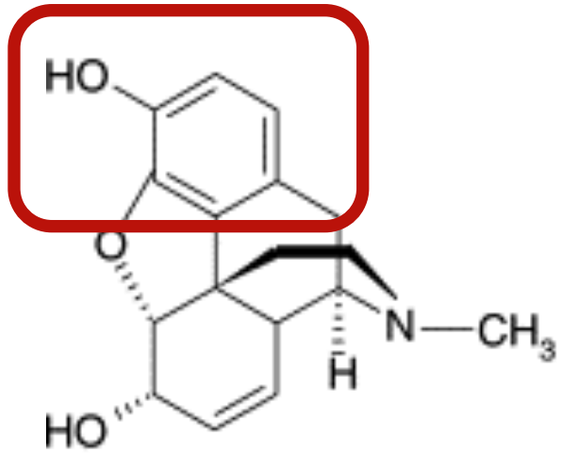
OPIOIDS

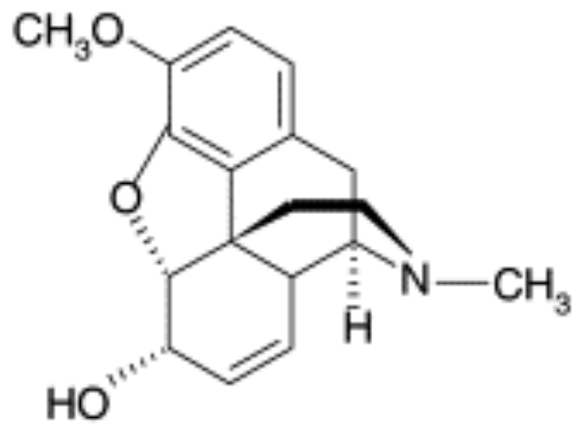
ALKALOIDS IN OPIUM

Heroin 2 -COCH3



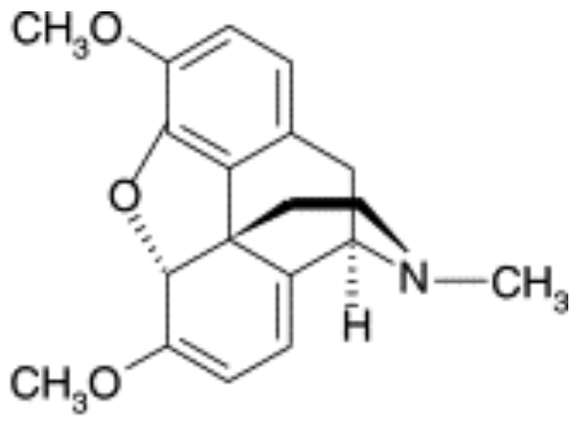
morphine

10%



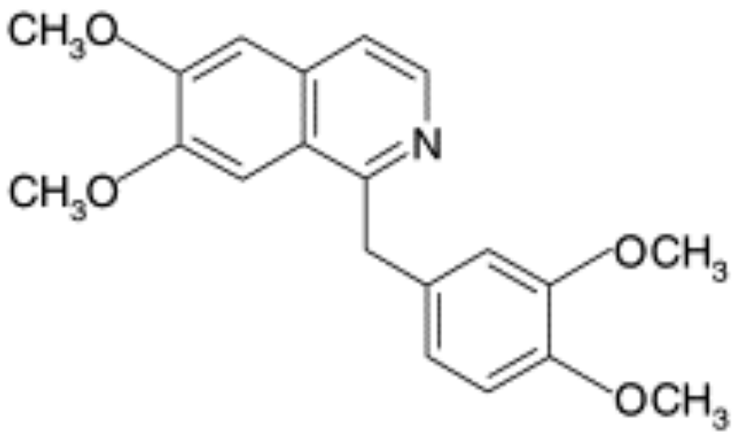
codeine

0,5%



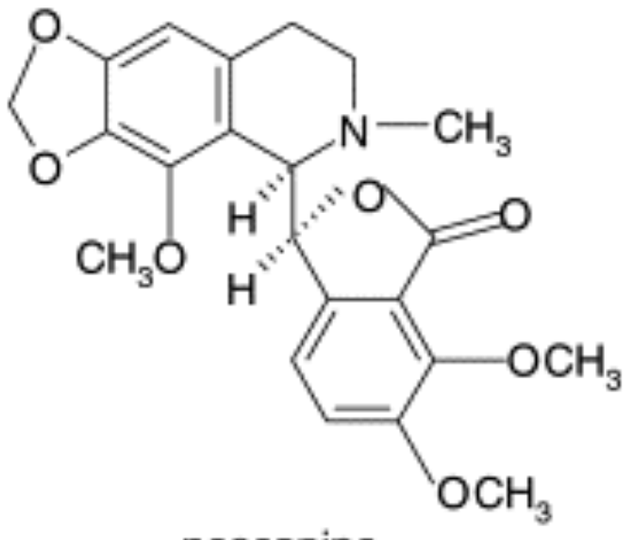
thebaine

0,2 %



papaverine

1 %



noscapine

6 %

% percentage in the opium juice

PHARMACOLOGICAL ACTIONS OF MORPHINE

Central Nervous System

Analgesia
Euphoria
Sedation
(Dysphoria and hallucinations)
Pupillary constriction
Nausea and vomiting
Respiratory depression
Depression of cough reflex
Tolerance and dependence

Gastrointestinal tract

Reduced motility and increased tone with:
Constipation
Contraction of biliary sphincter

Other actions

Histamine release with:
Urticaria and itching
Bronco constriction

Hypotension and bradycardia

Immunosuppressant effects

OPOIDS RECEPTORS AND THEIR LIGANDS

In the 1950s:
proposal of the presence of specific receptors for opioids

In the 1970s:
Proposal of the presence of three different receptors:

- mu receptors (from Morphine) MOR
- kappa receptors (from Ketocyclazocine) KOR
- delta receptors (from Deferent vessels) DOR

Isolation and characterization of endogenous ligands (endorphins):

- Beta-endorphins
- Dynorphins
- Enkephalins

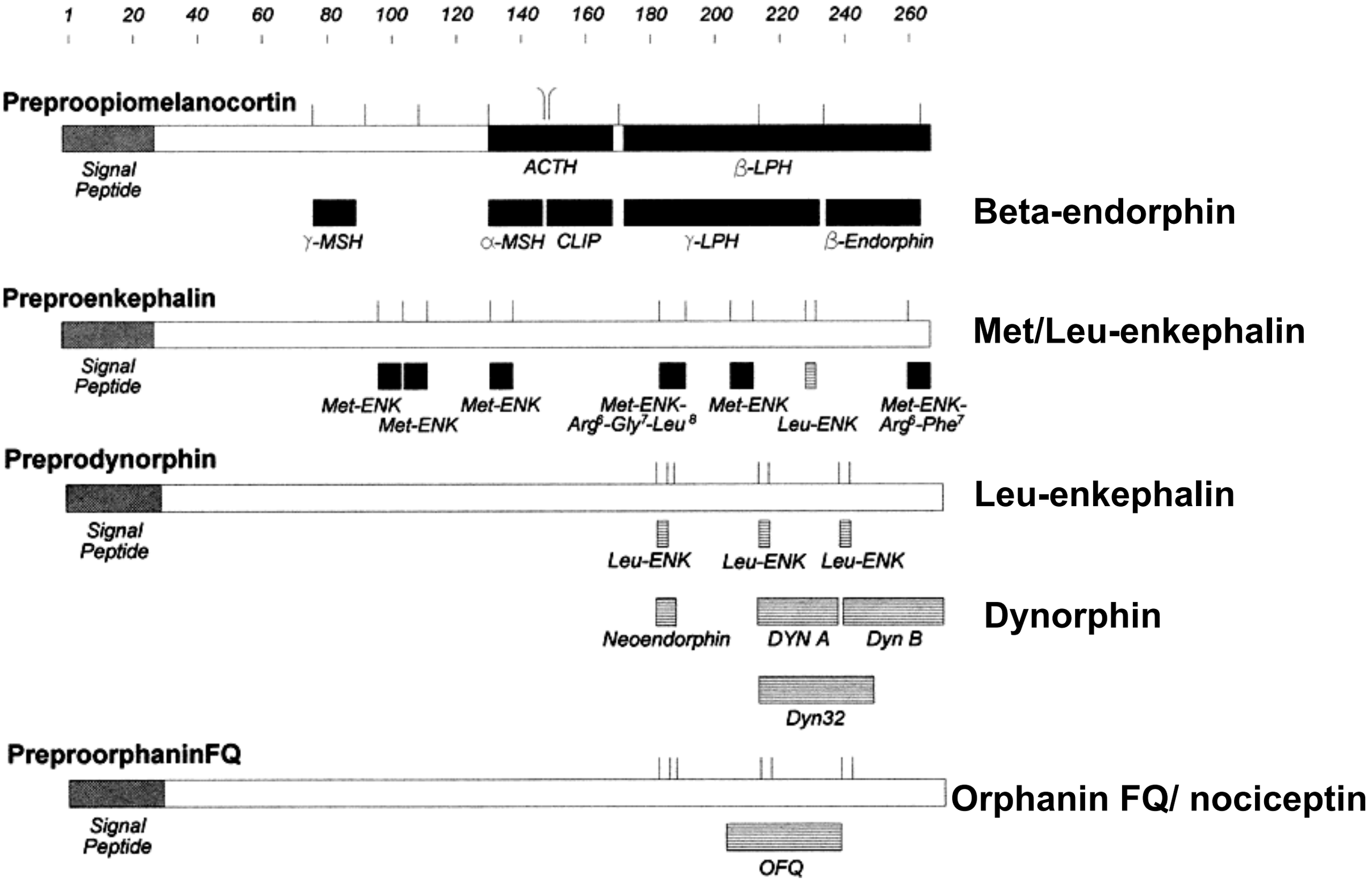
In the 1990s:

- Cloning of MOR, DOR and KOR GPCRs
- Identification of Orphanin FQ/ nociceptin receptor (no affinity towards naloxone)

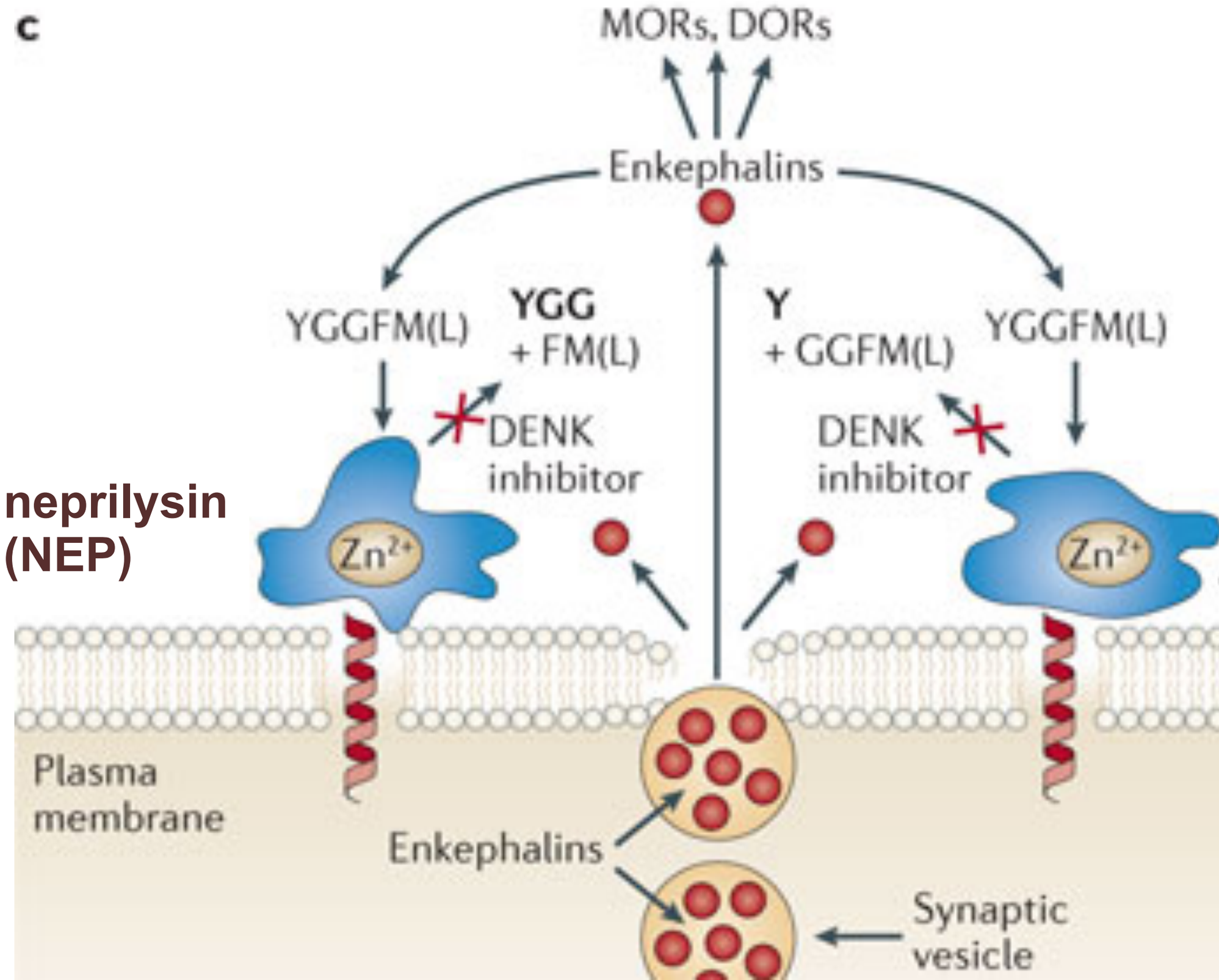
ENDOGENOUS OPIOID PEPTIDES

OFQ/N	Phe-Gly-Gly-Phe-Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-Leu-Ala-Asn-Gln
OFQ/N(1-11)	Phe-Gly-Gly-Phe-Thr-Gly-Ala-Arg-Lys-Ser-Ala
OFQ/N(1-7)	Phe-Gly-Gly-Phe-Thr-Gly-Ala
OFQ2	Phe-Ser-Glu-Phe-Met-Arg-Gln-Tyr-Leu-Val-Leu-Ser-Met-Gln-Ser-Ser-Gln
ppOFQ/N$_{160-187}$ (mouse)	Phe-Ser-Glu-Phe-Met-Arg-Gln-Tyr-Leu-Val-Leu-Ser-Met-Gln-Ser-Ser-Gln Arg-Arg-Arg-Thr-Leu-His-Gln-Asn-Gly-Asn-Val
Nocistatin (human)	Met-Pro-Arg-Val-Arg-Ser-Leu-Phe-Gln-Glu-Gln-Glu-Glu-Pro-Glu-Pro-Gly-Met-Glu-Glu-Ala-Gly-Glu-Met-Glu-Gln-Lys-Gln-Leu-Gln
Dynorphin A	Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Asn-Gln
[Leu^5]enkephalin	Tyr-Gly-Gly-Phe-Leu
[Met^5]enkephalin	Tyr-Gly-Gly-Phe-Met
β-Endorphin	Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Glm-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys-Asn-Ala-Ile-Ile-Lys-Asn-Ala-His-Lys-Lys-Gly-Gln
Endomorphin 1	Tyr-Pro-Trp-Phe-NH_2
Endomorphin 2	Tyr-Pro-Phe-Phe-NH_2

ENDOGENOUS OPIOID PEPTIDES: SYNTHESIS



METABOLISM OF ENDOGENOUS PEPTIDES



SELECTIVITY OF OPIOID LIGANDS

	MOR	DOR	KOR	NOR
ENDOGENOUS OPIOIDS				
Beta-endorphin	+++	+++	+	-
Leu-enkephalin	(++)	+++	+	-
Met-enkephalin	++	+++	+	-
Dynorphin	+	+	+++	-
Orphanin FQ/nociceptin	-	-	-	+++
RECEPTOR SELECTIVE				
Agonists	DAMGO	DPDPE	Enandoline	Ro64-6198
Antagonists	CTOP	Natrindole	Nor-binaltorphimine	SB 612111

MOR RECEPTORS SELECTIVE LIGANDS

AGONISTS	PARTIAL AGONISTS	ANTAGONISTS
Morphine (T1/2 = 2 h) Meperidine Levorphanol Fentanyl Tramadol* **Methadone** (T1/2 = 14-40 h)	**Buprenorphin** Nalbuphine Nalorphine	**Naloxone** Naltrexone

