Affective disorders and neuropathic pain as mutually influential factors: contribution of the opioid system

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A Adrià,

por su apoyo incondicional

A mi madre, mi abuelo y mi abuela,

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"Que tu deseo marque tu objetivo,
y tus acciones te dirijan hacia él"
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Abstract

Chronic pain is a major clinical issue producing huge economic and social burdens. Currently, chronic neuropathic pain treatment has limited efficacy and significant side effects. One of the reasons of this unmet clinical need is the insufficient knowledge of the exact mechanisms involved in the development and maintenance of neuropathic pain. Additionally, therapeutic approaches often overlook pain comorbidities that definitely impair nociceptive manifestations. Thus, addressing affective and cognitive disorders associated to neuropathic pain also represents an important challenge that may improve the efficacy of treatments. The high inter-individual variability in the neuropathic pain manifestations may lead to differential response of patients to treatments, and suggest the suitability of more personalized therapies rather than general guidelines. In the present thesis we have first studied the influence of behavioural traits on chronic neuropathic pain manifestations using different behavioural, electrophysiological and genetic approaches. The endogenous opioid system is a crucial therapeutic target for the management of moderate to severe nociceptive and inflammatory pain. However, the function of the opioid system during neuropathic pain is not well understood. Thus, we have evaluated the involvement of specific central and peripheral mu and delta opioid receptors populations modulating nociceptive, emotional, cognitive and neurochemical manifestations of chronic neuropathic pain. We have identified the endogenous delta opioid receptor as an interesting pharmacological target to limit nociceptive and affective phenotypes associated to neuropathic pain, whereas adverse consequences of mu opioid receptor activity after nerve injury were revealed.

Resumen

El dolor crónico es un problema clínico grave con una enorme carga económica y social. Actualmente, el tratamiento del dolor neuropático crónico presenta una eficacia limitada y efectos adversos significativos. Una de las razones de esta necesidad clínica insatisfecha es el escaso conocimiento de los mecanismos exactos que están involucrados en el desarrollo y mantenimiento del dolor neuropático. Además, los enfoques terapéuticos a menudo subestiman la importancia de las comorbilidades que acompañan al dolor, las cuales indudablemente deterioran las manifestaciones nociceptivas. Por ello, tratar los trastornos afectivos y cognitivos asociados al dolor neuropático también representa un desafío importante que puede mejorar la eficacia de los tratamientos. La alta variabilidad interindividual en las manifestaciones de dolor neuropático puede conducir a una respuesta diferencial de los pacientes a los tratamientos, y sugiere la idoneidad de terapias más personalizadas en lugar de pautas generales. En esta tesis hemos estudiado en primer lugar la influencia de los rasgos conductuales en las manifestaciones del dolor neuropático crónico utilizando aproximaciones conductuales, electrofisiológicas y genéticas. El sistema opioide endógeno es una diana terapéutica crucial para el tratamiento del dolor inflamatorio y nociceptivo moderado o intenso. Sin embargo, la función del sistema opioide en el dolor neuropático no está del todo clara. Por ello, hemos evaluado el papel de poblaciones específicas de receptores mu y delta opioides a nivel periférico y central en la modulación de las manifestaciones nociceptivas, emocionales, cognitivas y neuroquímicas del dolor neuropático. Así, hemos identificado el receptor delta opioide como una diana farmacológica interesante para restringir los síntomas nociceptivos y afectivos asociados al dolor neuropático, mientras que se demostraron las consecuencias adversas de la actividad del receptor mu opioide tras una lesión nerviosa.

Abbreviations

5-$HT_{1}/_{3}$: serotonin receptors 1/3

AMPA: α-amino-3-hydroxyl-5-methyl-4-isoxazeloproprionic acid

ATP: adenosine triphosphate

AUC: area under the curve

BDNF: brain-derived neurotrophic factor

CA1, CA3: specific regions of the hippocampus

cAMP: cyclic adenosine monophosphate

CB1: cannabinoid receptor type 1

CB2: cannabinoid receptor type 2

CCL2: chemokine (C-C motif) ligand 2 or monocyte chemoattractant protein 1 (MCP-1)

CD11B: cluster of differentiation molecule 11B

CD14: cluster of differentiation molecule 14

CeA: central amygdala

CFA: complete Freund's adjuvant

CNS: central nervous system

COX: cyclooxygenase (1, 2, 3)

CREB: cAMP response element-binding

CX3CR1: CX3C chemokine receptor 1 or fractalkine (CX3CL1) receptor

CXCL1: chemokine (C-X-C motif) ligand 1

DAMGO: D-Ala2, N-MePhe4, Gly-ol]-enkephalin (MOR agonist)

DOR: delta opioid receptor

DRG: dorsal root ganglion

DSM-V: Diagnostic and Statistical Manual of Mental Disorders

EOS: endogenous opioid system

ERK: extracellular signal-regulated kinase

fMRI: functional magnetic resonance imaging

GABA: gamma-Aminobutyric acid

Gadd45: Growth arrest and DNA-damage-inducible protein

GFAP: glial fibrillary acidic protein

GIRK: G protein-coupled inwardly rectifying potassium channels

GNTI: 5'-guanidinonaltrindole (KOR antagonist)

GPCR: G protein-coupled receptor

IASP: Association for the Study of Pain

IBA1: ionized calcium binding adaptor molecule 1

IL: interleukin (IL-1β, IL-6, IL-17, IL-18)

IFN-γ: interferon gamma

JNK_{1-3}: c-Jun N-terminal kinase 1-3

KOR: kappa opioid receptor

LTD: long-term depression

LTP: long-term potentiation

MAPK: mitogen-activated protein kinase

mGluR: metabotropic G-protein coupled glutamate

MMP-2: matrix metalloprotease 2

MOR: mu opioid receptor

NMDA: N-methyl-D-aspartate

NO: nitric oxide

NOR: nociceptin receptor

Npas4: Neuronal PAS Domain Protein 4

Nr3c1: Nuclear Receptor Subfamily 3 Group C Member 1

NSAID: Non-steroidal antiinflammatory drugs

ORL-1: opioid receptor like 1

P2X4: P2X purinergic receptor 4

PAG: periaqueductal grey matter

PBS: phosphate buffered saline (Na_2HPO_4/NaH_2PO_4/NaCl buffer)

PDYN: prodynorphin

PENK: preproenkephalin

PET: positron emission tomography

PG: prostaglandins (PGE_2, PGI_2)

PKA, PKB, PKC: protein kinase A, B, C

PNOC: pronociceptin

POMC: proopiomelanocortin

RVM: rostral ventromedial medulla

SNC80: 4-[(R)-[(2S,5R)-4-allyl-2,5-dimethylpiperazin-1-yl](3-methoxyphenyl)methyl]-N,N-diethylbenzamide (DOR agonist)

SNRIs: serotonin-norepinephrine reuptake inhibitors

TLR4: toll-like receptor 4

TNFα: tumor necrosis factor α

TRPV1: transient receptor potential vanilloid 1

Tsc22d3: TSC22 domain family protein 3

List of figures

List of tables

Index

INTRODUCTION

1. Physiology of pain

1.1 Definition

Pain is defined by the International Association for the Study of Pain (IASP) as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (Merskey and Bogduk, 1994). It is important to note that, as a subjective experience, the lack of expression or communication of pain does not preclude its existence, as happens in particular populations, such as newborns, unconscious people and demented people. Under physiological conditions, pain is aimed to alert from external or internal stimuli that can potentially induce tissue damage, so it has a clear protective role. However, pain can also be considered itself a disease, when it loses its warning function.

Perceived painful experience results from the integration of two main components (Aliaga *et al*, 2002):

- Nociceptive or sensorial: painful sensation secondary to the transmission of painful stimuli from nerves to the brain cortex.
- Affective or reactive: the suffering associated to pain, which depends on the cause of pain and the subjective assessment of the situation, the limitations that pain implies and the consequences. Many psychological factors can also modify the perception of the painful experience.

1.2 Classification

Pain has been classified in many ways considering the duration (acute, chronic), the intensity (mild, moderate, severe), the localization (cervical, spinal, pelvic, leg, arm, shoulder), the association to disease

(rheumatism, cancer, neuropathic) or the underlying pathophysiological mechanisms (nociceptive, inflammatory and neuropathic). For the purpose of the aims of this thesis, we focus on duration and pathophysiological classifications.

1.2.1 Acute vs chronic pain

The distinction between acute and chronic pain is due to a temporally issue, but also because of the essential differences in the physiological and pathophysiological mechanisms of these two pain modalities (Aliaga *et al*, 2002). Generally, acute pain is defined as an immediate sensorial event in the nociceptive system. Somatic or visceral tissue damage leads into acute pain that is maintained during the process of tissue healing. Thus, a good relationship exists between pain and injury. Chronic pain is described as a pain that lengthens beyond the injury and remains once the lesion disappears. Persistent pain is accompanied by physical, emotional, social or cognitive abnormalities that diminish the quality of life of patients (Aliaga *et al*, 2002).

The transition from acute to chronic pain has been the focus of intense study. Chronic pain is characterized by peripheral, spinal cord and cortical reorganization processes (Apkarian *et al*, 2013). In addition to neuronal mechanisms, the involvement of immune and glial cells in the development of chronic pain from acute tissue injury is also currently well accepted (Austin and Moalem-Taylor, 2010; Ren and Dubner, 2010; Scholz and Woolf, 2007). Further details of these mechanisms are described below, when reporting the pathophysiology of chronic neuropathic pain.

1.2.2. Classification based on pathophysiology

According to the pathophysiological mechanisms underlying pain, it can be divided into nociceptive, inflammatory and neuropathic pain (Cervero, 1991). Briefly, nociceptive pain refers to that caused by short and noxious stimuli, which are followed by transient stimulation of nociceptive pathways without significant tissue injury. In this case, painful sensation should continue as long as the stimulus is present.

By contrast, in inflammatory and neuropathic pain the injury triggers mechanisms of repair and the release of many molecules that produce pain and sensitize nociceptive fibres, by reducing their activation threshold. As a consequence of peripheral or central sensitization, inflammatory and neuropathic pain present sensory aberrations and the relationship between stimulus and painful response is almost completely lost. Once the process of healing has finished, inflammatory pain usually disappears, although in some cases it may persist leading to chronic pain. However, neuropathic pain usually lengthens beyond the injury and remains once the lesion disappears, thereby losing the protective role of pain (Costigan *et al*, 2009).

1.3 Nociceptors and nociceptive fibres

Nociceptors are sensory structures specialized in detecting different modalities of noxious stimuli and converting them into a membrane depolarisation and action potentials (Serra Catafau, 2007). They are located in peripheral terminals of afferent neurons responsible for the pain stimuli transmission. All primary afferent neurons have the cellular body in the dorsal root ganglion (DRG) and two axonal prolongations with pseudo-unipolar morphology. The central prolongation ends into

the dorsal horn of the spinal cord, whereas the peripheral axon ends in peripheral organs and constitutes the sensory fibre (Basbaum *et al*, 2009). There are two main types of nerve fibres conveying pain information: C fibres and Aδ fibres (Table 1). In both cases the stimuli come from the skin, muscle and joint tissues or certain visceral structures (Dubin and Patapoutian, 2010). They do not present a clear ending receptor structure and are commonly identified as free nerve endings. High activation threshold and multimodal stimuli detection are features of nociceptive fibres that differ them from other sensory fibres transmitting innocuous information (Serra Catafau, 2007; Woolf and Ma, 2007).

Aδ fibres are thinly myelinated, so they can conduct a first and fast (5-30 m/s) well-localized mechanical and cold pain signal. They can also convey information coming from intense mechanical or thermal stimulation (Basbaum *et al*, 2009). By contrast, C fibres are unmyelinated and conduct impulses at less than 2 m/s. They are related with slow, diffuse and long-lasting pain. According to the cytochemical content, they can be divided into non-peptidergic and peptidergic fibres. Both of them express the transient receptor potential vanilloid 1 (TRPV1), which responds to heat and capsaicin, but only peptidergic C fibres contain peptides such as substance P and calcitonin gene related peptide (Usoskin *et al*, 2015). While peptidergic C fibres mainly mediate thermal pain transmission, non-peptidergic C fibres are polymodal nociceptors that transmit noxious information regarding heat, cold, mechanical and chemical stimuli (Basbaum *et al*, 2009; Cavanaugh *et al*, 2009).

Table 1. Primary afferent axons arriving to the spinal cord (adapted from Serra Catafau, 2007)

Fibre	Myelin	Diameter (µm)	Velocity (m/s)	Function	Dorsal horn lamina
Aα	Yes	13-20	80-120	Propioception of skeletal muscle	III-VI
Aβ	Yes	6-12	35-75	Touch, Low Threshold MechanoReceptors (LTMR)	III-VI
Aδ	**Yes**	**1-5**	**5-30**	Touch and temperature perception	I, IIo and V
				Pain, Mechanical and cold nociceptors	**I, IIo and V**
C	**No**	**0.2-1.5**	**0.5-2**	**Polymodal nociceptors (Non-peptidergic C-fibres: mechanical, heat, cold and chemical pain)**	**IIi**
				Thermal nociceptors (Peptidergic C-fibres: pain)	**I-IIo**

The transduction of the nociceptive information starts in the periphery, where a noxious stimulus activates the nociceptor endings by stretching or bending the nociceptor surface or by promoting the activation of membrane ion channels. Anyhow, nociceptor activation induces the generation of action potentials that are transmitted to the spinal cord, where the signals are integrated and transmitted to other areas.

1.4 Ascending pain pathways and supraspinal integration

Nociceptive information arising in the periphery travels along the primary nociceptive neurons, whose soma are located in the DRG, and enters into the spinal cord by the dorsal roots. Following the dorsal root entry, nociceptive inputs travel within the zone of Lissauer before entering the grey matter of the spinal cord (substantia gelatinosa). Central terminals of Aδ fibres contact to second order neurons mainly placed in laminae I, II and V. Peptidergic C fibres mostly terminate in laminae I and outer II,

whereas non-peptidergic C fibres synapse with second order neurons in inner lamina II Figure 1 (Willis and Coggeshall, 2004). Excitatory amino acids (glutamate and aspartate) are the main neurotransmitters involved in these first relays, but neuropeptides (substance P and calcitonin gene related peptide) and purines (ATP) act as co-transmitters in peptidergic and non-peptidergic nociceptors, respectively, to enhance pain transmission (Julius and Basbaum, 2001).

