Molecular Basis of Anaesthesia and Analgesia

Novel targets for pain relief

BSc Surgery and Anaesthesia module

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Types of Pain

Overview of sensory nervous system and pain pathway

Introduction of key molecules in pain pathway

Pain killers

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Pain killers

Five senses

Audition
(hearing)

Vision

Olfaction
(smell)

Gustation
(taste)

Tactile sense
(touch, pain)

Types of Pain

Nociceptive Pain - mechanical, thermal, electrical…

Inflammatory Pain - ischaemia, infection…

Neuropathic Pain - nerve injury, maladaptive plasticity

Pain can be caused by wide range of stimuli

Temperature - heat, cold

Mechanical - pressure, friction edema

Chemical - gastric enzymes, histamines, caustic substances

Acute Pain vs. Chronic Pain

Acute Pain	Chronic Pain
Usually sudden, self-limiting < 6 months	May be sudden or gradual with periods of remission & exacerbation > 6 months
Precipitating event	May not be associated with injury
Resolves with treatment	Difficult to treat
Restless, anxious, crying	Depressed, withdrawn

Pain mechanisms

Same mechanism – different pain

Same pain – different mechanisms

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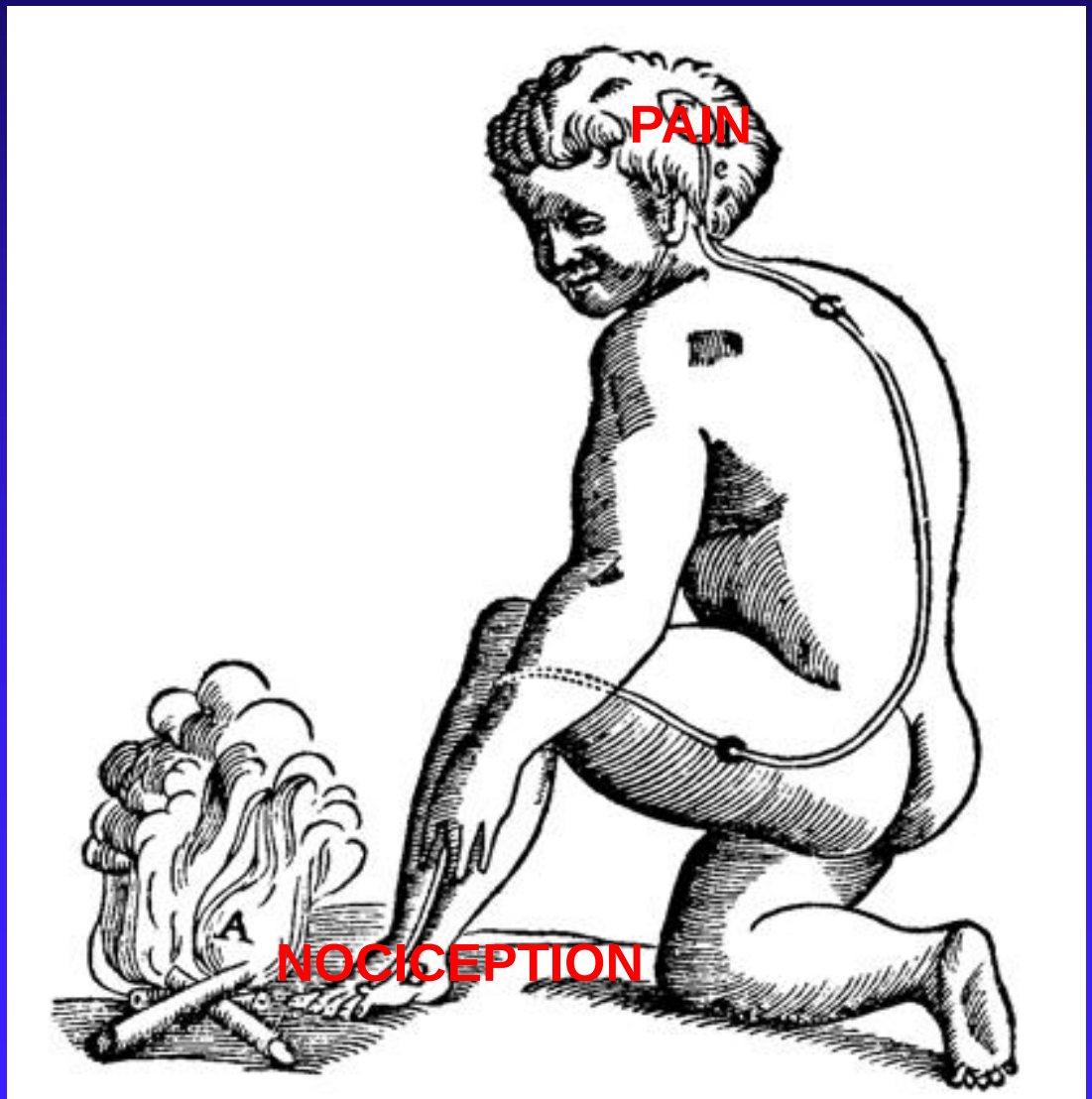
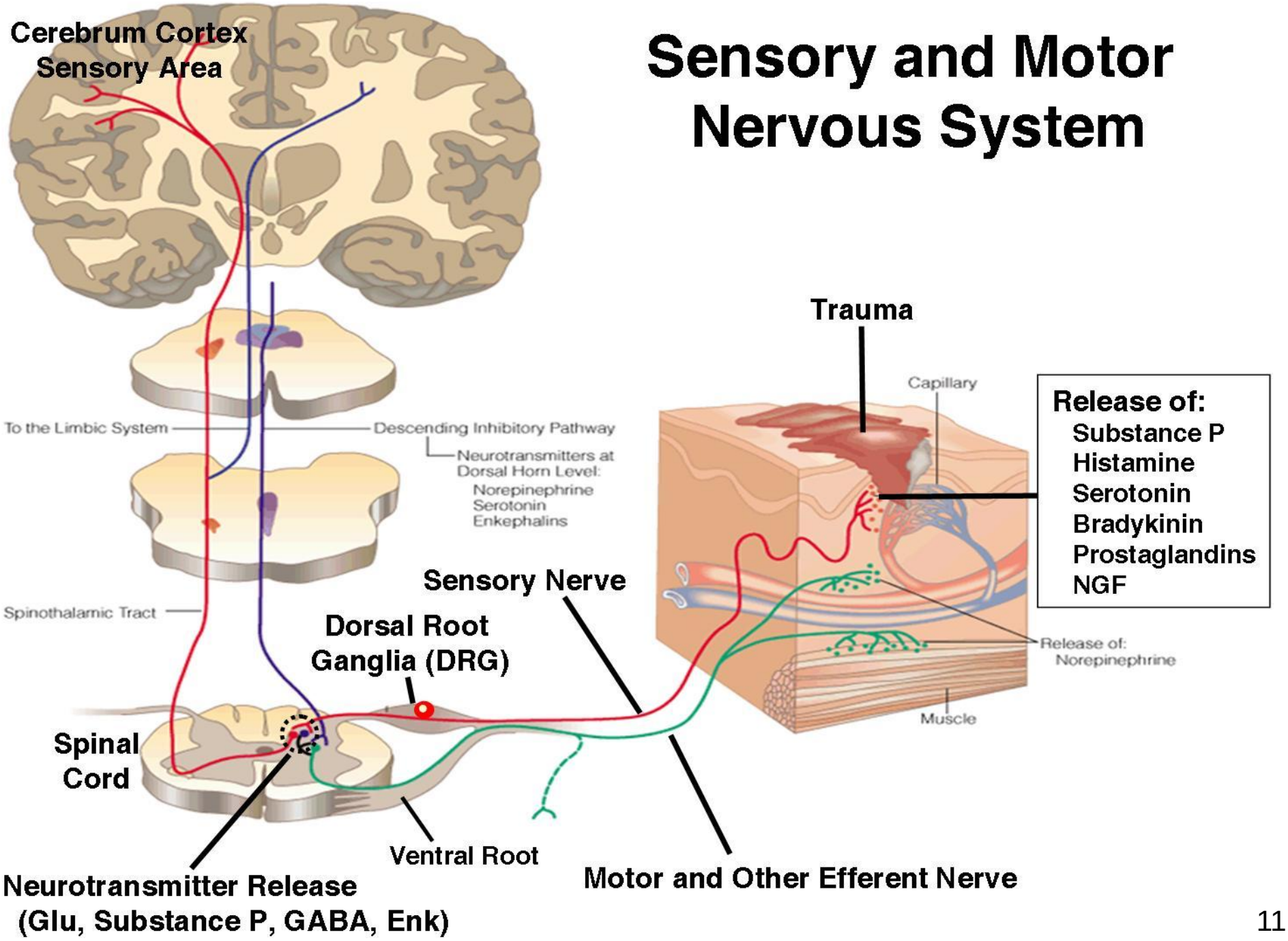
Descartes first described the human body in terms of a machine



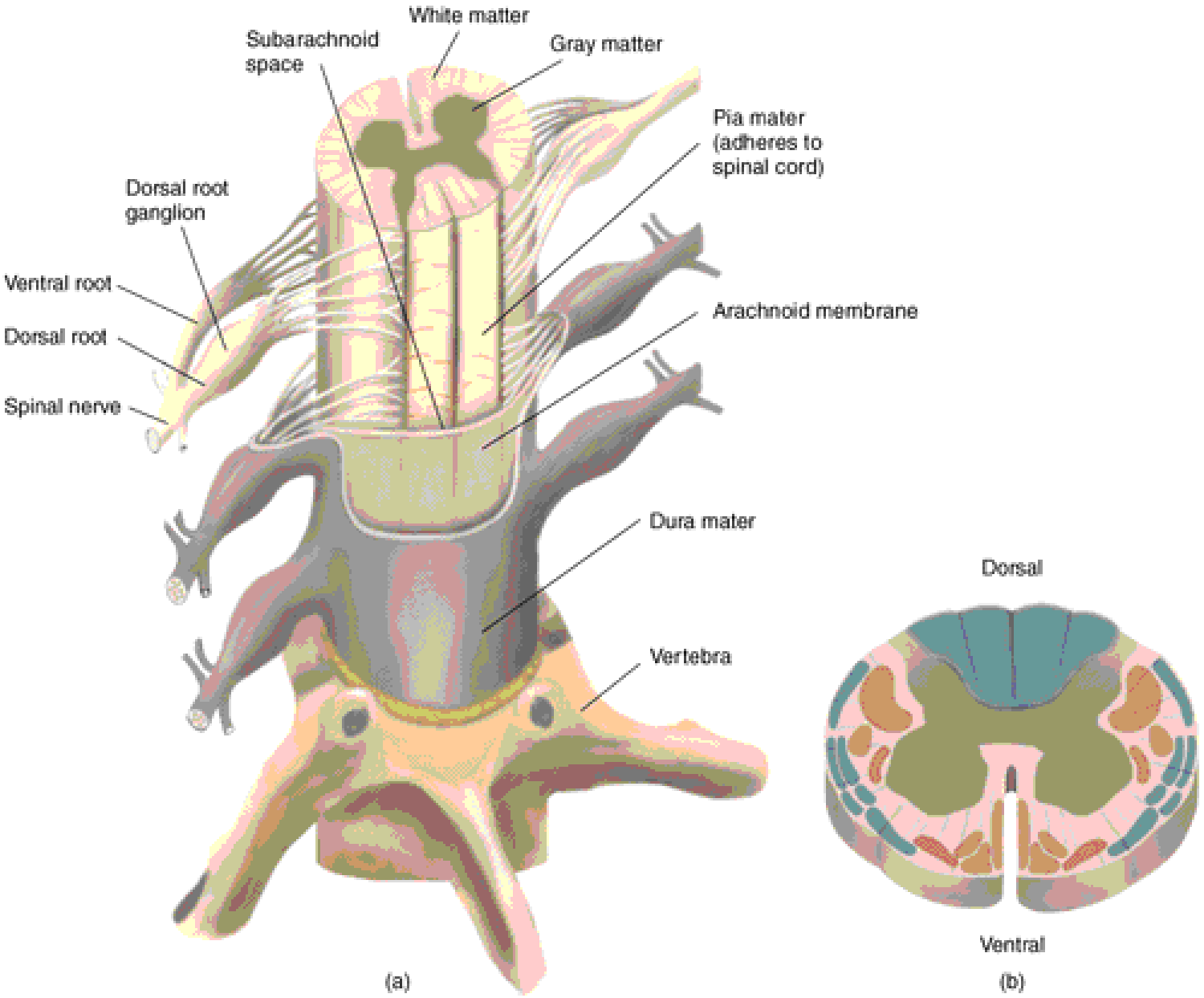
"fast moving particles of fire that disturb the filaments in the nerve"