* also inhibition of NA and 5-HT uptake

MECHANISM OF ACTION

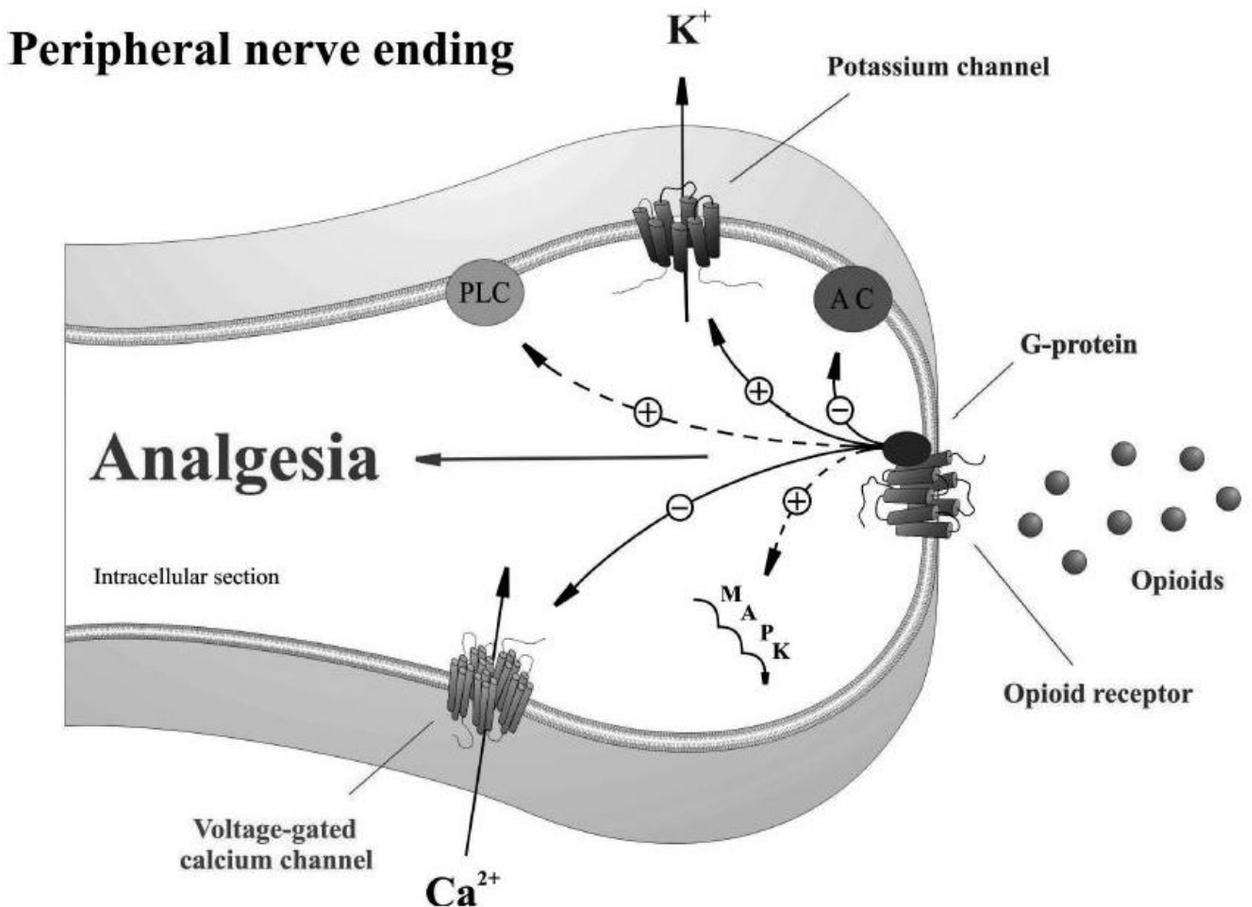
All four types of opioid receptors are Gi/o-protein coupled receptors

1. Adenynyl cyclase inhibition

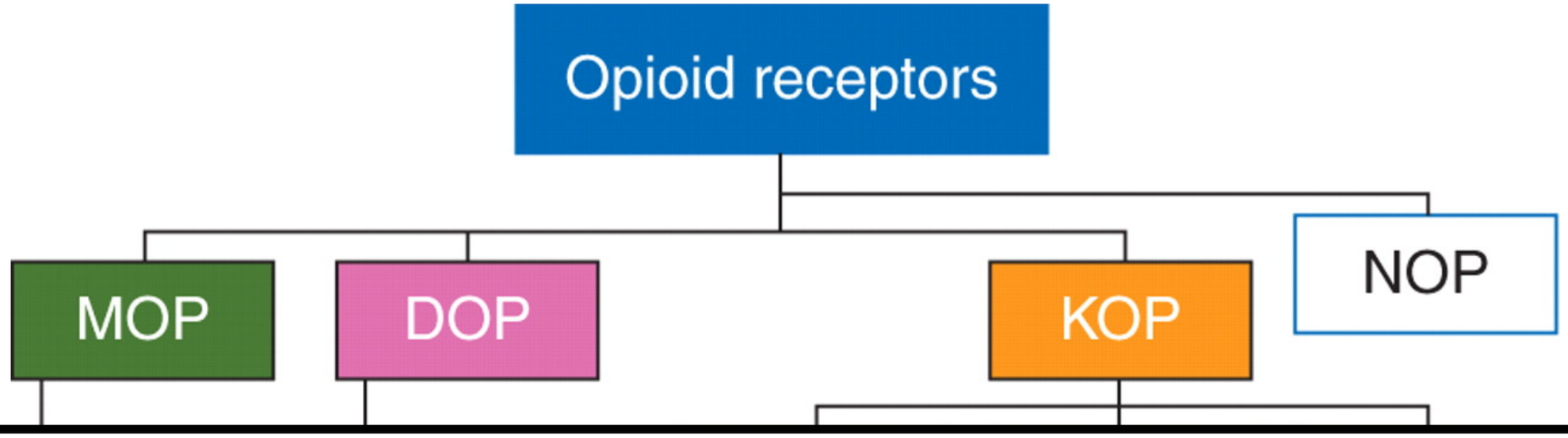
2. N and P/Q voltage-dependent calcium channel inhibition

3. Activation of GIRK (G protein-inhibited rectifying K+ channels)

4. Activation of MAP kinase pathway



CLASSIFICATION OF THE OPIOID RECEPTOR FAMILY



THE OPIOID SYSTEM PARADOX?

A large number of endogenous ligands (at least 11) converge on only a small number of opioid receptors (4 genes)

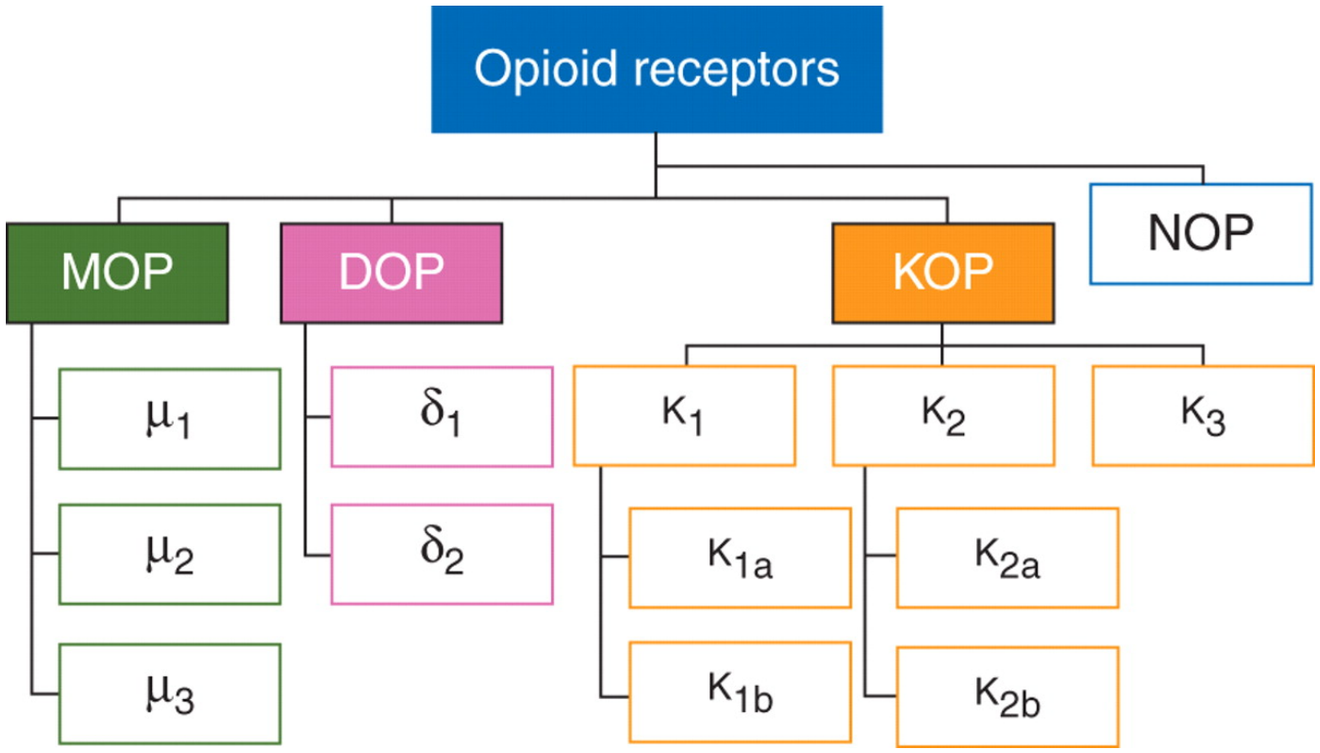
Endogenous ligands display poor selectivity towards opioid receptors (with the exception of Dynorphin A for KOR)

Several pharmacological evidence suggest the existence of multiple receptor subtypes

MOP antagonists (e.g. Naloxonazine) block morphine-induced analgesia but not alter respiratory depression, constipation or itching

On the other hand, knockout of MOP receptor inhibits all the MOP receptor associated activities

PHARMACOLOGICAL CLASSIFICATION OF THE OPIOID RECEPTOR FAMILY

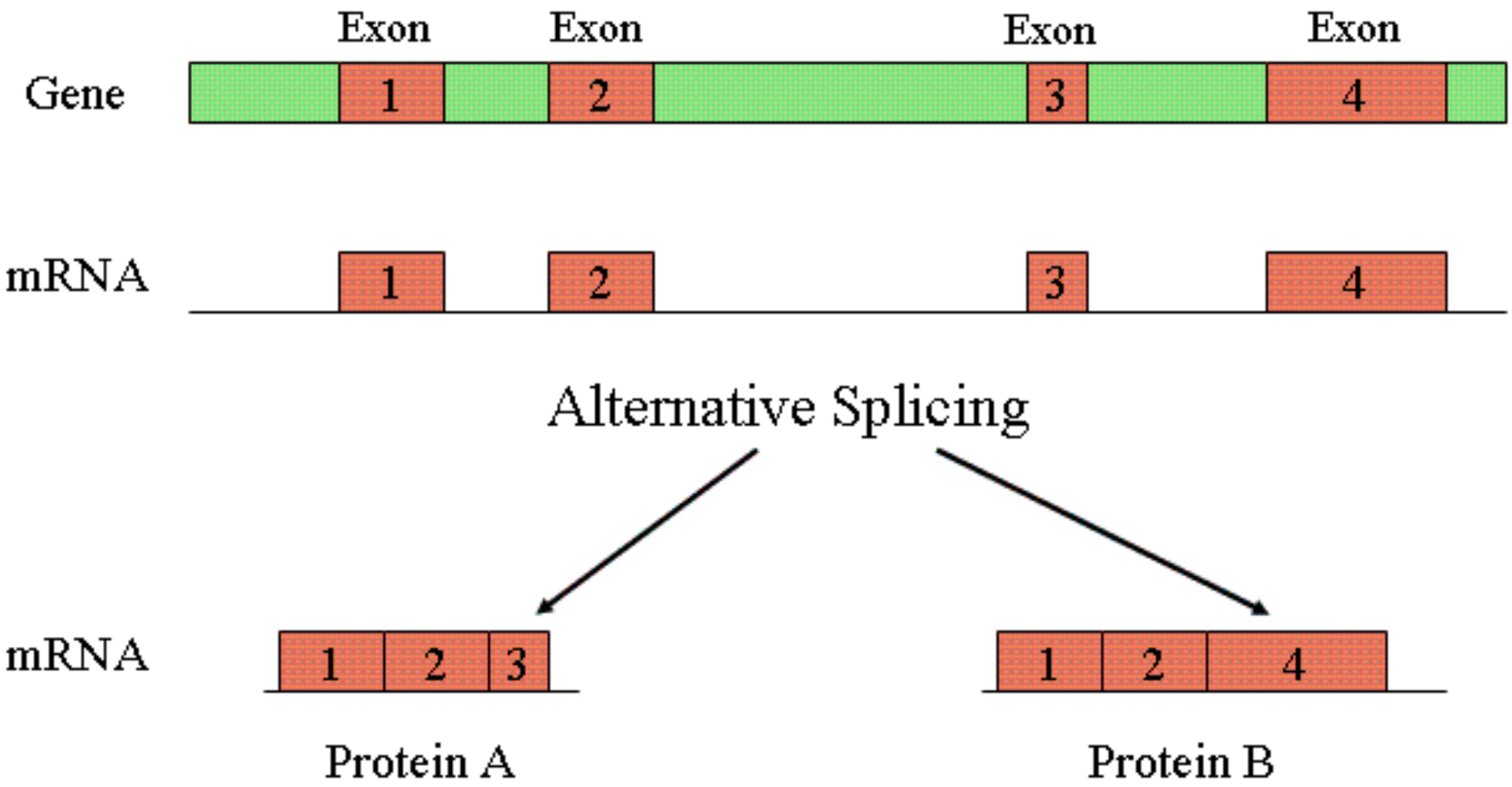


DO OPIOID RECEPTOR SUBTYPES EXIST?

Three alternative possible mechanisms:

- Alternative splicing of a common gene product
- Functional selectivity (biased agonism)
- Omo - and/or hetero-dimerization

