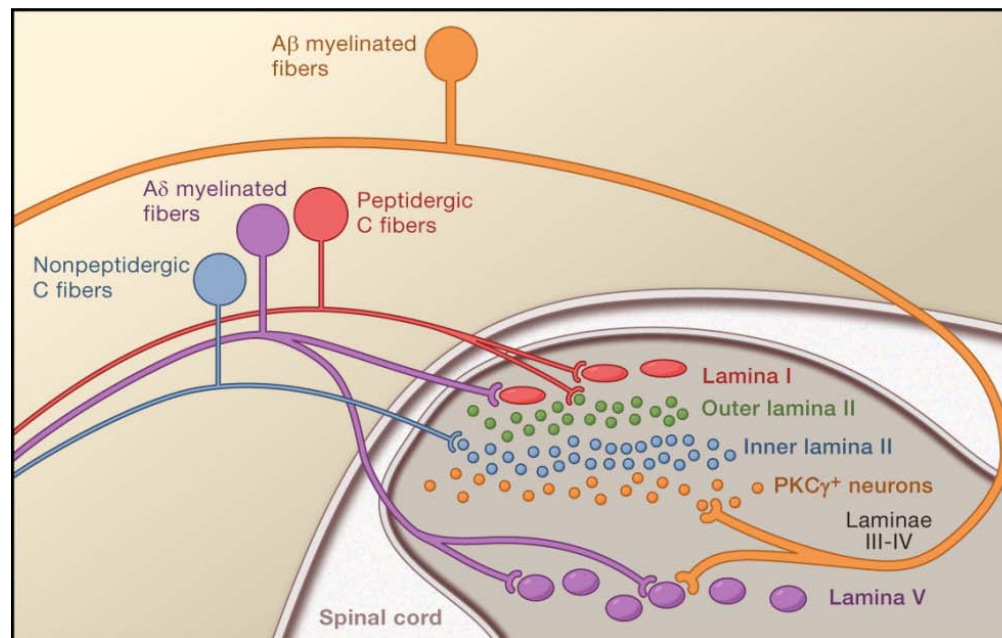


- **C-fibres** ( $\varnothing$  0,5-1  $\mu$ m, unmyelinated, conduction velocity <2m/sec) **nociceptive**



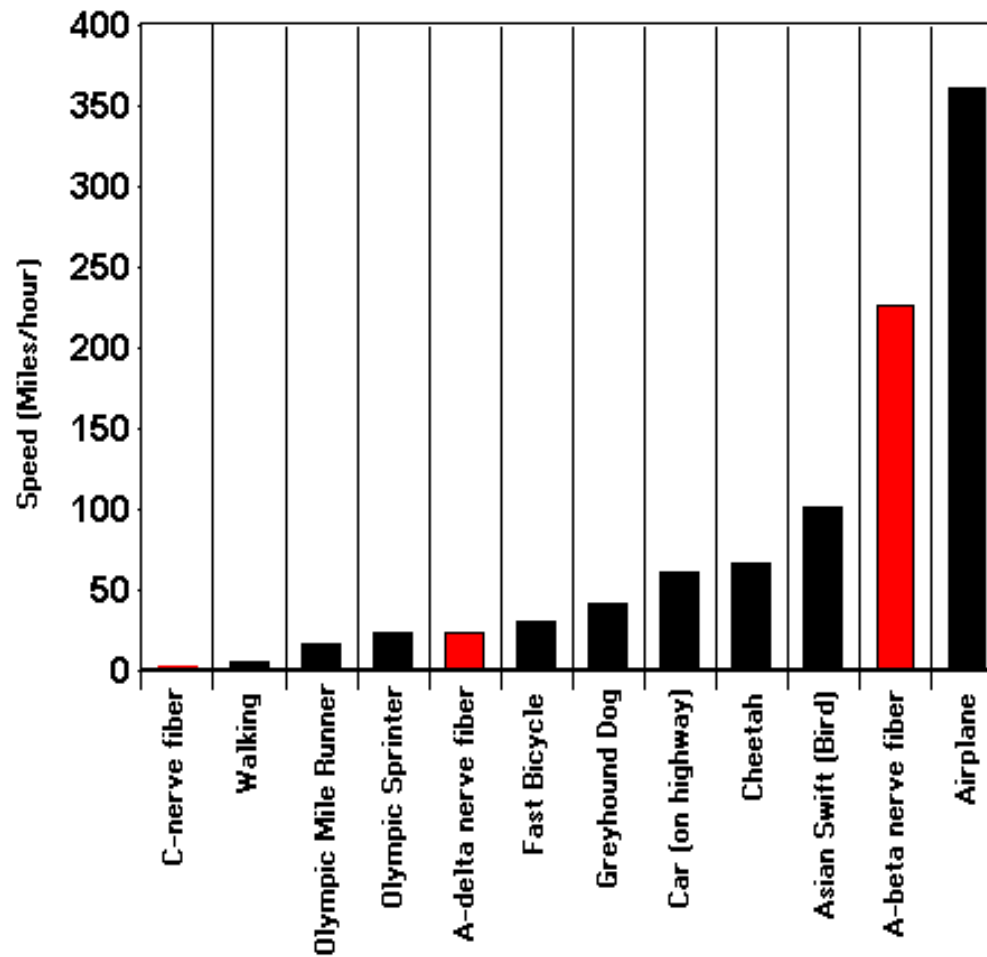
# Functional properties of primary afferents

- **A<sub>δ</sub>-fibres**, easy localized „first“ or fast pain 
- **C-fibres**, poorly localized, „second“ or slow pain   
*peptidergic*: release of neuropeptides, expression of TrkA,  
*non-peptidergic*: expression of neurotrophin receptors

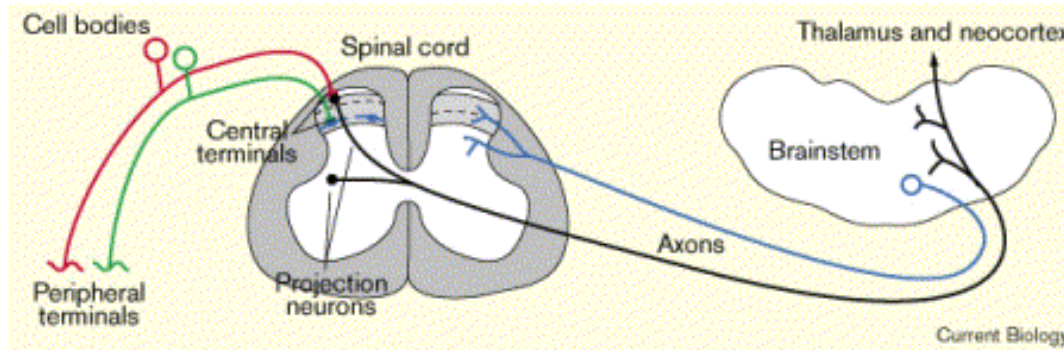


Signaling in both directions

# Functional properties of primary afferents

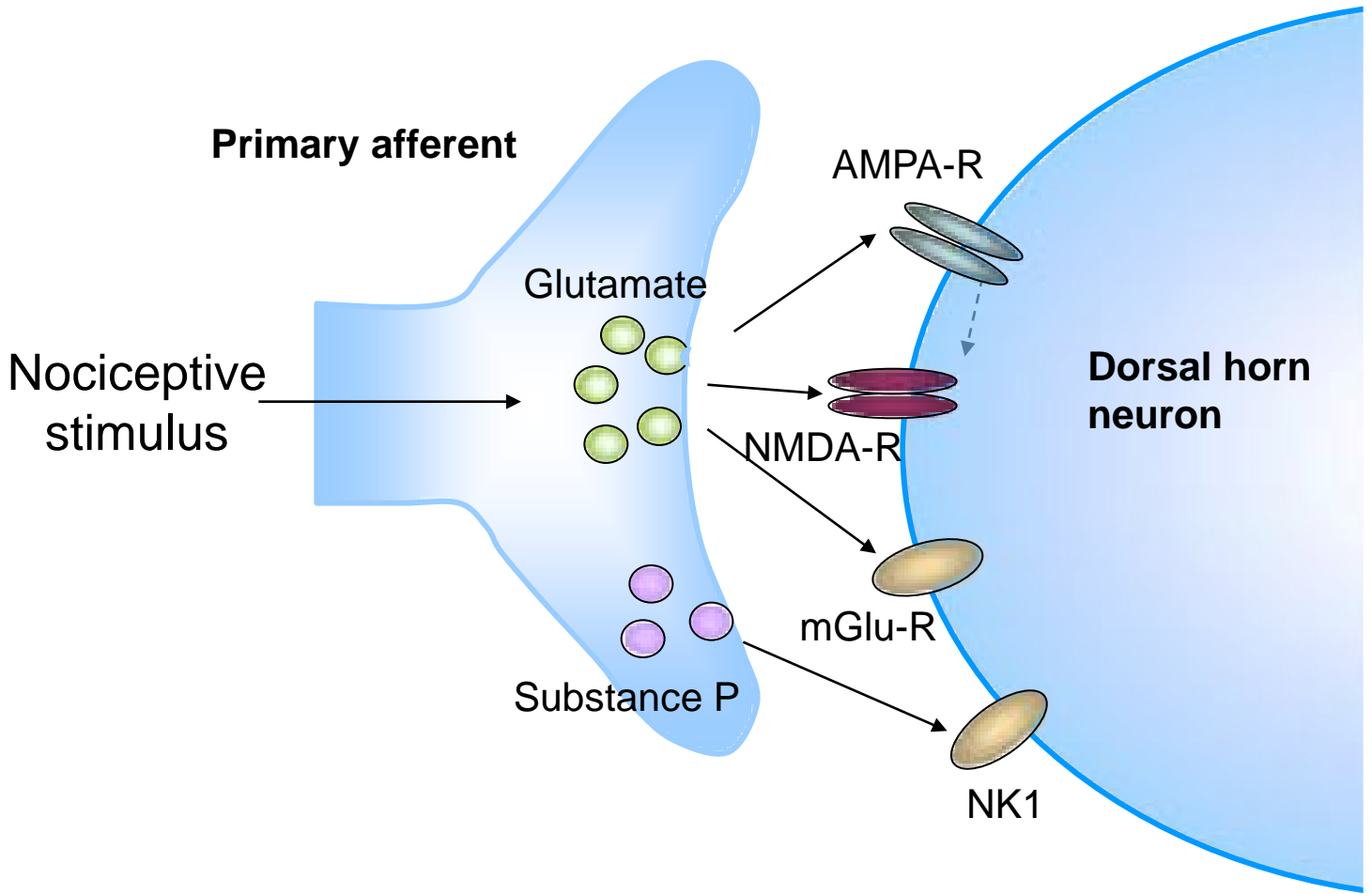


# Nociceptive transmission in the spinal cord



→ Transmission from primary to secondary nociceptive neurons (projection neurons) in the dorsal horn of the spinal cord by excitatory amino acids and neuropeptides