René Descartes
Le Traité de l'Homme (1664)

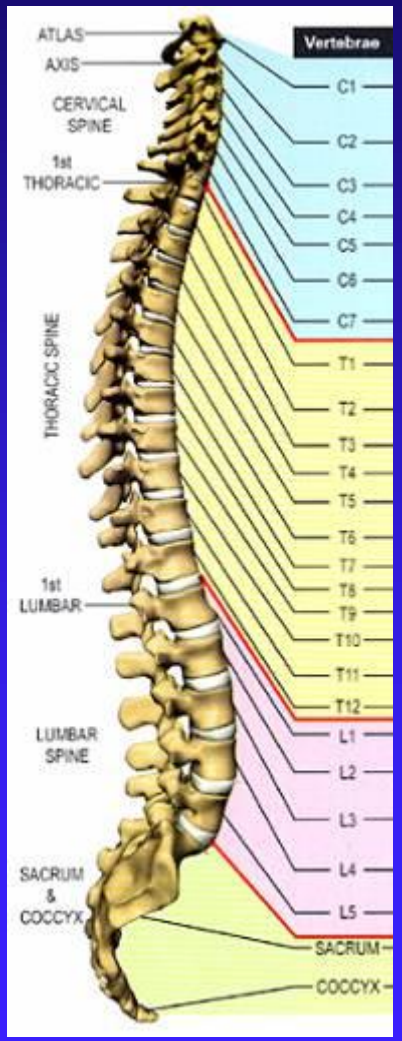
Sensory and Motor Nervous System



Spinal Cord and Dorsal Root Ganglion



Vertebral subluxation and innervation chart



Cervical

Median nerve (arms, hands)

Thoracic

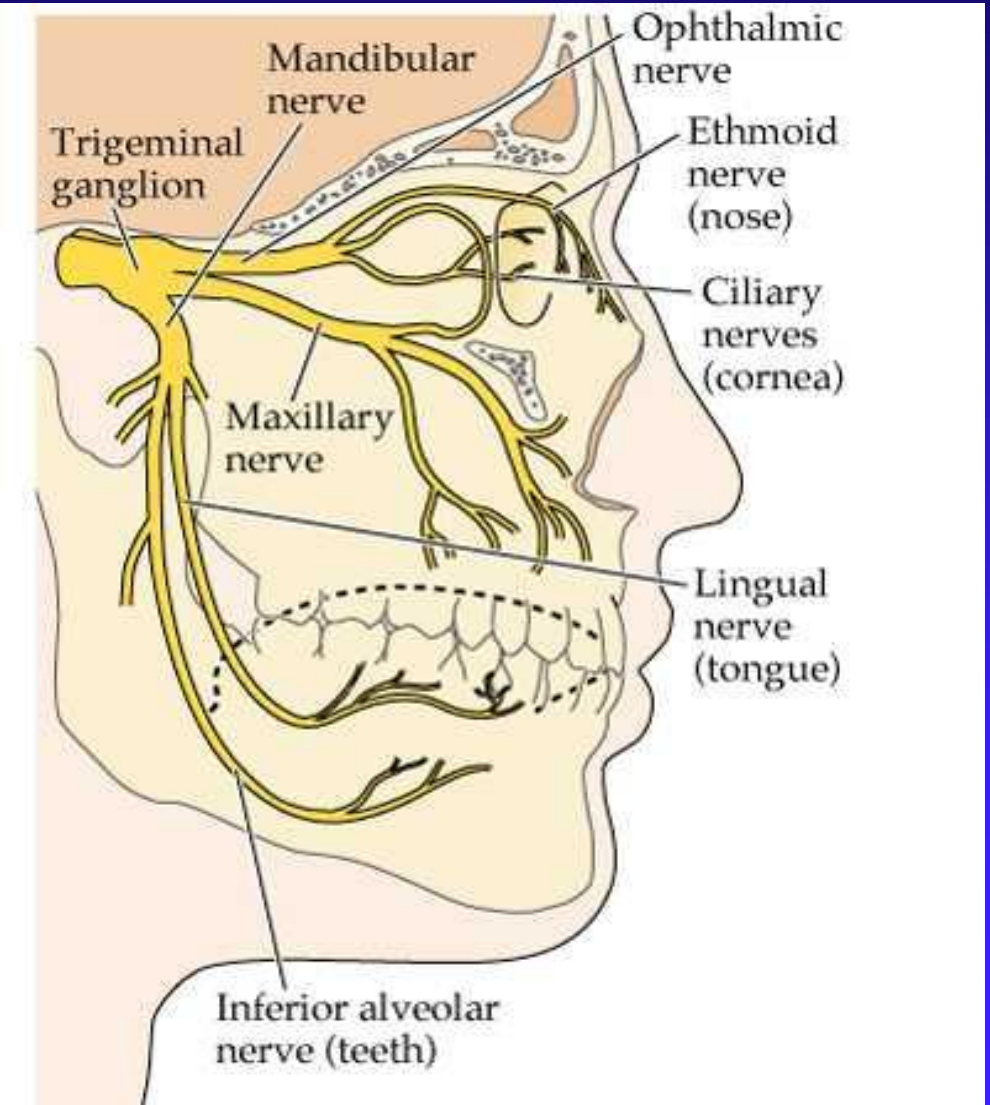
Gastric nerve (stomach)

Lumbar

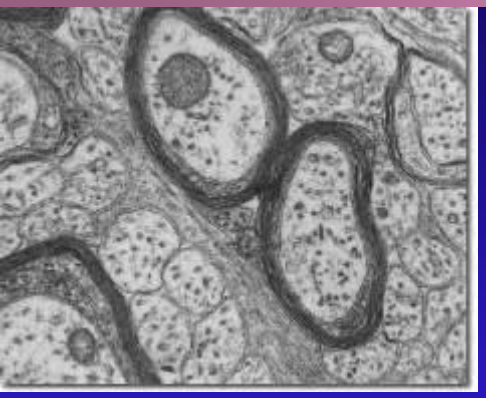
Sciatic nerve (legs, foots)

Sacral

Trigeminal ganglion neurons innervate facial nerves



Small diameter sensory neurons are responsible for nociception

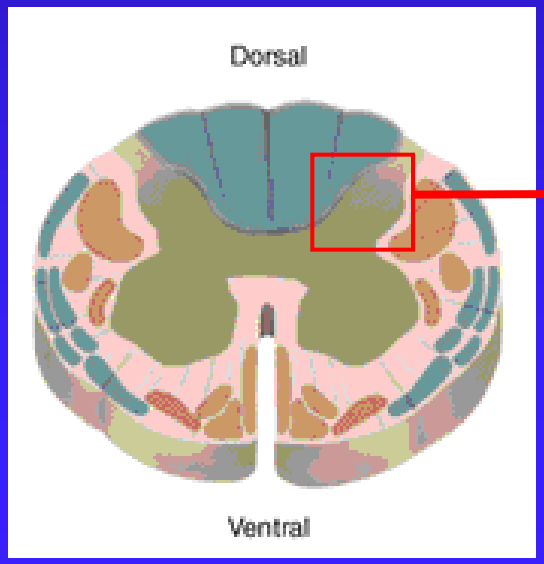


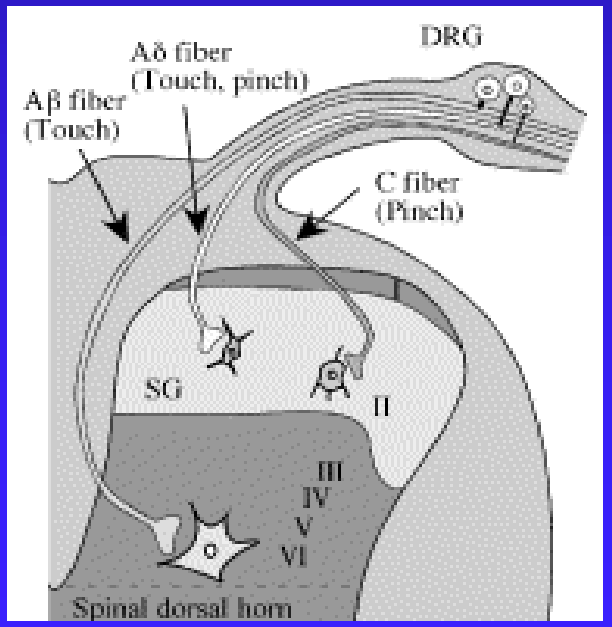
- Small diameter, unmyelinated **C fibre** neuron (chronic pain)
- Medium diameter, thinly myelinated **A-δ fibre** neuron (acute pain)
- Large diameter, myelinated **A-β fibre** neuron (touch)

Dorsal horn

The cells of the spinal cord are arranged in layers or laminae, six in the dorsal horn (I-VI), three in the ventral horn (VII-IX) and an additional column of cells clustered around the central canal as Lamina X

C nociceptive afferents terminate mainly in Laminae I and II
A-δ afferents terminate mainly in Laminae I. II and V
A-β afferents terminate mainly in Laminae V, VI