1. ALTERNATIVE SPLICING

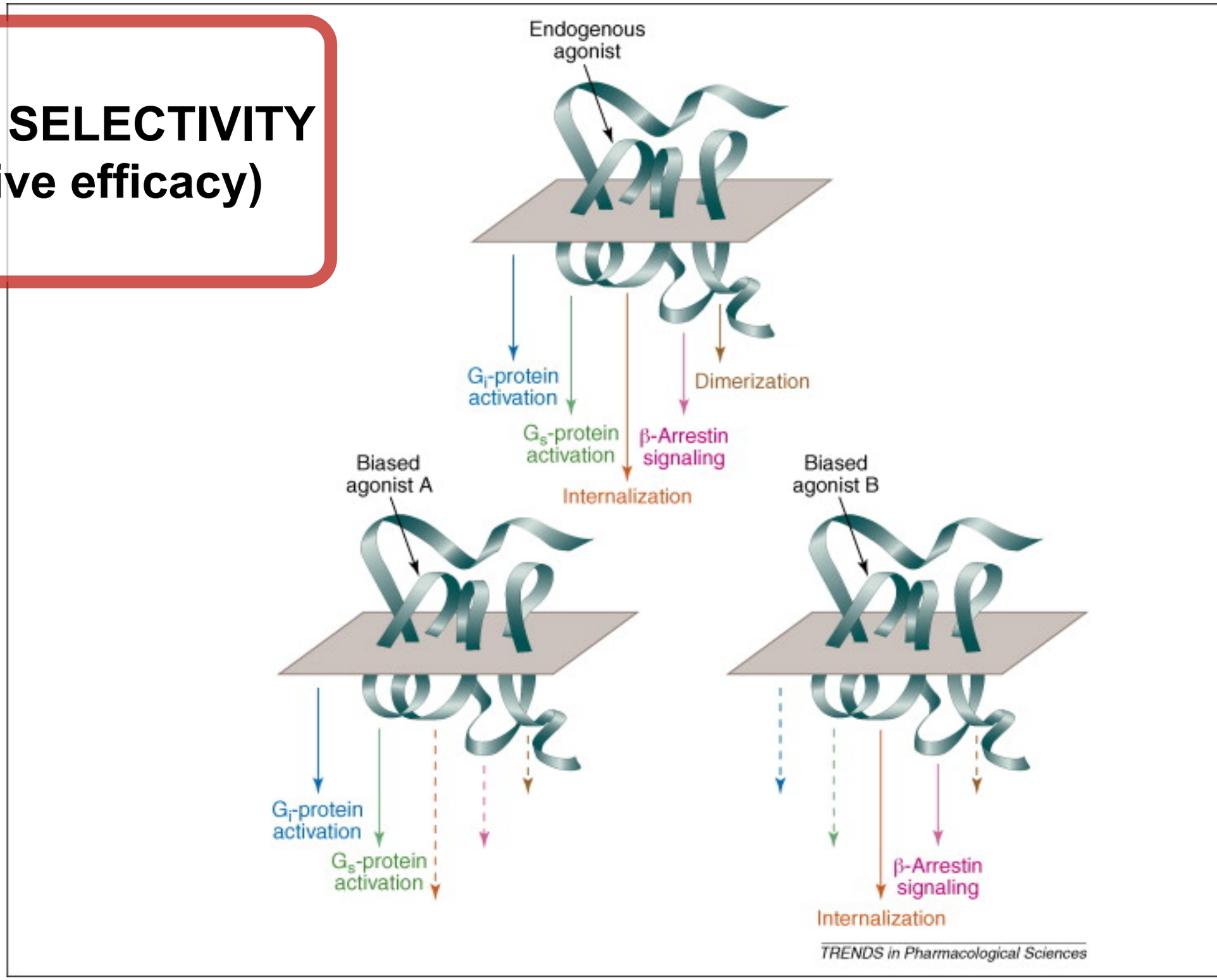


Exon 1 is necessary for the analgesic activity of morphine

Exon 2 is necessary for the analgesic activity of fentanyl

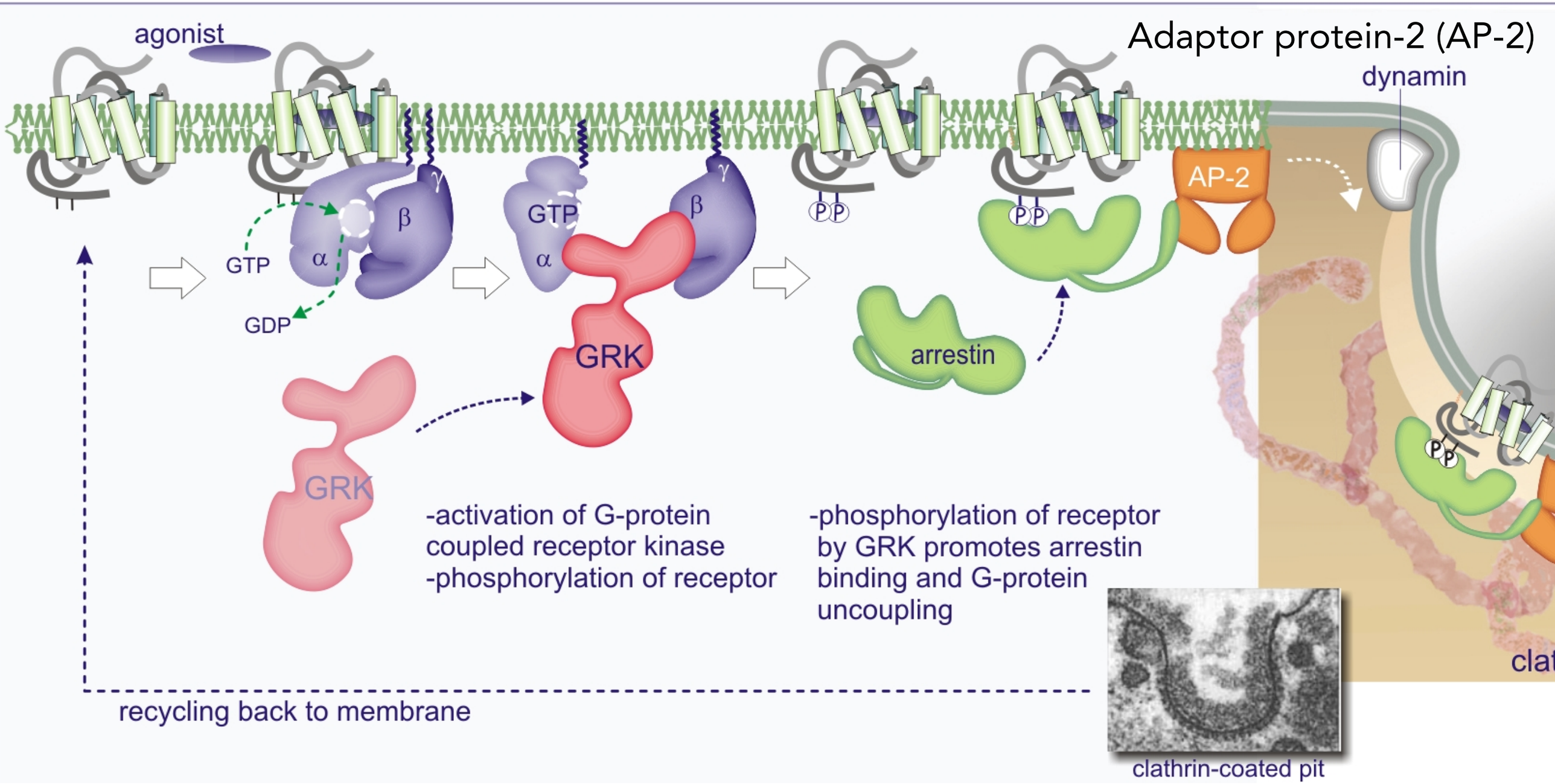
PROTEIN ISOFORMS

2. FUNCTIONAL SELECTIVITY (ligand-selective efficacy)



Different ligands influences which G protein associates with the receptor thus promoting distinct coupling efficiencies (distinct intracellular pathways)

DESENSITIZATION of GPCRs: molecular mechanisms



Role of G-protein coupled receptor kinase (GRK) and arrestins

Turning off the signal: desensitization of GPCRs function

Receptor **desensitization** is a reduced response of a receptor that follows a prolonged exposure to an agonist and it is due to uncoupling of a receptor from G proteins

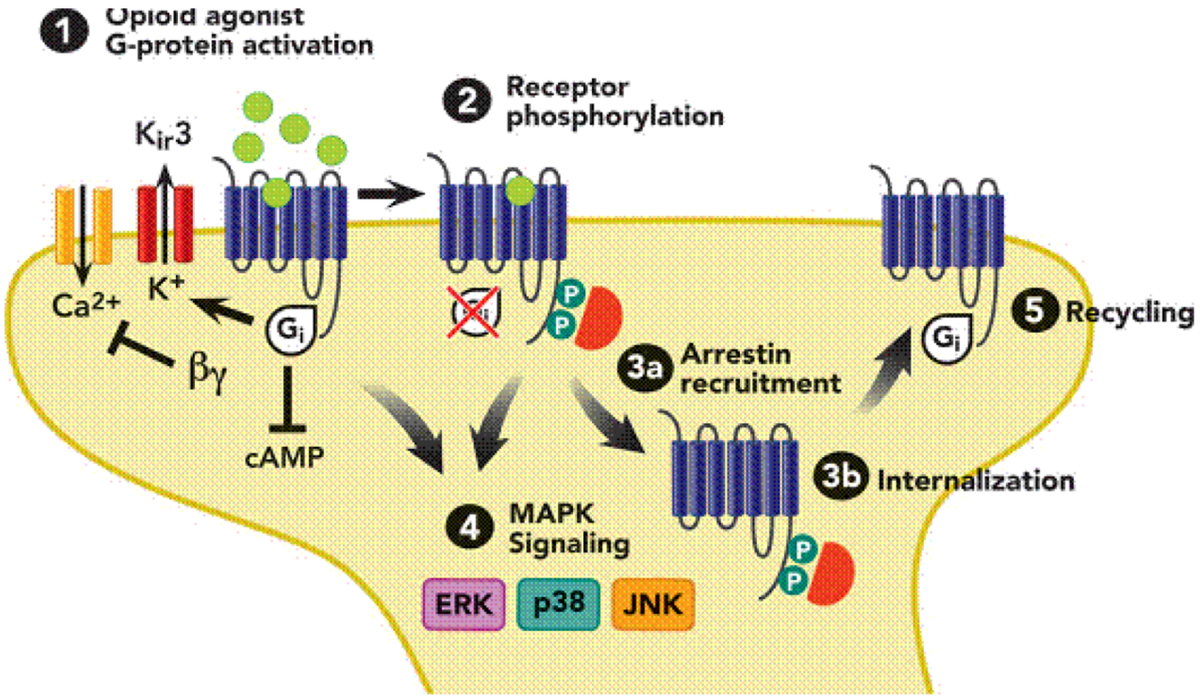
Desensitization also results from receptor **internalization**, the removal of receptors from a plasma membrane by endocytosis (**downregulation**)

Internalization can be followed by receptor recycling (**resensitization**) or lysosomal degradation

Desensitizantion can cause (pharmocodinamic) **tolerance**, the need to increase the drug dose to obtain the required effect

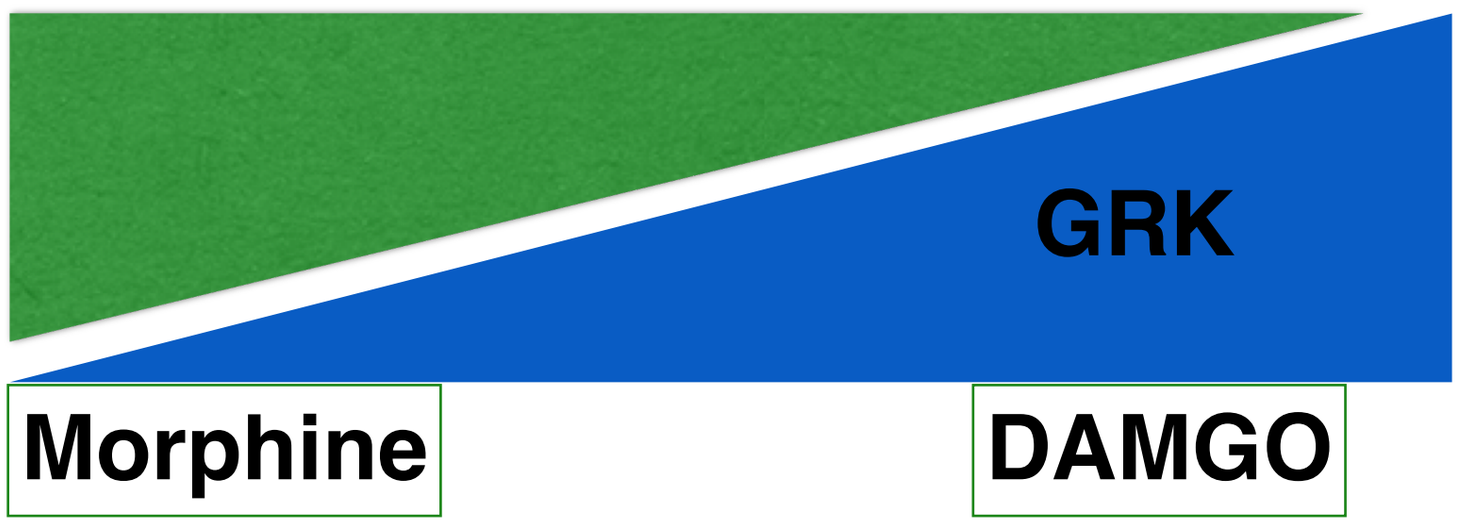
FUNCTIONAL SELECTIVITY

Selective ligands of opioid receptors can direct the receptor to favore one or more signaling events

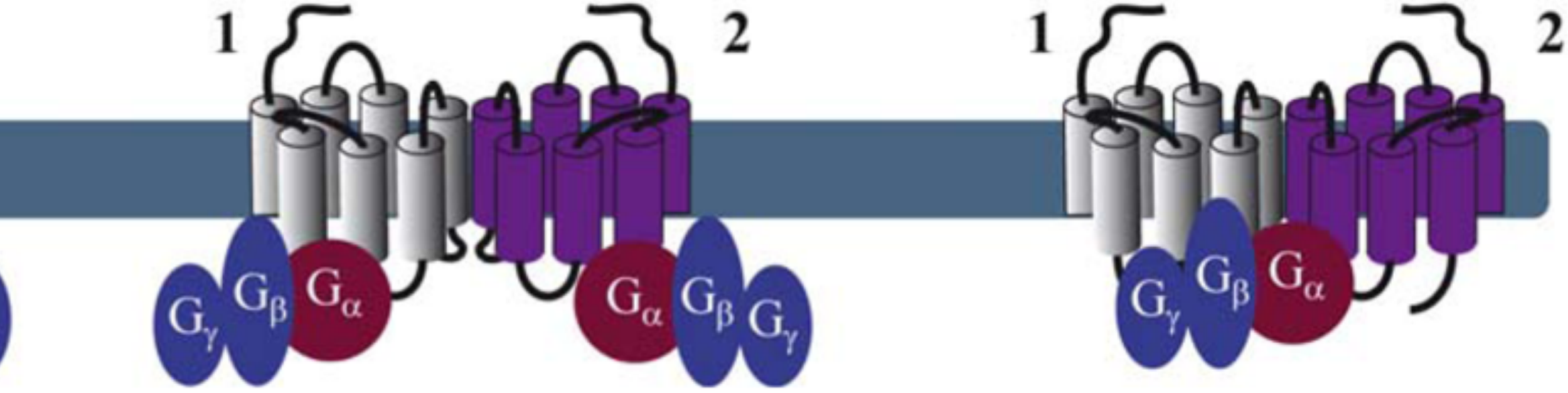


Morphine does not promote MOP receptor internalization and causes tolerance at high degree

In contrast, DAMGO causes robust internalization and low tolerance degree

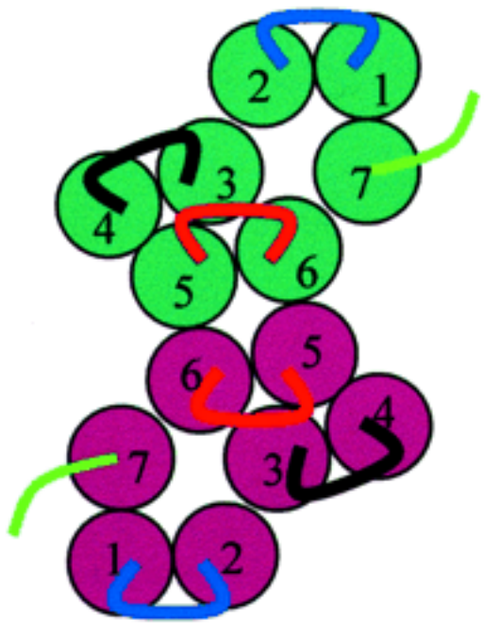


3. GPCR DIMERIZATION

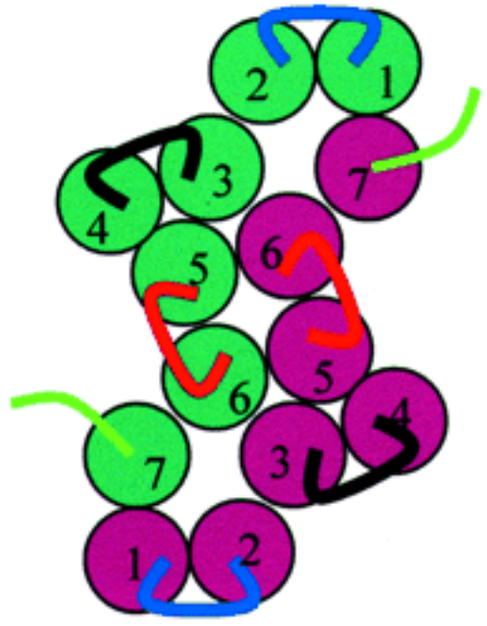


Potential GPCR dimer interfaces

Contact dimers



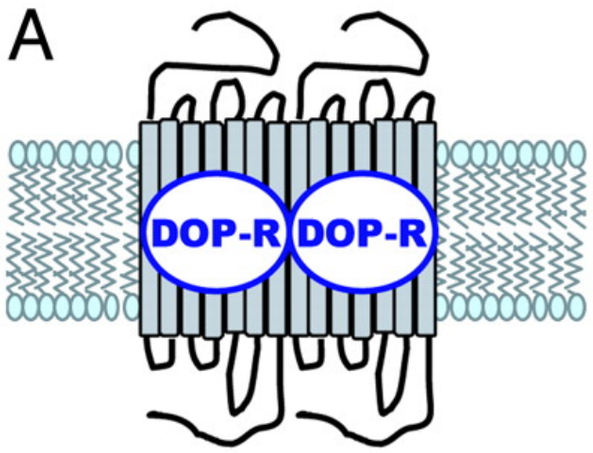
Domain dimer interfaces



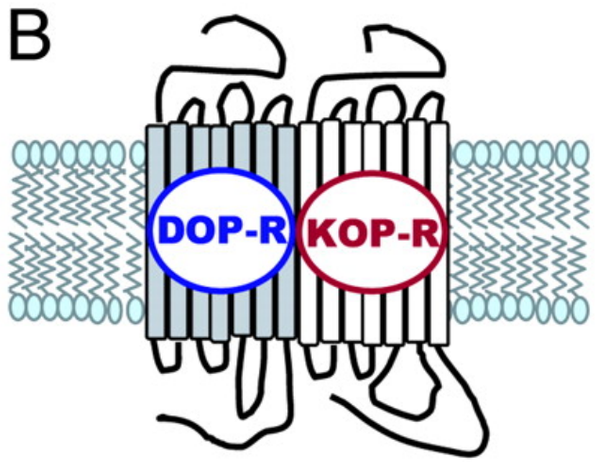
Dimerization affects signal transmission and desensitization

and can explain the differences in efficacy and in abuse potential of different ligands