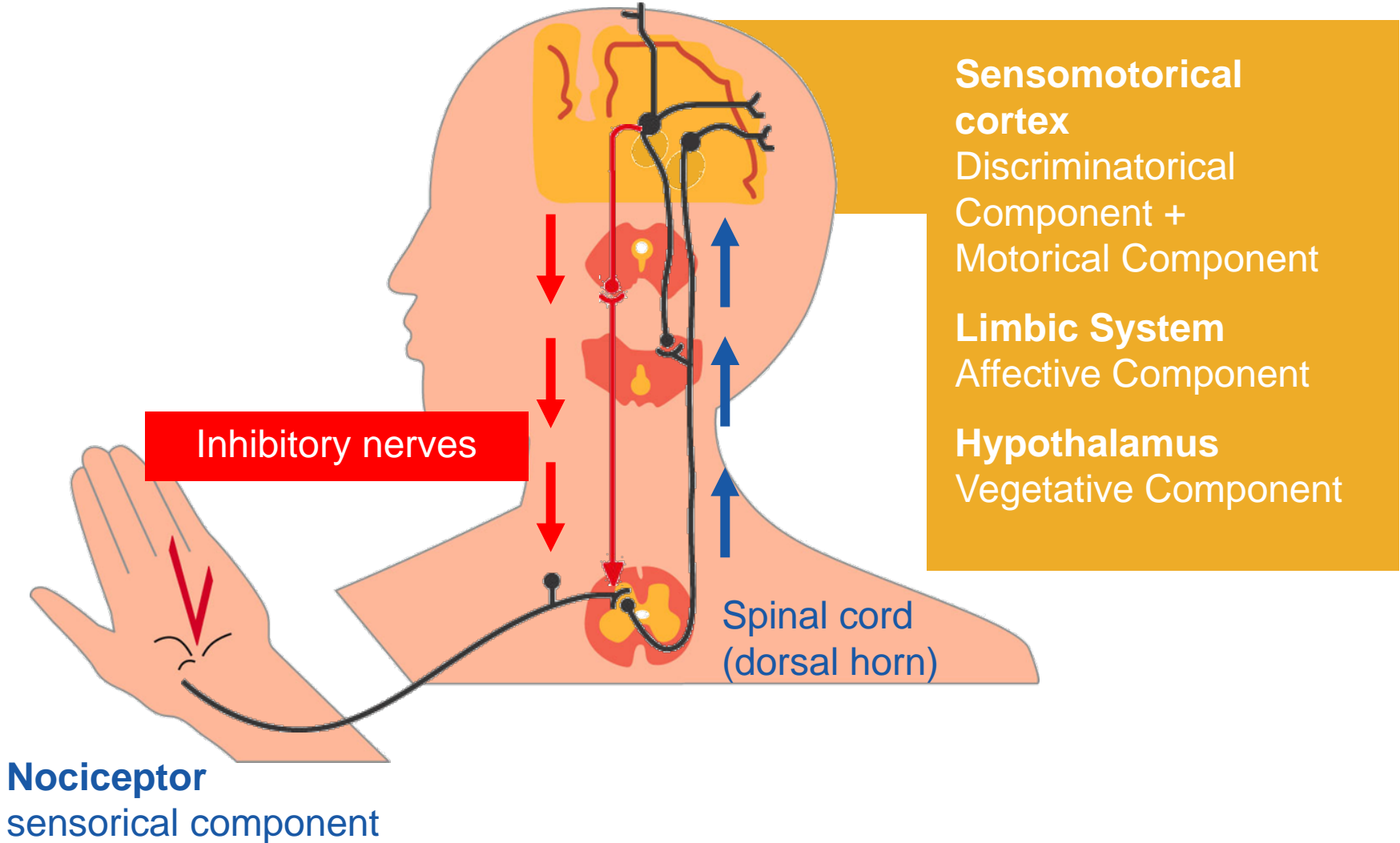
# Nociceptive transmission in the spinal cord



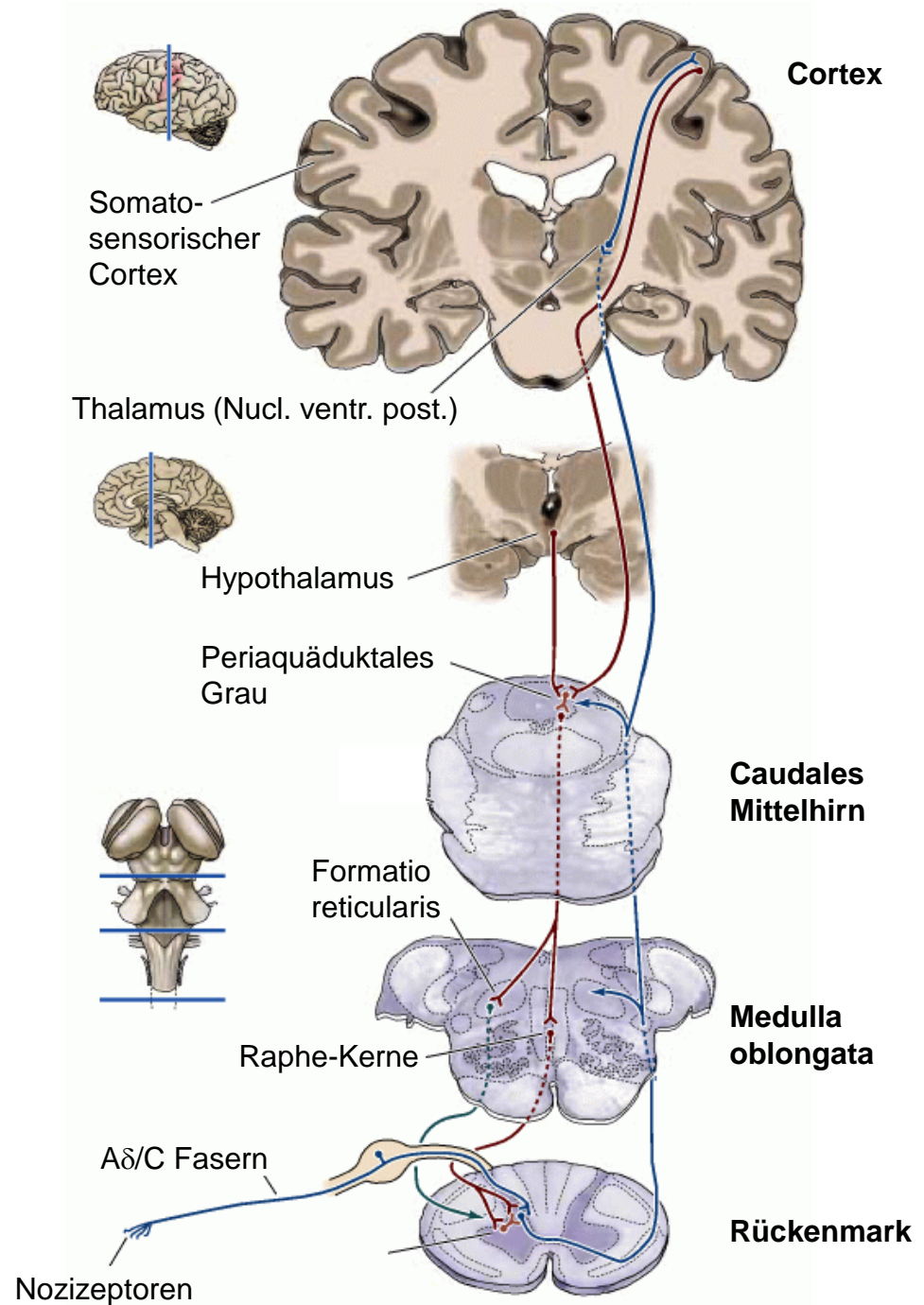
# Nociceptive neurons in the spinal cord

- **class 1 neurons** (low threshold, without nociceptive activity)
- **class 2 neurons** (wide dynamic range)
- **class 3 neurons** (high threshold, specifically nociceptive)
  
- Expression of „immediate early genes“
- Ascending tracts

# Nociceptive tracks

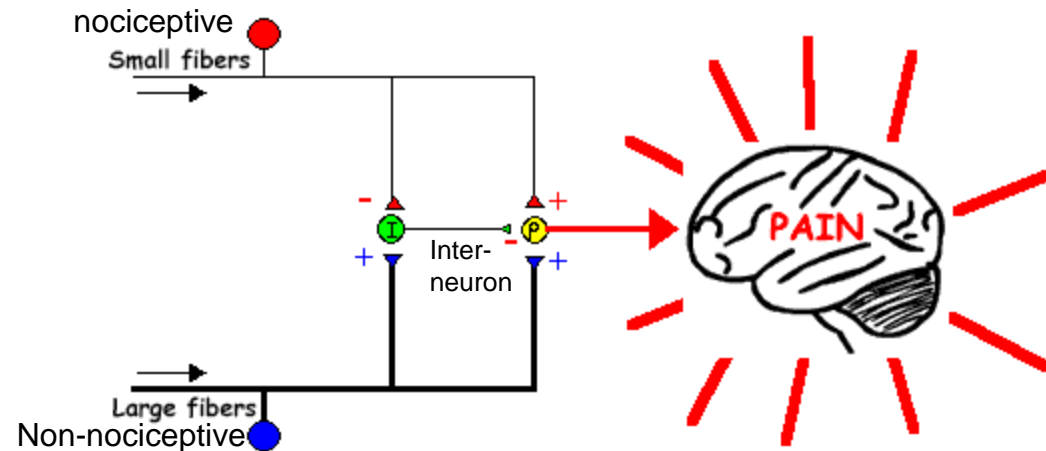


**Ascending nociceptive  
and  
descending  
antinociceptive system**



# Endogenous antinociceptive system

- Inhibitory Synapses
- Transmitter:
  - Endorphines and Enkephalines
  - Adenosine
  - GABA



Gate control theory, Wall and Melzack, 1965 , Science

Adjustment of central nociceptive threshold

- Segmental spinale and supraspinal inhibition
- Gate-control-hypothesis

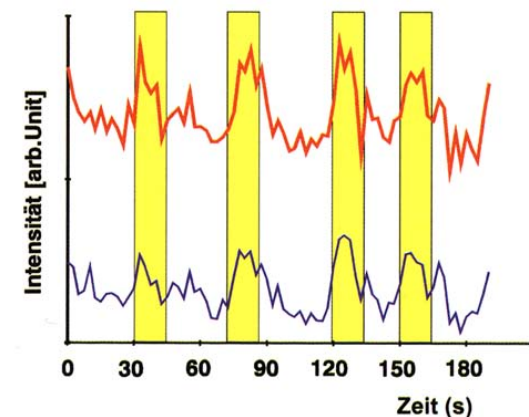
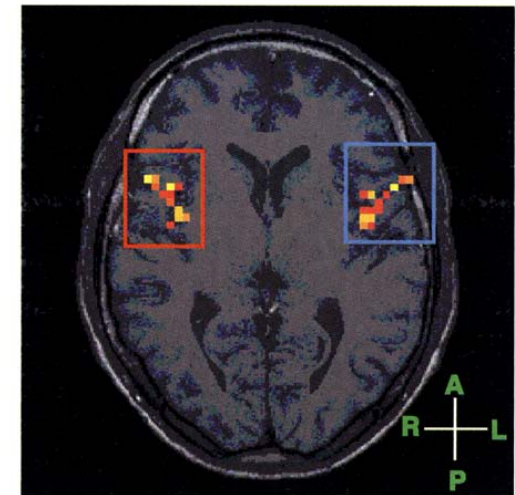
# Brain regions

## „Pain centres“

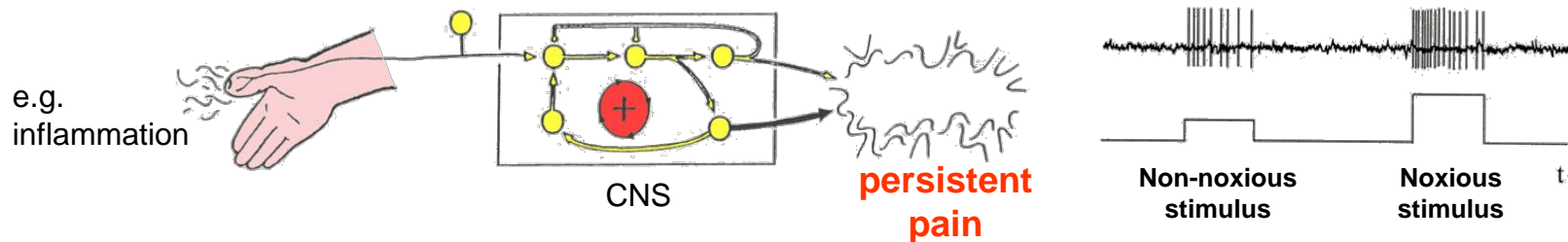
- Lateral system (sensory discriminating)
- Medial system (affective)

## Examinations

- Electrophysiological recordings
- Recording of evoked potentials
- Positron emission tomography (regional changes in blood circulation)
- Magnetic resonance imaging (regional changes in oxygen concentration)