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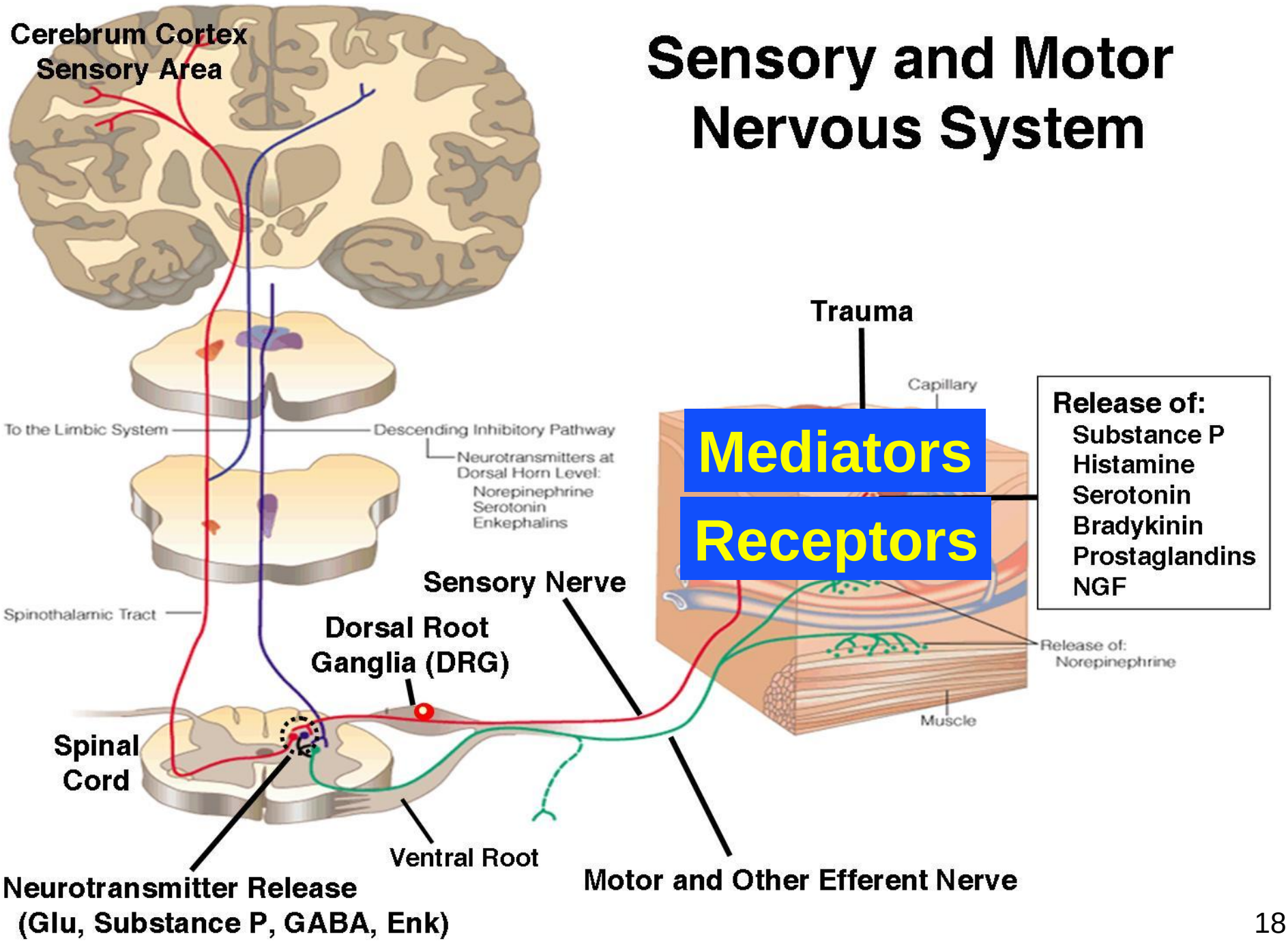
Sensory and Motor Nervous System



Pain inducers / Inflammation mediators

Bradykinin

Bradykinin

- polypeptide (Arg-Pro-Gly-Phe-Ser-Pro-Phe-Arg)
- made from Kininogen by proteolysis in blood in response to tissue damage/blood coagulation

Bradykinin receptors

- B1, B2
- 7-TM G-protein coupled receptors
- linked to Gq and Gi
- Activate phospholipase Cβ (PLCβ) and phospholipase A2 (PLA2)
- Activation of PLCβ leads to protein kinaseC (PKC) activation
- Activation of PLA2 causes production of arachidonic acid (AA) from the cellular phospholipid bilayer
- AA is converted into prostanoids

Pain inducers / Inflammation mediators

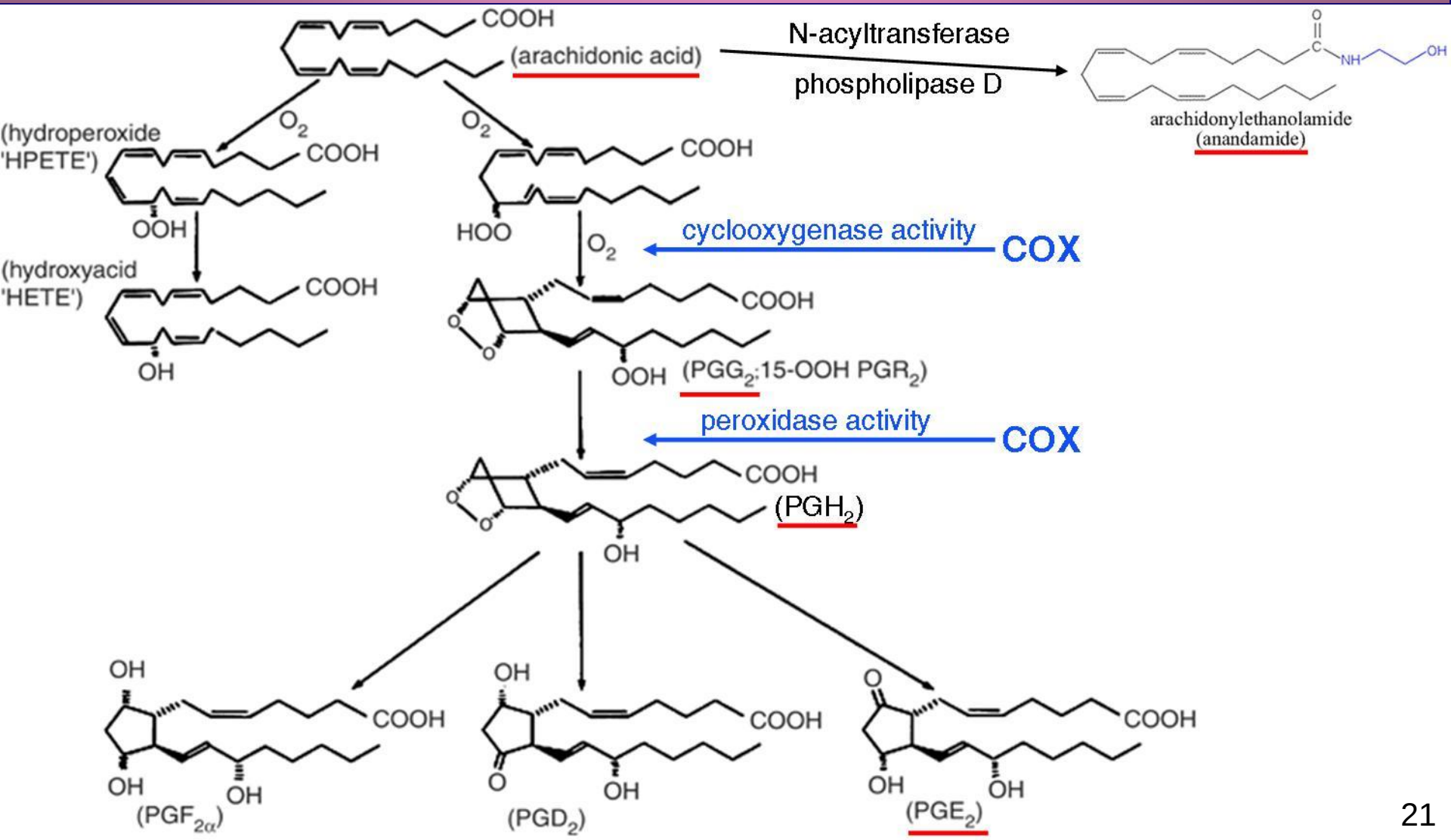
Prostanoids

Prostaglandins (PGE_2)

- Synthesized/released from Glanulocyte, Macrophage, and Nociceptive Neurons in response to tissue damage
- Binds to EP2 receptor expressed at free nerve endings

Cyclooxygenase: COX is the rate-limiting enzyme of PGE_2 synthesis
COX-2 can be induced by proinflammatory cytokines such as TNFα and IL-Iβ at the site of local inflammation

Prostaglandin synthesis pathways



Pain inducers / Inflammation mediators

Prostaglandin receptors

- DP1, DP2
- EP1, EP2, EP3, EP4
- FP
- IP

- 7-TM G-protein coupled receptors
- PGE2 binds to EP2 receptor expressed at free nerve endings
- Activates adenylate cyclase via Gs
- Increases intracellular cAMP
- cAMP leads to protein kinase A (PKA) activation
- Phosphorylates channels, Lower the thresholds of nociceptive neurons

Pain inducers / Inflammation mediators

NGF (Nerve Growth Factor)

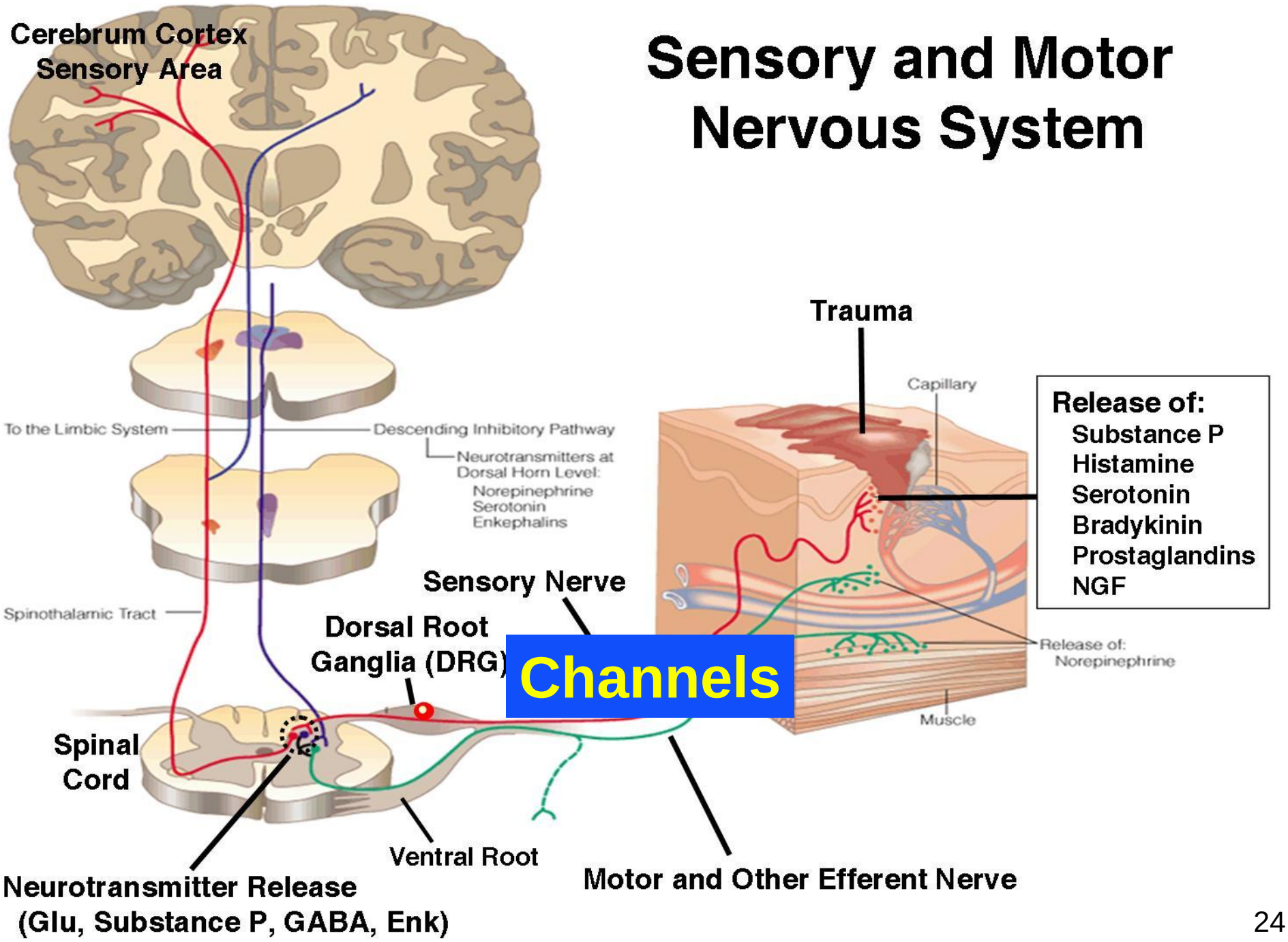
NGF
- Regulate the peripheral sensitivity of nociceptive neurons
- Increased NGF levels lead to mechanical and thermal hyperalgesia
- Increases functional sodium channel expression