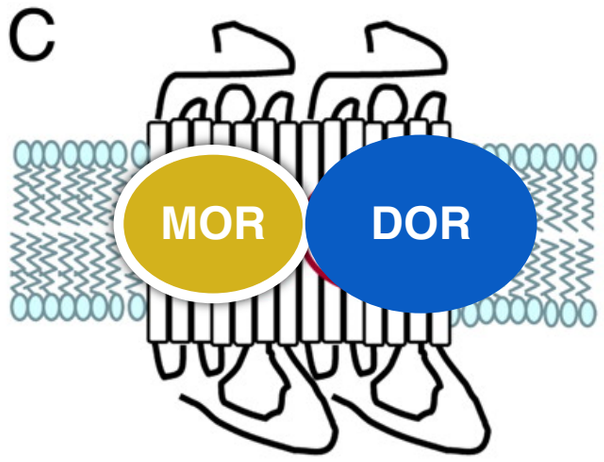
OMO- and HETERO-DIMERIZATION between Opioid receptor subtypes



No response: Agonists stimulation causes the dimer dissociation

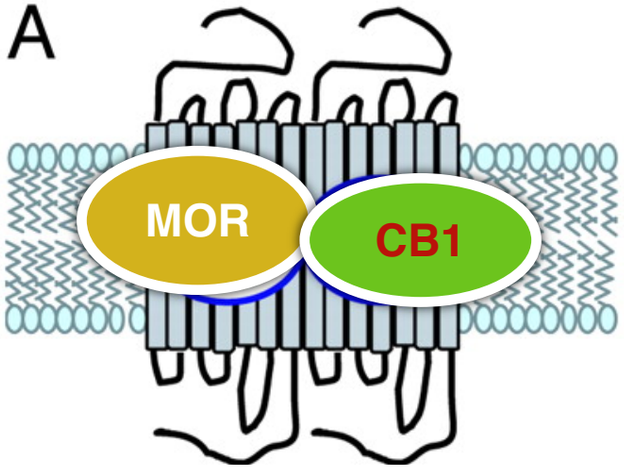


Strong response: Reduced internalization

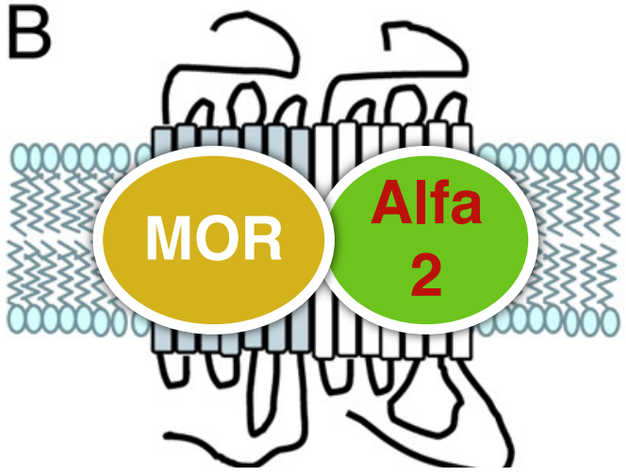


Different signal properties: in DOR absence, MOR dependent tolerance and dependence are reduced

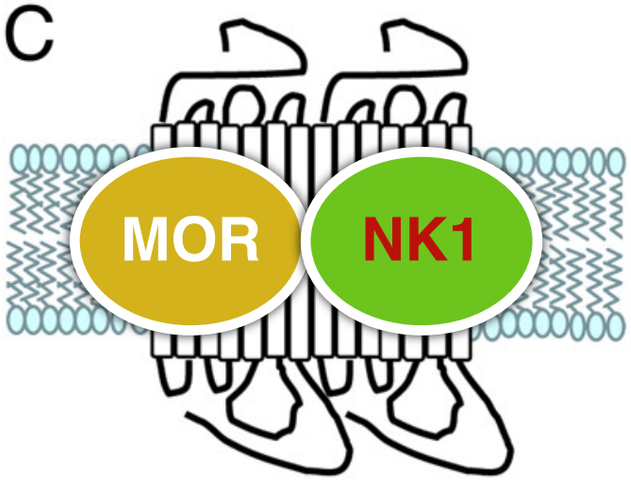
OMO- and HETERO-DIMERIZATION between GPCR classes



Enhances the potency of morphine

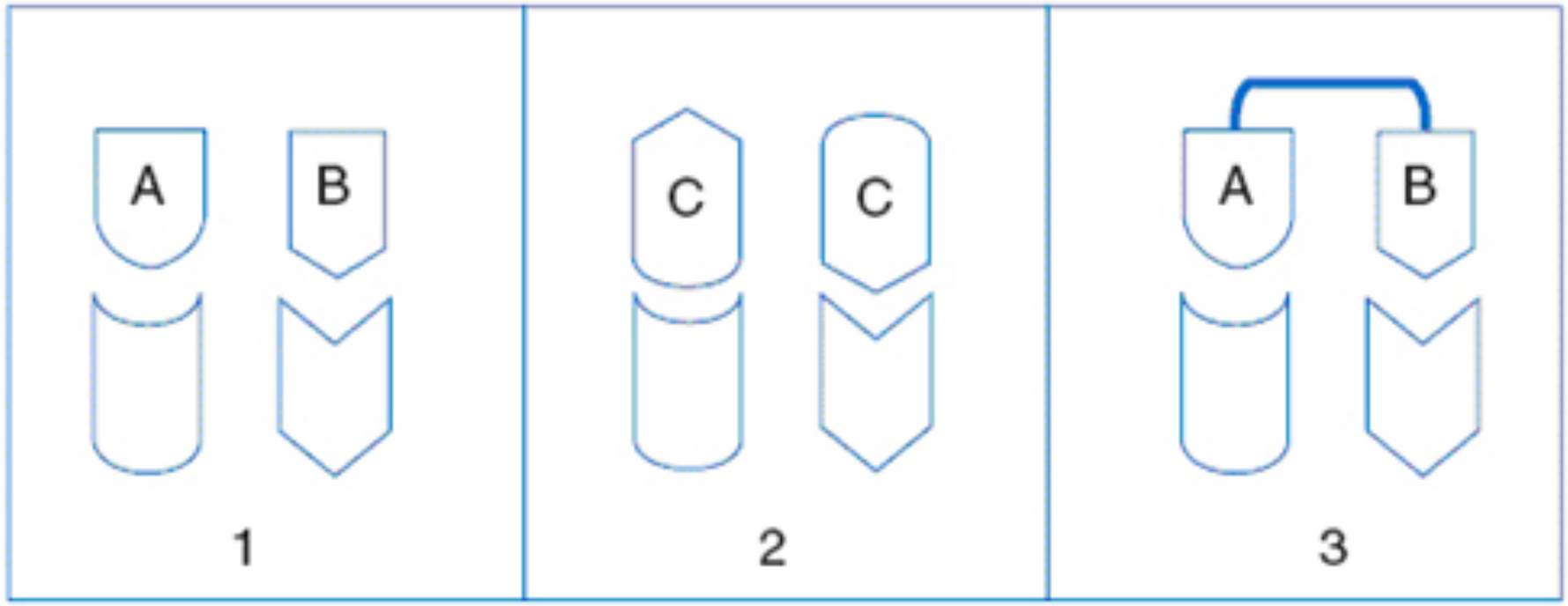


Potentiated phosphorylation of MAPK induced by morphine



SubP causes internalization

Multi-targeting



Administration of two different drugs:

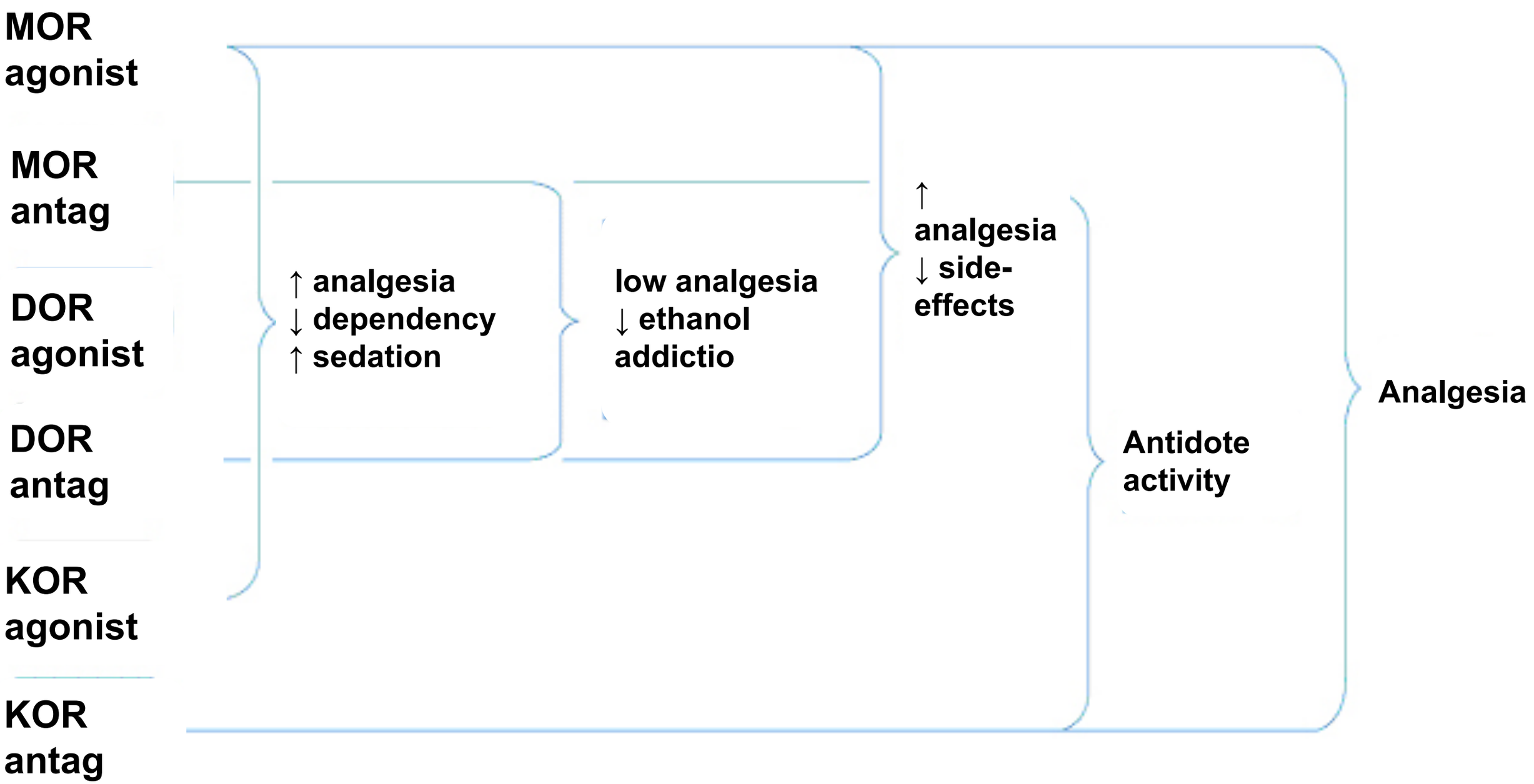
e.g.: MOP agonist (ossimorfone) and DOP antagonist (naltrindolo)

Administration of a non-selectiv but *bifuntional drug*

e.g.: butorfan (MOP and KOP agonists) - bifalin (MOP and DOP agonists)

Administration of a single *bivalent* ligand with two molecules linked by a spacer

Multi-targeting



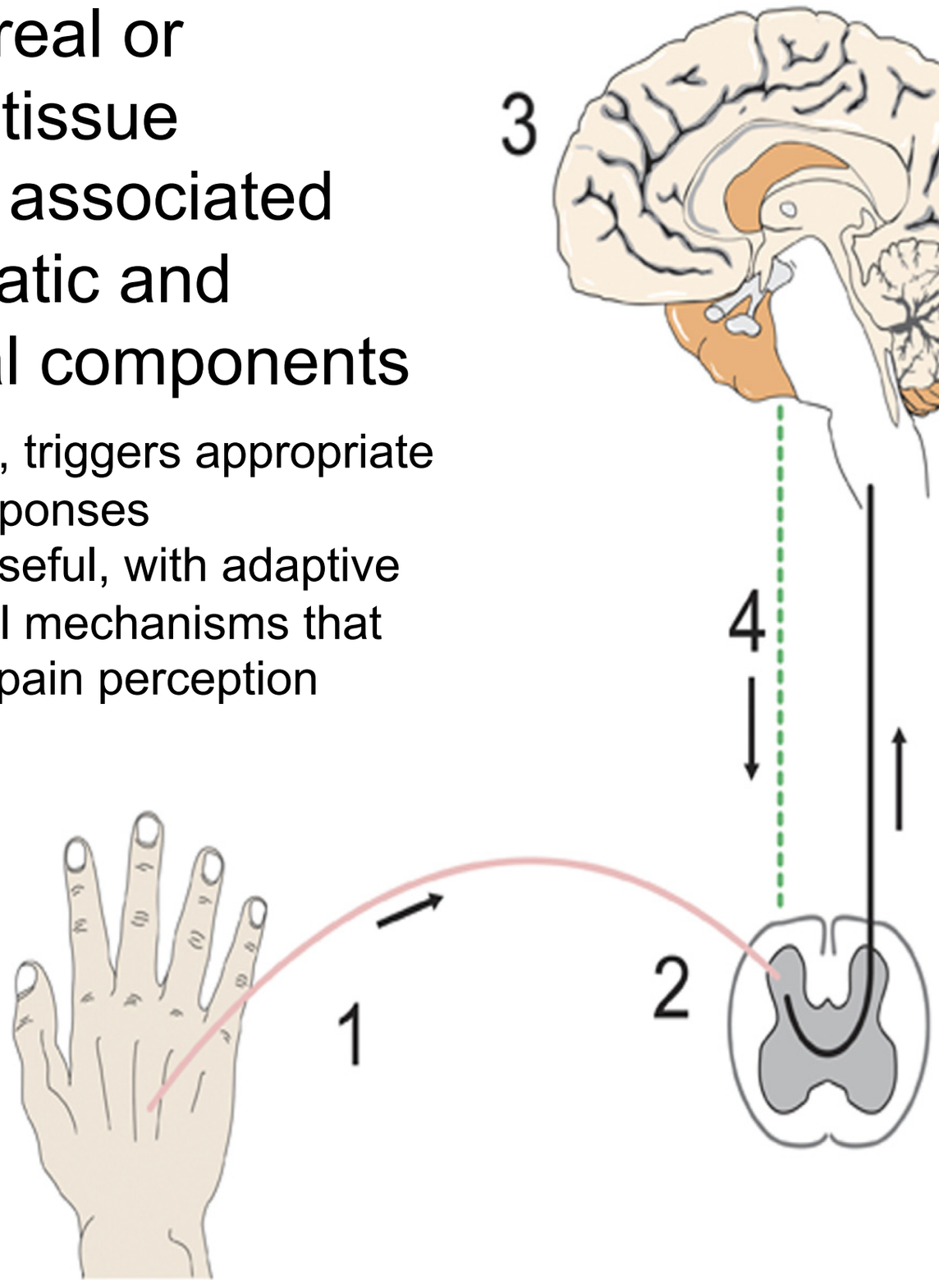
FUNCTIONAL EFFECTS ASSOCIATED WITH THE MAIN TYPES OF OPIOID RECEPTOR

	MOP	DOP	KOP	NOP
Analgesia				
supraspinal	+++	-	-	antag
spinal	++	++	+	++
peripheral	++	-	++	-
Respiratory depression	+++	++	-	-
Pupil constriction	++	-	+	-
Reduced gastrointestinal motility	++	++	+	-
Euphoria	+++	-	-	-
Dysphoria and allucinations	-	-	+++	-
Sedation	++	-	++	-
Tolerance and dependence	+++	-	-	-