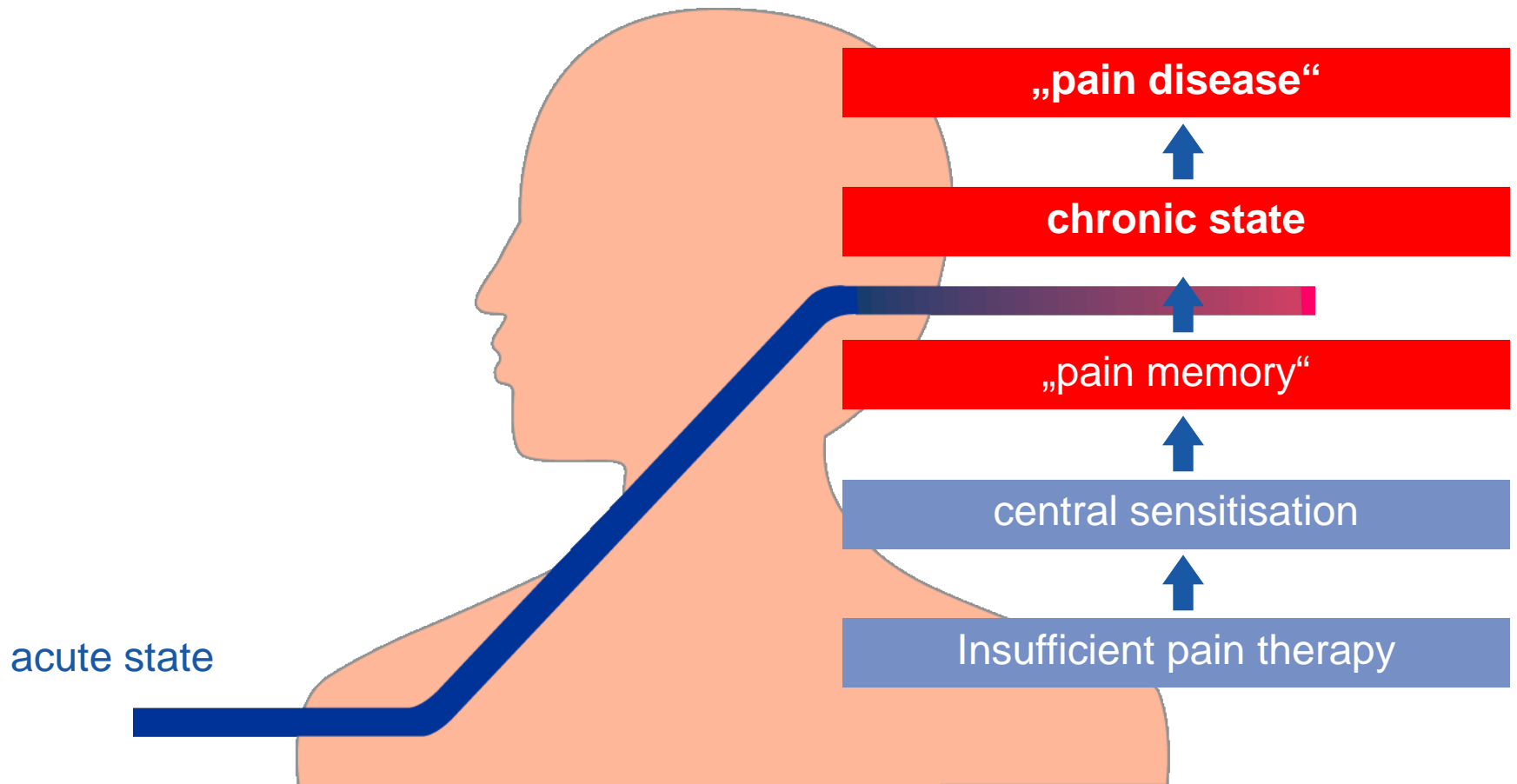


# Pathophysiological nociceptive pain



- synaptic plasticity, peripheral and central sensitization
- low threshold
- ⇒ *Hyperalgesia*: Increased response to a noxious stimulus
- ⇒ *Allodynia*: Pain due to a stimulus which does not normally provoke pain.
- ⇒ Persistent pain, increased pain sensitivity

# Chronification of pain



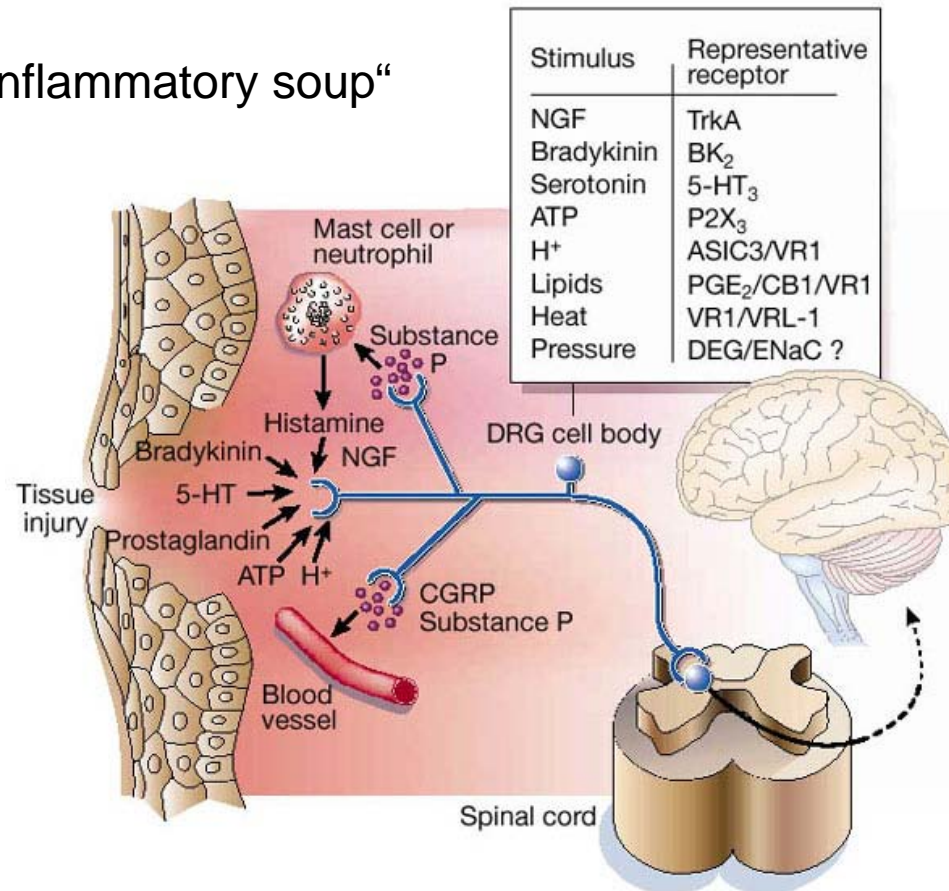
# Chronification of pain

- Chronic pain = Pain that lasts longer than 3 months
- „Pain disease“
- Pain remains after the initial injury has resolved.
- Pain can be a result of injury, disease or inflammation
- Peripheral or central disorder
- Peripheral and central sensitization

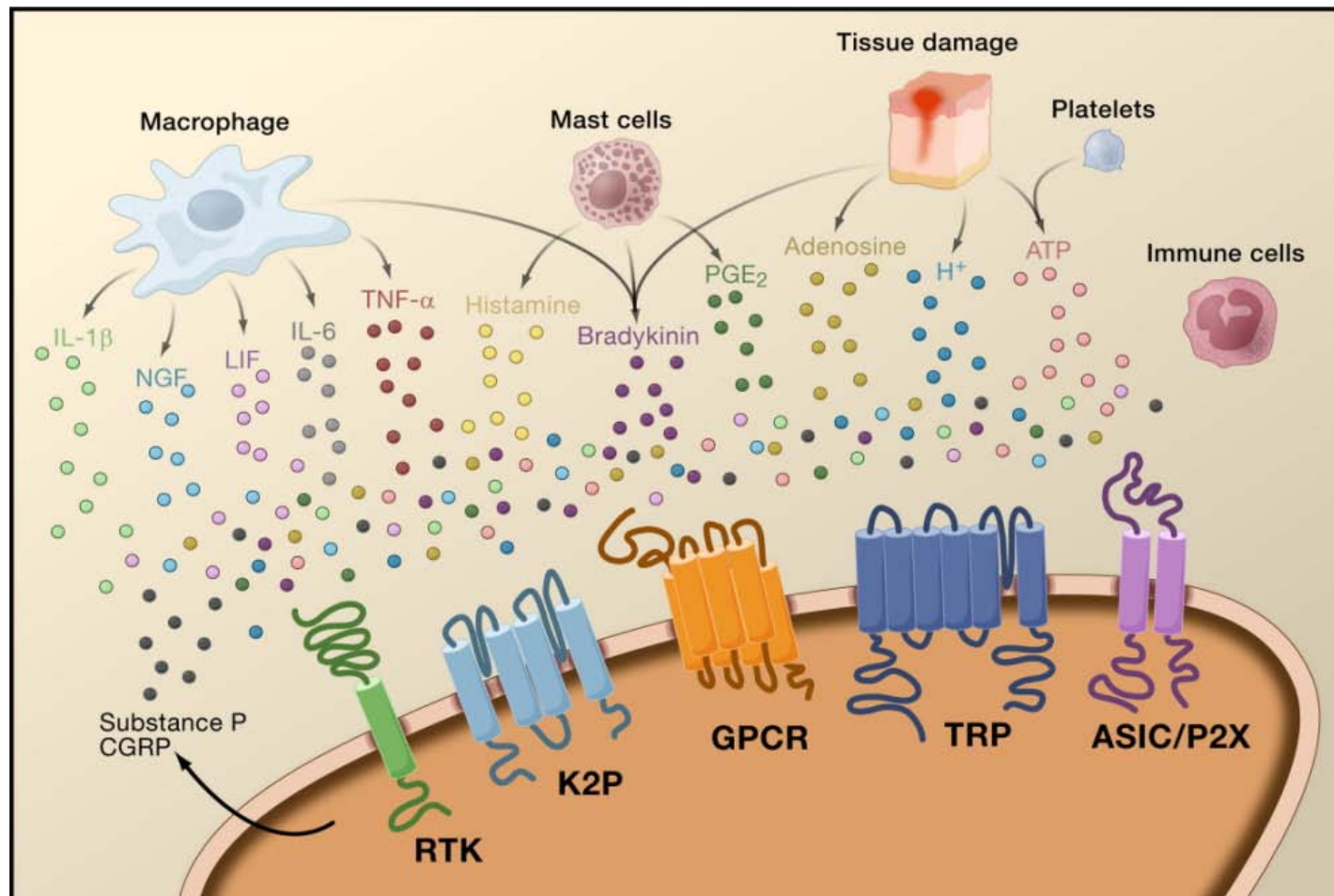
# Mediators of inflammation (Periphery)

„Inflammatory soup“

- Neurotransmitters
- Peptides
- Eicosanoids
- Neurotrophins
- Cytokines
- Chemokines



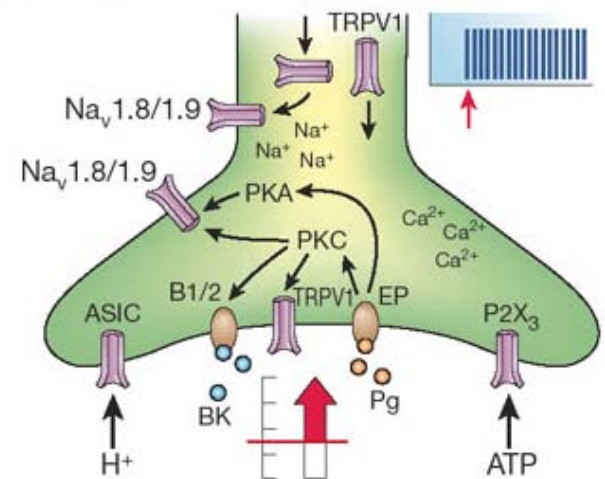
# Chemical mediators in the primary afferent



## Chemical mediators

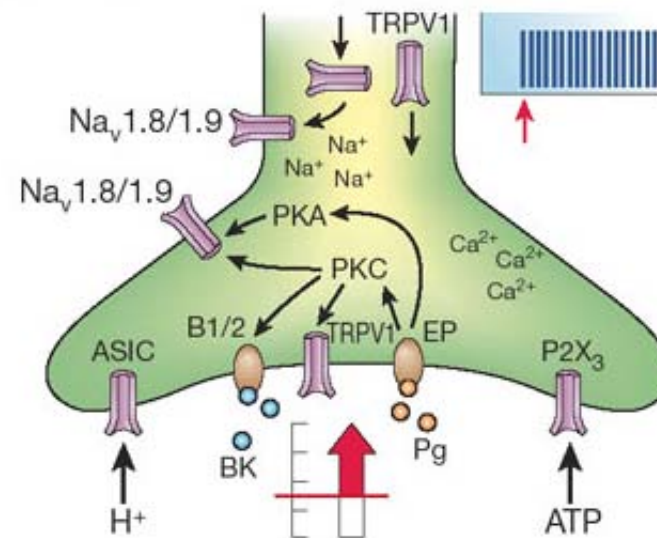
- Activation of G-protein coupled receptors or tyrosine kinase receptors on nociceptive terminals
- Phosphorylation of receptors and ion channels in the nociceptive terminals
- Expressional changes
- Change of thresholds and kinetics