TrkA
- Single-TM tyrosine kinase receptor for NGF
- Selectively expressed in unmyelinated nociceptive sensory neurons

BDNF (brain-derived neurorophic factor) → TrkB
NT-4/5

NT-3 → TrkC

Sensory and Motor Nervous System

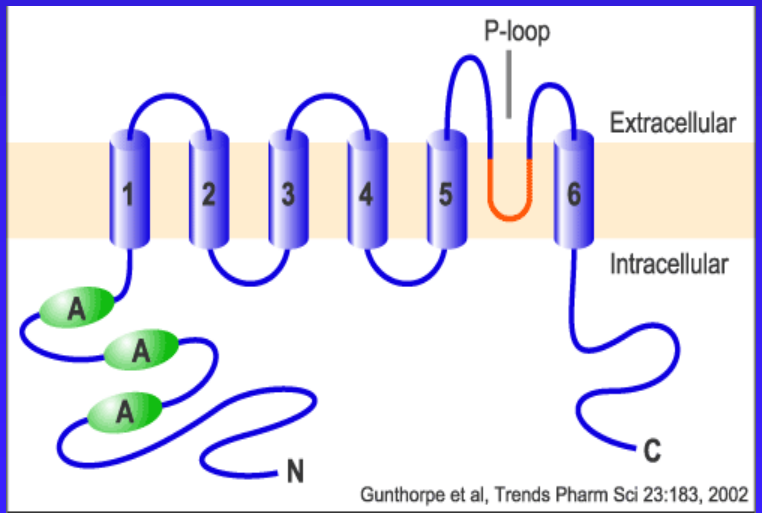


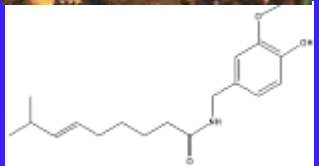
TRP Channels

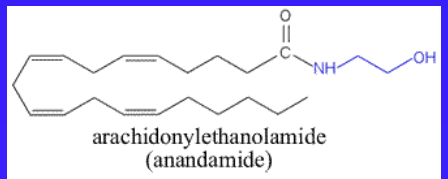
TRPV1 (VR1): Non-selective cation channel activated by capsaicin, noxious heat and low pH

Expressed in nociceptive primary sensory neurons

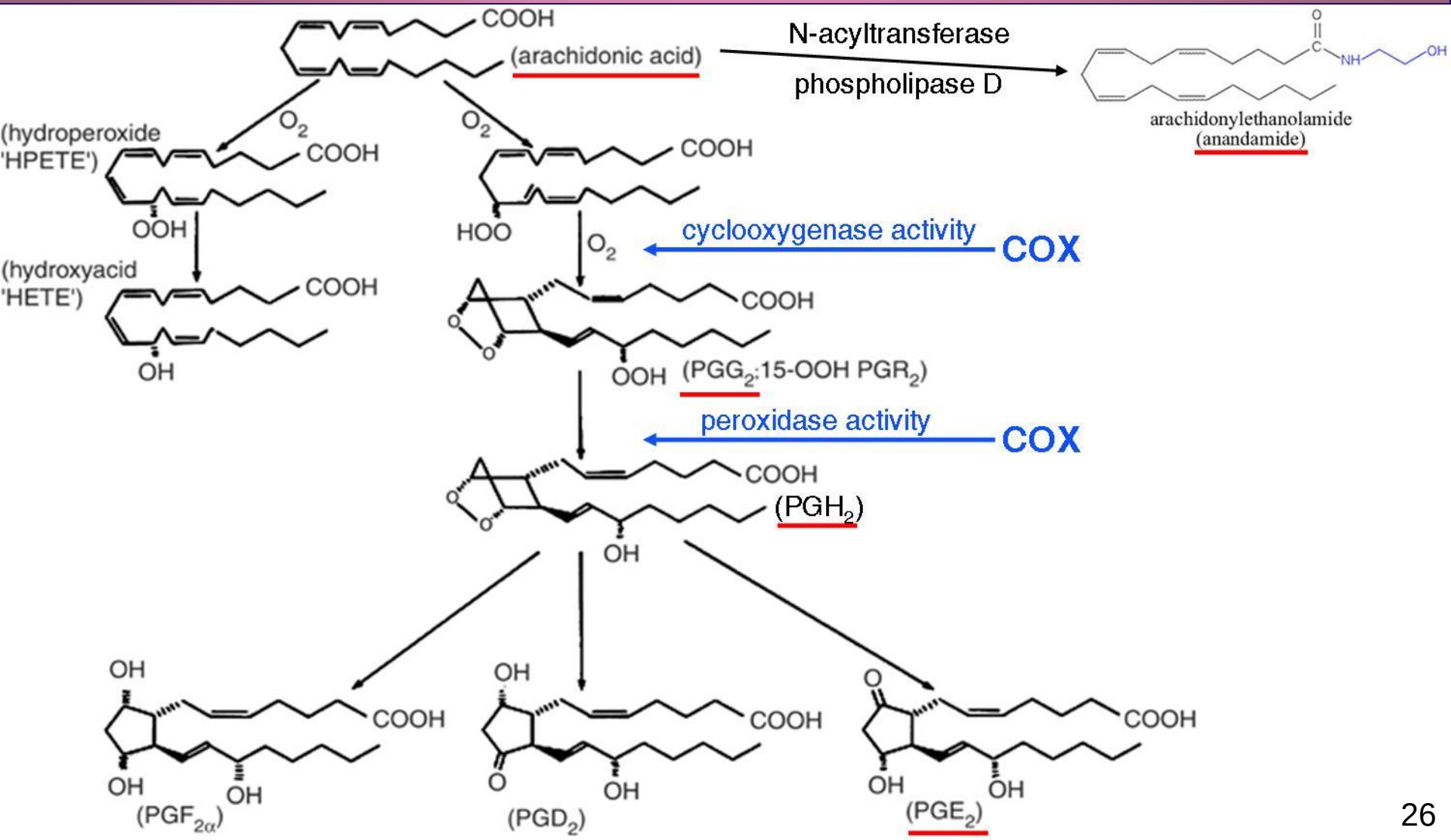
TRPV1 knockout mice are impaired in the detection of painful heat stimuli







Prostaglandin synthesis pathways



TRPV1 could be involved in the development of inflammatory heat hyperalgesia

TRPV1 (VR1): Non-selective cation channel activated by capsaicin, noxious heat and low pH

Expressed in nociceptive primary sensory neurons

TRPV1 knockout mice are impaired in the detection of painful heat stimuli

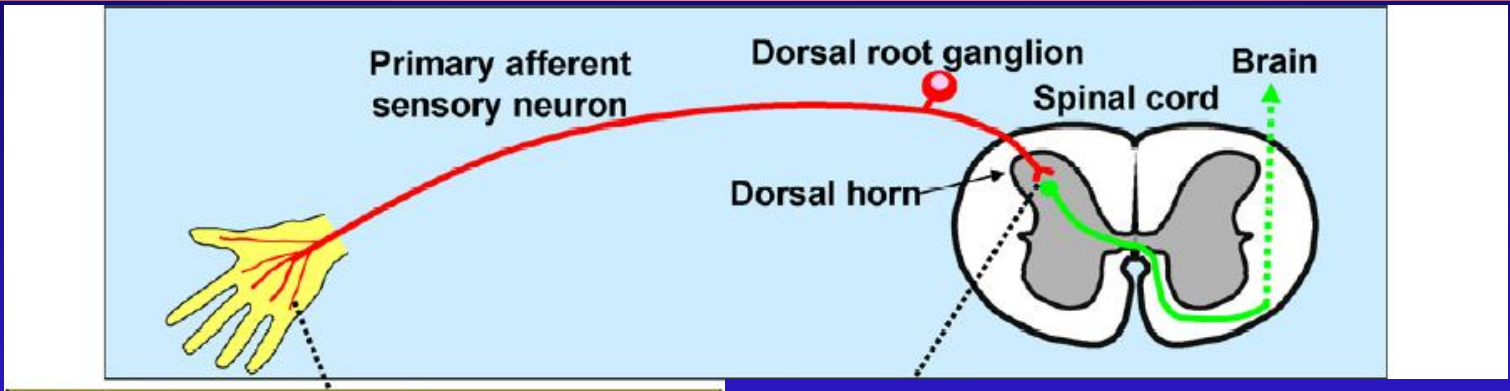
Induces depolarisation/excitation/transmitter release

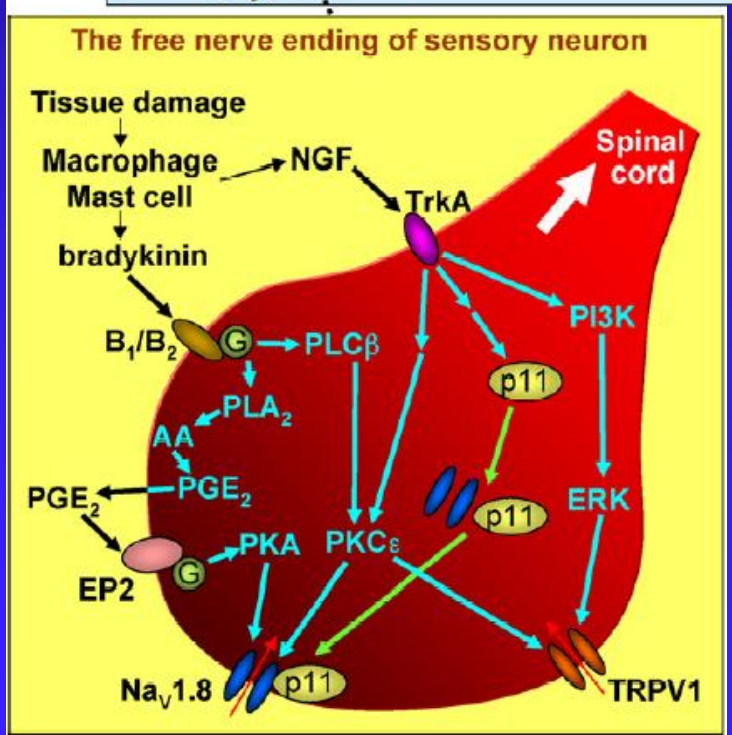
Acute TRPV1 activation induces burning pain sensation

The level of endogenous ligands (anandamide and lipoxygenase products) are increased during inflammation

Inflammatory mediators activate/sensitise it

Key molecules in pain pathways at peripheral nerves





TRPV1 is activated during inflammatory conditions

Inflammatory mediators (bradykinin, PGE2, NGF)