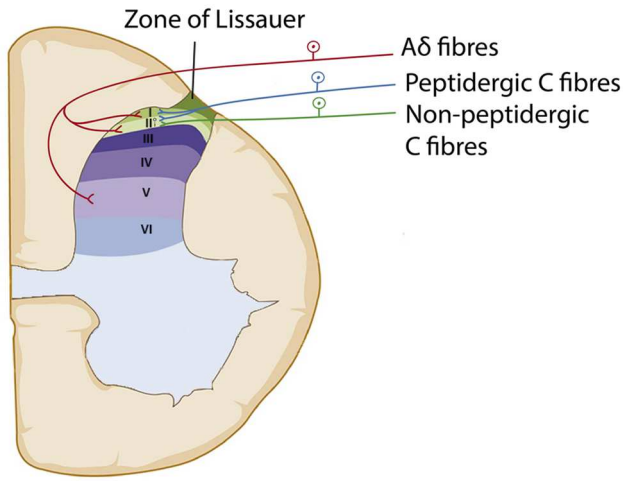

Figure 1. Termination sites of Aδ, peptidergic and non-peptidergic C fibres in the spinal cord.

Second order neurons decussate at the spinal level to the contralateral side and project nociceptive information directly to thalamic structures (spinothalamic tract) or indirectly making synapse first with the bulbar reticular formation (spinoreticular tract) or mesencephalic superior colliculus and periaqueductal grey matter (PAG, spinoencephalic tract). These three tracts constitute the anterolateral system, the main ascendant pathway involved in the transmission of nociceptive information to supraspinal areas. Painful ascending signals can relay in

lateral or medial thalamic nuclei to finally project to cortical areas, where the different components of pain are integrated. The thalamus is not merely a relay centre but is involved in processing nociceptive information. Generally, each group of thalamic nuclei has prominent functions in different components of pain perception. The ventral posterior nucleus is one of the lateral nuclei that includes the ventral posteromedial and posterolateral regions, which has somatotopic representations of the head and the trunk and limb, respectively. The posterior complex, which consists of many territories, is another lateral thalamic nucleus implicated in pain perception. On the other hand, the dorsomedial nucleus and intralaminar nuclei belong to medial thalamic structures involved in pain processing (Ab Aziz and Ahmad, 2006). The lateral pathway terminates in the primary and secondary somatosensorial, insular and orbitofrontal cortices and is associated with sensory-discriminative aspects of pain (location, intensity and quality). The medial system ends in the anterior cingulate cortex and the nucleus accumbens and is involved in the affective-motivational component of pain (unpleasant feelings, fear, anxiety) (Serra Catafau, 2007). The anterior cingulate cortex, in addition, is important for certain cognitive aspects of pain, including anticipation, attention and evaluation (Figure 1) (Seminowicz *et al*, 2004). The spinoreticular and spinomesencephalic tracts are also considered components of the medial system. Since these tracts send collaterals to several areas related to vegetative and homeostatic processes, such as the reticular formation, PAG, hypothalamus and superior colliculus (tectum), the medial system is also involved in autonomic reactions secondary to pain (Serra Catafau, 2007).

Subsequently, other prefrontal cortical areas and subcortical structures, such as the amygdala, have been included in the so-called “pain matrix”

or the “homeostatic afferent processing network” (Neugebauer *et al*, 2004). These brain areas may play a role in “secondary pain affect”, which includes the conscious awareness and cognitive evaluation of pain (Price, 2000). Purely nociceptive information reaches the central amygdala (CeA) directly from spinal cord and brainstem (parabrachial area, PB), thus bypassing the thalamus. Polymodal sensory, including nociceptive, inputs from thalamus and cortex (insular cortex and association cortices) target the lateral amygdala. Associative processing in the lateral-basolateral amygdala network is believed to attach emotional significance to sensory information and play an important role in fear and anxiety (Phelps and LeDoux, 2005). Highly processed affect-related information is then transmitted to the CeA, which can modulate pain behaviour through projections to descending pain control centres in the brainstem (Neugebauer, 2006) (Figure 2). The amygdala may also contribute to certain cognitive aspects of pain, since the neural circuit between basolateral amygdala and prefrontal cortex is crucial for decision-making based on reward expectancy, risk anticipation and punishment avoidance (Floresco and Ghods-Sharifi, 2006).

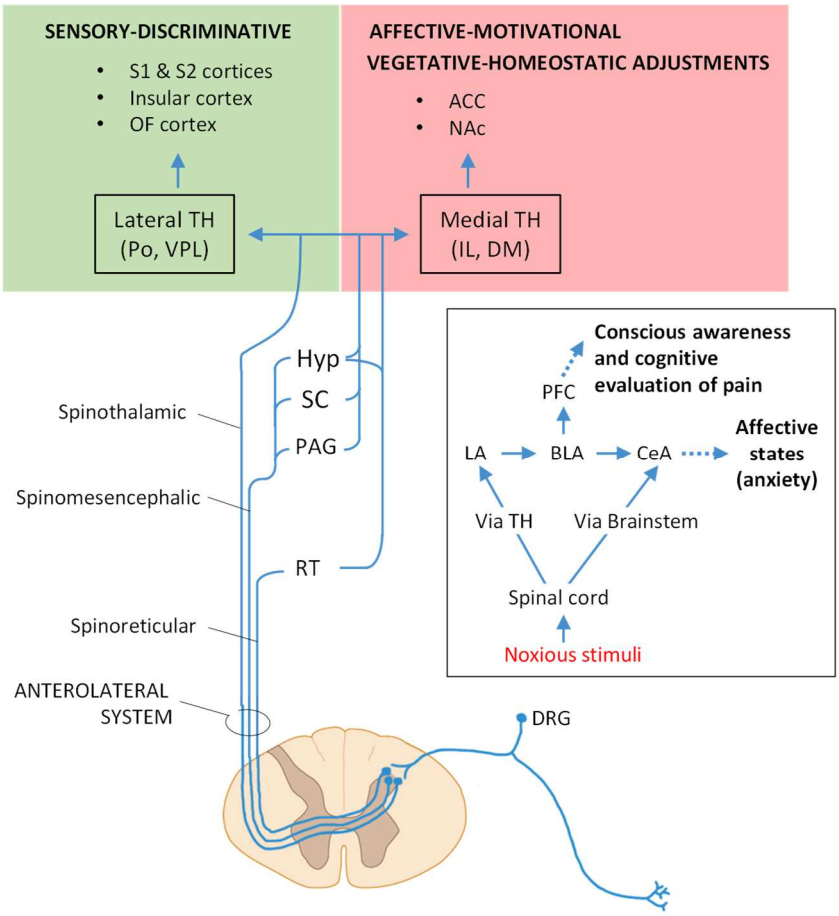

Figure 2. Ascending pain transmission and supraspinal integration of different aspects of pain. ACC, anterior cingulate cortex; BLA, basolateral amygdala; CeA, central amygdala; DM, dorsomedial nucleus; Hyp, hypothalamus; IL, intralaminar nuclei; LA, lateral amygdala; NAc, nucleus accumbens; OF, orbitofrontal cortex; PAG, periaqueductal grey matter; PFC, prefrontal cortex; RT, reticular formation; Po, posterior complex; S1, primary somatosensorial cortex; S2, secondary somatosensory cortex; SC, superior colliculus; TH, thalamus; VPL, ventroposterolateral nucleus. Adapted from (Neugebauer *et al*, 2009; Serra Catafau, 2007).

1.5 Descending control of pain

Once the nociceptive information arrives to the higher-level centres, it is integrated in order to elicit a complex physiological response in front of the noxious stimuli, and this information is modulated to reduce the intensity of the painful sensation. The main mechanisms for pain modulation are organized in the descending pathway (Figure 3). Several areas in the midbrain and the brainstem are involved: PAG, parabrachial nucleus, medullary reticular formation, locus coeruleus and rostral ventromedial medulla (RVM). Chemical neurotransmission of the neurons of these encephalic structures involves noradrenaline, serotonin, dopamine, opioid, cannabinoid and histamine, among others (Fields *et al*, 1991; Purves *et al*, 2012). They exert both excitatory and inhibitory effects on different sets of dorsal horn neurons. They can act by monosynaptic connections or intraspinal circuits on central terminals of primary nociceptive afferents, interneurons (excitatory and inhibitory), synaptic terminals of other descending pathways, and second order or projection neurons. These contacts control the balance between excitation and inhibition in the spinal cord.

PAG receives afferences from cortical (primary and secondary somatosensory as well as insular cortices) and subcortical (amygdala and hypothalamus) structures involved in pain processing, and triggers different analgesic descending systems (Purves *et al*, 2012). One of them is the classical PAG-RMV-dorsal horn pathway, which is predominantly serotonergic and opioid dependant. The spinal tract containing descending pathways from the RVM is the ipsilateral dorsolateral funiculus. Briefly, there are two main cell subpopulations in the RVM, OFF and ON cells, which exhibit phasic reciprocal changes in firing that precede nociceptor-elicited withdrawal reflexes (Fields *et al*, 1983; Fields

and Heinricher, 1985). Endogenous opioids released by PAG inhibit ON-cells and disinhibit OFF-cells. Activation of OFF-cells correlates with inhibition of nociceptive input, whereas ON-cell action is to facilitate nociceptive transmission in the dorsal horn of the spinal cord (Fields, 2004; Porreca *et al*, 2002). The facilitating or inhibitory effect at dorsal horn level will depend on the type of serotonergic receptor (inhibitory [5-HT_1] or excitatory [5-HT_3]) to which serotonin binds.

PAG also sends projections to locus coeruleus, parabrachial nucleus and medullary reticular formation, which represent the starting point of additional non-opioid analgesic pathways (Purves *et al*, 2012). For example, locus coeruleus neurons, once depolarised, release noradrenaline that hyperpolarizes nociceptive second order neurons by binding to α-adrenergic receptors.

Local circuits within the dorsal horn also play a role in modulating the nociceptive system. One of these systems was early proposed by Wall and Melzack, and was conceptualized under the called gate control theory of pain (Melzack and Wall, 1965), which actually is included in the afferent regulatory system of pain. This theory states that the activation of mechanoreceptors (Aβ fibres) can act on local interneurons to inhibit the transmission of information from C fibres to the dorsal horn projection neurons. This would explain how a mechanical stimulus, such as scratching, can temporarily give relief from pain in the same area.

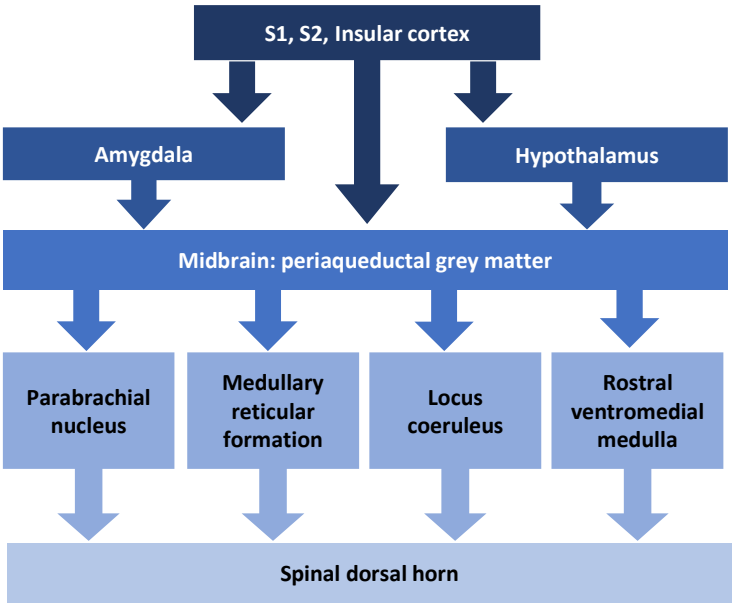

Figure 3. Descending control of pain. S1, primary somatosensory cortex; S2, secondary somatosensory cortex (adapted from Purves, 2012).

1.6 Management of pain

From a pharmacological point of view, drugs can be classified depending on the chemical structure, mechanism of action or the pharmacological effects. All these classifications often present some limitations, such as in the case of drugs with analgesic effects. Thus, we choose the classification that divides analgesics in primary and secondary (Table 2), as previously described (Aliaga *et al*, 2002). The primary are usually known as analgesics and are mainly used for this purpose. They basically include cyclooxygenase (COX) inhibitors and opioid agonists. The secondary analgesics were not developed for pain treatment and later have been used as analgesics. Many secondary analgesics are used as main analgesics for resistant pain or used as co-adjuvants.

Table 2. Classification of analgesic drugs according to their main therapeutic use (Aliaga *et al*, 2002)

Primary:
NSAID (ibuprofen, naproxen, metamizole, sulindac)
analgesic-antipyretic (paracetamol)
selective inhibitors of cox-2 (celecoxib)
opioid analgesics (morphine)
Secondary:
psychoactive drugs (benzodiazepines, antidepressants)
antiepileptic (gabapentin, pregabalin)
vasodilator and vasoconstrictor agents
glucocorticoids
local anaesthetics (lidocaine)
other (capsaicin, caffeine, guanethidine)

1.6.1 Primary analgesics

Non-steroidal antiinflammatory drugs (NSAID) inhibit COX, which converts arachidonic acid in prostaglandins (PG) and thromboxanes. Their analgesic effect is based on the reduction or prevention of nociceptor and spinal sensitization to algogens (e.g. bradykinin), due to the inhibition of PGE_2 and PGI_2 synthesis. In the case of paracetamol, its mechanism of analgesic action is not completely known. It is a weak inhibitor of COX_1 and COX_2 and it has been suggested that it may induce analgesia by acting on central COX_3, a COX_1 isoform, given that it has not peripheral anti-inflammatory effect (Aliaga *et al*, 2002).