⇒ **Peripheral Sensitization**



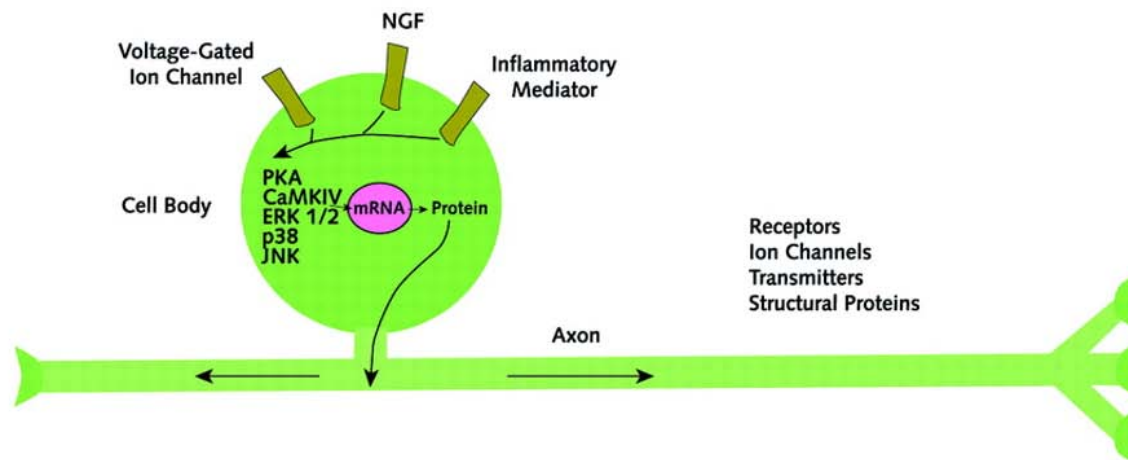
# Changes in the periphery

Peripheral	<p><b>Transduction</b> TRPV1, TRPV2, TRPV3, TRPM8 ASIC, DRASIC MDEG, TREK-1 BK<sub>1</sub>, BK<sub>2</sub> P2X<sub>3</sub></p>
	<p><b>Peripheral sensitization</b> NGF, TrkA TRPV1 Na<sub>v</sub>1.8 PKA, PKC isoforms, CaMK IV Erk1/2, p38, JNK IL-1β, cPLA<sub>2</sub>, COX2, EP1, EP3, EP4 TNFα</p>
	<p><b>Membrane excitability of primary afferents</b> Na<sub>v</sub>1.8, Na<sub>v</sub>1.9 K<sup>+</sup> channel</p>
	<p><b>Synaptic transmission</b> <i>Presynaptic</i> VGCC Adenosine-R (mGlu-R)</p>



# Processing in the DRG

## C. Transcriptional Change in the DRG



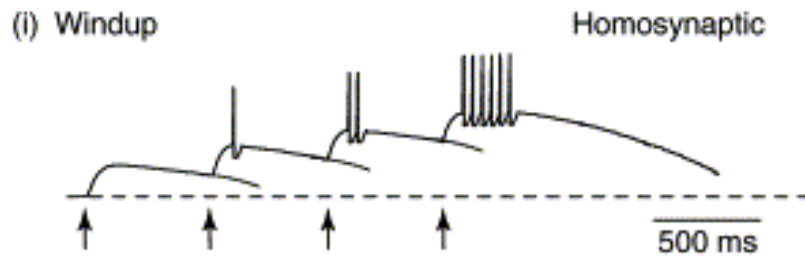
Woolf, C. J. Ann Intern Med 2004;140:441-451

# Central sensitization

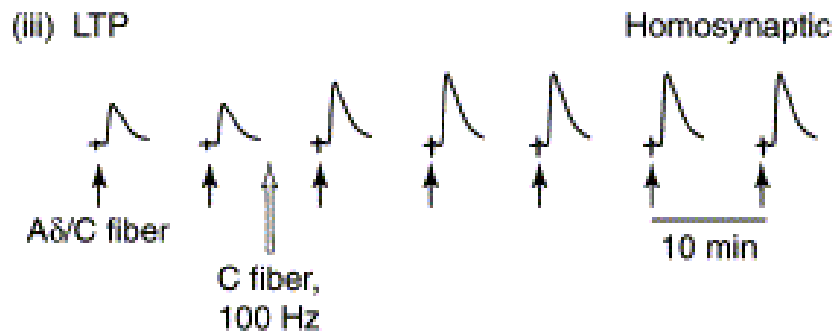
- ⇒ Increase of excitability of nociceptive neurons in the dorsal horn of the spinal cord
  - „Wind up“
  - Long-term-potential (LTP)
  - Inhibition of inhibitory neurons
  - Increase in intracellular  $\text{Ca}^{2+}$
  - Activation of immediate early genes (IEGs)
  
- ⇒ Persistent pain, secondary hyperalgesia

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Early onset



- Repetitive nociceptive stimulation
- „Accumulation“ of response

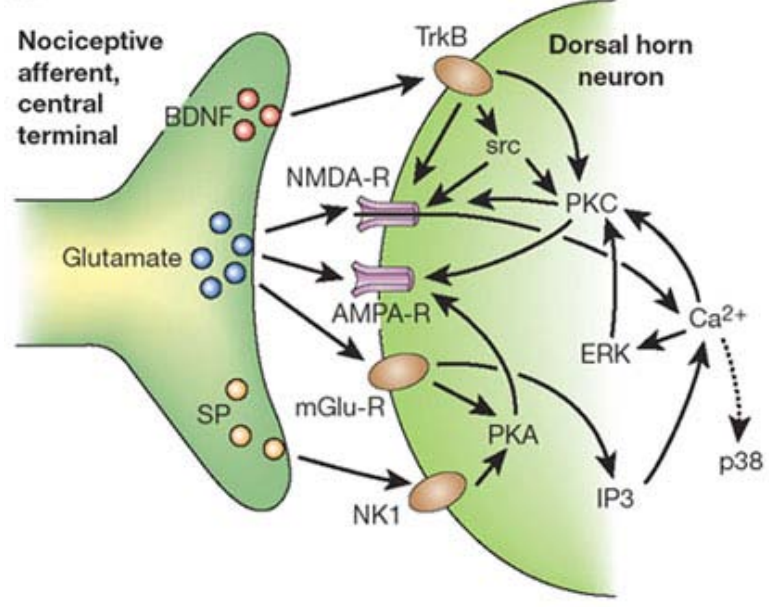


- High-frequency stimulation
- Increased longterm response (postsynaptic potential, postsynaptic current)

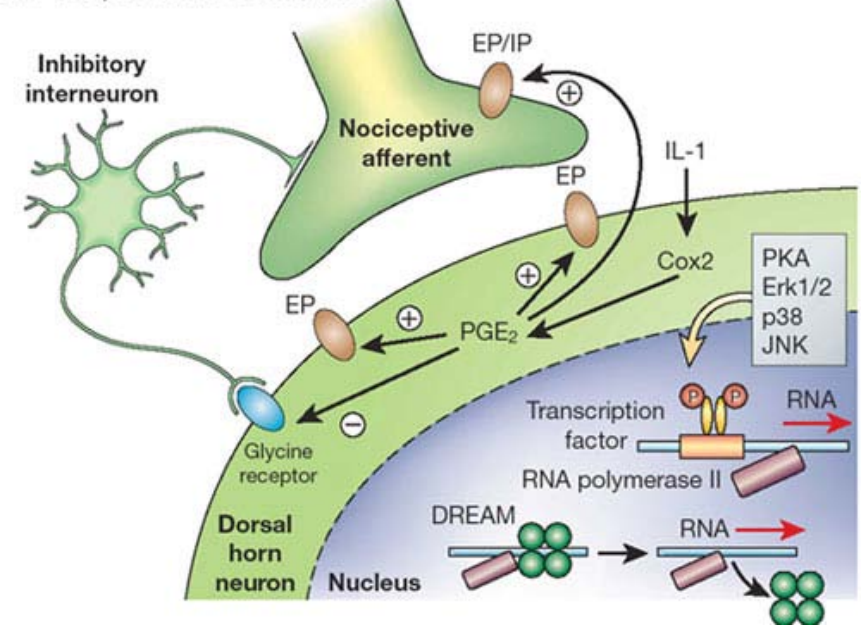


# Central sensitization

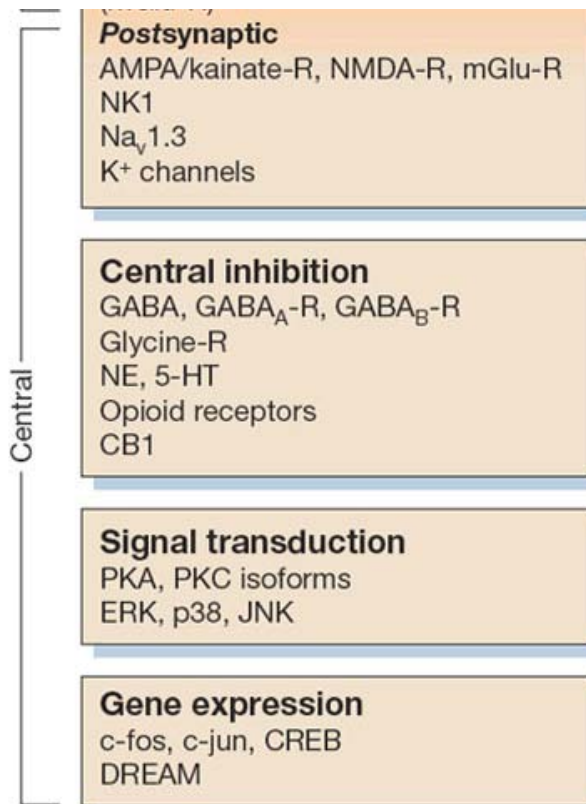
**a** Immediate central sensitization



**b** Delayed central sensitization



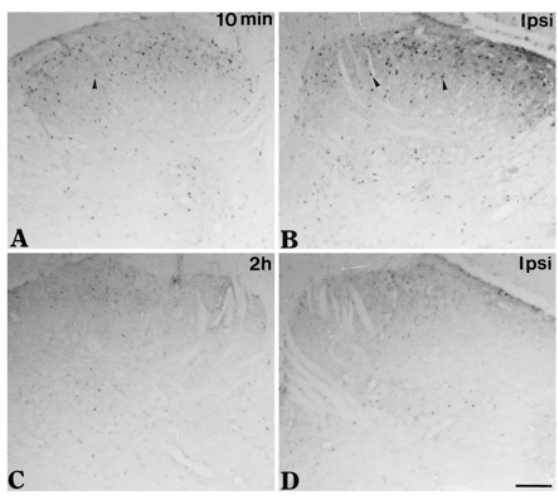
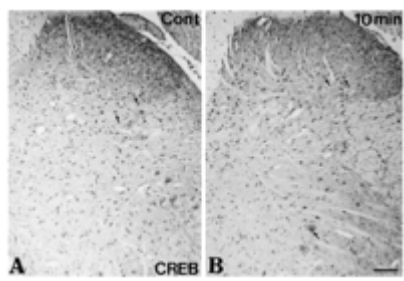
# Changes in the CNS



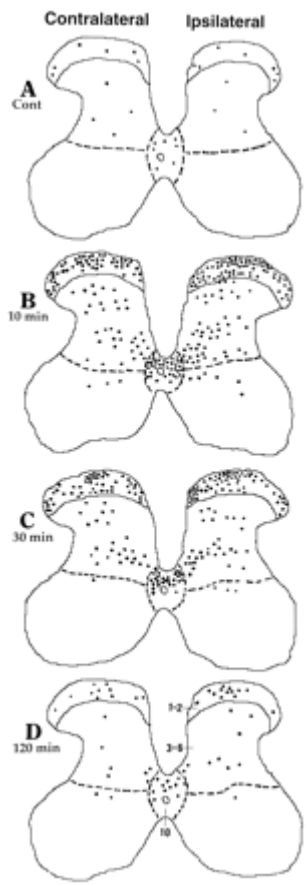
# Spinal plasticity

(after formalin injection into one hindpaw)

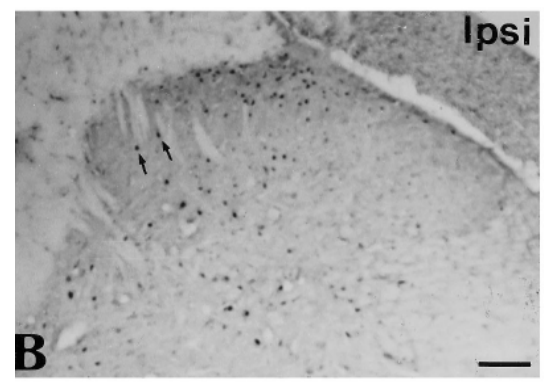
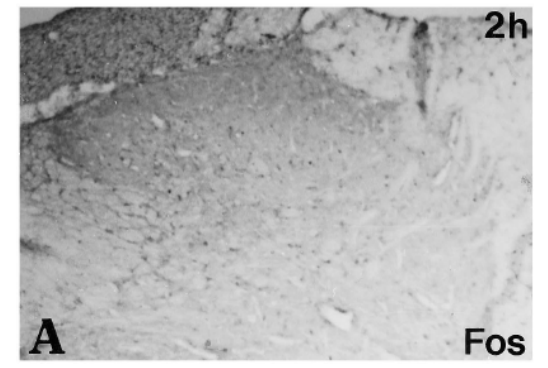
CREB