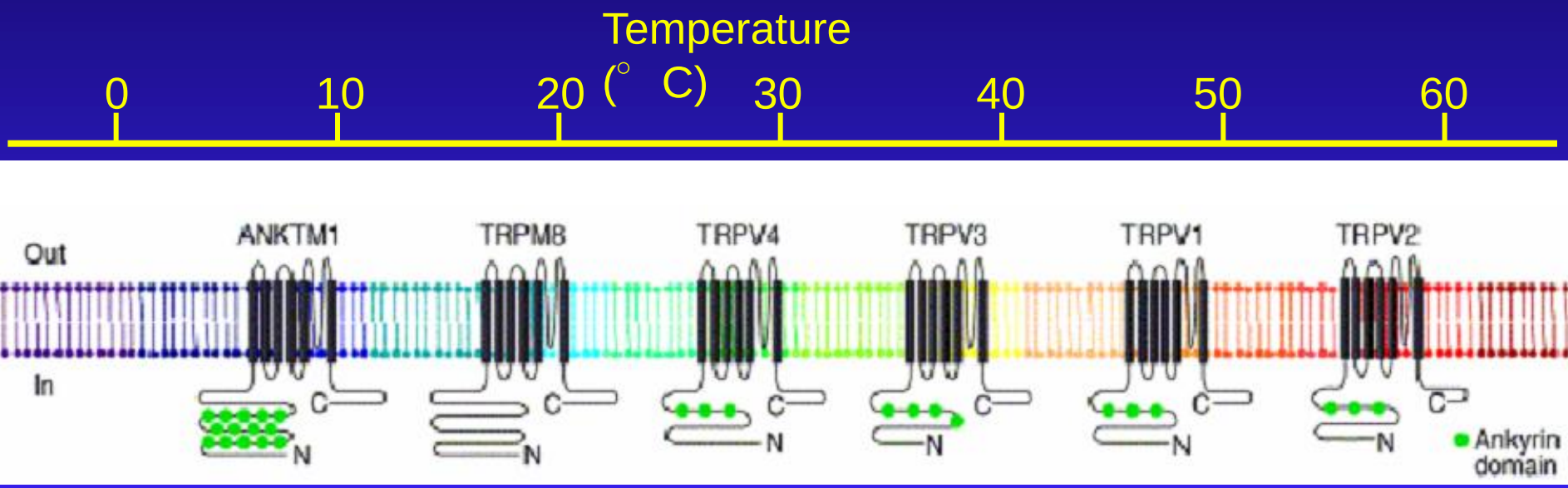
Post-translational modification (phosphorylation, PKA, PKC, PI3K, PIP2 removal)

Endogenous ligands (anandamide, lipoxygenase products)

Transcriptional changes
(splice variant - modified tetramer composition)

Reduced temperature threshold

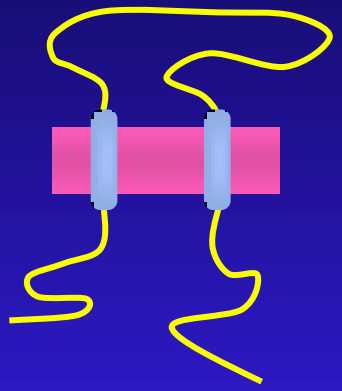
Thermosensitive TRP channels respond to a wide range of ambient temperatures



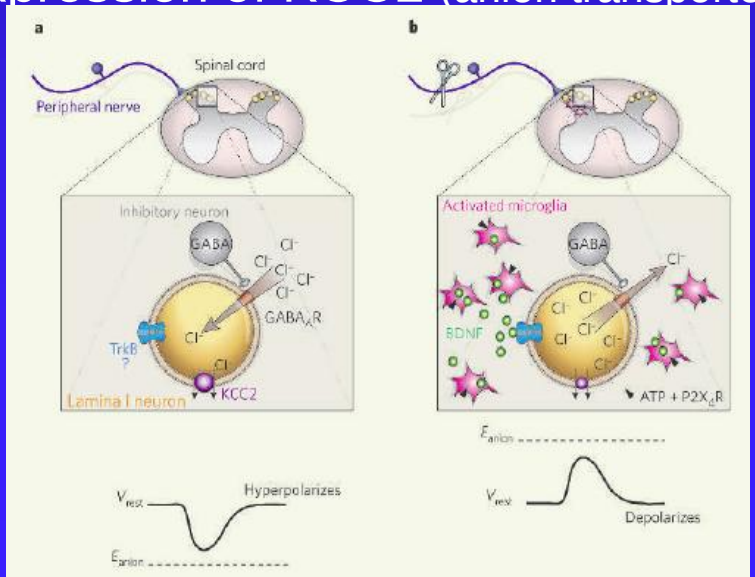
Temperature range	<17°	8-28°	>27°	>31or39°	>43°	>52°
Agonists		Menthol			Capsaicin	

ATP-gated channel

$P2X_3$: Non-selective cation channel activated by ATP.
Responsible for ATP-evoked nociceptor activation.
P2X3 knockout mice show deficiency in detection of some painful stimuli.



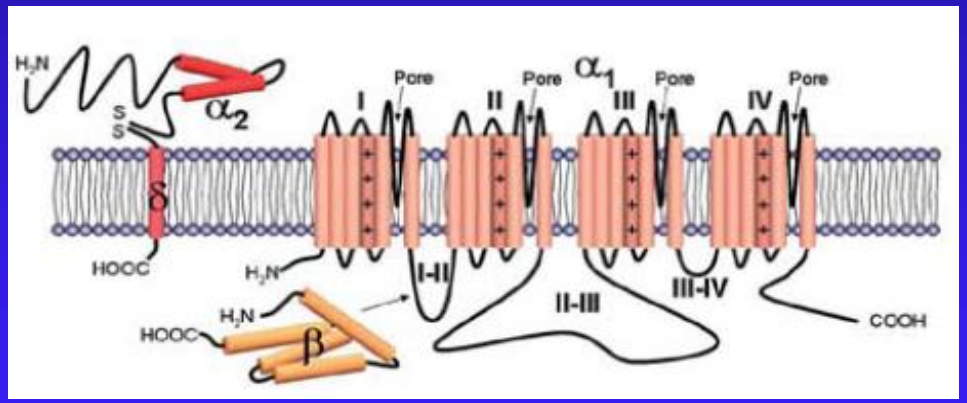
$P2X_4$: ATP activates microglial $P2X_4$ upon nerve injury.
Microglia releases Brain-Derived Neurotrophic Factor (BDNF).
BDNF acts on neurons to reduce the expression of KCC2 (anion transporter).
Increases of the intracellular Cl^-.
GABA works as excitatory transmitter rather than an inhibitory one.



Voltage-gated Ca^{2+} channels

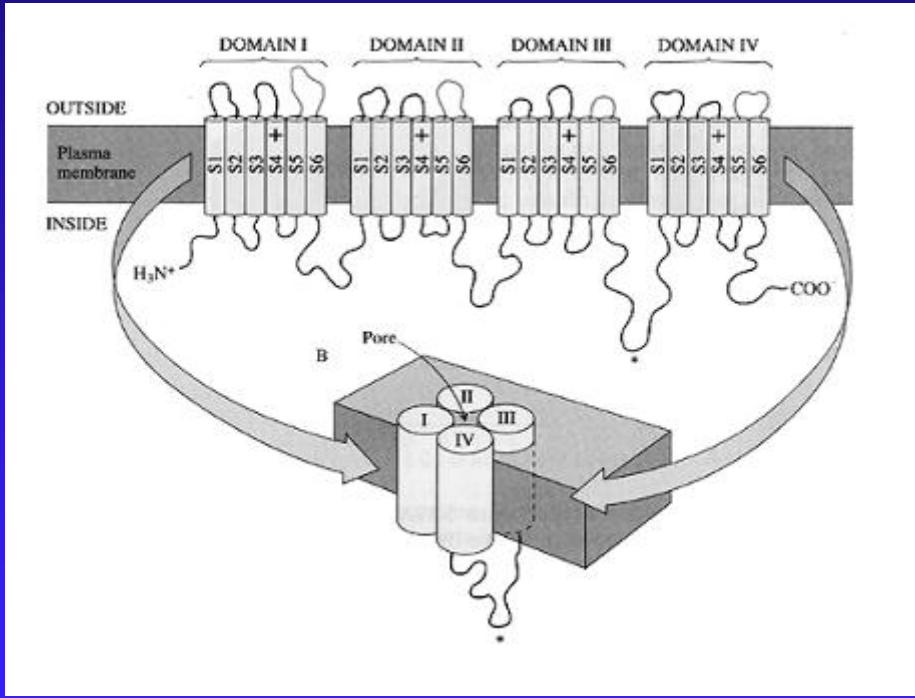
N-type: Blockers for N-type calcium channels prevent neurotransmitter release, and act as pain killer.

$Ca_V2.2$ knockout mice show reduced response to inflammatory and neuropathic pain.



Gabapentin: Originally developed for epilepsy
Binds and blocks the $\alpha 2\delta$ subunit of voltage-gated Ca^{2+} channel
Has analgesic effects in neuropathic pain

Voltage-gated Na^+ channels



- ~250 kDa

- 6 transmembrane segments X 4 domains (24 transmembrane regions)

- Voltage sensors in segment 4 in each domains

- 9 genes have been identified so far

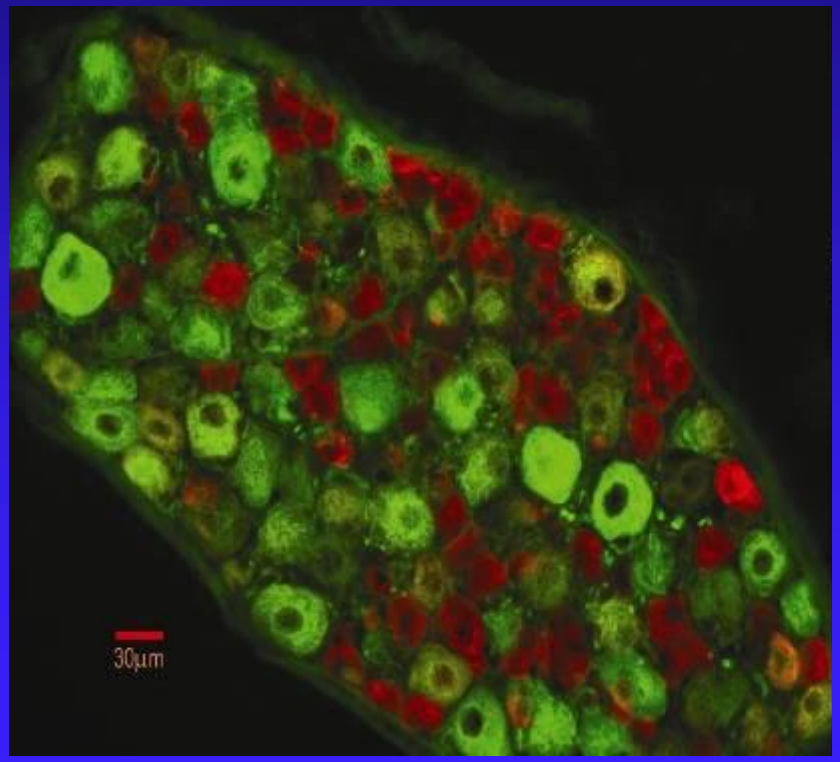
- Expressed mainly in neurons and muscle cells

Mammalian voltage-gated sodium channel α subunits

Type	Gene Symbol	Name	Primary Tissue	Present in DRG	TTX sensitivity
$Na_V1.1$	SCN1a	type I	CNS, heart	+	+
$Na_V1.2$	SCN2a	type II	CNS	+	+
$Na_V1.3$	SCN3a	type III	fetal brain	+	+
$Na_V1.4$	SCN4a	SkM1 (μ1)	skeletal muscle	-	+
$Na_V1.5$	SCN5a	SkM2 (H1)	heart	-	-
$Na_V1.6$	SCN8a	NaCh6	CNS, glial cells	+	+
$Na_V1.7$	SCN9a	PN1	SCG, CNS	+	+
$Na_V1.8$	SCN10a	SNS (PN3)	DRG	+	-
$Na_V1.9$	SCN11a	NaN (SNS2)	DRG	+	-
Na_X	SCN7a	NaG	sciatic nerve, lung	+	+(?)