Opioid analgesics act on classical mu opioid receptors (MOR), which are G protein-coupled receptors widely expressed at central and peripheral sites within the pain control circuits. MOR inhibit pain transmission at different levels of the ascending pain pathways (Stein and Machelska, 2011), supraspinal areas related to pain integration (Ossipov *et al*, 2010) and also participate in inhibitory and facilitating descending pathways by being recruited in PAG and RVM (Fields, 2004; Ossipov *et al*, 2010).

Basically they inhibit neuronal transmission by hyperpolarizing and decreasing transmitter release. Opioid drugs can be classified according to their affinity to opioid receptors and intrinsic activity or in accordance to their relative efficacy. Table 3 shows a classification of the main opioid analgesics considering both criteria. The route of administration depends on the specific opioid drug ranging from enteral to parenteral and transdermal (Aliaga *et al*, 2002). The role of opioids as painkillers is further described in section 4.5 (*role of endogenous opioid system in pain*).

Table 3. Classification of opioid analgesics according to their affinity to opioid receptors, intrinsic activity and relative efficacy (Aliaga *et al*, 2002)

Pure agonists (MOR agonists)
-High analgesic potency, used for moderate to severe pain • Morphine • Heroine • Oxicodone • Fentanyl and derivatives (remifentanyl) •Tramadol • Methadone -Low analgesic potency, used for mild to moderate pain • Codeine and dihydrocodeine • Dextropropoxyphene
Mixed agonist-antagonists (KOR agonists, MOR antagonists)
-Nalbuphine, butorphanol
Parcial agonists
-Buprenorphine (high intrinsic activity) -Pentazocine (low intrinsic activity)

1.6.2 Secondary analgesics

Antidepressants are drugs with proven analgesic properties, although this action is not extendable to the entire pharmacological group. Tricyclic antidepressants, especially amitriptyline, and serotonin-norepinephrine reuptake inhibitors (SNRIs), such as duloxetine, are those

that present greater analgesic power (McCleane, 2008; Micó *et al*, 2006). It should be noted that only some particular conditions of chronic pain are susceptible to being controlled with antidepressants. The medical condition that better responds to this pharmacological group is the chronic neuropathic pain, although encouraging results have been obtained in some cases of fibromyalgia (Acuña, 2008; Saarto and Wiffen, 2007). Serotonin and noradrenaline are two main transmitters released in the spinal cord by the descending analgesia system to inhibit pain transmission. Thus, the restoration of the descending inhibitory pathways by increasing spinal serotonin and noradrenaline levels constitutes the main mechanism of analgesic action of these drugs (Baron *et al*, 2010; Kremer *et al*, 2016).

Antiepileptic drugs are another pharmacological group considered as secondary analgesics. These drugs were developed to counteract seizures, which reflex neuronal hyperexcitability. Therefore, antiepileptics inhibit the excitability by blocking excitatory neurotransmission or favouring the action of inhibitory mediators such as gamma-Aminobutyric acid (GABA) (Aliaga *et al*, 2002). The potentiation of GABA-mediated inhibitory mechanisms includes the inhibition of GABA transaminase, GABA synergy, modulation of enzymes involved in the synthesis and metabolism of GABA and inhibition of GABA reuptake (Battistin *et al*, 1984). Regarding the inhibition of excitatory processes, a common mechanism shared by many antiepileptic drugs is the blockade of voltage gated cation (Na^+, Ca^{2+}) channels or the inhibition of α-amino-3-hydroxyl-5-methyl-4-isoxazeloproprionic acid (AMPA) or N-methyl-D-aspartate (NMDA) receptors-mediated responses (Tomić *et al*, 2018). These mechanisms are also responsible for the analgesic effect of antiepileptic drugs in some pain conditions, such as neuropathic pain

(gabapentin, pregabalin, carbamazepine, oxcarbacepine) and migraines (valproate, topiramate), which might share pathophysiological similarities with seizures (Aliaga *et al*, 2002).

Glucocorticoids are used as analgesics due to their potent and unspecific anti-inflammatory effect. The mechanism of action is based on their interaction with α glucocorticoid receptors that enhance the transcription of genes encoding anti-inflammatory proteins, such as the lipocortin I, secretory leukocyte protease inhibitor, antagonist of interleukin 1 receptor and interleukin 10. Glucocorticoids also inhibit the synthesis of pro-inflammatory genes by interacting with AP-1 and NF-κB transcription factors. These effects lead to the restriction of inflammatory responses and the subsequent attenuation of pain. Glucocorticoids are often administered by epidural or intraarticular route for non-cancer pain and usually by systemic route for cancer pain in combination with other analgesics (Aliaga *et al*, 2002).

Capsaicin produces analgesia by activating first and subsequent desensitisation of the TRPV1 following repeated administration. Topical application of capsaicin cream was effective in alleviating neuropathic pain, but its side effects, such as burning sensation, limit its use as first or second line treatment (Mason *et al*, 2004).

1.6.3 Cannabinoids as analgesics

The endogenous cannabinoid system is an important endogenous analgesic system. Cannabinoid drugs induce analgesic effects in multiple pain models including inflammatory and neuropathic pain. The action of both endogenous and exogenous cannabinoids is due to the interaction with cannabinoid receptor type 1 (CB1) and type 2 (CB2). CB2 receptors

are mainly expressed in immune and glial cells. Thus, CB2 receptor activation produces peripheral (Ibrahim *et al*, 2005) and spinal (Taylor, 2009) analgesic effect, without psychotropic effects. Analgesic action of CB1 receptors is predominantly mediated by the central inhibition of painful stimuli, with a lower peripheral involvement (Ledent *et al*, 1999; Meng *et al*, 1998). At the spinal level, CB1 receptors are found mainly in the dorsal horn. Most of the primary afferent neurons that express CB1 receptor mRNA are large diameter fibres involved in the non-nociceptive sensitivity (Hohmann and Herkenham, 1999). However, CB1 receptor is also expressed in nociceptive C fibres, where it inhibits the release of neurotransmitters involved in pain transmission (Drew *et al*, 2000; Wilson and Nicoll, 2002). At the supraspinal level, the endocannabinoid system inhibits pain transmission acting on the ascending pathways, mainly at the thalamus level (Martin *et al*, 1999) and modifies the subjective interpretation of pain by modulating neuronal activity in limbic structures, such as amygdala (Manning *et al*, 2003). CB1 receptors in the prefrontal cortex participate in stress-induced analgesia (Woodhams *et al*, 2017). Another central mechanism for endocannabinoid system-mediated antinociception is the modulation of the descending inhibitory pathways. CB1 receptors are recruited in the PAG and RVM where they inhibit GABA release and enhance OFF-cells activity (Marinelli *et al*, 2002). One of the limitations of the CB1 receptor agonists are the intrinsic psychotropic effects, which cannot be separated from the antinociceptive effects and are not tolerated by many patients (Burns and Ineck, 2006; Rodríguez de Fonseca *et al*, 2005). Consequently, CB2 agonists devoid of these side effects represent a potential analgesic target that has been extensively investigated in animal models of chronic pain in the last years (Ibrahim *et al*, 2005; Romero-Sandoval *et al*, 2008).

2. Neuropathic pain

2.1 Definition and classification

IASP defines neuropathic pain as pain that arises as a direct consequence of a lesion or diseases affecting the somatosensory system (Treede *et al*, 2008). According to the location of the injury, neuropathic pain is classified as central (from damage to the brain or spinal cord) or peripheral (from damage to the peripheral nerve, plexus, dorsal root ganglion, or nerve root). Neuropathic pain is also classified on the basis of the aetiology of the insult to the nervous system (Table 4) (Cousins *et al*, 2010).

Table 4. Classification of neuropathic pain (adapted from Cousins *et al*, 2010)

***Location**:*
peripheral (nerve, plexus, dorsal root ganglia, root)
central (spinal, brainstem, thalamus, cortex)
***Aetiology**:*
trauma
ischemia or haemorrhage
inflammation
neurotoxic
neurodegeneration
paraneoplastic
metabolic
vitamin deficiency
cancer

The reason why the same condition can be painful in some patients and painless in others remains unknown. Currently a comprehensive mechanism-based classification of neuropathic pain is not possible, because specific pain mechanisms in each patient cannot be always revealed. Moreover, one mechanism can be responsible for many different symptoms, and the same symptom can be caused by different mechanisms (Woolf and Mannion, 1999). There is not either a clear relationship between the symptoms and the causative disease. Thus, it is

not possible to determine the aetiology of neuropathic pain exclusively from the clinical characteristics of the pain (Attal *et al*, 2008).

2.1 Epidemiology

Neuropathic pain is usually underdiagnosed and undertreated. This common type of pain is associated with suffering, disability and impaired quality of life, which represents a large economic burden for the whole society and a challenge to health care. Thus, the current estimates of the direct and indirect costs for Europe run into the billions of euros (Breivik *et al*, 2013).

Nearly one in five Europeans (19%) suffers from chronic pain. The exact prevalence of neuropathic pain is unknown. According to general European population studies, 7–8% of adults currently have chronic pain with neuropathic characteristics (Bouhassira *et al*, 2008; Torrance *et al*, 2006). The prevalence is even higher in specific subpopulations (Table 5). Aged people, female gender and the prevalence of mental disorders may be susceptible factors to promote chronic neuropathic pain (Butler *et al*, 2013).

Table 5. Prevalence of neuropathic pain in European population (adapted from Cousins *et al*, 2010)

General population	7-8%	*Bouhassira et al., 2008; Torrance et al., 2006*
Specific subpopulations		
postsurgical herniotomy	10%	*Aasvang et al., 2008*
herpes zoster	8%	*Galil et al., 1997*
stroke	8%	*Andersen et al., 1995*
multiple sclerosis	28%	*Österberg et al., 2005*
spinal cord injury	67%	*Finnerup et al., 2001*
diabetes	26%	*Abbott et al., 2011*
HIV	50%	*Schütz and Robinson-Papp, 2013*
cancer	~20%	*Bennett et al., 2012*

2.2 Clinical characteristics

2.2.1 Nociceptive and sensorial manifestations

Neuropathic pain is characterized by altered sensation comprising negative and positive symptoms (Figure 4). Negative symptoms include deficits of different somatosensory qualities, such as tactile and thermal hypoesthesia or anaesthesia, pinprick hypoalgesia, and loss of vibratory sensation. These symptoms are uncomfortable but not painful. Positive symptoms refer to enhanced or painful sensations, which can be spontaneous or evoked by stimulation. Spontaneous positive symptoms include paraesthesia and dysesthesia, as well as paroxysmal and ongoing superficial pain. Two of the most typical clinical manifestations of patients with neuropathic pain are hyperalgesia and allodynia, two stimuli-evoked positive symptoms (Nickel *et al*, 2012). Definitions of these pain terms are listed in Table 6.

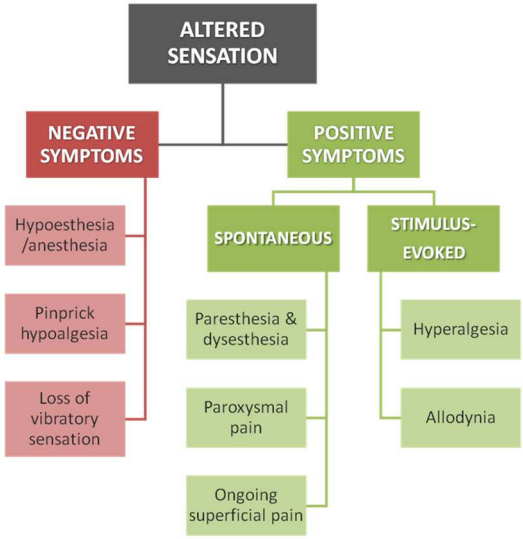

Figure 4. Nociceptive and sensorial manifestations of neuropathic pain (adapted from Nickel *et al*, 2012)**.**

Table 6. Definitions of common symptoms suggestive of neuropathic pain (Merskey and Bogduk, 1994)

Negative symptoms:	
Hypoesthesia	Decreased sensitivity to stimulation (tactile or thermal)
Anaesthesia	Lack of sensitivity to stimulation (tactile or thermal)
Hypoalgesia	Diminished pain response to a normally painful stimulus
Positive symptoms:	
Paraesthesia	An abnormal sensation
Dysesthesia	An unpleasant sensation
Paroxysmal pain	Intermittent spontaneous pain
Ongoing pain	Continuous spontaneous pain
Hyperalgesia	An increased response to a stimulus that is normally painful
Allodynia	Pain due to a stimulus that does not normally activate the nociceptive system

Another characteristic that can be used to distinguish nociceptive pain from neuropathic pain is the quality of the pain. There are plenty of descriptors referred by patients that can help clinicians to diagnose neuropathic pain: sharp, shocking, burning, shooting, pressing, pricking, pulsating, crushing, cramping dull, electric, radiating, stabbing, cold, penetrating or stinging, among many other (Merskey and Bogduk, 1994). Other symptoms and clinical findings (e.g., motor paresis, muscle cramps, and autonomic nervous symptoms) may also appear depending on the injury site.

2.2.2 Emotional manifestations

Neuropathic pain felt by patients is usually described as severe painful sensation that greatly influences their daily activities, such as walking, climbing stairs or housekeeping. Like other chronic pain conditions, neuropathic pain is frequently accompanied by emotional disorders with prevalence that range from 33% to 42% (Langley et al., 2013). Clinical studies consistently reported that these patients suffer fatigue, anxiety

and sadness, as well as high rate of depression and suicide (McWilliams *et al*, 2003; Nicolson *et al*, 2009; Soler *et al*, 2007). These comorbid affective disorders exert a major negative effect on their quality of life and negatively impact response to pain treatment.