PAIN PATHWAYS

Pain: a sensorial and emotional experience due to a real or potential tissue damage, associated with somatic and emotional components

Acute: useful, triggers appropriate protective responses
Chronic: unuseful, with adaptive and emotional mechanisms that can increase pain perception



Afferent nerves stimulated by noxiuos stimula (1)

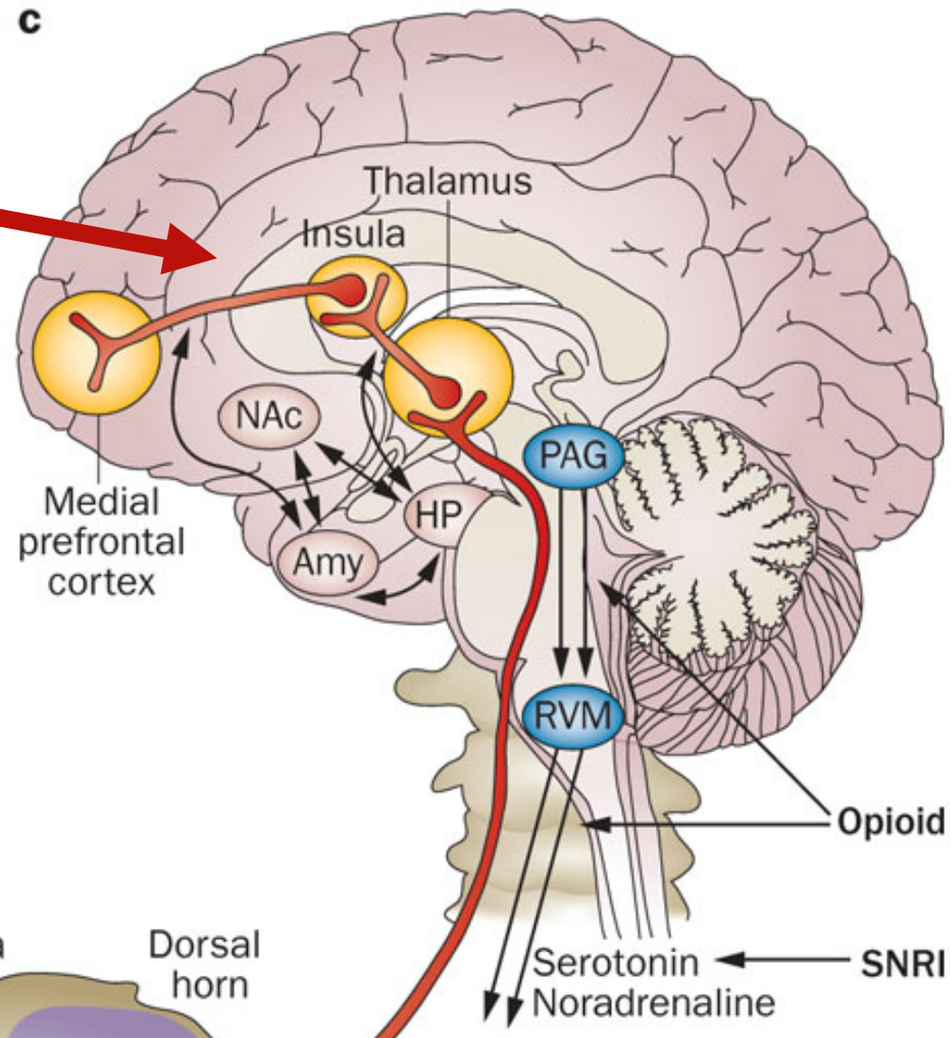
activate spinal neurones (2)

that take the informations to the sovraspinal centers (3)

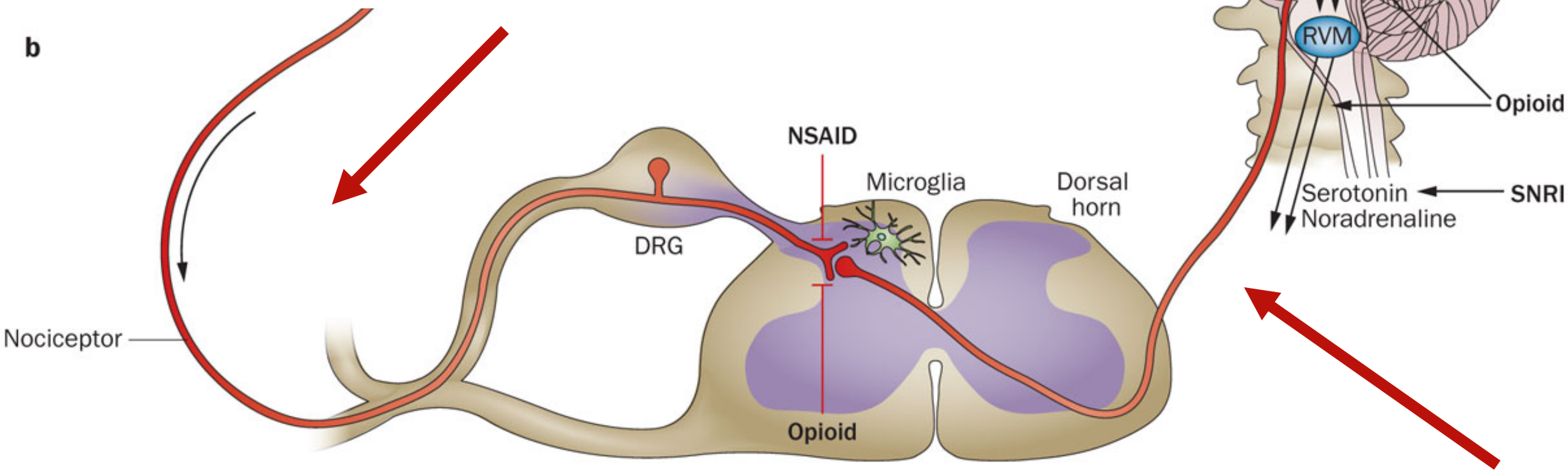
The SNC modulates the overall response through efferent control systems (4)

PAIN PATHWAYS

3th ORDER NEURON



1st ORDER NEURON



2nd ORDER NEURON

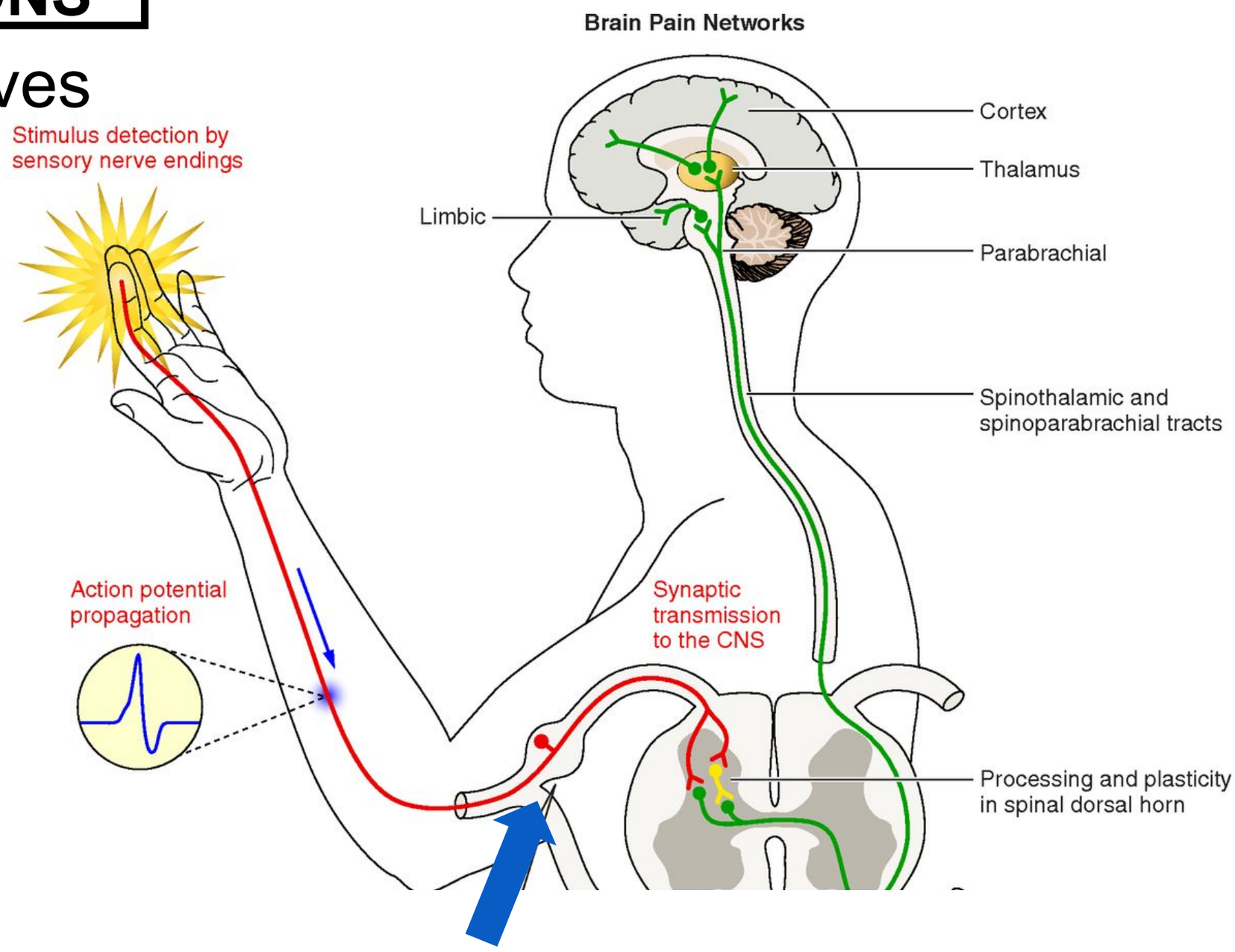
1st ORDER NEURONS

Primary sensory nerves
Nociceptors

Aδ fibers *- fast conductance speed:*

- small myelinated
-
- sensitive to mechanical noxiuos stimuli

localized pain



Dorsal root ganglion

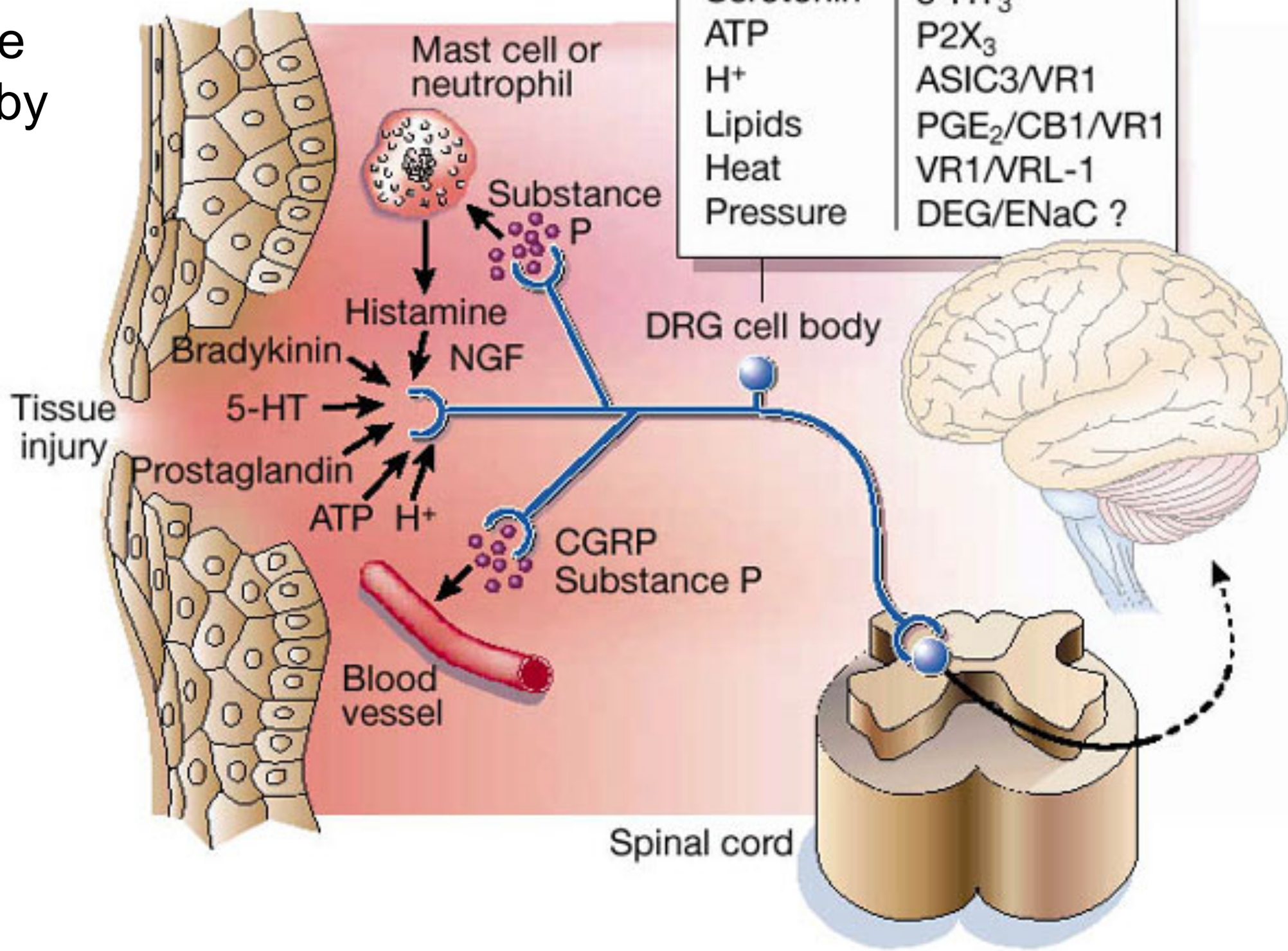
C fibers **-** *slow conductance speed*:

- small amyelinated
- sensitive to thermal changes, chemicals, pressure
- diffuse and strong pain