$Na_V1.8$-like immunoreactivity specifically localised in small diameter C fibre sensory neurons

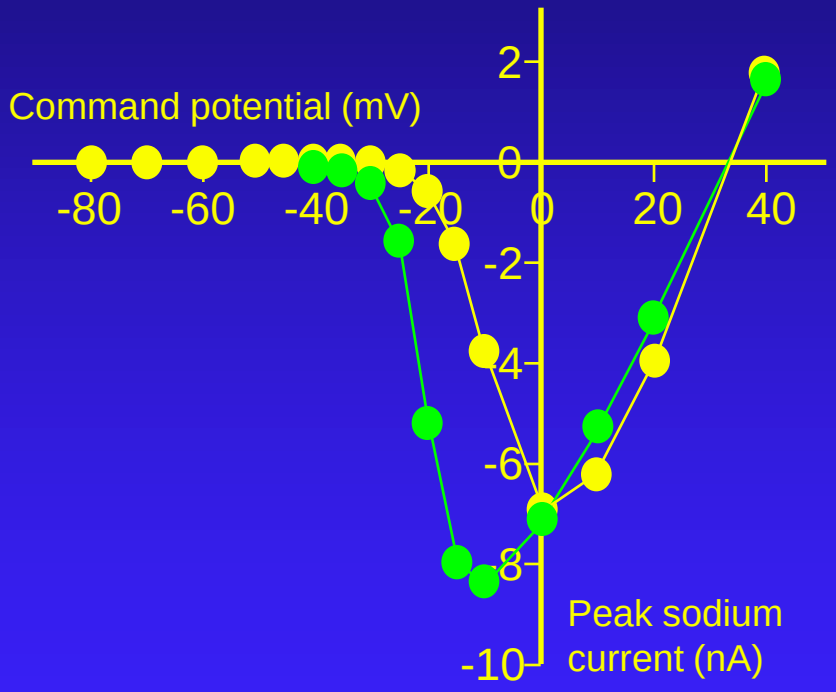
Rat Dorsal Root Ganglion



$Na_V1.8$

NF200 (a marker for large diameter A-β fibre neurons)

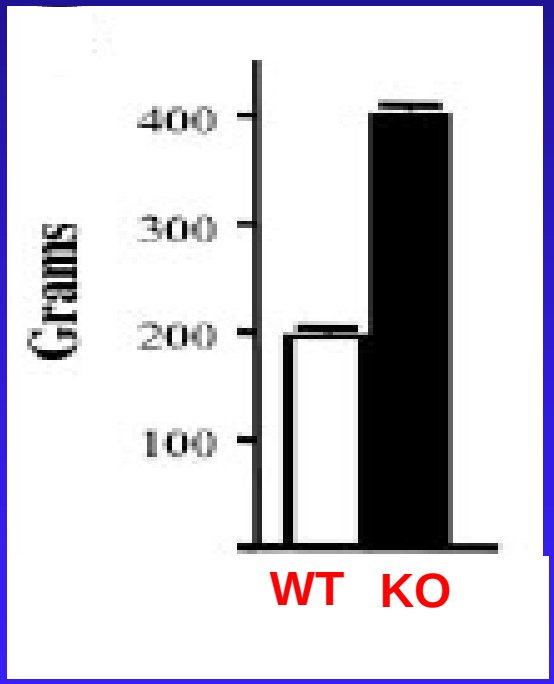
PGE_2 increases TTX-resistant Na^+ current and causes a hyperpolarizing shift of its activation curve in DRG neurons



- control
- 1 μM PGE_2

S. England *et al.* (1996) *J. Physiol.*

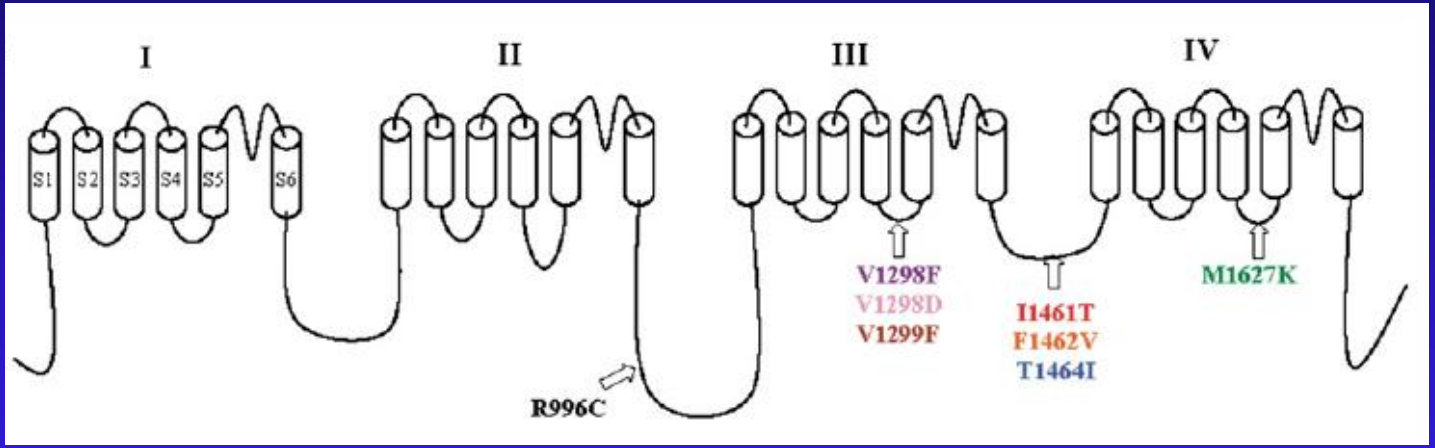
$Na_V1.7$ Knock-Out mice show reduced responses to mechanical stimuli



Nassar *et al.* PNAS (2004) **101**(34): 12706-12711

The locations of the identified human mutations in $Na_V1.7$ which cause paroxysmal extreme pain disorder

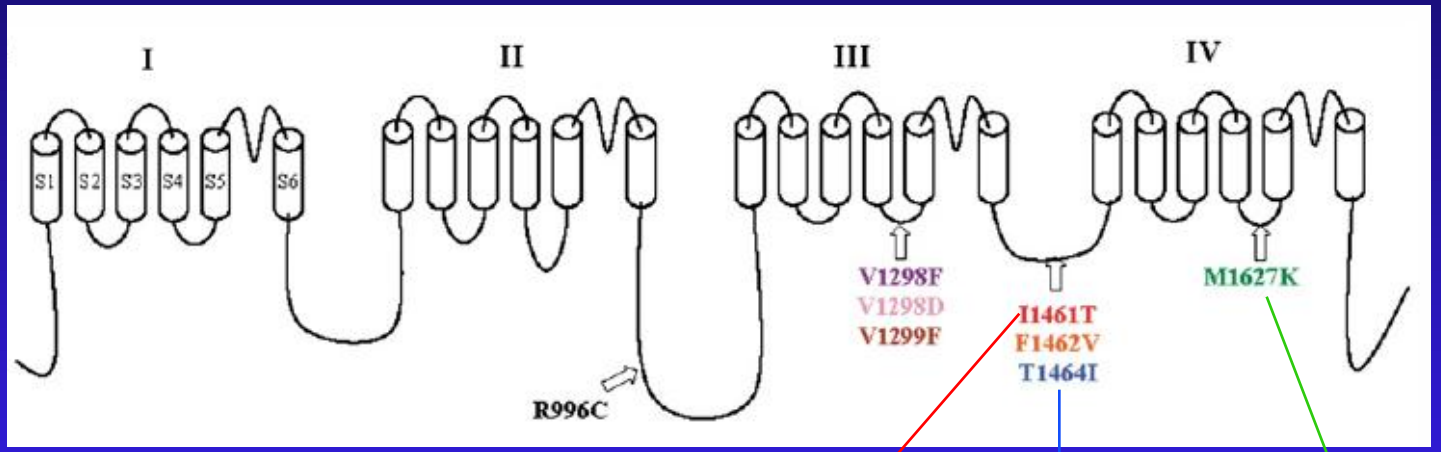
(gain of function mutation)



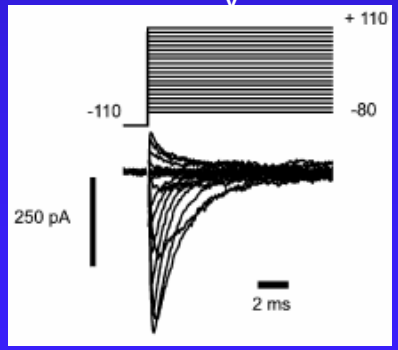
Paroxysmal extreme pain disorder, previously known as familial rectal pain, is an autosomal dominant paroxysmal disorder of pain and autonomic dysfunction. The distinctive features of this disorder are paroxysmal episodes of burning pain in the rectal, ocular, and mandibular areas accompanied by autonomic manifestations such as skin flushing.