Multiple animal studies using different neuropathic pain models showed increased anxiety-like behaviours in mice with a peripheral nerve injury (Narita *et al*, 2006a, 2006b; La Porta *et al*, 2016; Roeska *et al*, 2008). The development of depressive-like behaviour following a nerve injury was also reported in preclinical studies, as shown by increased behavioural despair in the forced swimming test and decreased sucrose preference (indicative of anhedonia) in neuropathic animals (Gonçalves *et al*, 2008; Leite-Almeida *et al*, 2009; La Porta *et al*, 2016; Suzuki *et al*, 2007; Wang *et al*, 2011). However, no effect of nerve injury on behavioural despair was previously reported using the tail suspension test (Benbouzid *et al*, 2008b; Hasnie *et al*, 2007), suggesting paradigm-related differences in the assessment of neuropathic pain-induced depressive-like behaviour.

Many evidences point to the amygdala as an important neural substrate of the interaction between pain and emotion (Meagher *et al*, 2001). Neuroimaging pain studies using positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) have revealed amygdala hyperactivation in humans (Petrovic *et al*, 1999) and animals (Paulson *et al*, 2002). Pain-related amygdala plasticity has also been reported in animal models of neuropathic pain (Ikeda *et al*, 2007). Enhanced membrane excitability and increased neurotransmission at the CeA synapses through NMDA receptor-independent synaptic plasticity were revealed in neuropathic rats (Ikeda *et al*, 2007). Pain-related changes in other amygdala projecting areas, such as the prefrontal

cortex, have also been suggested to contribute to the emotional and emotion-based cognitive consequences of chronic pain (Neugebauer *et al*, 2009).

The causal relationship between persistent pain and negative affective states is difficult to establish because of their reciprocal influence. Experiencing pain contributes to a negative affective state and, conversely, a negative affective state magnifies and worsens pain perception. Thus, an integrative therapeutic approach targeting also emotional comorbidities of neuropathic pain seems crucial to improve the quality of life of these patients.

2.2.3 Cognitive manifestations

Chronic pain is commonly associated with the impairment of cognitive functions (prevalence of 11.4%), which makes difficult its clinical management (Langley *et al*, 2013; Moriarty *et al*, 2011). Dysfunction of a wide range of cognitive outputs have been reported in chronic pain patients including attention, concentration, speed processing, memory, learning, psychomotor ability, decision-making and executive function (Apkarian *et al*, 2004; Dick *et al*, 2002; Muñoz and Esteve, 2005). The negative impact of neuropathic pain on the attention capability (Low *et al*, 2012), recognition and working memory (Kodama *et al*, 2011; Leite-Almeida *et al*, 2009; Ren *et al*, 2011), cognitive flexibility (Moriarty *et al*, 2016), decision-making (Neugebauer *et al*, 2009) and hippocampal-dependent fear extinction (Mutso *et al*, 2012) has also been demonstrated in rodents, providing further support for the human reports in clinical settings.

Strong resemblances and associations have been found between pain and cognitive processing. Some neuroanatomical substrates involved in cognition, such as the medial prefrontal cortex and the hippocampus, also participate in pain processing, which suggests a reciprocal modulation of each other. Central sensitization is a mostly accepted cellular model of pain hypersensitivity (Woolf, 2011). On the other hand, long-term potentiation (LTP) is a synaptic plasticity phenomenon involved in learning and memory (Collingridge *et al*, 2010; Neves *et al*, 2008). Striking similarities in the mechanisms of central sensitization and LTP were revealed after pain induction and during the maintenance phase of chronic pain (Ji *et al*, 2003), raising the possibility of shared cellular/molecular substrates between pain and cognition. In addition, the presence of LTP has been demonstrated in many pain-related central nervous system (CNS) areas, such as the spinal cord (Sandkühler, 2007), primary somatosensory cortex (Wang *et al*, 2010a), anterior cingulate cortex (Lu *et al*, 2014), insular cortex (Liu *et al*, 2013; Qiu *et al*, 2013), the amygdala (López de Armentia and Sah, 2007) and the hippocampus (Liu and Chen, 2009; Zhao *et al*, 2009).

Several theories have arisen regarding the mechanisms that mediate cognitive impairment in persistent pain. One of them claims that the neurochemical mediators and neuroplastic changes produced under chronic pain may alter neural circuitries and interfere with cognitive functioning (Hart et al., 2000). In its favour, many authors reported functional synaptic plasticity in cognition-related brain areas under neuropathic pain conditions. Several models of peripheral nerve injury showed LTP deficits (Kodama *et al*, 2007; Liu and Chen, 2014; Ren *et al*, 2011; Tanabe *et al*, 2008) and short-term plasticity impairment in hippocampal synapses (Mutso *et al*, 2012; Ren *et al*, 2011), which may

correlate with cognitive deficits. Impaired LTP in the hippocampus was also observed in streptozotocin-induced diabetic neuropathy models (Biessels *et al*, 1998; Kamal *et al*, 2000). Similarly, the loss of long-term depression (LTD) and reduced LTP were reported in the anterior cingulate cortex in peripheral nerve injury-induced neuropathic pain models (Li *et al*, 2010; Wei *et al*, 1999; Zhao *et al*, 2006). Other pain-related functional changes were observed in the anterior cingulate cortex of neuropathic animals, such as increased excitatory synaptic transmission and enhanced neuronal excitability (Xu *et al*, 2008). Besides these functional changes, peripheral nerve injury induces various forms of structural plasticity in the amygdala, prefrontal cortex, primary somatosensory area, anterior cingulate cortex, insular cortex and the hippocampus of neuropathic animals (Gonçalves *et al*, 2008; Mutso *et al*, 2012; Ren *et al*, 2011; Seminowicz *et al*, 2009). Therefore, it is reasonable to suggest that chronic pain-induced cortical and hippocampal plasticity may be a triggering factor of cognitive impairment under such conditions.

2.2.4 Other manifestations

Beside emotional disorders and cognitive impairment, sleep disturbances (prevalence of 37%-60%), pain-related fear or deficits in social behaviour are other important comorbid manifestations of neuropathic pain (Langley *et al*, 2013). These symptoms may be independent of sensorial manifestations (Dimitrov *et al*, 2014). Thus, a further effort must be made to evaluate these comorbid symptoms in animal models of neuropathies.

Therefore, it is of great importance the design and development of therapeutic strategies that not only tackle nociceptive alterations, but also deal with the comorbid manifestations associated with long-lasting pain experience.

2.3 Mechanisms of neuropathic pain after peripheral nerve injury

Neuropathic pain is characterized by pain in the absence of stimulus and reduced nociceptive thresholds so that normally innocuous stimulus produces pain. Knowledge of the cellular and molecular mechanisms of neuropathic pain has advanced with the development of many experimental models of nerve injury (Kumar *et al*, 2018). These models have shown that the development of neuropathic pain involves not only neuronal alterations, but also immune and glial cells that share reciprocal signalling pathways with neurons, as described below.

2.3.1 Peripheral and central sensitization

Mechanisms perpetuating neuropathic pain can be divided in six types of maladaptive changes in the peripheral, central and autonomous nervous system: sensitization of nociceptors, abnormal ectopic excitability of affected neurons, pronociceptive facilitation at the spinal dorsal horn, disinhibition of nociception at the spinal inhibitory network, sympathetically maintained pain, and CNS reorganization processes (Figure 5) (Nickel *et al*, 2012).

Sensitization of nociceptors

A great variety of sensitizing agents are released after nerve injury, leading to the overactivation of nociceptors and lowering their activation threshold. These endogenous substances comprise inflammatory

mediators (bradykinin, prostaglandins and other derivates eicosanoids), neurotransmitters (excitatory amino acids, neurokinins, serotonin, noradrenaline, histamine), growth factors (nerve growth factor), protons and lipid metabolites (lysophosphatidic acid) (Julius and Basbaum, 2001; Ueda, 2008). These agents act on different receptors and alter intracellular signalling including second messengers (cyclic adenosine monophosphate, cAMP), protein kinases A and B (PKA, PKB), mitogen-activated protein kinase (MAPK), and nitric oxide signalling pathways (Hucho and Levine, 2007). The TRP ion channel family (TRPV1, TRPA1, TRPM8, among others) sense a broad repertoire of harmful signals released following neve injury, and therefore play a crucial role in the nociceptor sensitization process (Basso and Altier, 2017). Specifically, the well-known dysregulated expression and function of TRPV1 after peripheral nerve injury (Hong and Wiley, 2005) has been recently attributed to upregulated deubiquitinase USP5 under such conditions (Stemkowski *et al*, 2016). In summary, all these factors contribute to functional and structural changes in peripheral nociceptors, which perpetuate pain experience.

Abnormal ectopic excitability of affected neurons

After nerve injury, the concentration of voltage-gated sodium (Nav1.1 to Nav1.9) and calcium channels rises at the site of injury and in the whole axon (Luo *et al*, 2001; Yang *et al*, 2018). Consequently, a significant increase of sodium and calcium currents occurs in these neurons leading to spontaneous discharges of primary afferent sensory fibres and the release of substance P and glutamate, that further sensitize nociceptors (Hong *et al*, 2004; Misawa, 2012). Spontaneous discharge of Aδ and C fibres results in lancinating and burning pain, whereas paraesthesia and

dysesthesia are caused by altered excitability in Aβ fibres (Nickel *et al*, 2012).

The two abovementioned mechanisms usually lead to spontaneous painful sensations, whereas enhanced stimulus-evoked painful sensations, such as hyperalgesia and allodynia, are clinical signs mainly related to central sensitization (Woolf and Mannion, 1999).

Pronociceptive facilitation at the spinal dorsal horn

The major excitatory neurotransmitter of the pain system is glutamate and both ionotropic and metabotropic glutamate receptors are involved in the transmission of peripheral pain signals (Nickel *et al*, 2012). Activation of AMPA receptor in the dorsal horn neurons mediates the basic response to acute painful stimuli. Under pathological conditions, such as in neuropathic pain, ongoing nociceptive input triggers several mechanisms that result in LTP of noxious stimuli and hyperexcitability of spinal dorsal horn projecting neurons (D'Mello and Dickenson, 2008). Calcitonin gene related peptide and substance P released from C fibre terminals promote the disinhibition of spinal NMDA receptors and the ensuing calcium-dependent neurochemical changes in the postsynaptic neurons (Sandkühler, 2009; Suzuki *et al*, 2003). There are three classes of metabotropic glutamate receptors. Receptors of group I (mGluR1 and 5) activate phospholipase C (PKC), thereby enhancing synaptic transmission and neuronal discharge. In contrast, group II (mGluR2 and 3) and III (mGluR 4, 6, 7 and 8) receptors inhibit the adenylyl cyclase and reduce transmission of nociceptive signals (Pan *et al*, 2008). As a response to ongoing nociceptive input, metabotropic glutamate receptors of group I elicit adaptive changes (e.g. PKC-mediated activation of NMDA receptors,

activation of MAPK pathways, upregulation of c-fos expression, crosstalk with 5-HT_{2A} receptors) that contribute to synaptic plasticity (Aira *et al*, 2012; Vincent *et al*, 2016; Xie *et al*, 2017). Inflammatory mediators released from activated microglia also contribute to the pathologically enhanced pain signalling (Inoue *et al*, 2004), which will be described in more detail in section 2.3.2. As a result, nociceptive central neurons become activated not only by low level stimulation of C and Aδ fibres, but also by Aβ fibres. This fact is reflected in widespread peripheral receptive fields of nociceptive fibres and exaggerated stimulus-evoked painful sensations like hyperalgesia and allodynia (Nickel *et al*, 2012).

Increased spinal release of dynorphin also contributes to neuropathic pain by enhancing pain transmission in the spinal dorsal horn (Ossipov *et al*, 2000). The mechanisms of dynorphin-induced pronociceptive effects are diverse. Elevated levels of spinal dynorphin modulate NMDA receptor activity and promote further release of excitatory transmitters (glutamate, substance P and calcitonin gene related peptide) from primary afferent neurons, contributing to LTP at C-fibres synapses (Bian *et al*, 1999; Gardell *et al*, 2002; Labombarda *et al*, 2008). The activation of spinal bradykinin receptors by elevated spinal dynorphin has been correlated with maintenance of neuropathic pain-induced hypersensitivity (Bannister *et al*, 2014). Dynorphin-induced production of PGE_2 in the spinal cord (Koetzner *et al*, 2004) also contributes to its pronociceptive effect.

Disinhibition of nociception at the spinal inhibitory network

Dorsal horn projecting neurons play a pivotal role in pain transmission and their activity is modulated by several factors. Descending serotonergic, noradrenergic and dopaminergic pathways originating from

the PAG, locus coeruleus, and the RVM inhibit dorsal horn nociceptive neurons mainly by recruiting the endogenous opioid system (Nickel *et al*, 2012). Inhibitory interneurons within the dorsal horn constitute local circuits that also play a role in inhibiting nociceptive transmission (Nickel *et al*, 2012). Animal models of neuropathic pain showed a reduced activity and efficacy of descending inhibitory pathways (Zimmermann, 2001). Similarly, chronic pain is accompanied by a loss of glycinergic and GABAergic spinal inhibitory network (Zeilhofer, 2008). These adaptive mechanisms account for the disinhibition of the nociceptive input and the increased pain sensitivity.

Sympathetically maintained pain

Sympathetic nervous system interacts with the somatosensory system by direct and indirect mechanisms. The coupling between nociceptive afferent fibres and efferent sympathetic signalling at DRG level has been shown in animal studies and humans (Nickel *et al*, 2012). Histological studies showing sympathetic sprouting into DRGs provide evidence for increased coupling of sympathetic fibres to DRG neurons following peripheral nerve lesions (Shinder *et al*, 1999). Moreover, the expression of α-adrenoceptors on primary nociceptive fibres has also been demonstrated after nerve injury (Sato and Perl, 1991). Several studies suggested that sympathetic activity may directly induce nociceptive activation (Baron *et al*, 2002; Torebjörk *et al*, 1995). Therefore, these adaptive mechanisms may account for sympathetically-maintained pain in neuropathic pain syndromes.