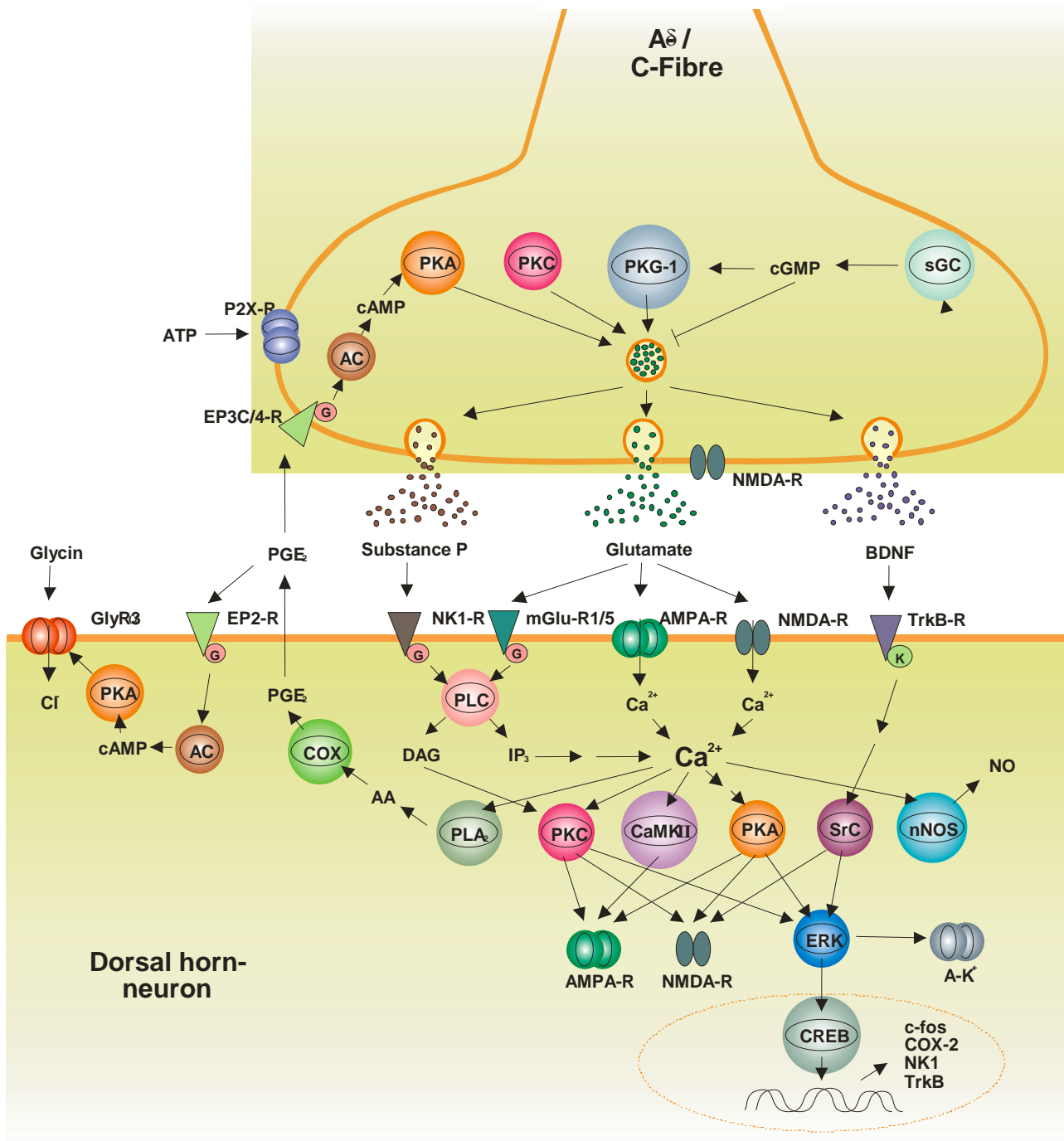


phospho-CREB



c-Fos



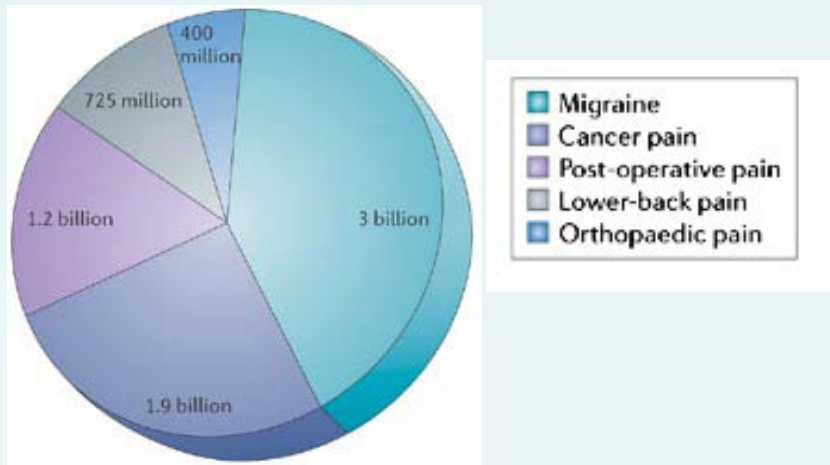




# Pharmacotherapy of pain and inflammation

World-wide:

Prevalence of chronic pain  
<25 years of age 17.3 %  
>65 years of age 42 %



Estimated worldwide size of pain submarkets  
Total market for pain drugs: 7.25 billion US\$

Gershell and Goater *Nature Reviews Drug Discovery* 5, 889–890 (2006) |

In Germany:

~12-15 Mio people with chronic pain  
~17% of people in a telephone survey

From this 17%

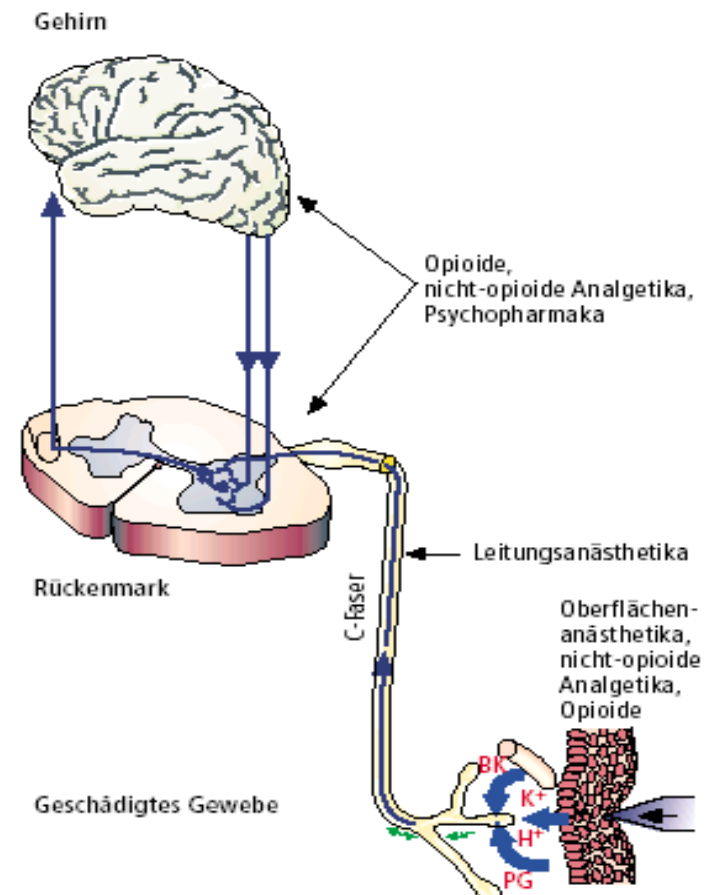
- 38% had problems with social activities
- 14% lost their job
- 20% with additional depressions