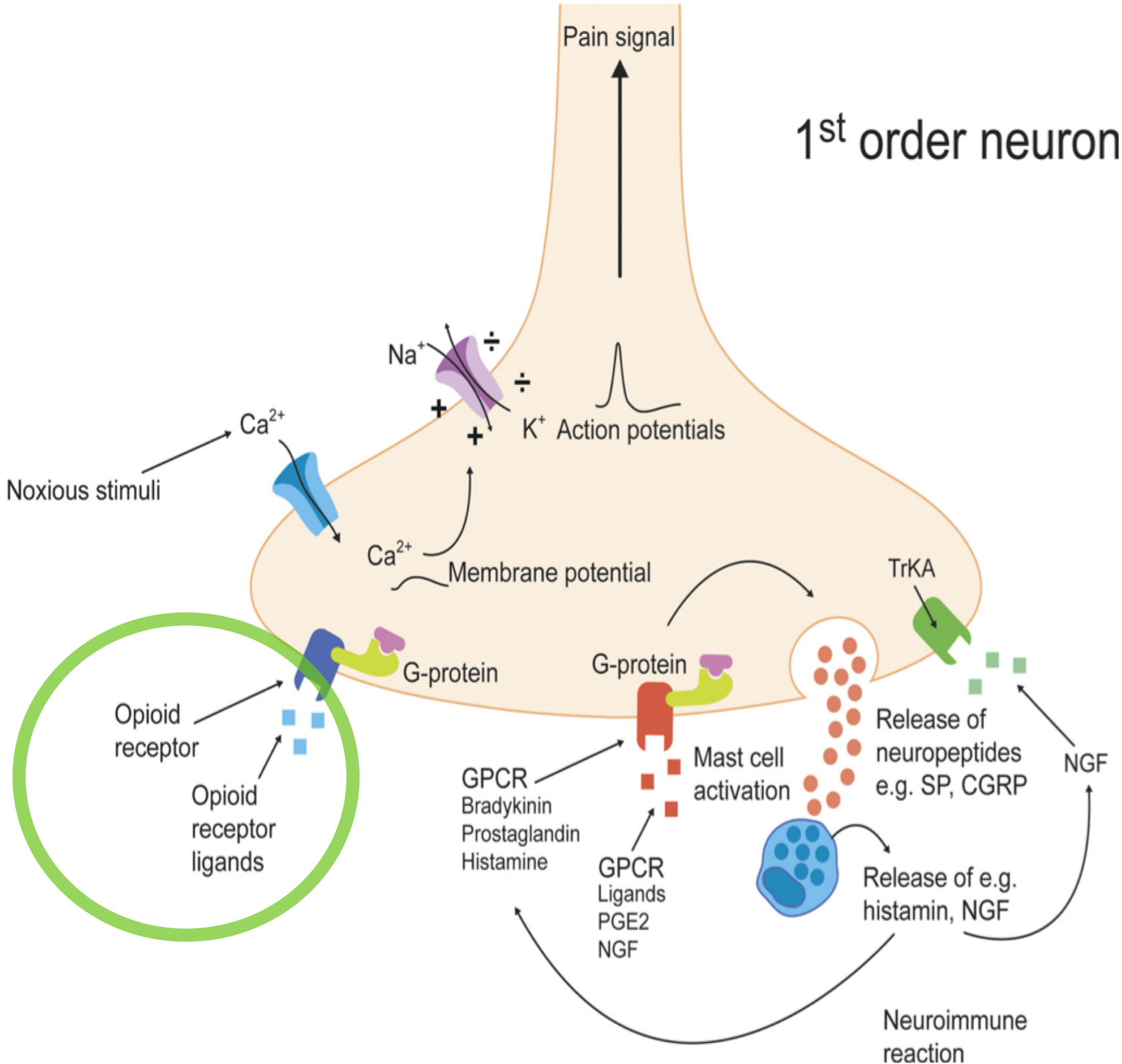
1st ORDER NEURONS

Nociceptors are activated also by mediators released after tissue injury

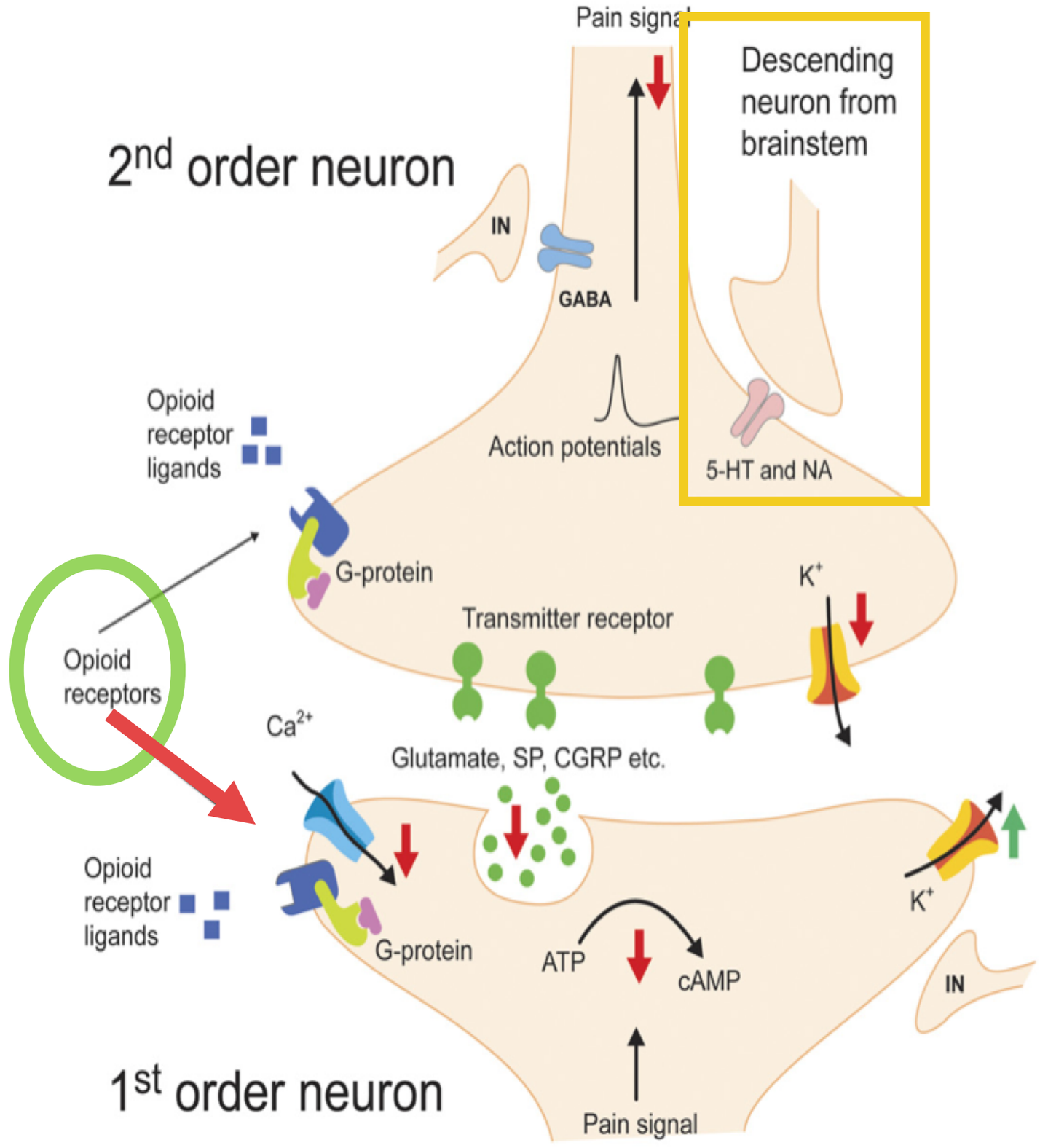
Stimulus	Representative receptor
NGF	TrkA
Bradykinin	BK_2
Serotonin	$5\text{-}HT_3$
ATP	$P2X_3$
H^+	ASIC3/VR1
Lipids	PGE_2/CB1/VR1
Heat	VR1/VRL-1
Pressure	DEG/ENaC ?



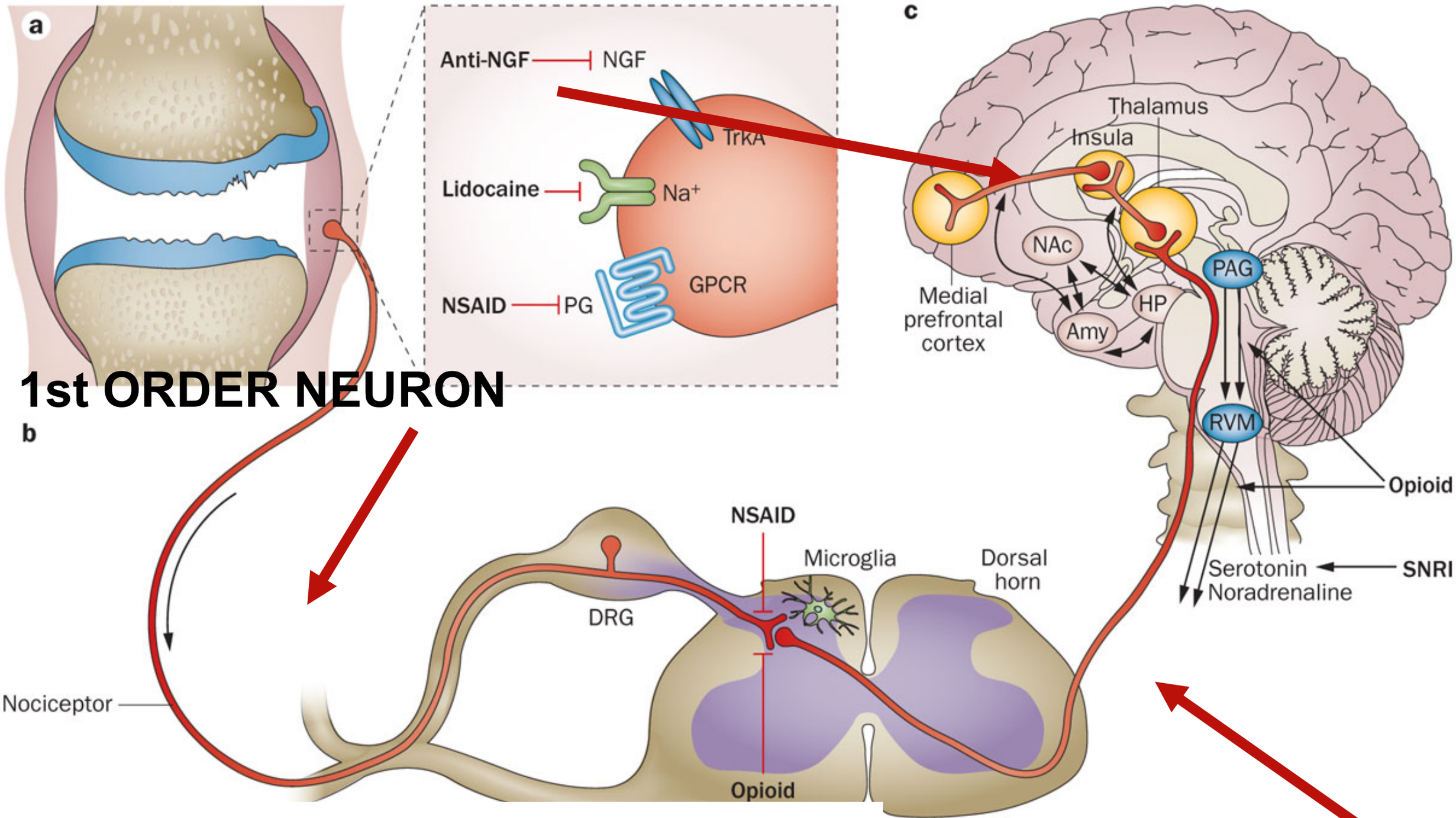
1st ORDER NEURONS



1st and 2nd order neurons



PATHWAYS

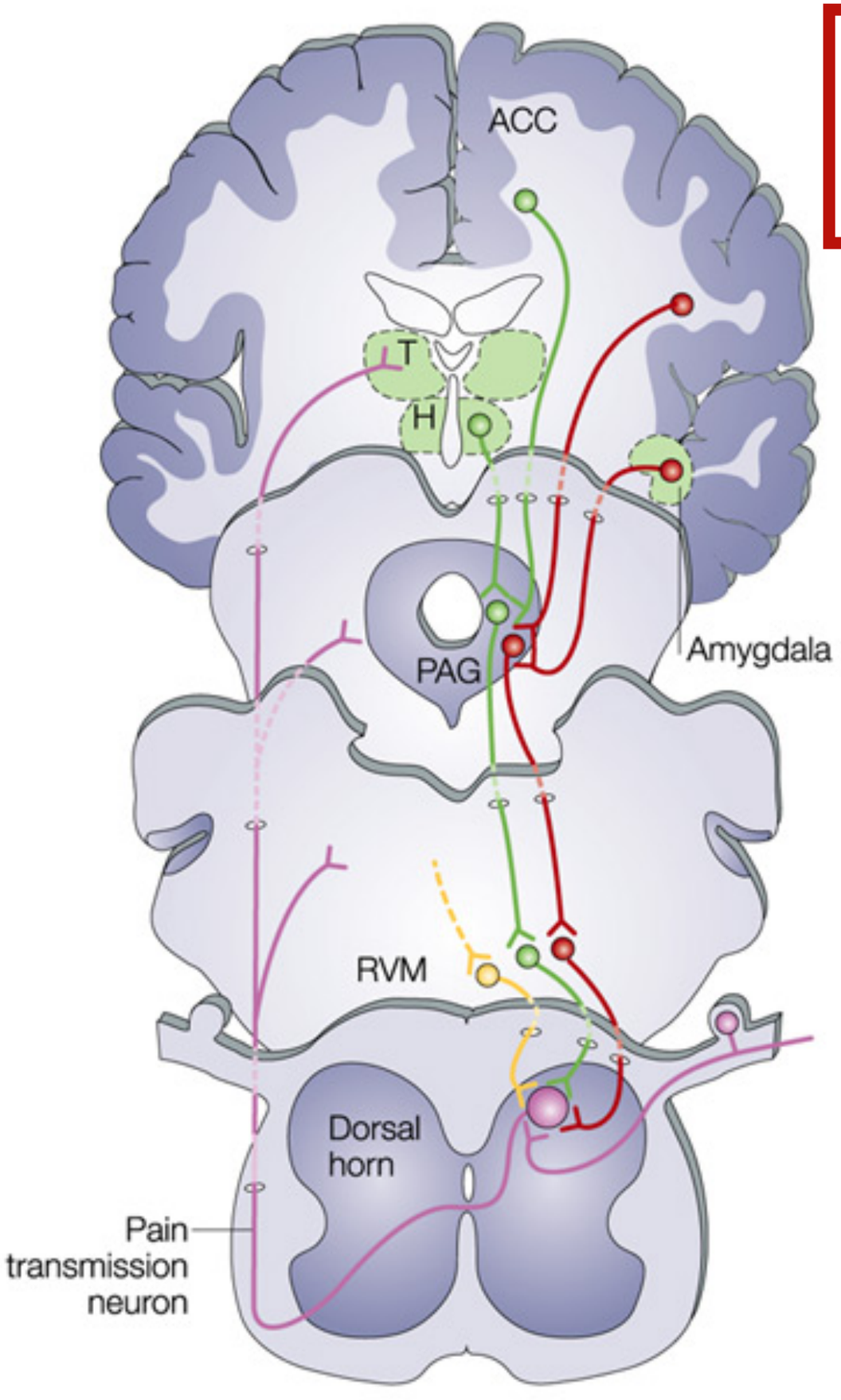


1st ORDER NEURON

2nd ORDER NEURON

3th ORDER NEURON

THE TOP-DOWN PATHWAY:
the efferent control systems



facilitatory (ON cells, red)

Yes, pain!

inhibitory (OFF cells, green)

serotonergic (yellow)

No pain....

anterior cingulate cortex (ACC)

hypothalamus (H)

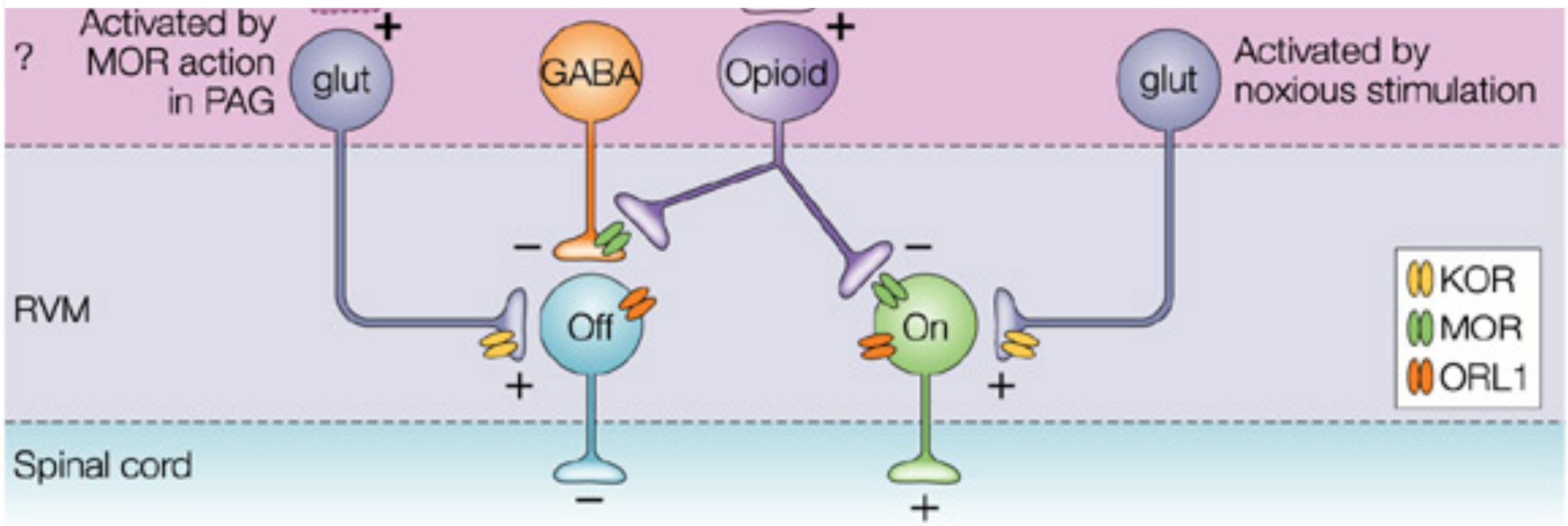
thalamus (T)

periaqueductal grey (PAG)