The sympathetic system can also regulate vasomotor activity and inflammation. Under neuropathic pain conditions, increased sympathetically-mediated vasomotor activity impairs oxygenation and

leads to acidotic environment, in which protons act as a potent nociceptive stimulus (Birklein *et al*, 2000; Kurvers *et al*, 1995). Bradykinin-induced plasma extravasation, a key step in inflammatory processes, directly depends on sympathetic activity (Miao *et al*, 1996). All these mechanisms may indirectly contribute to sympathetically-maintained pain.

CNS reorganization processes

Finally, adaptive changes in the CNS have also been reported in neuropathic pain syndromes and experimental animal models. Cortical reorganization processes, including the primary somatosensory cortex, the somatosensory thalamus and motor cortices were revealed in patients with phantom limb pain (Flor *et al*, 1995), patients with complex regional pain syndrome (Maihöfner et al., 2003) and in rats following a partial sciatic nerve ligation (PSNL) (Brüggemann et al., 2001). These changes in the somatotopic representation areas of specific body parts lead to phenomena that cannot be explained by peripheral mechanisms, such as referred sensations in the amputated limb by tactile stimulation of other body parts (Ramachandran *et al*, 1992), hemisensory phenomena and neglect-like symptoms (Frettlöh *et al*, 2006) and allodynia/hyperalgesia in adjacent innervation areas of that of damaged nerve (Brüggemann *et al*, 2001).

Aside cortical reorganization, neuroplastic changes in thalamus and brain stem nuclei may also occur in neuropathic pain. PET studies revealed reduced regional cerebral blood flow in the contralateral thalamus in patients with mononeuropathy and post-traumatic neuropathic pain (Hsieh *et al*, 1995; Iadarola *et al*, 1995), which may reflect a protection mechanism against ongoing nociceptive input. Physiological and

biochemical changes in PAG and RVM under neuropathic pain conditions have been also reported (Seifert and Maihöfner, 2009; Vanegas and Schaible, 2004). More recently, maladaptive dendritic spine plasticity within dorsal horn neurons was observed in spinal cord and peripheral nerve injury models as well as in streptozotocin-induced peripheral diabetic neuropathy (Tan *et al*, 2009, 2011, 2012).

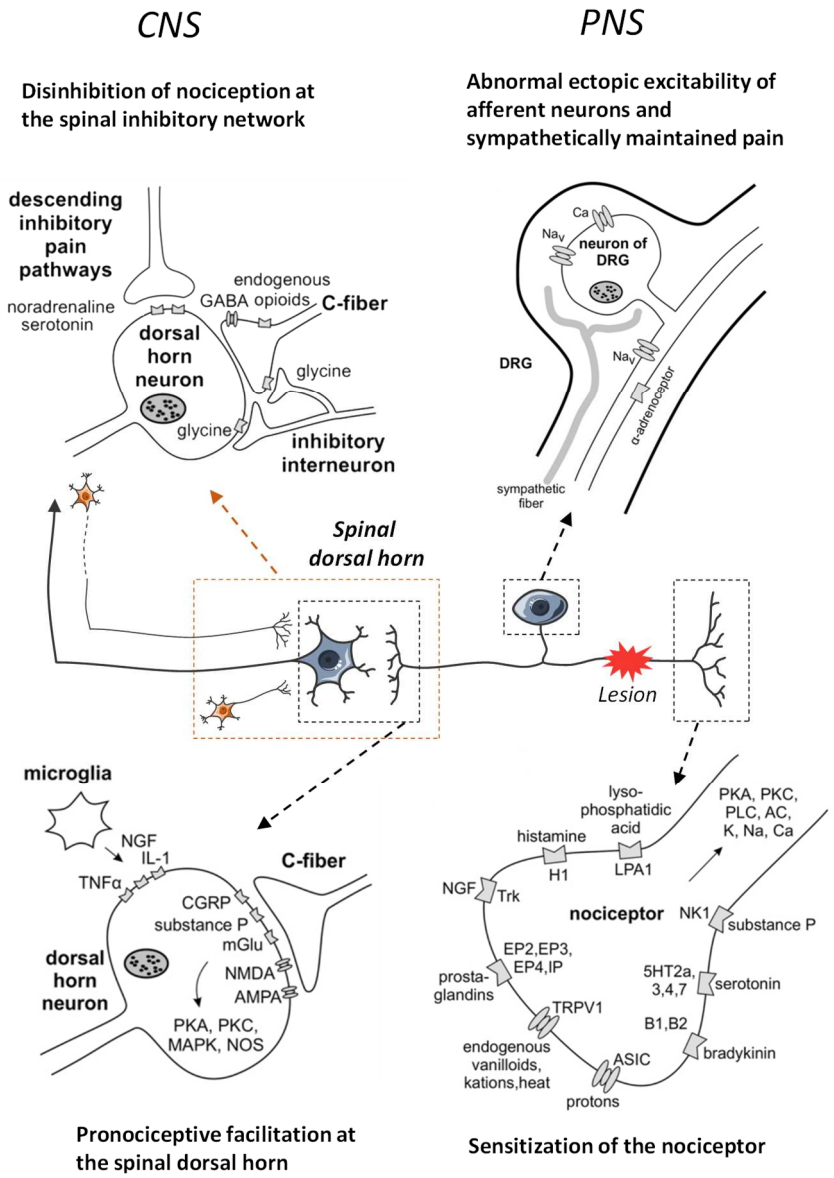

Figure 5. Synopsis of molecular mechanisms contributing to neuropathic pain. 5HT2a,3,4,7, serotonin receptors; AC, adenylate cyclase; AMPA, α-amino-3-hydroxyl-5-methyl-4-isoxazeloproprionic acid receptor; ASIC, acid-sensing cation channel; B1-B2, bradykinin receptors 1 and 2; Ca, calcium; CGRP, calcitonin gene related peptide; CNS, central nervous system; DRG, dorsal root ganglia; EP2-4 and IP, prostaglandin receptors; H1, histamine receptor 1; IL-1, interleukin 1; K, potassium; LPA_1, lysophosphatidic acid receptor 1; MAPK, mitogen-activated protein kinase; mGlu, metabotropic glutamate receptors; Na, sodium; Nav, voltage gated sodium channel; NGF, nerve growth factor; NK1, substance P receptor; NMDA, N-methyl-D-aspartate receptor; NOS, nitric oxide synthase; PKA, protein kinase A; PKC, protein kinase C; PNS, peripheral nervous system; TNFα, tumor necrosis factor α; Trk, NGF receptor; TRPV1, transient receptor potential vanilloid 1 (adapted from Nickel et al., 2012).

2.3.2 Neuroimmune interactions and neuropathic pain

Great body of evidence has shown that the pathogenesis of neuropathic pain is not restricted to an aberrant neuronal activity. Immune and glial cells also play an important role in the establishment and maintenance of neuropathic pain, as well as proinflammatory mediators released after nerve injury. Indeed, the similarities between neuropathic pain and neuroimmune disorders are increasingly accepted (Scholz and Woolf, 2007).

At peripheral level, damaged primary sensory neurons release “endogenous danger signals” as well as nociceptive and vasoactive mediators (substance P, bradykinin, nitric oxide and calcitonin gene related peptide) that activate resident mast cells and macrophages and promote the infiltration of circulating T lymphocytes, monocytes and neutrophils to the site of injury (Scholz and Woolf, 2007). Activated immune cells release several pro-inflammatory mediators, including

cytokines (TNFα, IL-1β, IFN-γ, IL-6, IL-17), chemokines (CXCL1, CCL2), nociceptive substances (histamine, bradykinin), reactive oxygen species, PGE_2 and PGI_2 and effectors of the complement cascade (C3a and C5a). These mediators promote neuroimmune activation and sensitise primary afferent neurons, contributing to pain hypersensitivity (Austin and Moalem-Taylor, 2010; Ellis and Bennett, 2013). At DRG level, damaged neurons, activated satellite glial cells and infiltrating blood-derived immune cells interacts with each other to enhance painful sensitivity, by releasing pro-inflammatory and algesic mediators and by disrupting tissue homeostasis (Capuano *et al*, 2009; Hu *et al*, 2007; Hu and McLachlan, 2002; Morin *et al*, 2007; Xie *et al*, 2009).

Sustained nociceptive input from peripheral tissues as well as the release of nociceptive and pro-inflammatory mediators from central terminals of primary afferent neurons activate not only spinal postsynaptic neurons, but also trigger glial reactivity (Deleo *et al*, 2004; Ren and Dubner, 2010). Activated microglia is characterized by the expression of several markers, such as type 3 complement receptors (CR3 or the subunit CD11B), ionized calcium binding adaptor molecule 1 (IBA1), cluster of differentiation molecule 14 (CD14) and toll-like receptor 4 (TLR4) as well as the activation of p38 MAPK, extracellular signal-regulated kinase (ERK) isoforms 1 and 2, and the Src-family kinases (Src, Lck and Lyn) (Jin *et al*, 2003; Katsura *et al*, 2006; Zhuang *et al*, 2005). In turn, microglial activation leads to further release of pro-inflammatory cytokines (IL-1β, IL-6, IL-18, TNFα), chemokines, brain-derived neurotrophic factor (BDNF), PGE_2, and upregulated expression of enzymes (inducible nitric oxide synthase, COX_2), adhesion molecules, proteases (cathepsin S) and membrane receptors (P2X4, CB2, CX3CR1). All these mediators acting in the dorsal horn increase neuronal excitability and are crucial for the pain

enhancing role of microglia (Ren and Dubner, 2010; Scholz and Woolf, 2007). Microgliosis is followed by further activation and spread of inflammation by astrocytes, which are involved in the maintenance of neuropathic pain. When activated, astrocytes are characterised by hypertrophy, increased production of intermediate filaments, glial fibrillary acidic protein (GFAP), vimentin and/or nestin and the activation of JNK pathway (Austin and Moalem-Taylor, 2010). Reactive astrocytes release pro-inflammatory cytokines (IL-1β and TNFα), glutamate, D-serine, substance P, ATP, nitric oxide, CCL2, prostaglandins, IFN-γ and the matrix metalloprotease 2 (MMP-2) that cleave and activate IL-1β released from microglia (Benarroch, 2010). Overall, changes in the astrocyte network signalling that occur during neuroinflammation disturb the two-way interaction between astrocytes and neurons. This disturbance results in increased neuronal excitability and enhanced and prolonged synaptic pain transmission. Figure 6 shows interactions between neurons and glial cells in the dorsal horn of the spinal cord. Neuronal-glial interaction has also been reported in supraspinal sites after peripheral nerve or spinal cord injury, including the ventroposterolateral nucleus of the thalamus (Saab and Hains, 2009), RVM (Cunha and Dias, 2009), PAG (Mor *et al*, 2010) and hypothalamus (Takeda *et al*, 2009).

Infiltration of haematogenous macrophages and T-cells in the spinal cord has been also demonstrated in models of peripheral neuropathic pain (Cao and DeLeo, 2008; Hu *et al*, 2007; Zhang *et al*, 2007b). Therefore, immune cells not only contribute to peripheral sensitization of nociceptors, but also interact with glial cells in the spinal cord to increase the excitability of the dorsal horn neurons, thus contributing to the maintenance of neuropathic pain.

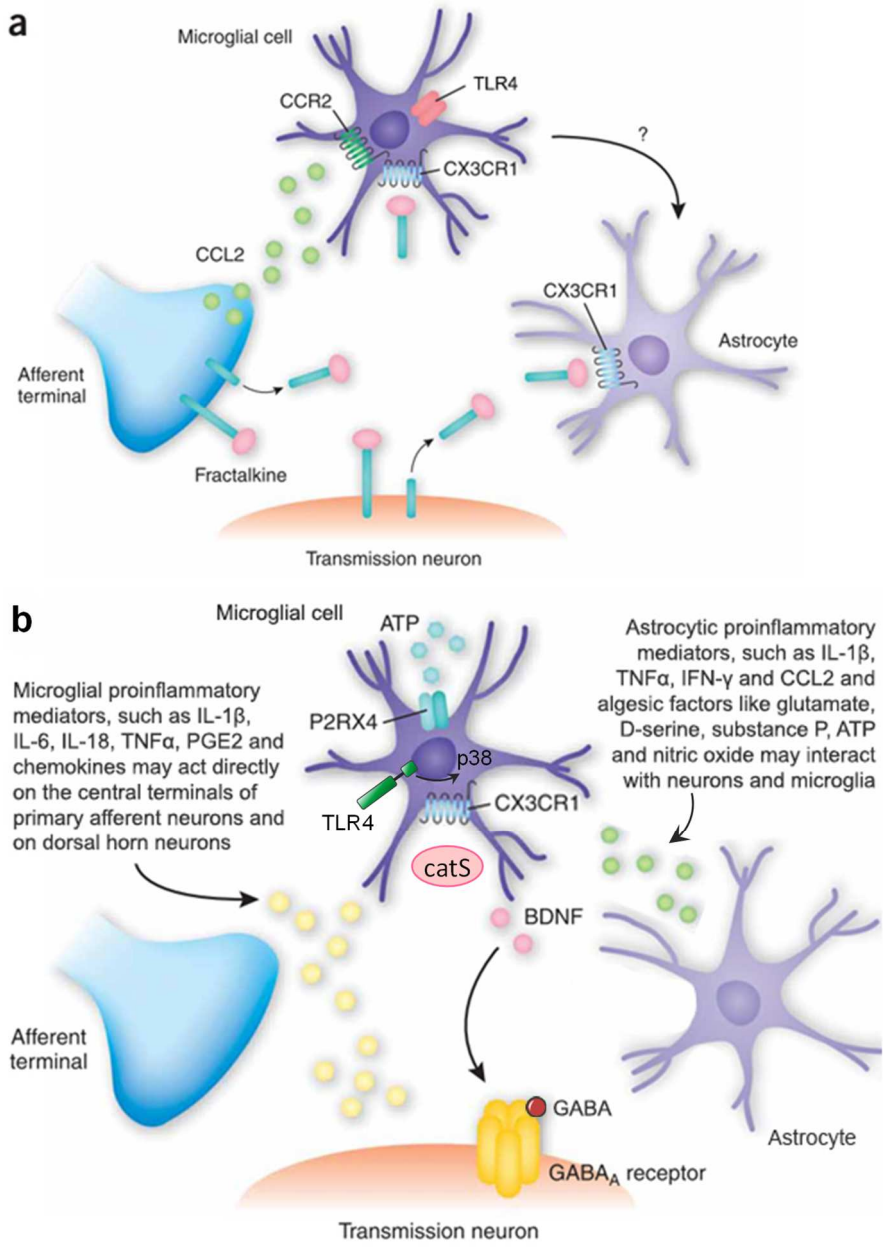

Figure 6. Recruitment and activation of spinal microglia and astrocytes. (a) Microglial recruitment depends on signalling pathways involving TLR4 and on the chemokine CCL2 acting on CCR2. The neuronal protein fractalkine has a chemokine domain that can be cleaved from its membrane-bound portion. Both bound and soluble fractalkine have chemokine function and may attract

microglia as well as astrocytes by acting on CX3CR1. Because the microglial response to nerve injury precedes the proliferation of astrocytes, a direct path of communication may exist between these two glial cell types to coordinate their sequential temporal patterns of activation. (b) Main features of activated spinal glial cells. ATP, adenosine triphosphate; BDNF, brain-derived neurotrophic factor; catS, cathepsin S; CCL2, C-C motif chemokine ligand 2; CCR2, C-C chemokine receptor type 2; CX3CR1, CX3C chemokine receptor 1; GABA, gamma aminobutyric acid; P2RX4, P2X purinoceptor 4; TLR4, toll-like receptor 4 (adapted from Scholz and Woolf, 2007).