Breivik et al., *Eur J Pain*, 2006 10(4),287

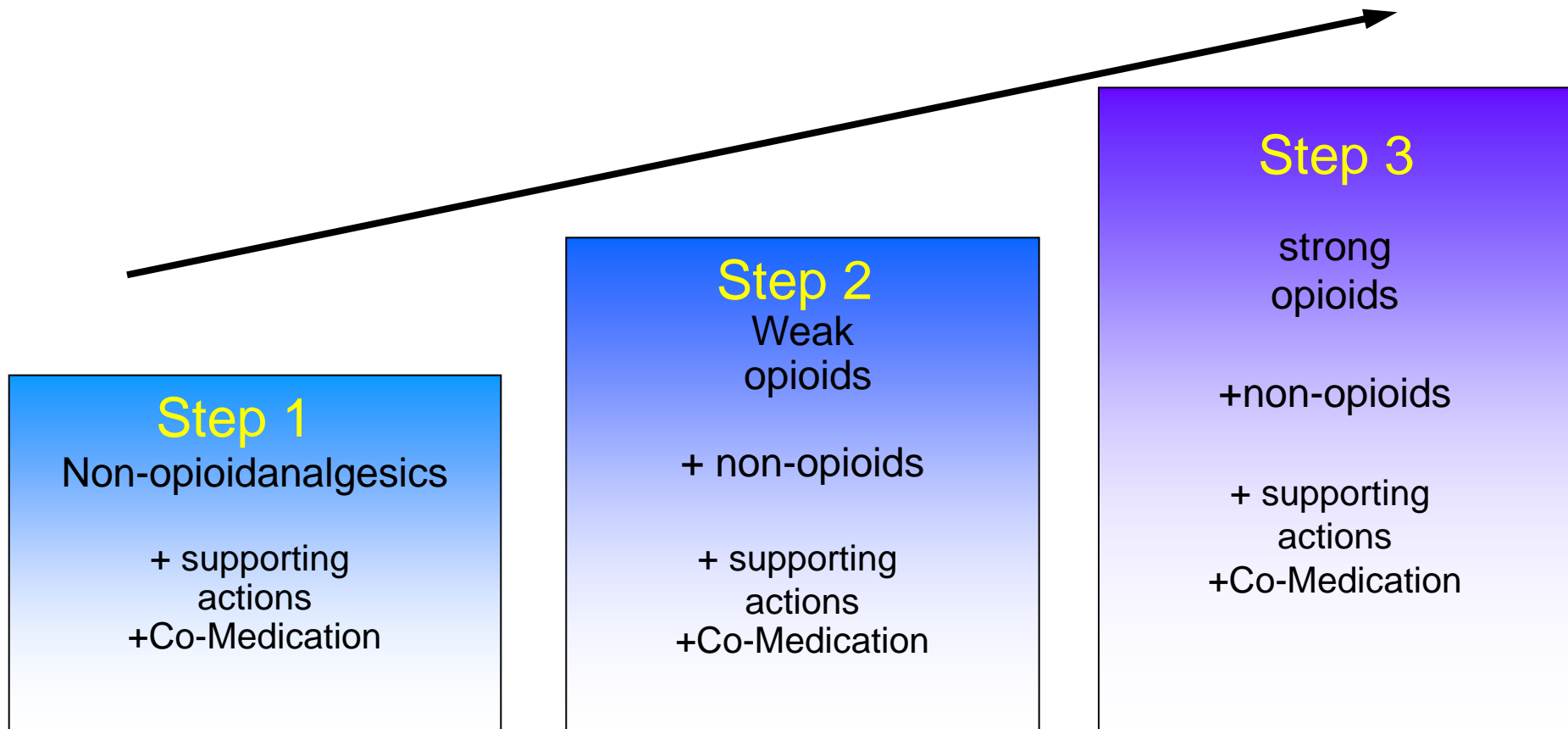
# Analgesics

**Non-Opioid-Analgesics**  
central and peripheral effects

**Opiod-Analgesics**  
mainly central effects

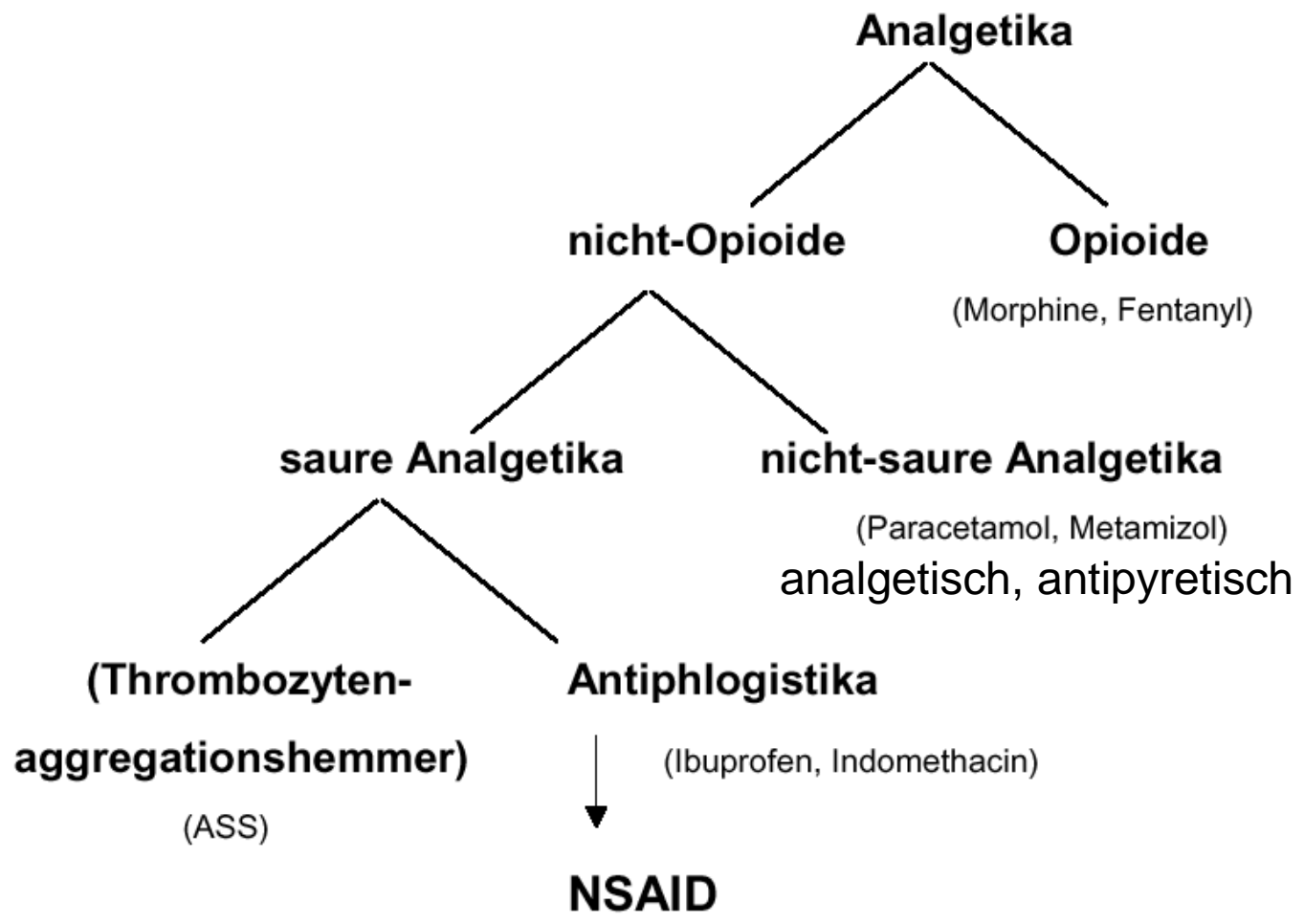


## WHO-analgesics ladder



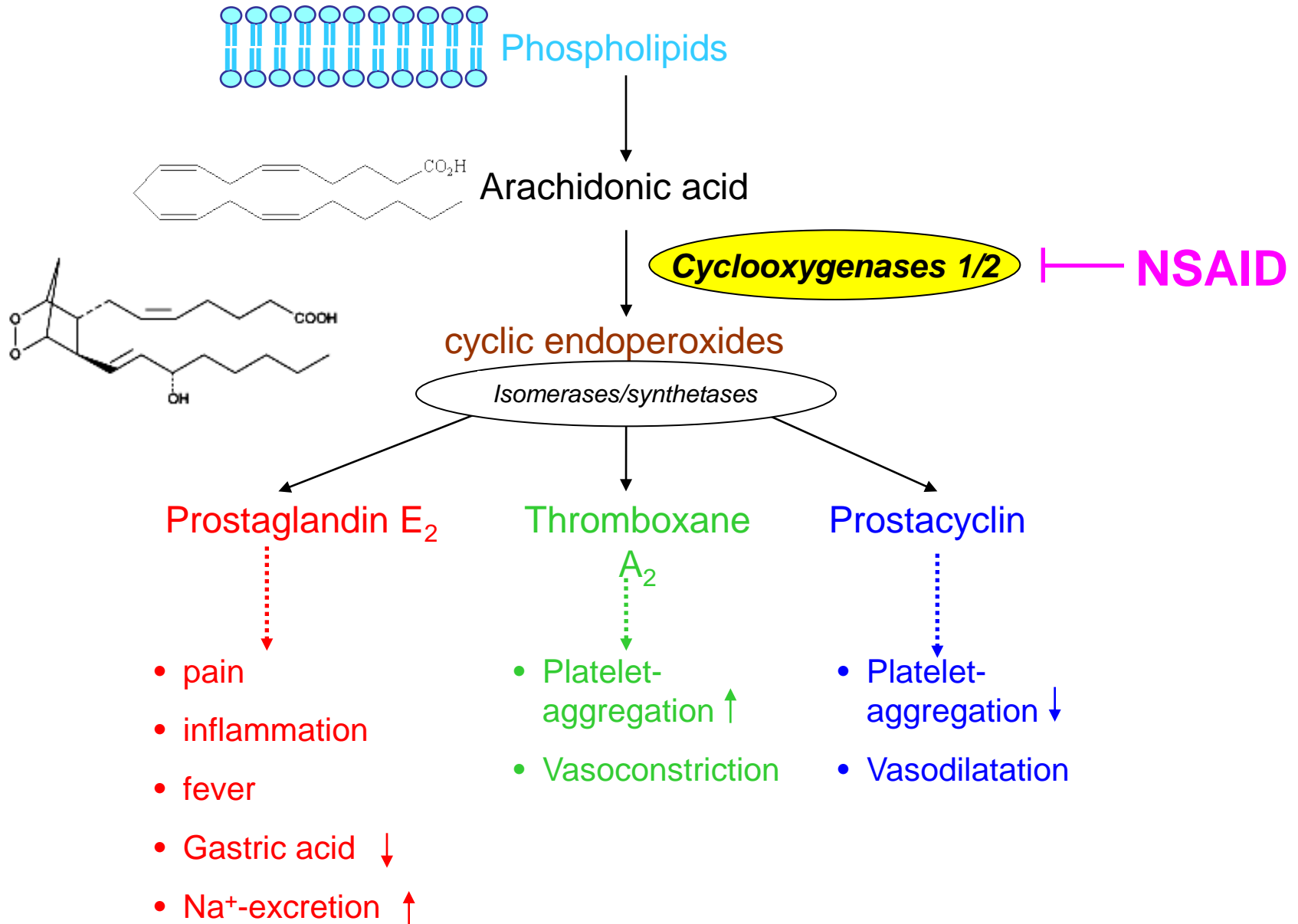


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Non-steroidal anti-inflammatory drugs

# Arachidonic acid-cascade



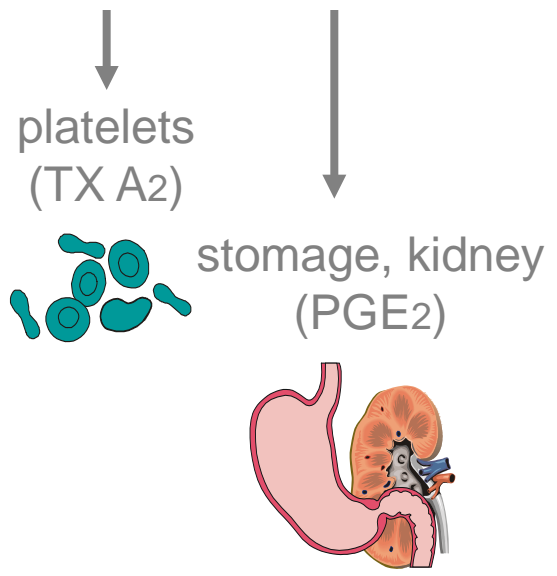
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Physiolog. Stimulus

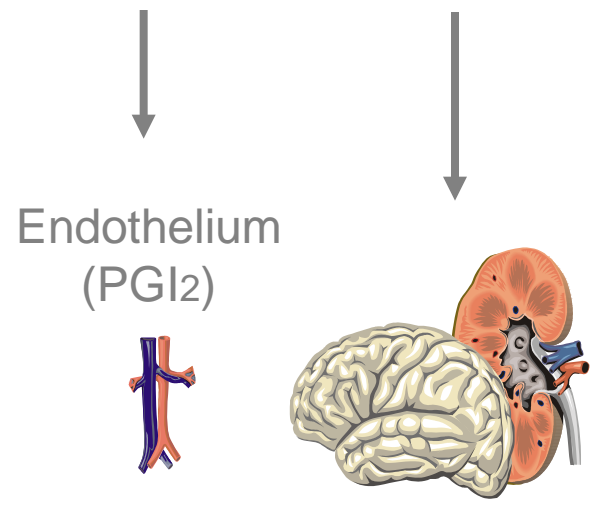
Physiolog. Adaptation

Inflamm. Stimulus

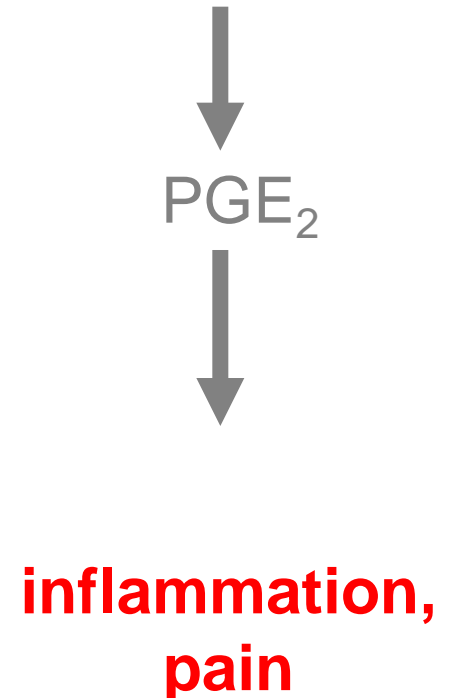
**COX-1**  
constitutive



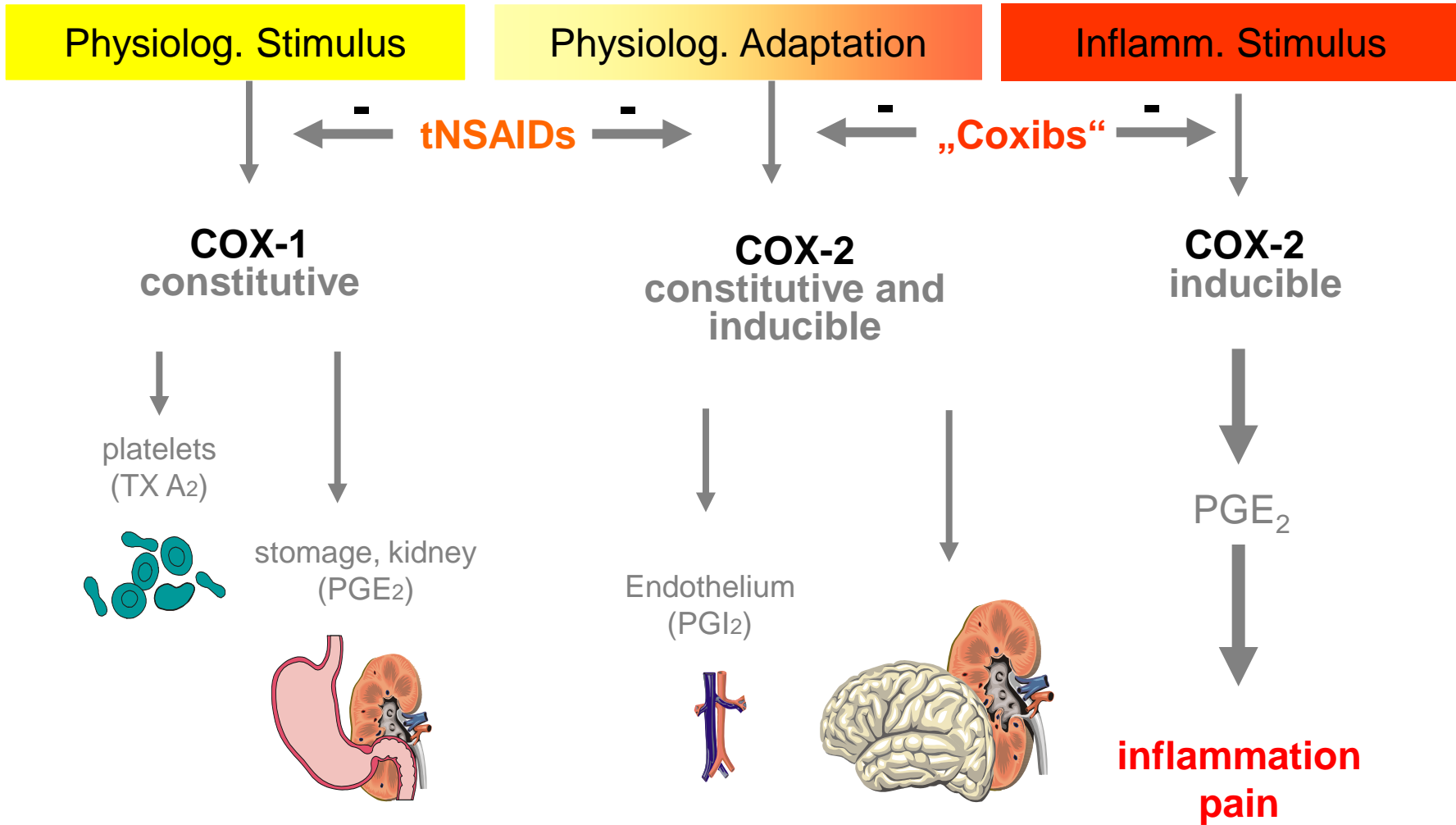
**COX-2**  
constitutive and inducible



**COX-2**  
inducibile



# COX inhibitors



⇒ Gastrointestinal side effects



# Non-steroidal anti-inflammatory drugs (NSAIDs)

## Traditional NSAIDs

- Acetylsalicylic acid
- Ibuprofen
- Diclofenac
- Inhibition of Cyclooxygenase (COX) 1 + 2
- analgesic, antipyretic and antiphlogistic