The locations of the identified human mutations in $Na_V1.7$ which cause paroxysmal extreme pain disorder

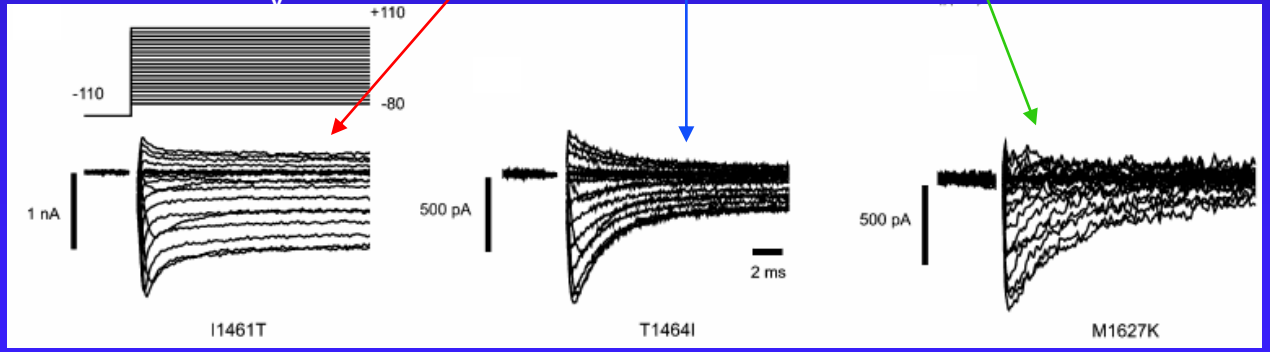
(gain of function mutation)



WT $Na_V1.7$



mutant $Na_V1.7$



The locations of the identified human mutations in $Na_V1.7$ which cause the complete inability to sense pain

(loss of function mutation)

2298 T deletion

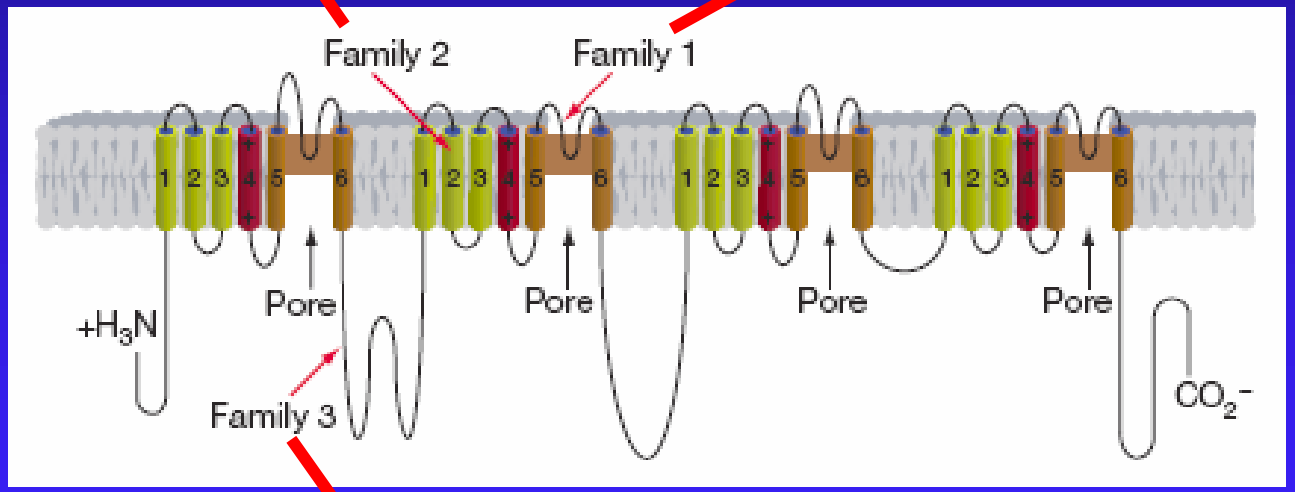
GCT ATA GGA → GC ATA GGA

Ala Ile Gly Ala STOP

2691 G → A

TGG → TGA

Trp STOP

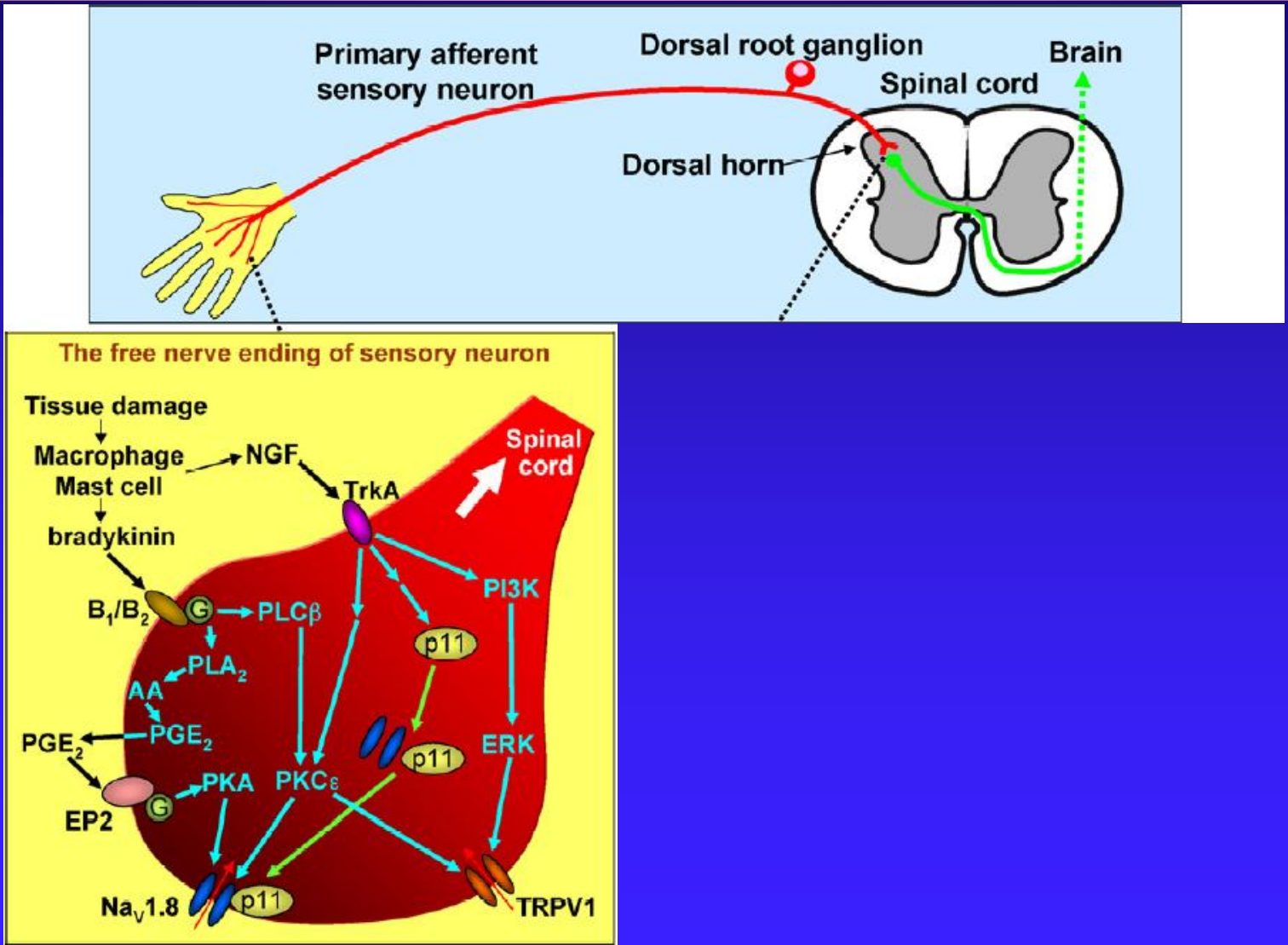


1376 C → G

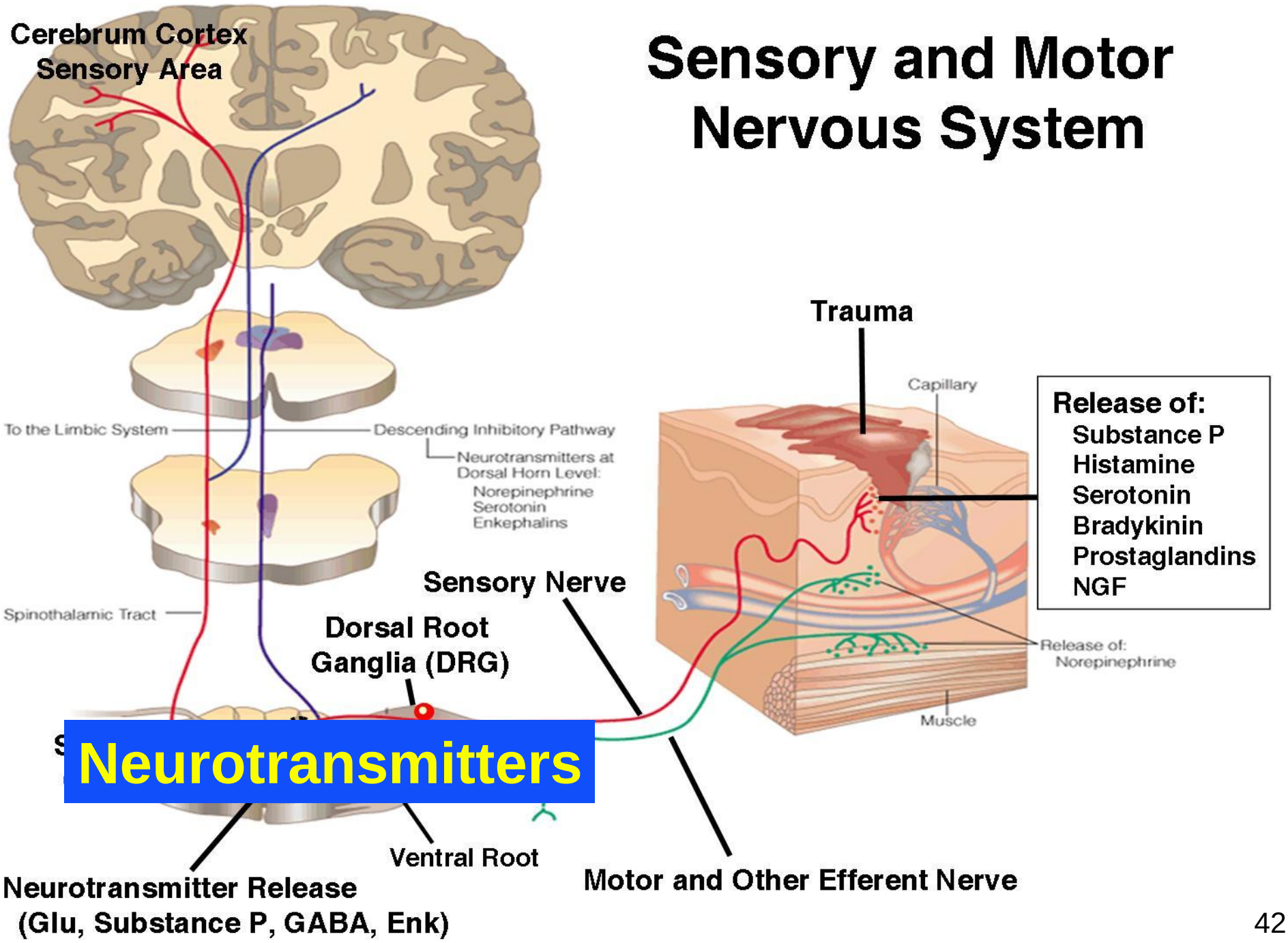
TCA → TGA

Ser STOP

Key molecules in pain pathways at peripheral nerves



Sensory and Motor Nervous System



Neurotransmitters
Glutamate

- Excitatory neurotransmitter
- Released from postsynaptic terminals of sensory neurons at dorsal horn
- Variety of receptors