2.4 Therapeutic approaches for neuropathic pain

Neuropathic pain is often underdiagnosed and undertreated. Epidemiological surveys have shown that many patients with neuropathic pain do not receive appropriate treatment (Attal *et al*, 2011; Torrance *et al*, 2013) and none of the available treatments prevent the development of neuropathic pain nor completely eliminate it when established (Woolf and Mannion, 1999). In addition, the inter-individual variability of neuropathic pain mechanisms and symptoms as well as the emotional and cognitive comorbidities further complicate the management of this clinical entity. Therefore, treatments are often directed to lessen pain and help the patients to cope with their symptoms by means of psychological or occupational therapy, rather than to supress the pain.

The current management of neuropathic pain comprises pharmacological and nonpharmacological therapies. Some reports suggest benefits of several non-drug therapies such as exercise (Sherry *et al*, 1999), transcutaneous electrical nerve stimulation (Kumar and Marshall, 1997), percutaneous electrical nerve stimulation (Ghoname *et al*, 1999) and

graded motor imagery (Moseley, 2004), as well as cognitive behavioural therapy or supportive psychotherapy (Evans *et al*, 2003). Electrical nerve stimulation is effective against neuropathic pain by modulating the inhibitory influence of pain transmission, enhancing the inhibitory GABAergic signalling at the spinal dorsal horn and inhibiting spontaneous discharge (Cui *et al*, 1997; Guan *et al*, 2010).

Regarding pharmacotherapy, neuropathic pain is usually refractory to traditional pain therapies (Costigan *et al*, 2009). Systemic drugs approved for neuropathic pain treatment include tricyclic antidepressants, SNRIs, antiepileptics and opioids, while topically administered lidocaine and capsaicin are only indicated for peripheral neuropathic pain (Figure 7) (Attal *et al*, 2010; Baron *et al*, 2010). Other drugs, such as cannabinoids, are used as analgesics for this purpose despite not being approved.

Some tricyclic antidepressants (amitriptyline) and SNRIs (duloxetine) have analgesic effects that are independent of their antidepressant effect. The restoration of descending inhibitory pathways by elevating the endogenous levels of serotonin and noradrenaline constitutes the main mechanism of analgesic action of these drugs (Baron *et al*, 2010; Kremer *et al*, 2016). Another mode of action of tricyclic antidepressants that contributes to their analgesic effect is the blockade of sodium channels, thus inhibiting abnormal ectopic excitability of afferent neurons (Dick *et al*, 2007). Different studies evidenced an involvement of the opioid system in the action of antidepressants on neuropathic pain (Kremer *et al*, 2016). Both the identity and the location of the opioid receptors implicated in antidepressants' action have been studied. The activation of MOR in the spinal cord and of DOR at supraspinal levels seemed to be required for the antiallodynic effect of antidepressants

(Marchand *et al*, 2003), although more recent research showed a preferential involvement of DOR rather than MOR (Benbouzid *et al*, 2008a; Choucair-Jaafar *et al*, 2014). However, the link between monoaminergic and opioid systems remains unclear. The respective location of adrenergic and opioid receptors is a critical point. If both receptors were to be expressed by the same cells, direct interactions might be possible. Indeed, a functional cross-talk between MOR and α2A adrenoceptors, with inhibition of one receptor by the other, has been proposed (Vilardaga *et al*, 2008). On the other hand, if adrenergic and opioid receptors are on different cells, a cascade mechanism implying opioid peptide synthesis and/or opioid receptor regulation would be more likely. Accordingly, antidepressant treatment may increase the production of opioid peptides in the spinal cord and in some supraspinal structures (Binder *et al*, 2004; Böhm *et al*, 2006; Hamon *et al*, 1987) and it may increase the densities of MOR and DOR binding sites in the spinal cord (Hamon *et al*, 1987). However, the impact of chronic antidepressant treatment on the opioid system in neuropathic pain conditions is still to be addressed. Antidepressant drugs can also attenuate proinflammatory and favour anti-inflammatory cytokine production in neuropathic pain (Sud *et al*, 2008; Zhu *et al*, 2008), even though the exact mechanism is still to be detailed. In addition to the analgesic effects, their antidepressant properties may be also beneficial due to the emotional comorbidities associated to chronic neuropathic pain.

Gabapentin and pregabalin are two antiepileptic drugs commonly used for neuropathic pain treatment. Both compounds prevent transmitter release through a direct inhibition of the $\alpha_2\delta_1$ subunit of the voltage gated calcium channels. Thus, gabapentinoids contribute to improve neuropathic pain by inhibiting neuronal transmission at the level of the

spinal dorsal horn, reducing excitability of afferent neurons and favouring descending control of pain (Patel and Dickenson, 2016).

Opioids are effective and widely used in clinical management of chronic pain. However, opioid compounds are often discouraged in neuropathic pain due to its uncertain efficacy and its potential for the development of tolerance and other important side effects, as it is further discussed in section 4.5.2. Briefly, the analgesic effects of opioid agonists in neuropathic pain are mediated by reducing the excitability of afferent neurons, increasing descending and segmental inhibition of pain transmission in the dorsal horn and modulating pain integration in supraspinal areas (Nadal *et al*, 2013).

Capsaicin and lidocaine patches have been shown to relieve localized neuropathic pain (Gilron *et al*, 2006), but the therapeutic gain is modest against placebo and the level of evidence is lower than for systemic drugs (Attal *et al*, 2010). Capsaicin alleviates neuropathic pain by activating and desensitising TRPV1 receptors in the nociceptors, thus reducing peripheral sensitization. Lidocaine analgesic effect in neuropathic pain conditions is due to its properties as a sodium channel blocker. It reduces neuronal depolarization and therefore attenuates nociceptor excitability and ectopic discharges (Baron *et al*, 2010).

Recent preclinical studies revealed the crucial role of the endocannabinoid system in the development and maintenance of neuropathic pain (reviewed in Maldonado et al., 2016). These studies have provided important findings, showing the potential analgesic effect of cannabinoid agonists in different neuropathic pain models and identifying specific targets in the endocannabinoid system to develop more effective and safe drugs (Maldonado *et al*, 2016). Although

moderate evidence supports the use of cannabinoid compounds in neuropathic pain, some clinical trials with oromucosal spray Sativex® (containing delta-9-tetrahydrocannabinol and cannabidiol) showed beneficial effects of these compounds in different chronic neuropathic pain syndromes (Hoggart *et al*, 2015; Serpell *et al*, 2014). Cannabinoids inhibit pain transmission by reducing neurotransmitter release in the dorsal horn, thus inhibiting neuronal transmission, and by stimulating the descending inhibitory pathway, and modify other components of pain perception acting in cortical and limbic areas (Nadal *et al*, 2013).

Last international therapeutic guidelines for neuropathic pain recommend antiepileptic (pregabalin and gabapentin) and antidepressants drugs (amitriptyline and duloxetine) as first line therapy (Finnerup *et al*, 2015). They restrict the clinical use of opioid drugs with low efficacy/side effect profile such as tramadol to second line therapy for neuropathic pain, in the same group than lidocaine and capsaicin patches (for peripheral neuropathies), while strong opioids are relegated to third line therapy (Finnerup et al., 2015). According to this systematic review and meta-analysis, cannabinoids showed weak recommendations against use for neuropathic pain treatment due to their small size effect and low tolerability and safety, whereas the evaluation of NMDA receptor antagonists and tapentadol lead to inconclusive results.

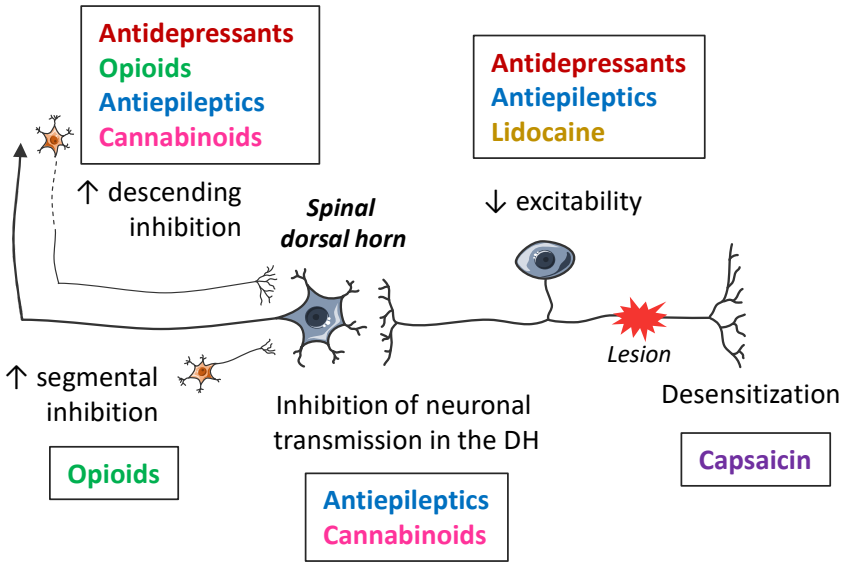

Figure 7. Summary of the modes of action of available pharmacological therapies for neuropathic pain.

2.5 Experimental models for neuropathic pain evaluation

Animal models of neuropathic pain have been crucial in the last decades to improve our understanding of the mechanisms underlying the pathophysiology of this disease and to test novel druggable targets to design new therapeutic strategies for clinical use (Bridges *et al*, 2001). Many experimental animal models of neuropathic pain caused by damage to central or peripheral nervous system have been developed. They can be classified into four categories, namely nerve injury models, drug-induced neuropathic pain, disease-induced neuropathy and miscellaneous ones. Table 7 summarises the main neuropathic pain experimental models. A schematic view of the site of injury of the most used peripheral nerve injury models is depicted in Figure 8.

Table 7. Classification of neuropathic pain models (adapted from Kumar et al., 2018)

1. Nerve injury
Central pain
• ***Spinal cord injury***
Excitotoxins, Contusion, Photochemical model
• ***Spinal hemisection***
• ***Thalamic syndrome***
Peripheral pain
• ***Complete lesion***
Sciatic nerve transection (neuroma model)
Brachial plexus avulsion
• ***Partial lesion***
Sciatic nerve chronic constriction injury (CCI)
Partial sciatic nerve ligation (PSNL)
Spinal nerve ligation (SNL)
Photochemically induced ischemia in sciatic nerve
Cuffing of sciatic nerve
Caudal trunk resection
Spared nerve injury (SNI)
Sciatic cryoneurolysis
Sciatic inflammatory neuritis
Trigeminal neuralgia
2. Drug-induced neuropathy
• ***Anti-cancer agents***
Vincristine
Cisplatin
Taxanes
• ***Anti-retroviral drugs***
Didanosine
Zalcitabine
Stavudine
3. Disease-induced neuropathy
Diabetes (streptozotocin-induced peripheral diabetic neuropathy)
Cancer pain model
HIV-induced
Post herpetic neuralgia model
4. Miscellaneous
Ethanol consumption/withdrawal-induced neuropathy
Pyridoxine (vitamin B6)-induced neuropathy
Inherited-induced neuropathies (Charcot-Marie-Tooth)
Uremic peripheral neuropathy (end stage kidney disease)

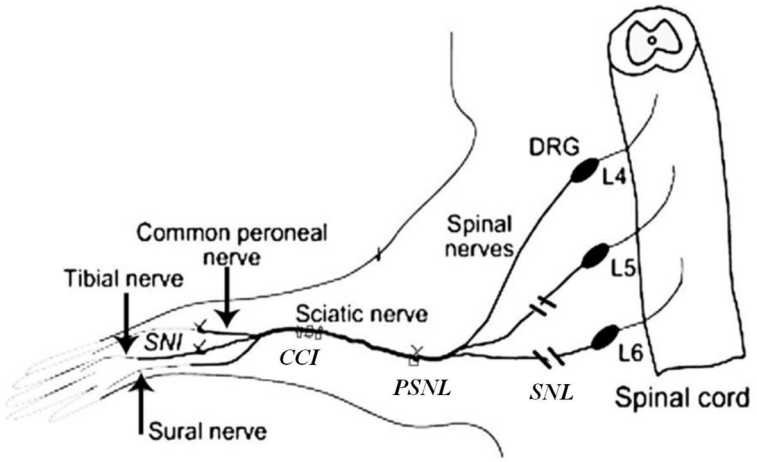

Figure 8. Schematic drawing of the most used peripheral nerve injury models. SNI, spared nerve injury; CCI, chronic constriction injury; PSNL, partial sciatic nerve ligation; SNL, spinal nerve ligation (Kumar *et al*, 2018).