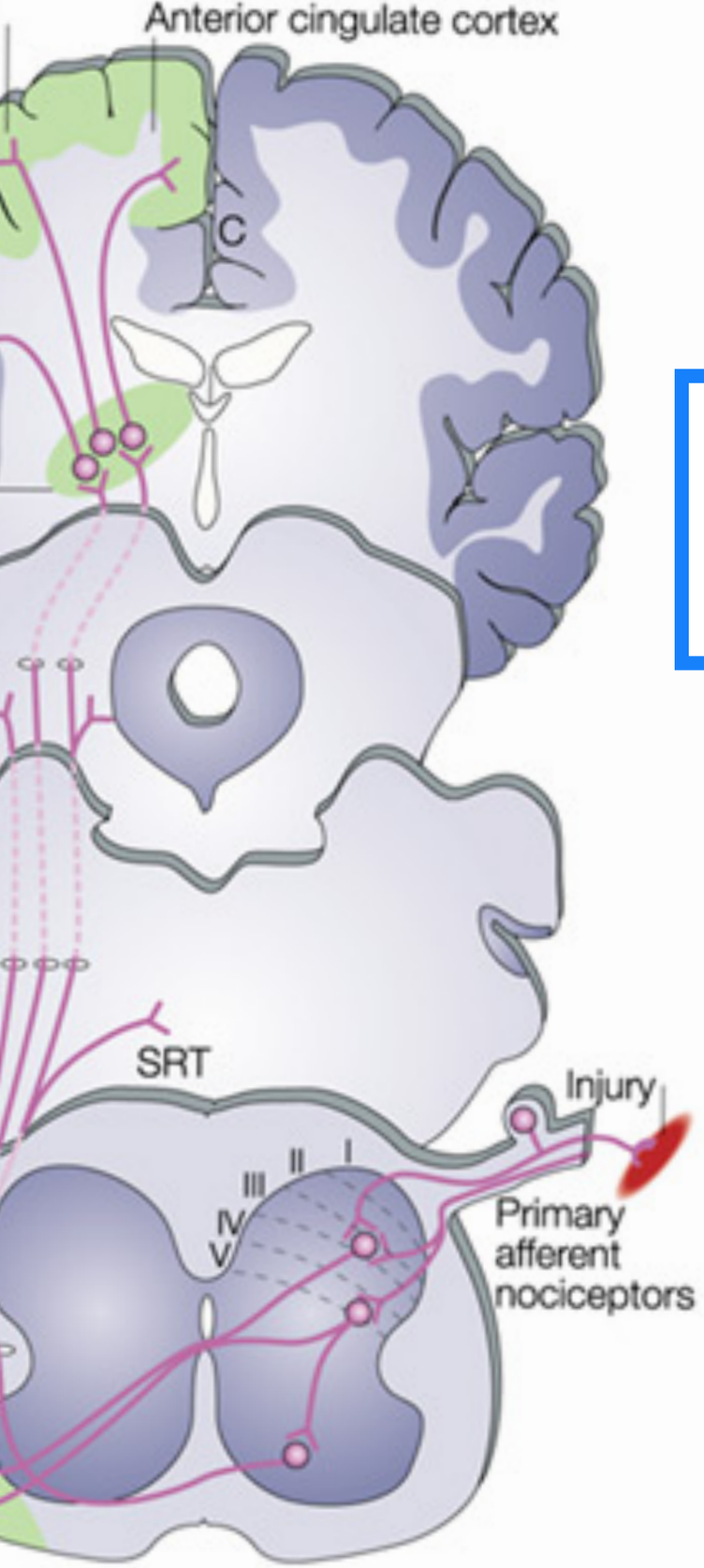
rostral ventromedial medulla (RVM)

Within the RVM

MOR agonists produce anti-nociceptive effects by inhibiting ON cells and disinhibiting OFF cells



No pain.... Yes, pain!



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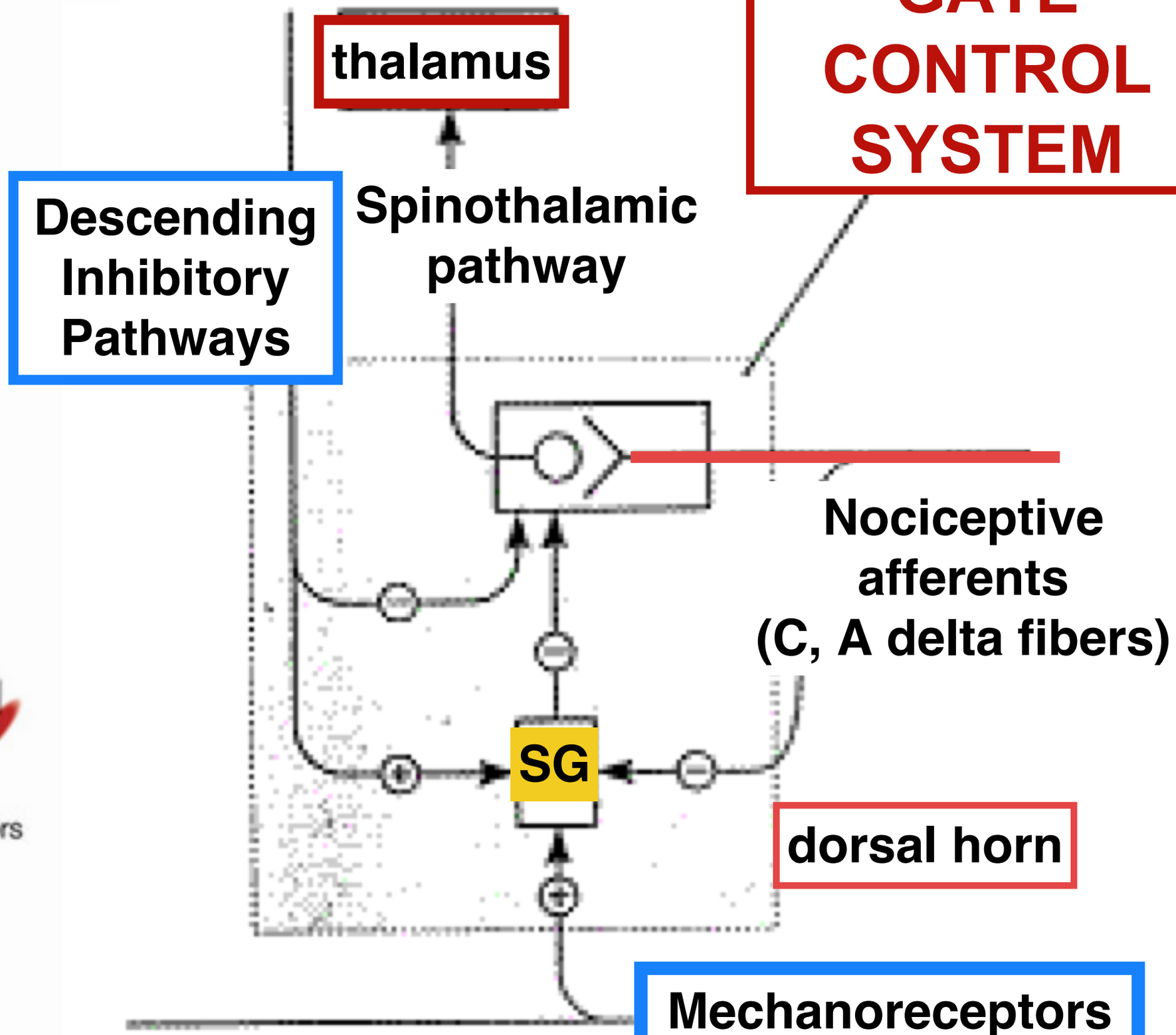
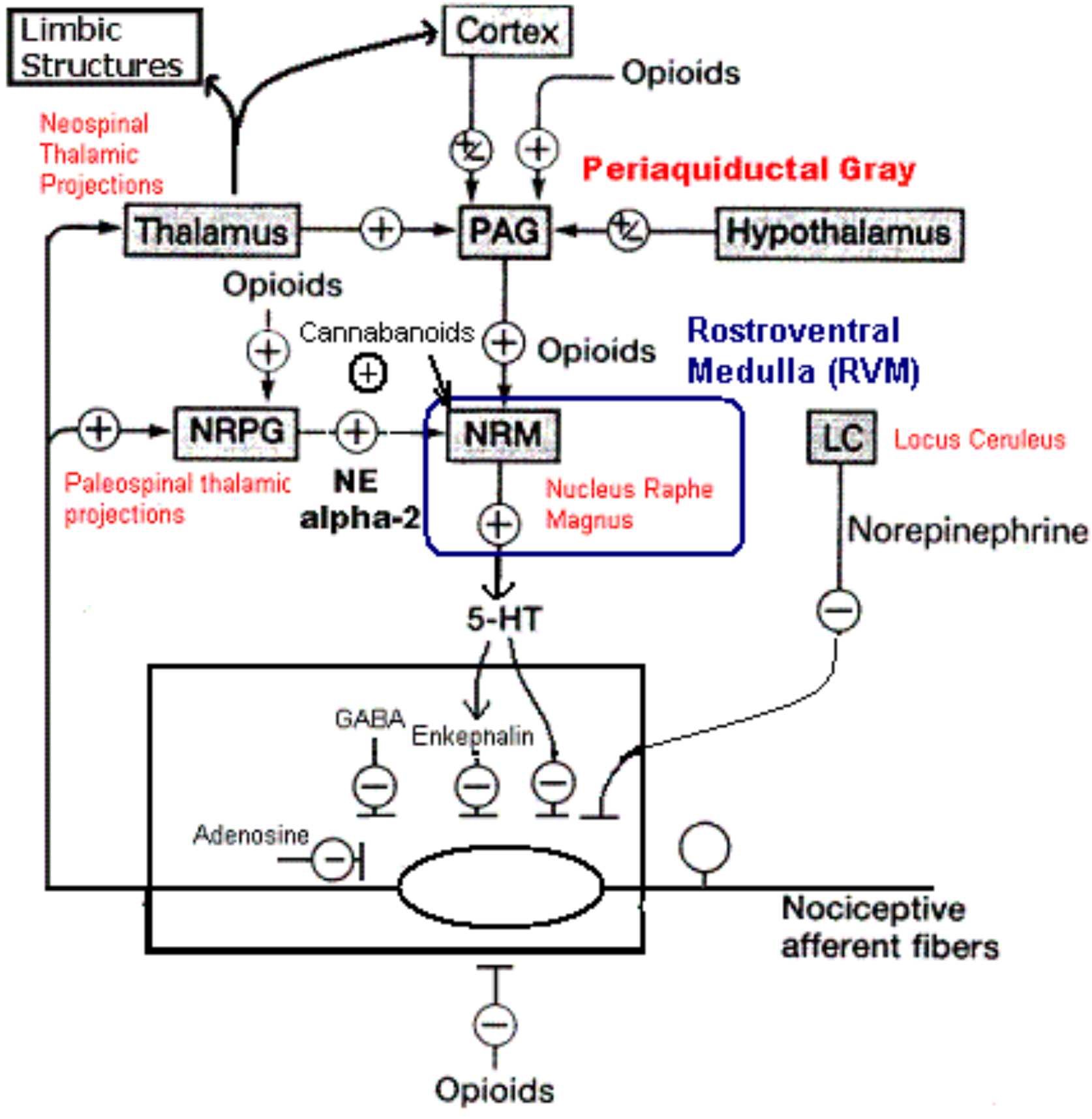
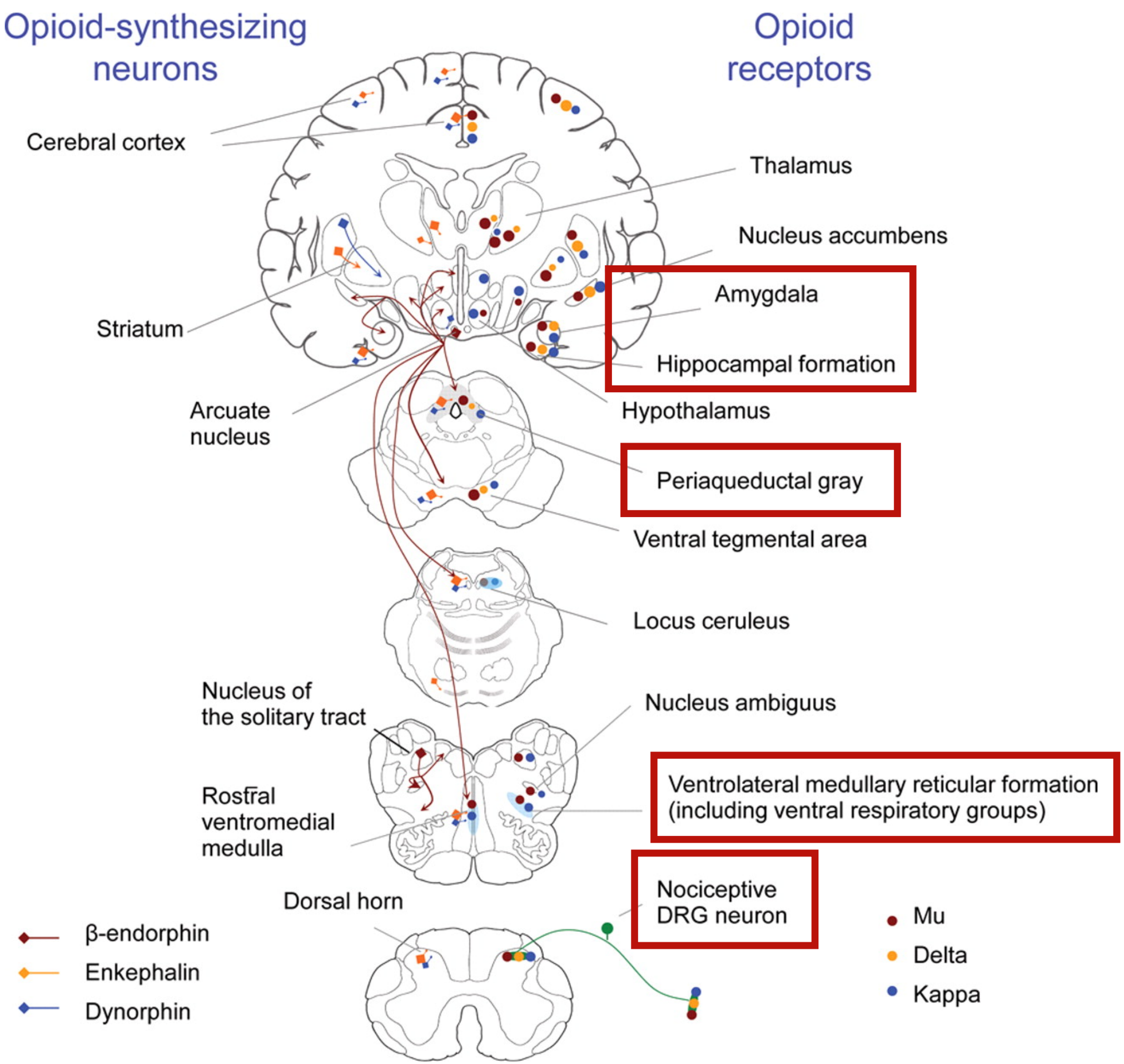