## Coxibs

- Celecoxib (Celebrex®)
- Etoricoxib (Arcoxia®)
- selective inhibition of COX 2
- ⇒ less gastrointestinal side effects

2004, 2005: withdrawal of Rofecoxib (Vioxx®) and Valdecoxib (Bextra®) (cardiovascular side effects)

2007: suspension of Lumiracoxib (Prexige®) (liver toxicity)

# Non-steroidal anti-inflammatory drugs (NSAIDs)

## Indications

- pain
  - headache, tooth pain
  - Migraine
  - Rheumatic pain
- fever
- inflammation

## Side effects

- gastrointestinal  
(less in case of COX-2 selective)
- kidney disturbances
- Cardiovascular (Coxibs)



# Non-steroidal anti-inflammatory drugs (NSAIDs)

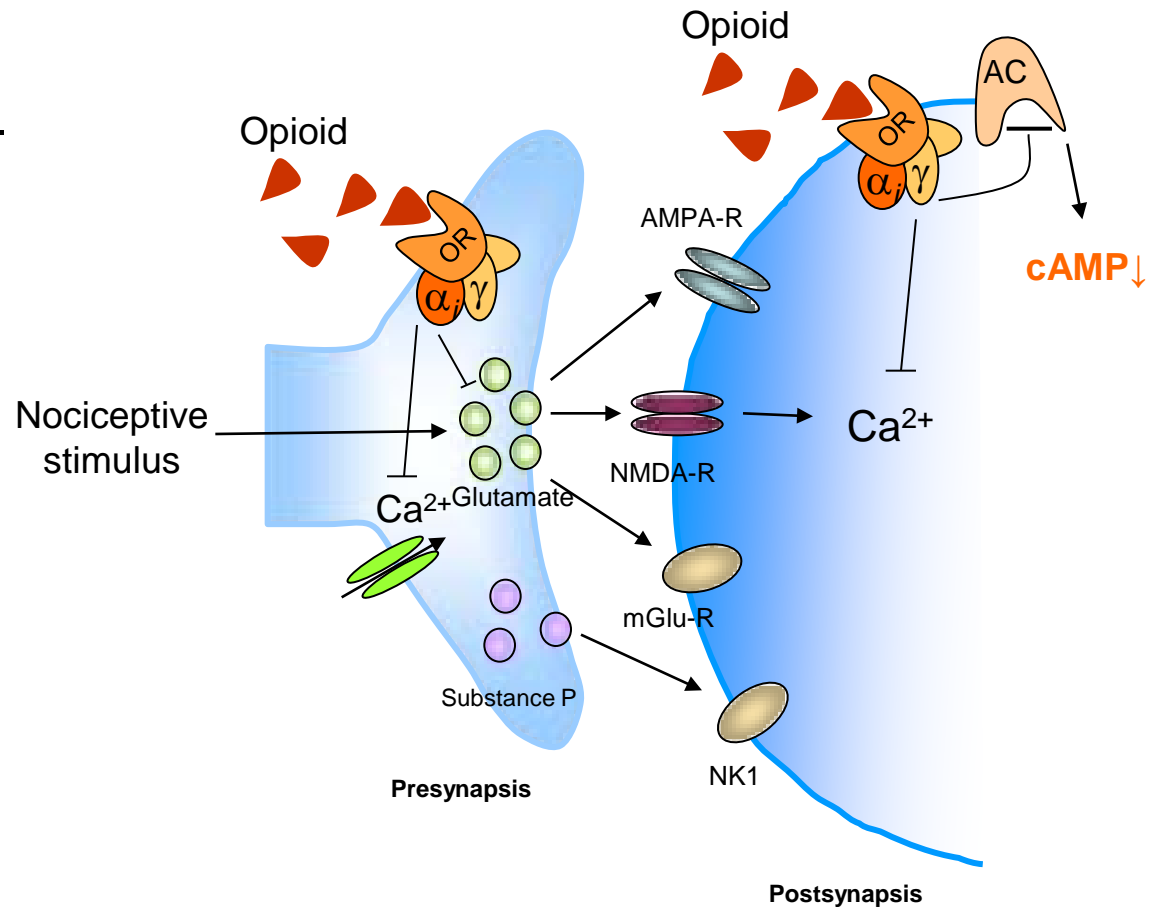
effective analgesics, but:

From 1000 patients an average of

- 100 patients develop ulcera
- 10 patients develop stomage bleeding
- 1 patient dies

# Opioid-analgesics

- Agonists of  $\mu$ ,  $\kappa$ ,  $\delta$ -opioid-receptors
- Activation of the endogenous antinociceptive system
- cAMP intracellular  $\downarrow$
- Inhibition of transmitter release





## Indications

- Severe and worst pain
  - Postsurgical
  - Traumatic
  - Tumor pain
- Morphine
- Tramadol (Tramal®)  
weak  $\mu$ -agonist
- Codein (cough)

# Effects of Opioid-Analgesics

## central

- Analgesia
- Sedation
- sickness, emesis
- Euphoria
- Respiratory depression  
*(in case of pain minor)*
- Decreased cough
- itching
- Decreased blood pressure
- Tolerance
- addiction

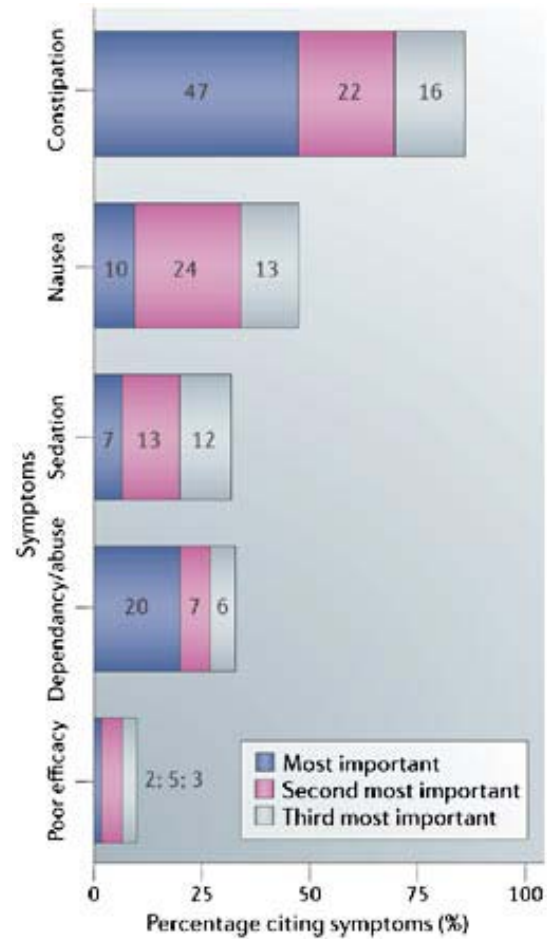
## peripheral

- Analgesia
- Obstipation
- Inhibition of bile flow
- anuresis
- Release of histamin, itching

Most Opioids are under control of the **Narcotics law (BtMG)**, to avoid abuse



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# Pain research-necessary?

- ~ 12-15 Mio patients suffering from chronic pain in Germany  
⇒ Analgesics = most common pharmaceuticals  
(93 Mio prescriptions 2003)
- Long-term use of analgesic is particularly associated with severe side effects
- A number of patients is not sufficiently treated with the currently available drugs



Urgent need of analgesics with new and alternative mechanism of action



in vitro models



Animal models

# Nociceptive animal models

**Species:** In most cases rats and mice

**General principle:** motoric reaction to a stimulus as a parameter for the extent of nociception

**Stimuli:**

- thermal
- mechanical
- chemical

⇒ More than one method necessary to increase significance

# Motorical testing: Rotarod-Test

- Animals are placed onto a rotating rod
- Latency until the animals fall from the rod



# Measurement of acute thermal nociception

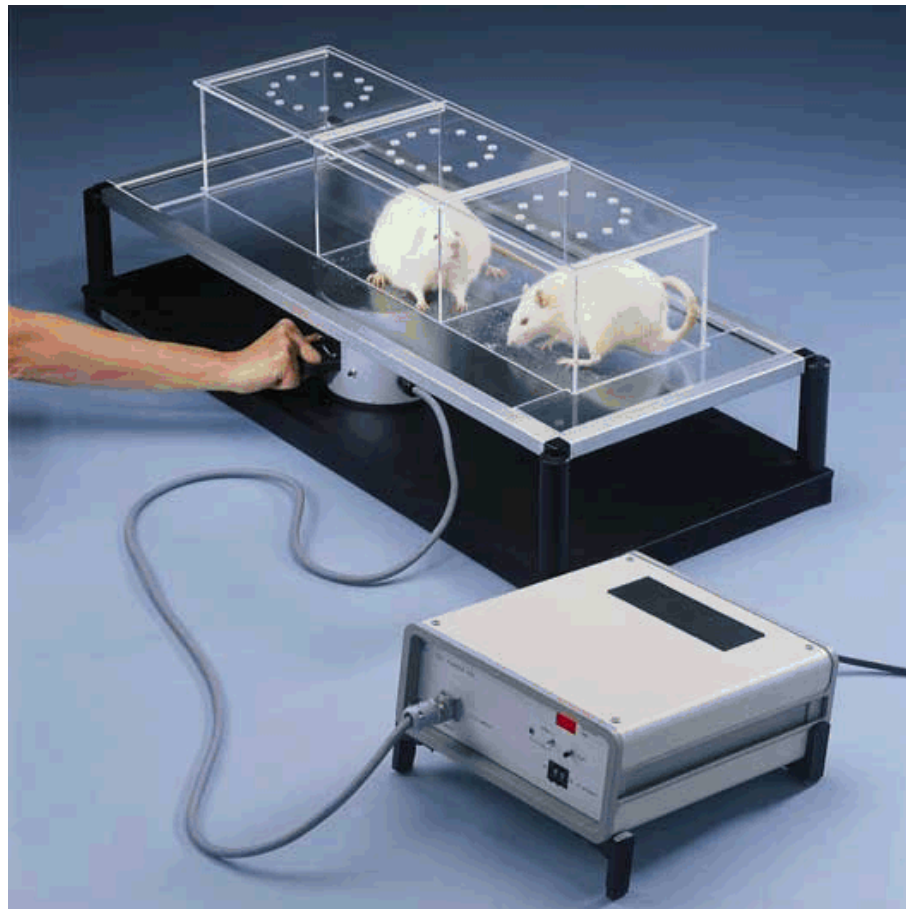
## Hot Plate-Test

- Animals are placed onto a 52°C warm Plate
- Latency until licking the paw/jumping



# Measurement of thermal Hyperalgesia: Hargreaves-Test

- Induction of paw inflammation by injection of zymosan
- Stimulation of the inflamed paw by a thermal stimulus
- Paw withdrawal latency