Although animal models of neuropathic pain do not completely mimic a human lesion and its consequences, they are useful since they can reproduce most of the acute and long-term pathophysiological mechanisms arising following the damage of the nervous system and can provide essential information for the development of future therapies.

3. Influence of personality traits in neuropathic pain manifestations

There is a lack of relationship between the aetiology of neuropathic pain and the symptoms (Woolf and Mannion, 1999), as discussed above. In fact, only some individuals subjected to nerve injury actually develop chronic pain (Kehlet *et al*, 2006). Once neuropathic pain is established, pain intensity and analgesic response are also high variable among patients with the same condition. These individual differences could be explained by environmental, personality and genetic factors. Human twin studies of chronic pain syndromes were performed to unravel these possibilities and most of them demonstrated at least moderate heritability (Mogil, 2012). Large-scale association studies revealed a wide variety of genes that are potentially associated with both experimental and clinical pain states (Mogil, 2012).

Interestingly, clinical evidences support that personality traits of patients contribute to magnify the high inter-individual variability of neuropathic pain manifestations. Personality traits contribute to determine our emotional-driven states, which in turn play a key role modulating pain (Asghari and Nicholas, 2006). A reciprocal relationship has been reported between pain and negative affect. Chronic pain not only lead to emotional alterations, but pain can be positively and negatively modulated by personality traits such as sociability, anxiety and depression as summarized in Figure 9.

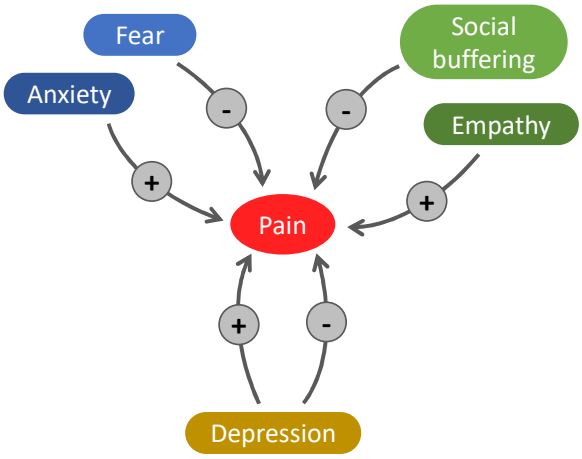

Figure 9. Schematic view of the effect of personality trait-dependant emotional states on pain modulation according to preclinical and clinical data.

3.1 Anxiety and fear

In order to understand the effect of emotional traits on pain modulation, it is worth noting the difference between two qualitatively distinct emotional states: fear and anxiety. Fear is an immediate alarm reaction to present threat, that induces high levels of arousal and mobilizes the organism to take action (fight/flight response). In contrast, anxiety is a future-oriented emotion produced by relatively diffuse threat and characterized by negative affect and apprehensive anticipation, which leads to hypervigilance and somatic tension (e.g. muscle tension) that facilitates sensory receptivity (Rhudy and Meagher, 2000a).

Animal studies suggested that exposure to conditioned or unconditioned fear decreases pain sensitivity by a mechanism known as “stress-induced analgesia” (Basbaum and Fields, 1984; Bodnar *et al*, 1980), a phenomenon linked to the release of endogenous opioids (Terman *et al*, 1984). The same inhibitory effect of fear on pain reactivity was later generalized to humans (Janssen and Arntz, 1996; Rhudy *et al*, 2004).

Clinical studies also revealed a divergent effect of fear and anxiety on pain intensity modulation, since experimentally induced-anxiety (Arntz *et al*, 1994; Ploghaus *et al*, 2001; Rhudy and Meagher, 2000a, 2003) and high anxiety sensitivity (Keogh and Mansoor, 2001) increased nociception. These results agreed with those obtained from patients with generalized anxiety or post-traumatic stress disorder, whose hypervigilant states led to increased attention to pain, thus amplifying pain intensity (Barlow *et al*, 1996; Defrin *et al*, 2008). Although the negative influence of anxiety on pain perception was consistently reported in human, conflicting results were obtained from preclinical research. Thus, enhanced nociceptive behaviour to the subcutaneous injection of formalin was shown in rats with experimentally-induced anxiety (Andre *et al*, 2005). However, rats with a genetic predisposition to high anxiety-related behaviour showed reduced pain response to thermal stimuli (Jochum *et al*, 2007). A possible explanation for the apparently contradictory relationship between pain and anxiety could be the existence of relevant and irrelevant anxiety in the reaction to pain according to the source of anxiety, as previously suggested (Weisenberg *et al*, 1984). When the source is related to pain experience, anxiety can exacerbate pain perception, while if the source is related to something else, anxiety may reduce pain sensitivity (Weisenberg *et al*, 1984).

Less is known about the involvement of anxiety trait in the manifestations of chronic pain syndromes. Anxiety trait increased mechanical hypersensitivity in neuropathic rats during the chronic phase of pain (Roeska *et al*, 2009). In humans, different anxiety sensitivities did not modify pain intensity of patients with chronic low back pain (Asmundson and Norton, 1995). However, high anxiety sensitivity patients were more negatively affected by their pain experience (greater

cognitive disruption, anxiety, fear and negativity of affect) compared to patients with medium or low anxiety sensitivity (Asmundson and Norton, 1995). Therefore, the consideration of emotional and cognitive aspects of pain in pre-clinical and clinical research is necessary for a comprehensive evaluation of any possible target to modulate pain. Further studies are required to elucidate the effect of anxiety on emotional and cognitive aspects of neuropathic pain.

The brain areas responsible for the influence of anxiety and fear on pain sensitivity are not well known, although several evidences strongly support a crucial role of the amygdala in the emotional-affective dimension of pain (Ikeda *et al*, 2007; Neugebauer *et al*, 2004, 2009). The amygdala plays a key role in the formation of fear-related memories and emotional processing (Phelps and LeDoux, 2005) and contains several nuclei, including the lateral, basolateral and central nuclei, which are important for sensory processing (Neugebauer *et al*, 2009). Strong neuronal responses to peripheral nociceptive stimuli have been reported in the CeA, defined as the 'nociceptive amygdala' (Neugebauer *et al*, 2004). Indeed, increased excitability of CeA neurons has been reported in arthritic (Neugebauer *et al*, 2003), visceral (Han and Neugebauer, 2004) and neuropathic pain models (Gonçalves and Dickenson, 2012; Ikeda *et al*, 2007), as well as in patients with generalized anxiety, social phobia, panic and post-traumatic stress disorder (Etkin and Wager, 2007).

3.2 Depression

Complex and reciprocal relationships also exist between depression and pain. Chronic pain can promote the appearance of depressive symptoms, while on the other hand, depression can modulate pain-related behaviours (Kroenke *et al*, 2011). However, whether depression exerts a

positive or negative influence on pain perception has not been yet clarified.

Large body of preclinical research was performed trying to clarify this topic, but they brought out conflicting results. Multiple animal models of depression were used in these studies inducing depressive-like behaviour by different experimental procedures (unpredictable chronic mild stress and bilateral olfactory bulbectomy) or by using a specific rat strain with genetical predisposition to depressive-like behaviour (Wistar-Kyoto). Some authors showed that modelled depression resulted in enhanced nociceptive responses under physiological conditions (Burke *et al*, 2010, 2013; Nagakura *et al*, 2009). However, divergent effects of depression on evoked and spontaneous pain behaviours have been reported, since pre-established depression state exacerbated formalin-induced spontaneous licking behaviours, but it attenuated heat and mechanical evoked nociception (Shi *et al*, 2010a, 2010b; Wang *et al*, 2010c). The distinctive effects of depression depending on pain modality were in accordance with the human data obtained on patients with major depressive disorders (Bär *et al*, 2005). Other human studies also supported the inconsistent effect of depression on physiological nociception observed in animals. Two studies agreed that patients with depressive disorders showed decreased nociceptive responses (Bär *et al*, 2006; Schwier *et al*, 2010), whereas another revealed the opposite effect of depression enhancing nociception under normal conditions (Chiu *et al*, 2005).

Some preclinical research was performed in the last years to evaluate the involvement of depression in the manifestations of chronic pain. The conflicting results concerning the influence of depression on pain reactivity obtained in naïve state were also observed within

inflammatory or neuropathic pain syndromes. Unpredictable chronic mild stress-induced depression decreased the perceived intensity of painful stimulation in rats exposed to complete Freund's adjuvant (CFA)-induced inflammatory pain (Shi *et al*, 2010b) and spinal nerve ligation-induced neuropathic pain (Shi *et al*, 2010a). However, modelled depression was also shown to enhance mechanical and cold allodynia as well as heat hyperalgesia under both chronic inflammatory (Kim *et al*, 2012; Wang *et al*, 2012) and neuropathic pain conditions (Bravo *et al*, 2012; Burke *et al*, 2013; Zeng *et al*, 2008). To our knowledge, no clinical studies have addressed so far specifically this issue. Based on the controversial preclinical results, further comprehensive studies should be performed to understand the role of depression in the predisposition to develop chronic pain.

Several mechanisms acting in distinct neuroanatomical substrates have been proposed to account for the positive interaction between pain and depression. The melatonin system in the anterior cingulate cortex through modulation of NMDA receptor was suggested to play a role in the mechanisms of comorbidity between depression and pain (Wang *et al*, 2012; Zeng *et al*, 2008). Increased accumulation of phosphorylation/activation of the ERKs and a decrease in neuronal density in the anterior cingulate cortex were also proposed to contribute to the pain-enhancing effect of depression (Bravo *et al*, 2012). The upregulation of the brain indoleamine 2,3-dioxygenase 1 and the subsequent altered tryptophan metabolism was observed in bilateral hippocampus in both models of depression and chronic pain, and was thus suggested as a regulatory mechanism underlying their comorbidity (Kim *et al*, 2012). Furthermore, depression-induced enhanced nociception was inversely correlated with monoamine (serotonin and 5-

hydroxyin-doleacetic acid) levels in the hippocampus, amygdaloid cortex and the hypothalamus (Burke *et al*, 2010). Changes in the expression of neuroinflammatory genes in the amygdala may also account for the enhanced cold allodynia under neuropathic pain conditions (Burke *et al*, 2013). Interestingly, no mechanisms underlying pain-attenuating effect of depression have been suggested.

3.3 Sociability

The importance of social factors to modulate pain perception is widely accepted in humans (Sturgeon and Zautra, 2016). Social interactions can provide support and be related to pain attenuation or enhance pain behaviour by emotional contagion. The amelioration of aversive stimuli by the presence of a supportive accompanying person (i.e., social buffering) can reduce acute pain ratings (Brown *et al*, 2003) and emotional expressions of fear (Epley, 1974). Greater social support has been associated with lower pain intensity in response to experimental stimuli, both if the supportive is physically present or not (Montoya *et al*, 2004). Social buffering can also reduce pain and outcomes in chronic pain patients. Clinical studies revealed that social relationships may improve coping responses and overall function in chronic pain, promoting pain-specific resilience (Sturgeon and Zautra, 2016). Meaningful social ties may play a protective role by engaging neural networks associated with more adaptive responses to pain, such as reward circuitry (Younger *et al*, 2010). Social support also protects patients against pain-related exacerbations in negative mood (Onoda *et al*, 2009). However, the effect of social support on pain seems to depend on the beliefs about potential threat of pain, context of the pain, level of stress associated with the

painful event, and the communication and relationship status between individuals (Jackson *et al*, 2009; Krahé *et al*, 2013).

On the other hand, the observation of another's pain can trigger it in the observer (Craig *et al*, 1975; Craig and Weiss, 1971). In addition to representing a physical sensation, pain elicits psychological and behavioural responses that serve as social cues with communicative functions. The ability of others to perceive such social cues is commonly called empathy (Martin *et al*, 2014). Many studies have investigated the neuroanatomical substrates of human empathy. Shared activation of the rostral anterior cingulate cortex and anterior insular cortex, cortical areas associated with motivational-affective dimensions of pain, was observed in individuals experiencing pain first-hand or watched someone in pain (Botvinick *et al*, 2005; Morrison *et al*, 2004; Rainville *et al*, 1997; Singer *et al*, 2004). These findings suggest that empathy does not require the use of memory, mentalization, and cognitive reasoning.

It is commonly assumed that psychosocial aspects of pain can only be studied in human beings, but recent data from preclinical studies is beginning to challenge this assumption. Increasing reports support the ability of housing, social buffering and emotional contagion (a form of empathy) to modulate pain sensitivity and pain behaviour in mice and rats (Martin *et al*, 2014). When rodents are isolated, their behaviour, including pain behaviour, is drastically altered. Autotomy after dorsal rhizotomy is frequent in male rats housed in isolation, but it was almost completely prevented by co-housing with a female rat (Berman and Rodin, 1982). A few studies have shown that social isolation decreases pain sensitivity and increases analgesic responding by enhancing μ-opioid activity (Becker *et al*, 2006; Coudereau *et al*, 1997). At the opposite,

crowding can affect pain sensitivity and opioid analgesia with different outcomes depending on the modality of the stimulus (Pilcher and Browne, 1982). A more recent study revealed an overwhelming effect of housing and the identity of cagemates on chronic pain behaviour, even counteracting genetic predisposition to pain (Raber and Devor, 2002). The contact with rats genetically predisposed to neuropathic pain was sufficient to enhance neuropathic pain in rats exposed to a neuroma (Raber and Devor, 2002). However, this phenomenon does not seem to be due to a behavioural contagion, since it was apparently mediated via olfaction (Raber and Devor, 2002).

Contact with conspecifics and the subsequent affiliative behaviours were reported to decrease pain sensitivity in an opioid-dependent manner in rodents (D'Amato, 1998; D'Amato and Pavone, 2012). Social approach behaviour to a conspecific in pain decreases pain behaviours in the affected mouse (Langford *et al*, 2010b). Another evidence of social buffering is the modulation of painful environmental threats by the presence of other animals. Thus, the presence of naïve rat blocked freezing behaviour of a test rat in response to a foot-shock (Kiyokawa *et al*, 2004).