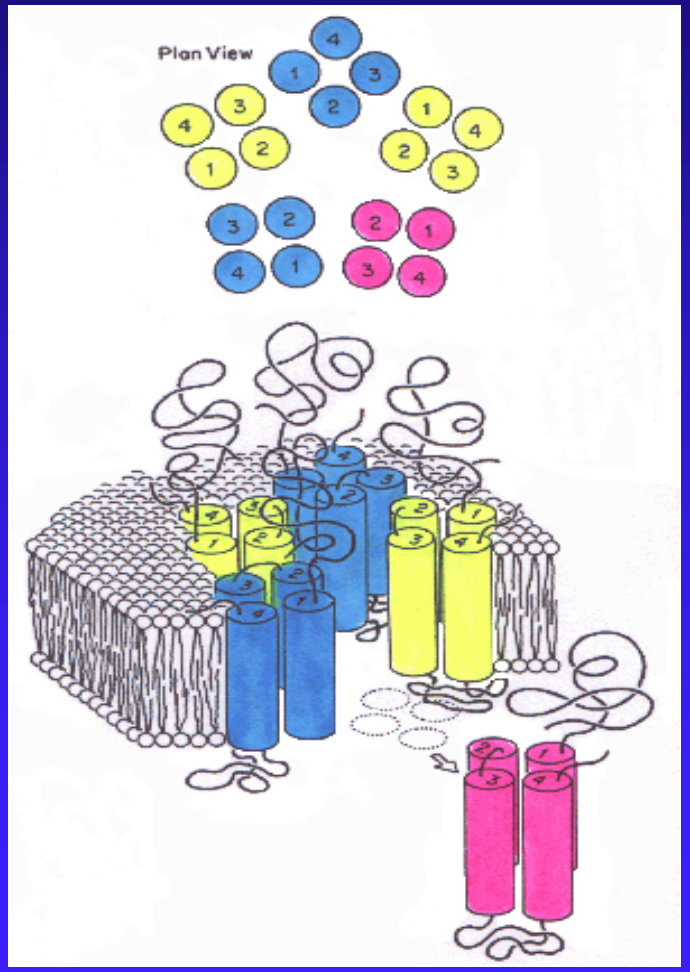
Ionotropic	AMPA	GluR1-4 (GluRα1-α4)	
	Kinate	GluR5-7 (GluRβ1-β3) KA1/2 (GluRγ1/γ2) GluRδ1/δ2	
	NMDA	NA2A-D (GluRε1-ε4) NR1 (GluRζ) NR3A/3B (GluRκ1),	
metabotropic	mGluR group I	mGluR1/5	Gq/G_{11} – PLCβ (IP3/DAG)
	mGluR group II	mGluR2/3	Gi – AC (cAMP$\downarrow$)
	mGluR group III	mGluR6-8	Gi – AC (cAMP$\downarrow$)

Neurotransmitters
GABA (γ-amino butyric acid)

Interneurones in laminae I, II and III are GABA-rich, and mediate gate control in the dorsal horn by synapsing on neurons that contain Glutamate and substance P

GABA-A receptor: ligand-gated Cl^- channel

GABA-B receptor: 7-TM G-protein coupled receptor



Neurotransmitters

Peptidergic neurotransmitters

Substance P

CGRP (calcitonin gene-related peptide)

VIP (vasoactive intestinal polypeptide)

Somatostatin

Bombesin

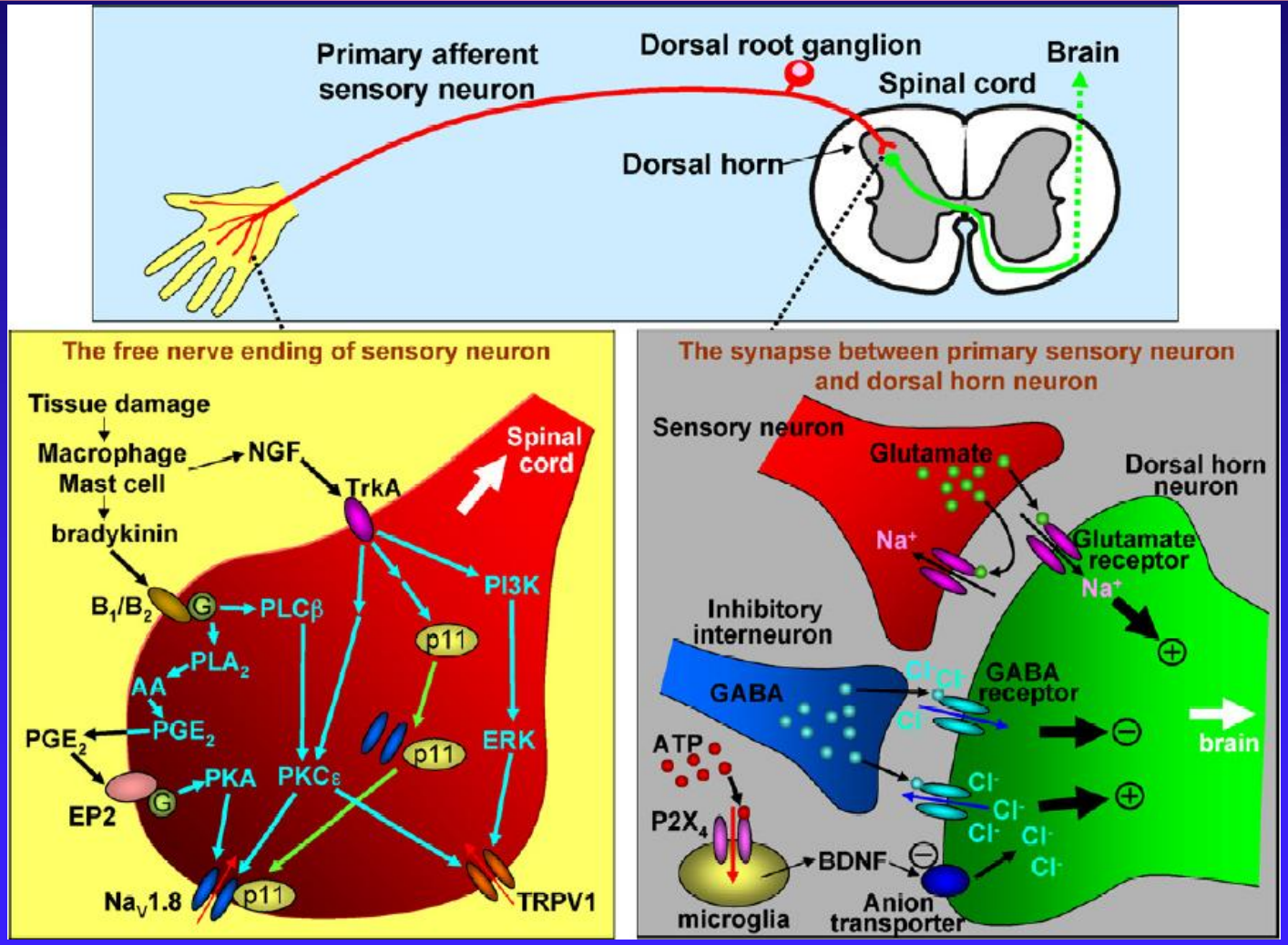
Galanin

Neuropeptide Y

Cholecystokin

PACAP (Pituitary adenyl cyclase activating peptide)

Key molecules in pain pathways at peripheral nerves



Okuse K (2007) Int J Biochem Cell Biol. 39(3): 490-496

Types of Pain

Overview of sensory nervous system and pain pathway

Introduction of key molecules in pain pathway

Pain killers

Current analgesics
NSAIDs (Non-Steroid Anti Inflamatory Drugs)

Prevent prostaglandin biosynthesis by inhibiting cyclo-oxygenase (COX), the crucial enzyme in the initial synthesis of prostaglandins

irreversible inactivation of COX
- Aspirin (irreversibly acetylates COX)

reversible competitive inhibition
- Ibuprofen and Mefenamic acid

reversible non-competitive inhibition
- Paracetamol (no anti-inflammatory effect)

COX-1: makes prostaglandins vital for protecting the stomach through mucus production, and maintenance of renal blood flow

COX-2: the inducible form that mediates the pain of inflammation by sensitising peripheral nociceptors

COX-3: splice variant of COX-1 (retaining intron 1), target for Paracetamol

Current analgesics
Opiates

Opioids/morphine act by stimulating μ, δ, and κ receptors

Orphan receptor called ORL-1 (for "opiate-like receptor 1")
- 60% sequence homology with the other opiate receptors
- An endogenous ligand (nociceptin/orphanin F2)

Morphine as pain killer

μ and δ receptor activation causes decrease of cyclic AMP production, and hyperpolarisation of membranes by stimulating an inward rectifying potassium channel. This hyperpolarisation decreases the release of neurotransmitters from the nerve cell, as such release depends on opening of voltage-sensitive calcium channels

Opiate receptors are expressed in brain, spinal cord, and peripheral nerves

Current analgesics
Opiates

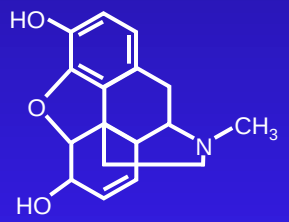
Opium (morphine): an extract of the poppy plant

Why should the brain have receptors for an extract of the opium poppy?

- the brain must have receptors for compounds produced by the body (endogenous compounds) which happen to share a chemical similarity with morphine

Enkephalins (from the Greek meaning "in the head“)

Tyr-Gly-Gly-Phe-Met
Tyr-Gly-Gly-Phe-Leu



morphine

enkephalins/ endorphins

Endorphins (endogenous morphine-like molecule)

α-Endorphin

Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr

γ-Endorphin

Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu

β-Endorphin

Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys-Asn-Ala-Ile-Ile-Lys-Asn-Ala-Tyr-Lys-Lys-Gly-Glu

Up-and-coming medications

COX-2 (Cyclo-Oxygenase 2) Inhibitors: Celebrex (released in 1999, Pfizer)
Vioxx (withdrawn in 2004, Merck)

Unlike the other COX inhibitors such as Aspirin, COX-2 specific inhibitors do not block the COX-1 enzyme that protects the stomach lining, thus do not produce the potential stomach problems

darbufelone (Pfizer)
CS-502 (Sankyo)
LAS 34475 (Almirall Profesfarma)
LAS 34555 (Almirall Profesfarma)
S-33516 (Servier)
SD 8381 (Pharmacia)
BMS-347070 (Bristol-Myers Squibb)
MK-966 (Merck)
L-783003 (Merck)
T-614 (Toyama)
D-1367 (Chiroscience)
L-748731 (Merck)
CT3 (Atlantic Pharmaceutical)
CGP-28238 (Novartis)
BF-389 (Biofor/Scherer)
GR-253035 (Glaxo Wellcome)
6-dioxo-9H-purin-8-yl-cinnamic acid (Glaxo Wellcome)
S-2474 (Shionogi)

Up-and-coming medications

Sodium Channel Blockers

Co102862 (CoCensys): currently undergoing pre-clinical development for neuropathic pain and epilepsy

4030W922, 4991W93, GW-286103(GSK): Phase II

$Na_V1.8$ regulator : IX-4000 (Ionix-Varnalis) Lead Discovery & Optimization

Through rational design methods the company has identified drug-like compounds which disrupt the interaction of $Na_V1.8$ with one of these regulatory proteins – p11

IX-4000 compounds have been demonstrated to specifically down-regulate functional expression of $Na_V1.8$ in neuronal cells

Up-and-coming medications

Calcium Channel Blockers

Ziconotide (Elan): under regulatory review at t
Developed from cone snail w-conotoxin
Targets N-type calcium channels

Gabapentin (Neurontin): originally developed for epilepsy
Binds $\alpha 2\delta$ subunit of voltage-gated calcium channel, and blocks the channel
Has analgesic effects in neuropathic pain

Up-and-coming medications

P2X receptor antagonist: AZD9056 (AstraZeneca)

$P2X_7$ antagonist

into phase II clinical trials for rheumatoid arthritis

Up-and-coming medications

Nicotinic ACh receptor agonists: ABT-594 (Abbott Laboratories)

Epibatidine, an skin extract from the skin of South American tree frogs, can block pain 200 times more effectively than morphine but can not be used for human due to its toxicity

Epibatidine's structure resembled nicotine

ABT-594, an experimental compound for Alzheimer's Disease, is very similar in structure to Epibatidine, and also has pain-killing properties

Finished Phase I-II development