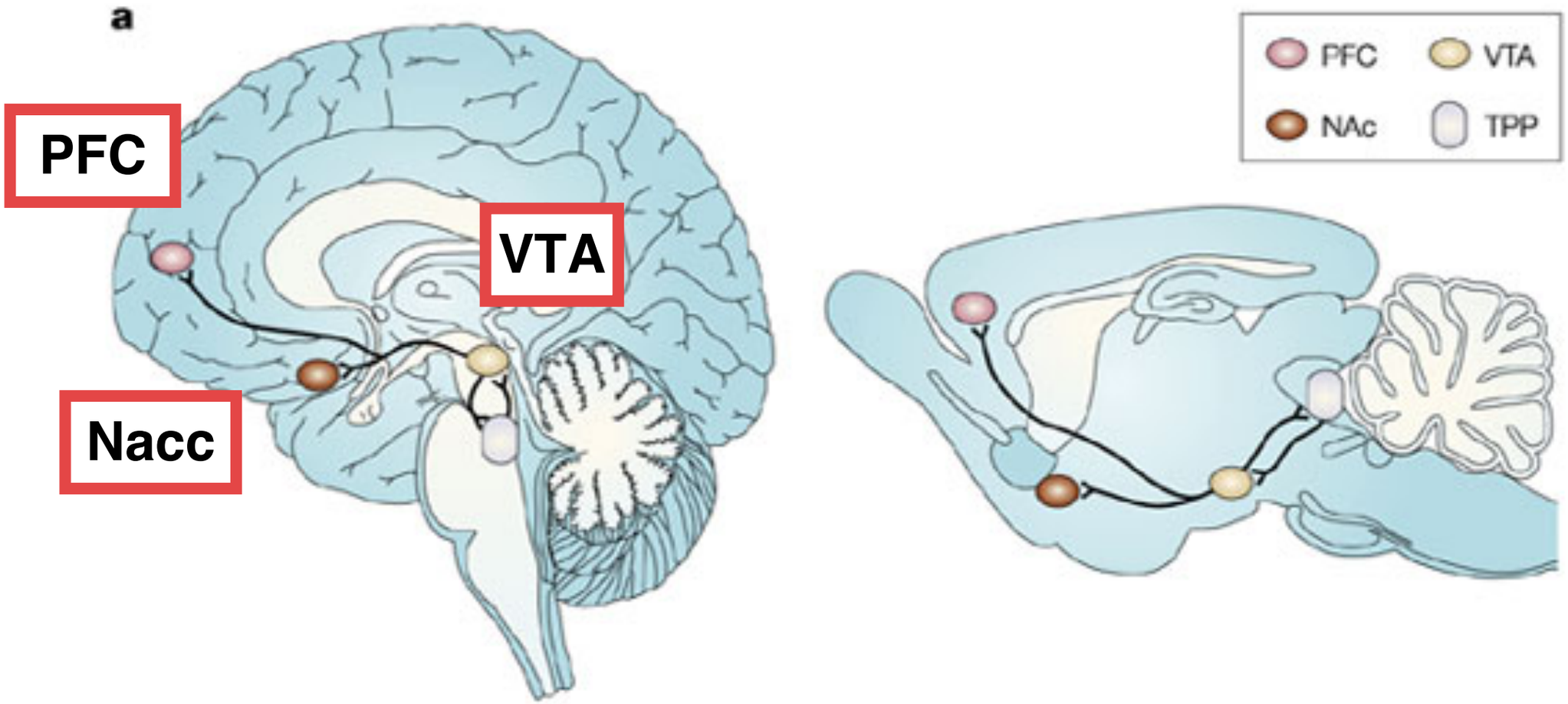
GS substantia gelatinosa

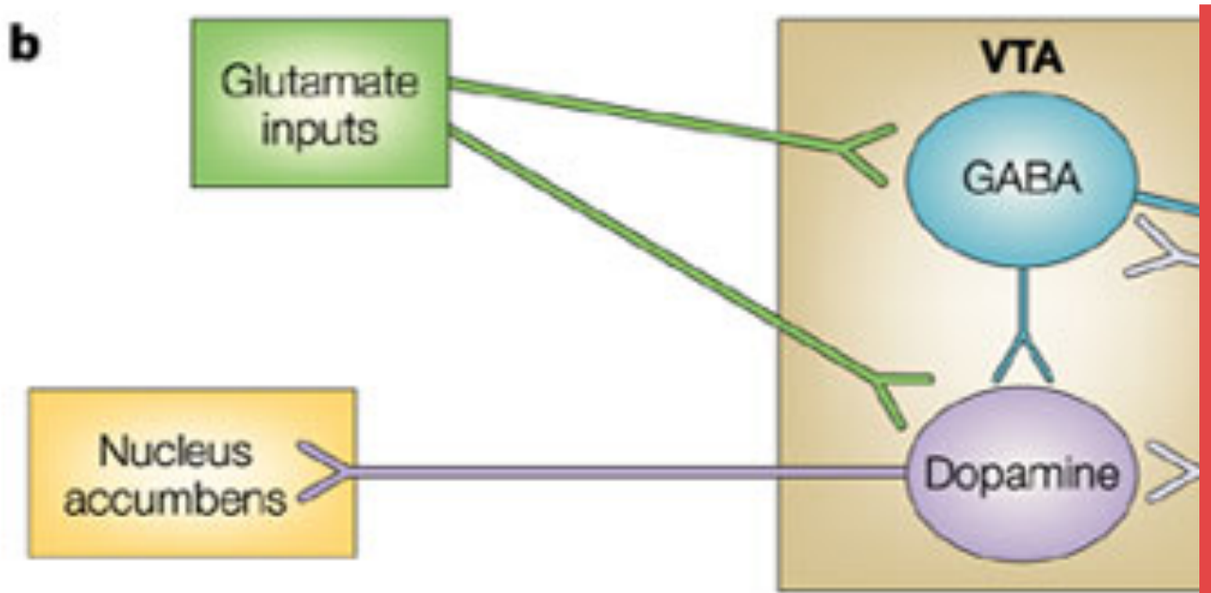
Pain pathways: an overview




Opioid-synthesizing neurons
Opioid receptors
Cerebral cortex
Thalamus
Nucleus accumbens
Striatum
Amygdala
Hippocampal formation
Arcuate nucleus
Hypothalamus
Periaqueductal gray
Ventral tegmental area
Locus ceruleus
Nucleus of the solitary tract
Nucleus ambiguus
Rostral ventromedial medulla
Ventrolateral medullary reticular formation (including ventral respiratory groups)
Dorsal horn
Nociceptive DRG neuron
β-endorphin
Enkephalin
Dynorphin
Mu
Delta
Kappa

THE MESOLIMBIC DOPAMINE PATHWAY:
THE REWARD CIRCUIT AND THE BASIS FOR DRUG ABUSE





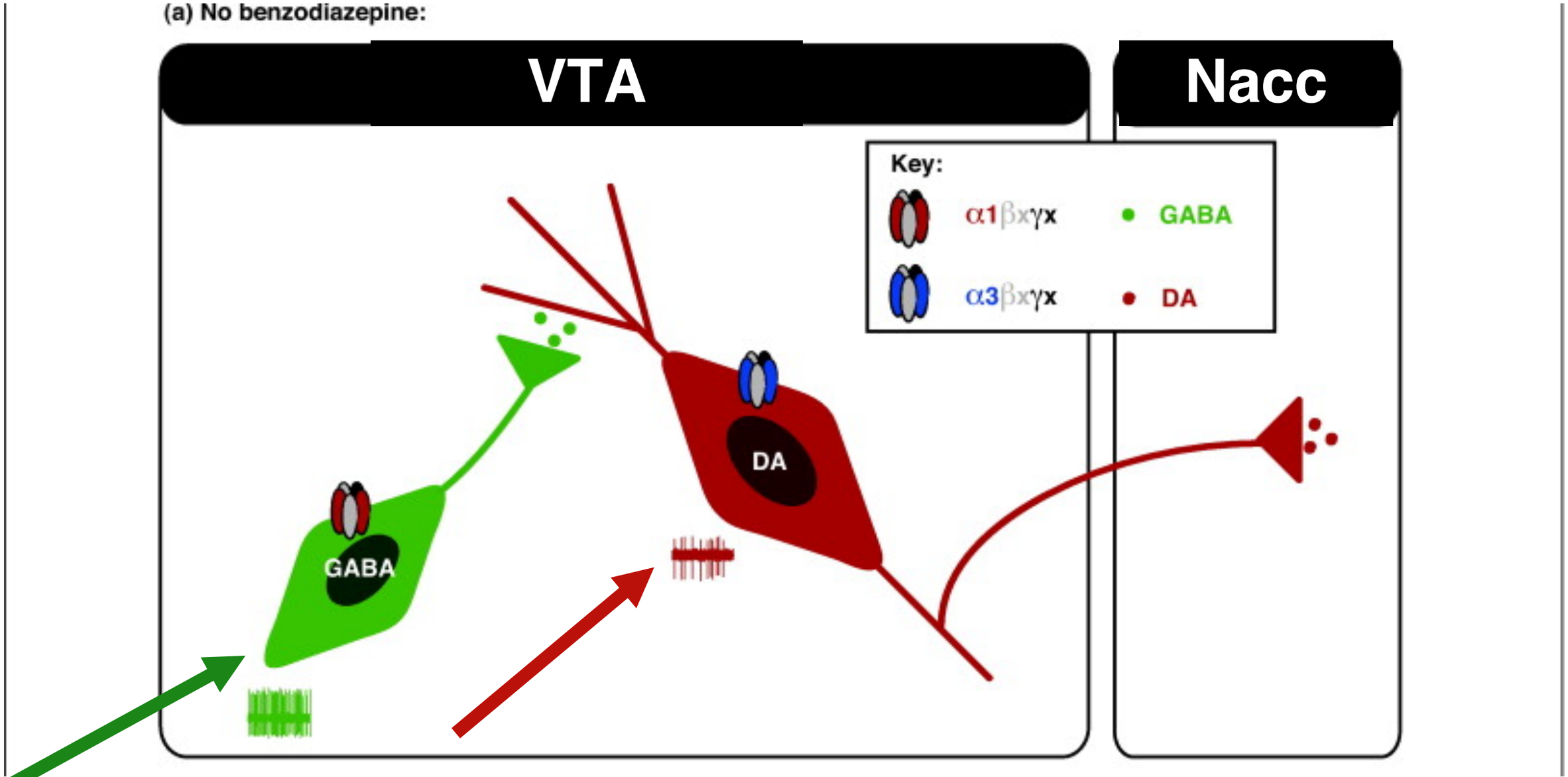
VTA ventral tegmental area

Nacc nucleus accumbens

PFC prefrontal cortex

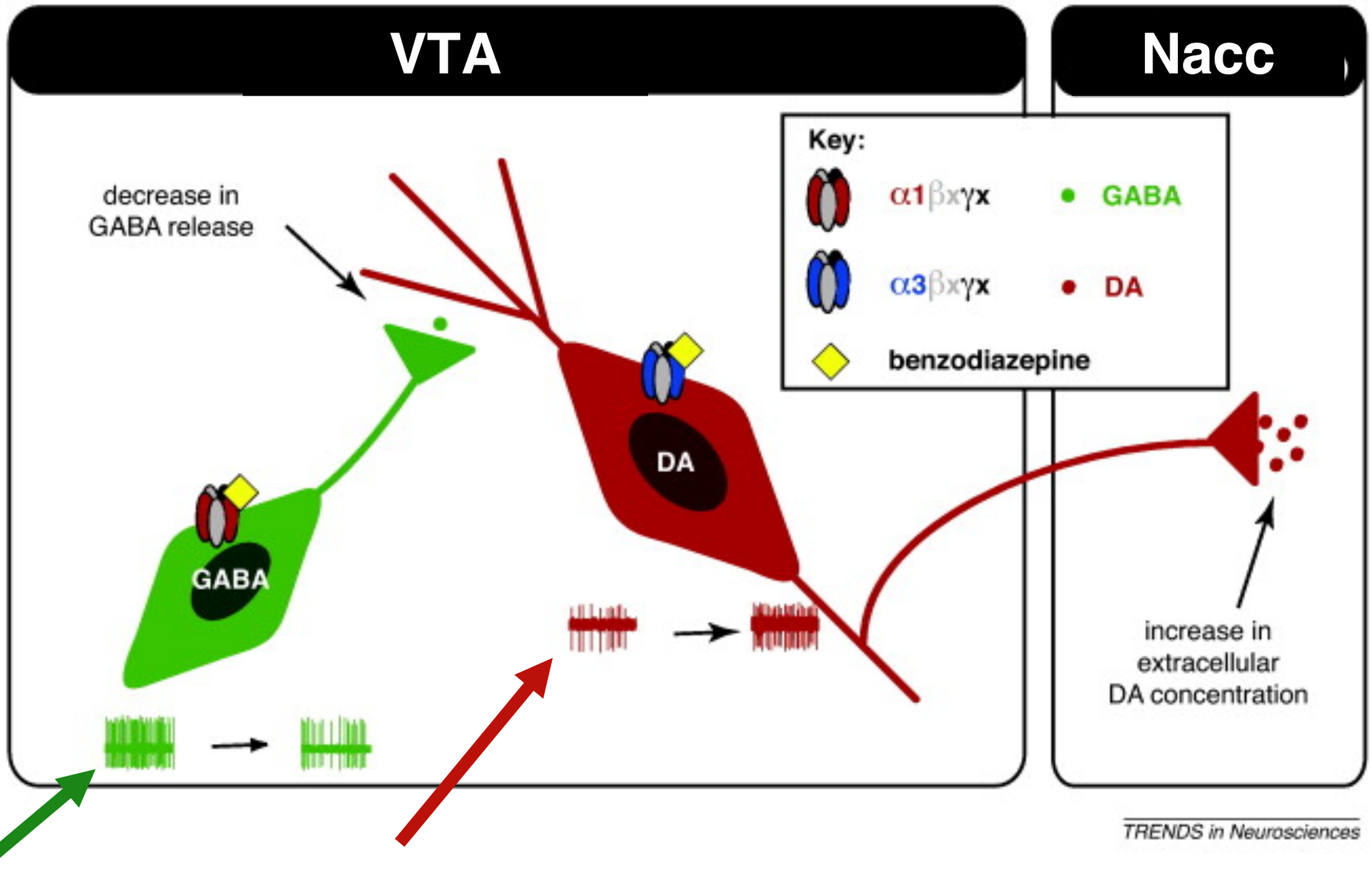
THE MESOLIMBIC DOPAMINE PATHWAY: THE REWARD CIRCUIT AND THE BASIS FOR DRUG ABUSE

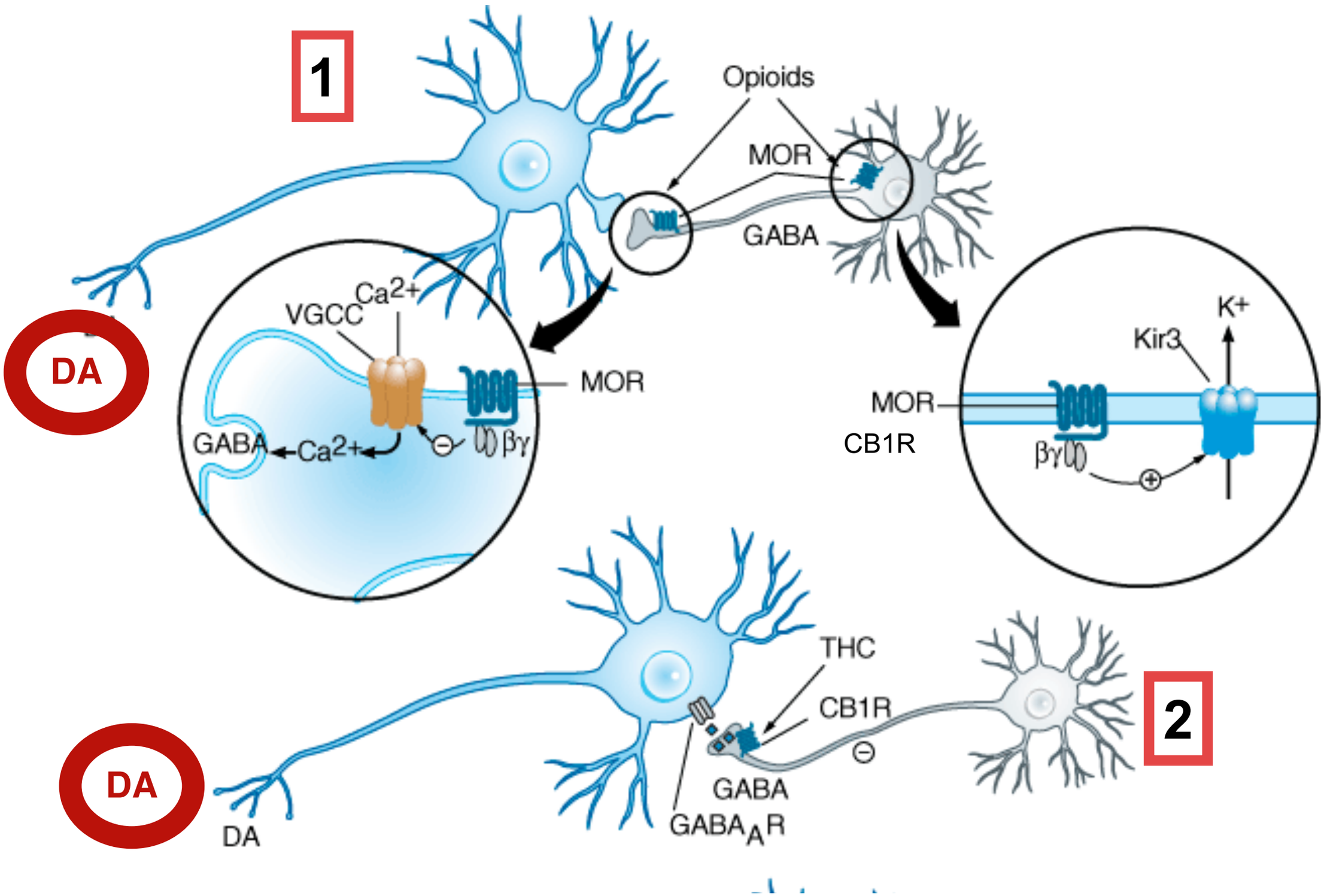
The dopaminergic neurons activity in the VTA is negatively controlled by the basal activity of GABAergic neurones



All drugs that cause disinhibition of dopaminergic neurons are potentially drug of abuse

Disinhibition mechanism of dopaminergic neurons in the VTA by benzodiazepines





Disinhibition of dopaminergic neurons in the VTA by opioid (1) and cannabinoid (2) receptors expressed on GABAergic neurons