# Measurement of mechanical Hyperalgesia

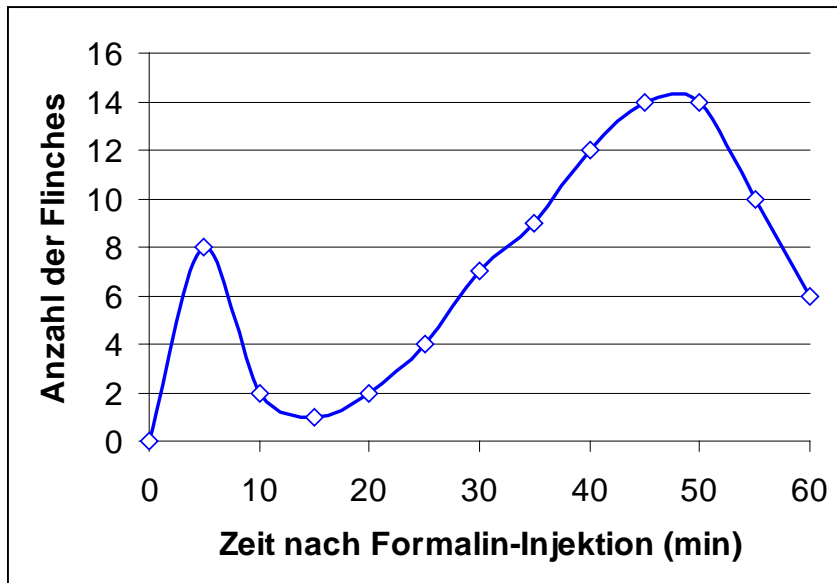
## Dynamic Plantar-Test

- Induction of paw inflammation by injection of zymosan
- Stimulation of the inflamed paw by a steel rod
- Paw withdrawal latency



# Measurement of tonic pain : Formalin-Test

- Injection of Formalin into one hind paw  
⇒ “Flinches” (rats)  
licking (mice)
- Counting Flinches / licking over 60 min or  
45 min, respectively
- Nociceptive response in 2 phases

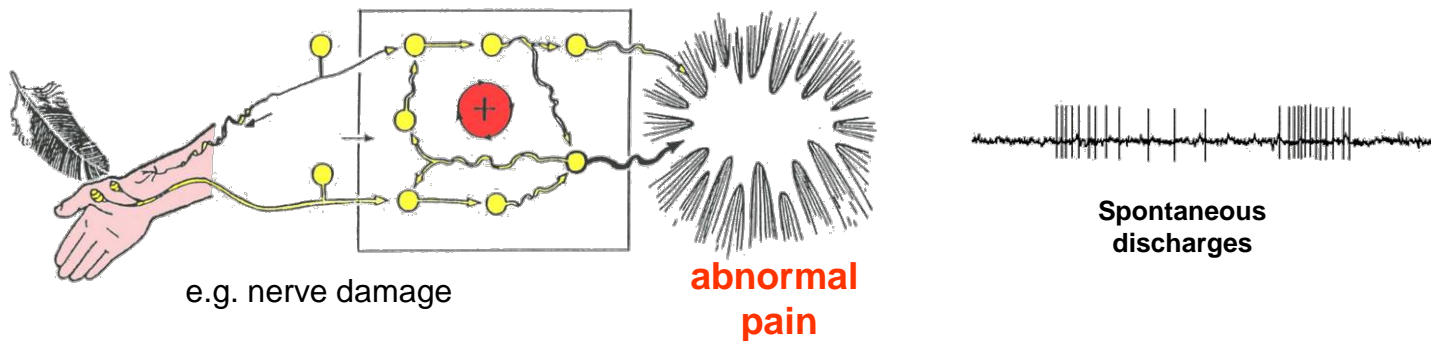


Phase 1 (0 - 10 min): *acute pain*

Phase 2 (11 – 60 min): *tonic pain*

# Neuropathic pain

## Neuropathic pain



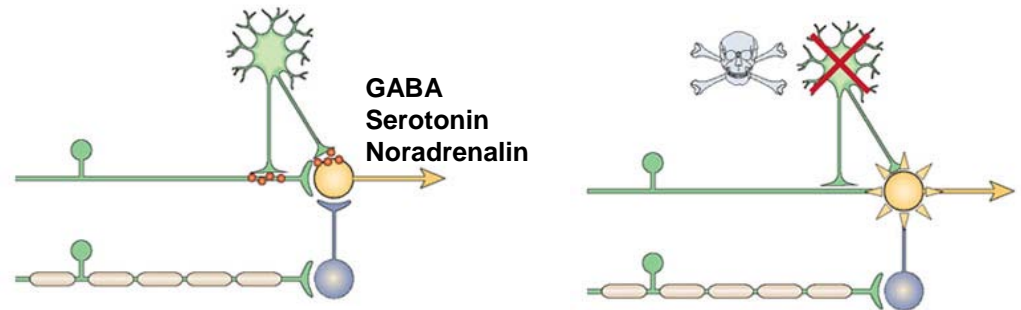
Abnormal pain:

- spontaneous or stimulus-independent pain  $\Rightarrow$  Shooting, burning, prickling, electrical
- Stimulus-dependent pain  $\Rightarrow$  allodynia, hyperalgesia



## Mechanisms

- Loss of inhibition



*J. Scholz and C.J. Woolf, Nature Neuroscience 2002*

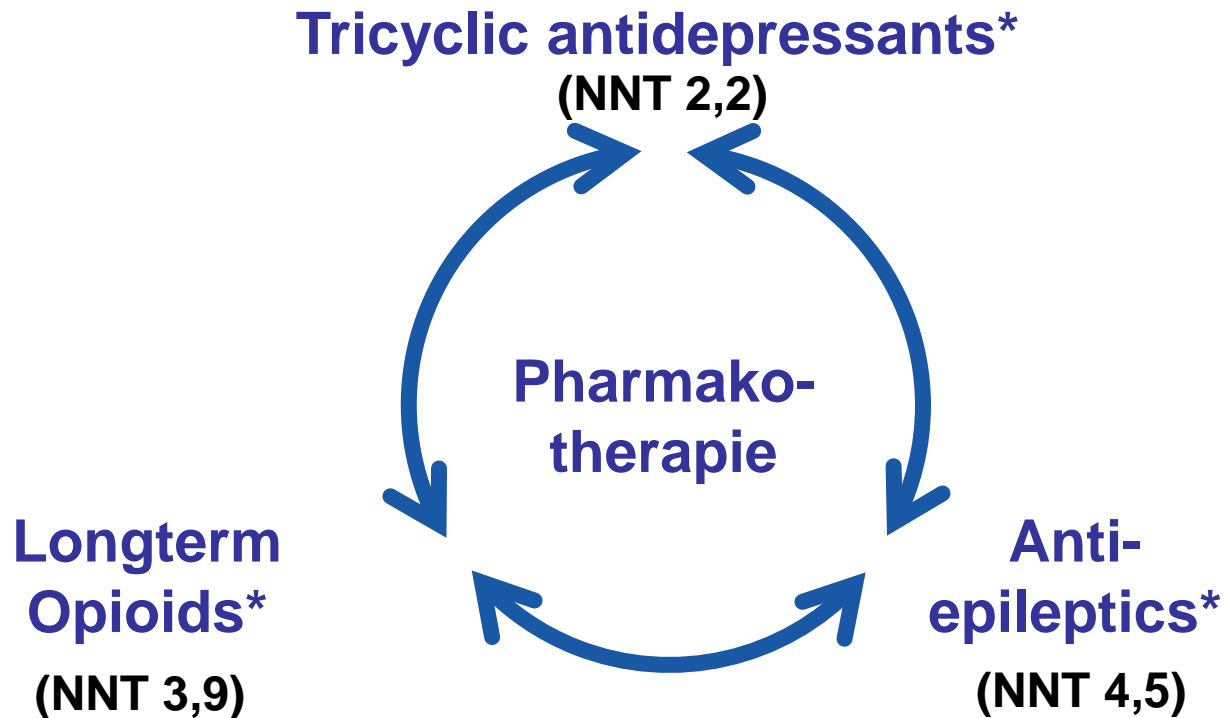
- Afferent discharge
- Upregulation of spinal dynorphin
- Redistribution of  $\text{Na}_v1.8$  sodium channels



- ~ 300.000 people suffer from severe neuropathic pain

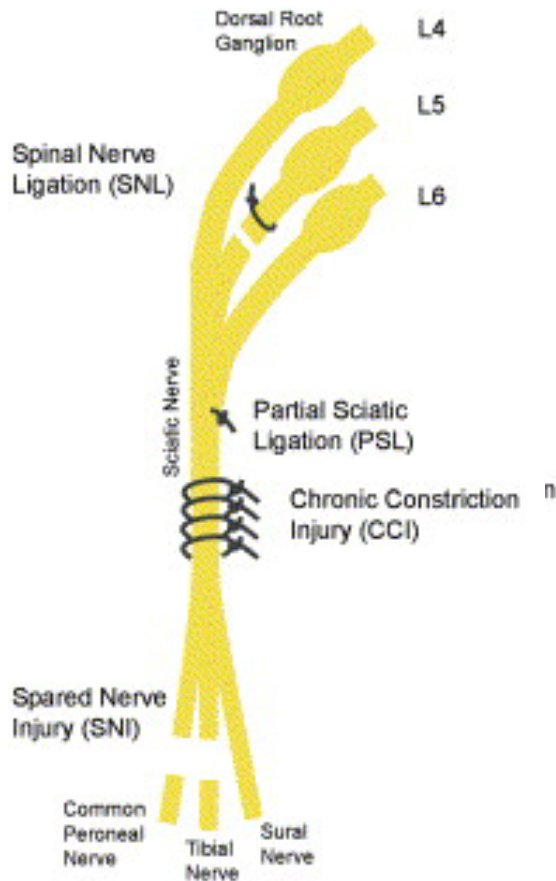
## Causes:

- Herpes zoster
- Diabetes
- Mechanical nerve damage (amputation, herniated disk, accident)
- Alcohol

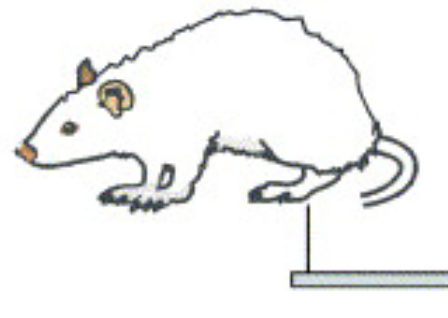


\* (in Mono- or combinatorial therapie)

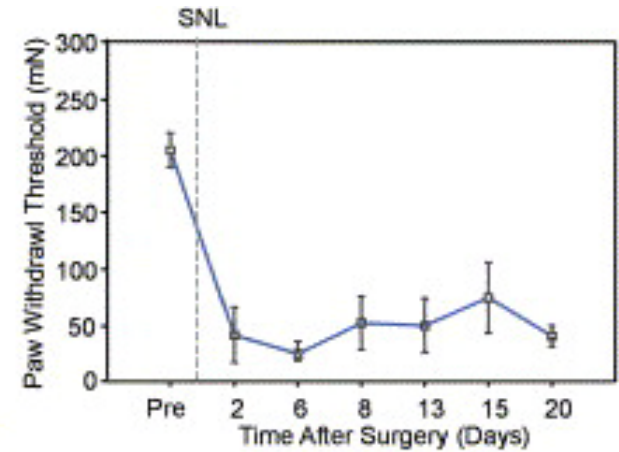
# Animal models of neuropathic pain



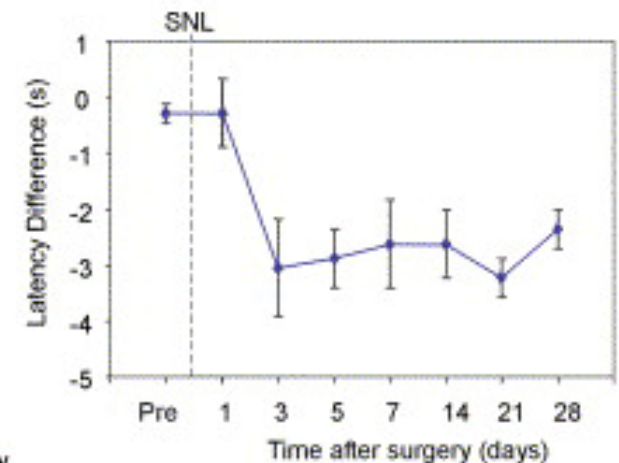
**A** Animal Models of Neuropathic Pain



**B** Von Frey Test of Mechanical Threshold



**C** Radiant Heat Test of Thermal Sensitivity



# Summary

## Physiological Pain

Protection and warning, high threshold

## Pathophysiological Pain

- Inflammatory pain  $\Rightarrow$  Hyperalgesia, Allodynia  
 $\rightarrow$  Treatment is necessary to avoid „pain memory“: Opioids, non-Opioids
- Neuropathic Pain  $\Rightarrow$  Allodynia  
 $\rightarrow$  Treatment is insufficient: Opioids, TCA, AE

## Research for new analgesics is important

Traditional analgesics exhibit a number of side effects

Some patients cannot be sufficiently treated