The ability to share emotional states relies on a so-called perception-action mechanism (Preston and de Waal, 2002) that includes mimicry and/or emotional contagion. Emotional contagion is a form of empathy that can operate without the presence of evolved 'theory of mind' (Hatfield *et al*, 1993). A variety of animals may be able to socially transfer emotional states to others. Thus, mice have the ability to transmit pain status between cagemates, resulting in contagious pain hypersensitivity, only when both mice in the dyad are in pain, while no effects are

observed among strangers (Langford *et al*, 2006). This study showed that the transmitting sensory modality was vision, as blockade of vision, but not of other sensory modalities including touch, olfaction, and audition was effective in blocking the phenomenon (Langford *et al*, 2006). In fact, it is now known that mice and rats display facial expressions of pain, which combined with other body cues have been suggested to be the primary drivers of pain contagion effects (Keating *et al*, 2012; Langford *et al*, 2010a; Sotocinal *et al*, 2011).

4. Endogenous opioid system

Opioid receptors, their endogenous peptide ligands and the enzymes involved in their metabolism comprise the endogenous opioid system. This system is extensively distributed through the CNS and peripheral tissues. The wide distribution of the endogenous opioid system is determinant to its involvement in multiple physiological responses including control of pain, emotional behaviour, learning and memory functions or regulation of reward circuitry, among others (Bodnar, 2017).

4.1 Discovery of the endogenous opioid system

The isolation and purification of morphine from opium in 1806 by Friedrich Sertürner established the starting point for the modern pharmacognosy. For the first time, the main active principle of a plant was isolated and could be used in therapeutics. After this discovery, the pharmacology of natural substances advanced quickly with the identification and isolation of different plant compounds with a great spectrum of activities. This fact generated the possibility of using these compounds to investigate their effects, identify their mode of action, and use them both as therapeutic drugs and as chemical template to develop new drugs. At the end, it allowed the identification of opioid receptors almost one hundred and seventy years later.

In the decade of 1960s, it became apparent that opioid drugs were likely to exert their actions at specific receptor sites (McClane and Martin, 1967). In 1971, a new radiobinding methodology was described to analyse the association between the morphine derivative levorphanol and the brain tissue, suggesting that opioid receptors were in specific membrane fractions of brain homogenates (Goldstein *et al*, 1971). Few

years later, three laboratories simultaneously succeeded in demonstrating the existence of opioid binding sites in the CNS (Pert and Snyder, 1973; Simon *et al*, 1973; Terenius, 1973). The first definitive evidence that opioid receptors did not form an homogeneous population was presented by Martin and his colleagues in 1976, who identified MOR and KOR (Martin *et al*, 1976). The DOR was identified one year later in mouse vas deferens (Lord *et al*, 1977). With those discoveries, researchers reasoned that the opioid receptors might be the binding sites of endogenous neurotransmitters. Soon, the first endogenous opioid compounds that bind to opioid receptors, named enkephalins, were isolated from guinea pigs brain extracts and identified based on the determination of the amino acid sequence (Hughes *et al*, 1975). One year later, endorphin, a second endogenous opioid peptide derivative from enkephalin sequence was isolated (Cox *et al*, 1976). Finally, dynorphins were identified in 1979 (Goldstein *et al*, 1979). The demonstration of the existence of the opioid receptors and the endogenous opioid ligands, was the first step of the endogenous opioid system characterization. During the mid-1990s, the molecular characterization and cloning of the different opioid receptors widely improved the knowledge and advances in this system (Kieffer, 1995).

4.2 Opioid receptors

Three classical opioid receptors have been identified and cloned in experimental animals and humans (Kieffer, 1999): mu (MOR), delta (DOR) and kappa (KOR) opioid receptors. A non-classical opioid receptor, the nociceptin or orphanin receptor (NOR or opioid receptor like 1, ORL-1) was identified later on and was accepted to be part of the opioid receptors family (Bunzow *et al*, 1994; Mollereau *et al*, 1994).

Opioid receptors belong to the superfamily G protein-coupled receptor (GPCR). They are seven-transmembrane domain proteins with an extracellular N-terminal domain and an intracellular C-terminal domain. Each receptor is encoded by a unique gene (*Oprm1*, *Oprd1*, *Oprk1*, *Oprl1*) but present a high homology in the sequence identity in both, transmembrane (73%-76%) and intracellular domains (63%-66%). In contrast, a large divergence is reported in the extracellular N-domains (34%-40% identity) (Al-Hasani and Bruchas, 2011; Pogozheva *et al*, 2005; Toll *et al*, 2016). The crystal structures for the inactive and active state of each receptor have been identified with atomic-level details, which allow the definition of the unique opioid binding pockets that maintain ligand preferences (Figure 10) (Granier *et al*, 2012; Manglik *et al*, 2012; Thompson *et al*, 2012; Wu *et al*, 2012). These findings provide insight into how different agonists distinctly alter receptor conformations to direct downstream intracellular cascades, which may ultimately lead to more effective pharmacological treatments.

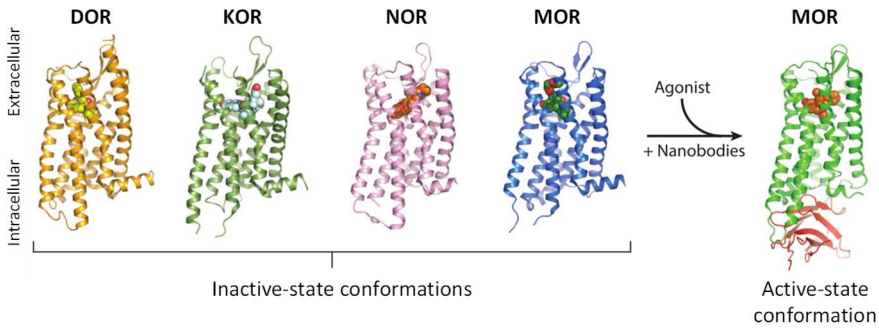

Figure 10. **Opioid receptor family.** Crystal structures of the inactive state of all four opioid receptors (DOR, KOR, NOR, and MOR). When an opioid agonist enters the binding pocket of its cognate receptor, a conformational change in the transmembrane domains allows for intracellular effector molecules to bind

and activate signalling cascades that modulate neural function. The addition of stabilizing nanobodies to the crystal preparation has elucidated the active state of MOR (Corder *et al*, 2018).

Opioid receptors are coupled to pertussis toxin sensitive G-proteins ($G_{\alpha i}$ and $G_{\alpha o}$). Activation of opioid receptors leads to the dissociation of the G protein into two different subunits, G_{α} and $G_{\beta\gamma}$, which subsequently engage a variety of effectors and intracellular signalling cascades that typically depress neural functions. Thus, $G_{\beta\gamma}$ subunit positively modulates the G protein-coupled inwardly rectifying potassium channels (GIRK) (Torrecilla *et al*, 2002; Wickman and Clapham, 1995) and inhibits N-, P/Q- and L-type voltage-gated calcium channels (Zamponi and Snutcht, 1996). In turn, the G_{α} subunit inhibits adenylate cyclase activity and reduces the cAMP (Law *et al*, 2000). These processes lead to neuronal hyperpolarization and inhibition of neurotransmitter release, which result in reduced neuronal excitability. Although these were considered the primary actions of opioid receptors in the nervous system, more recent studies have shown that phosphorylated GPCRs recruit β-arrestin, which is a key signal effector at these receptors, mediating an array of cellular and behavioural responses. Phosphorylated arrestin-bound GPCR complexes trigger critically important downstream signalling cascades, including the MAPK cascade (Al-Hasani and Bruchas, 2011). These MAPKs, which consist of three major proteins [extracellular signal regulated kinase 1 and 2 ($ERK_{1/2}$), c-Jun N-terminal kinase 1–3 ($JNK_{1–3}$), and p38], notably modulate cell proliferation, differentiation, apoptosis, transcription factor regulation, ion channel regulation, neurotransporter regulation, and protein scaffolding (Raman *et al*, 2007). Arrestin also regulate the G protein signalling through desensitization and

internalization of the opioid receptors (Corder *et al*, 2018). It was generally accepted that internalised receptors were inactive (Bohn *et al*, 1999), but more recent studies have shown that opioid receptors may still signal, including from endosomal compartments (Eichel *et al*, 2016; Irannejad *et al*, 2013). Figure 11 summarises the basic signalling properties of the four opioid receptors.

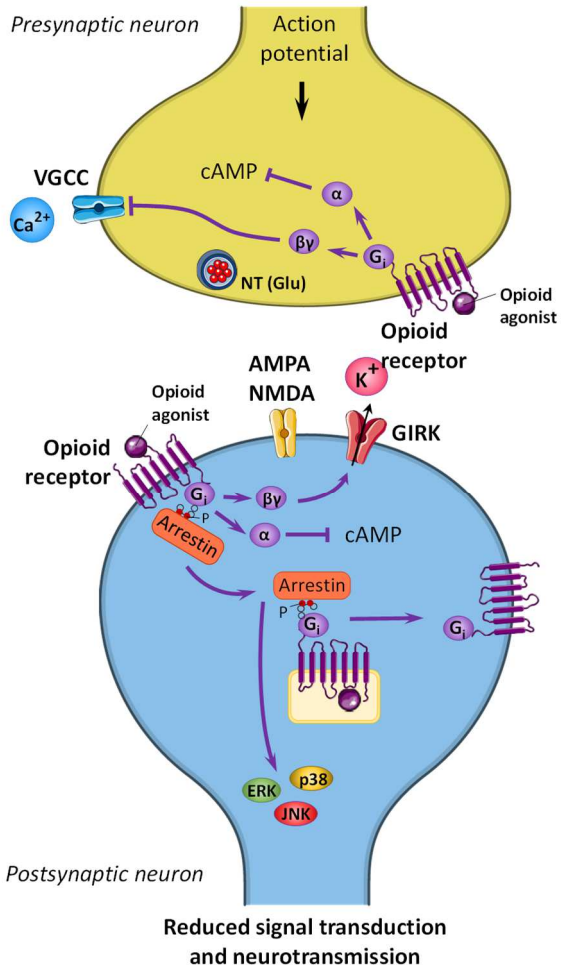

Figure 11. Opioid modulation of signalling and synaptic transmission. Activation of opioid receptors promotes dissociation of inhibitory G_{α} and $G_{\beta\gamma}$

protein subunits. G_{α} subunits suppress adenylate cyclase, and $G_{\beta\gamma}$ subunits presynaptically inhibit voltage-gated calcium channels opening and postsynaptically activate GIRK channels, resulting in reduced neurotransmitter release and membrane hyperpolarization, respectively. Thus, G proteins mediate the inhibitory action of opioid signalling on neurotransmission. Additionally, agonist binding to opioid receptors causes conformational changes that promote recruitment of arrestin effector signalling cascades. Arrestin signaling is required both for internalization of opioid receptors and for kinase activities. AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; cAMP, cyclic adenosine monophosphate; ERK, extracellular signal regulated kinase; GIRK, G protein-coupled inwardly rectifying potassium channels; JNK, c-Jun N-terminal kinase; NMDA, N-methyl-D-aspartate receptor; NT, neurotransmitter (Glutamate); P, phosphate; VGCC, voltage-gated calcium channels.

Opioid receptors are widely distributed all over central and peripheral nervous system (Mansour *et al*, 1988; Stein, 1993). Importantly, each receptor has a distinct expression pattern throughout the brain, where they are expressed primarily in the cortex, limbic system, and brain stem (Mansour *et al*, 1994; Neal *et al*, 1999). Ligand autoradiography studies have determined the opioid binding sites (receptor protein), whereas *in situ* hybridization studies characterised the distribution of cell bodies expressing opioid receptors, based on the detection of mRNA (Le Merrer *et al*, 2009). The sites of opioid receptor expression (mRNA) generally match the distribution of binding sites (protein), suggesting that many neurons synthesizing opioid receptors are local neurons. Binding sites for the three opioid receptors overlap in most structures, but some structures exhibit higher expression of one receptor over the others. **MOR** is the most expressed opioid receptor in the amygdala, but not in the CeA, thalamus, mesencephalon, and some brain stem nuclei. **KOR** is the most represented receptor in the basal anterior forebrain, including

the claustrum and endopiriform cortex, olfactory tubercle, striatum (caudate putamen and nucleus accumbens), preoptic area, hypothalamus, and pituitary gland. **DOR** is the most abundant receptor in the olfactory tract (olfactory bulbs, anterior olfactory nucleus, olfactory tubercle, medial amygdala) and in the cortices, including whole neocortex and regions of the amygdala that derive ontogenically from the cortex (basolateral, cortical, and median nuclei), and is also highly expressed in the striatum (Figure 12) (Lutz and Kieffer, 2013; Le Merrer *et al*, 2009).

Opioid receptors also show mostly divergent expression in spinal cord dorsal horn and DRG neurons. MOR are mainly present in thermo nociceptive transmission pathways, that is, in small diameter C-fibres and superficial lamina I and outer II of the dorsal horn (Scherrer *et al*, 2009). Conversely, DOR are expressed in myelinated Aβ primary afferents, where it inhibits mechanical transmission, as well as in deeper laminae of the dorsal horn (inner II, III, IV and V) (Bardoni *et al*, 2014; François and Scherrer, 2018). Only a percentage of myelinated neurons positive for the calcitonin gene related peptide, which belong to polymodal Aδ fibres, co-express MOR and DOR receptors (François and Scherrer, 2018; Wang *et al*, 2010b).

Opioid receptors have also been found in immune cells and various peripheral tissues including gastrointestinal system, dermis and epidermis (around hair follicles), bone, joint tissue and in dental pulp (Bigliardi and Bigliardi-Qi, 2014). In these tissues, they are located in sensory and sympathetic fibres where they modulate different physiological effects (Mansour *et al*, 1988; Przewłocki and Przewłocka, 2001).

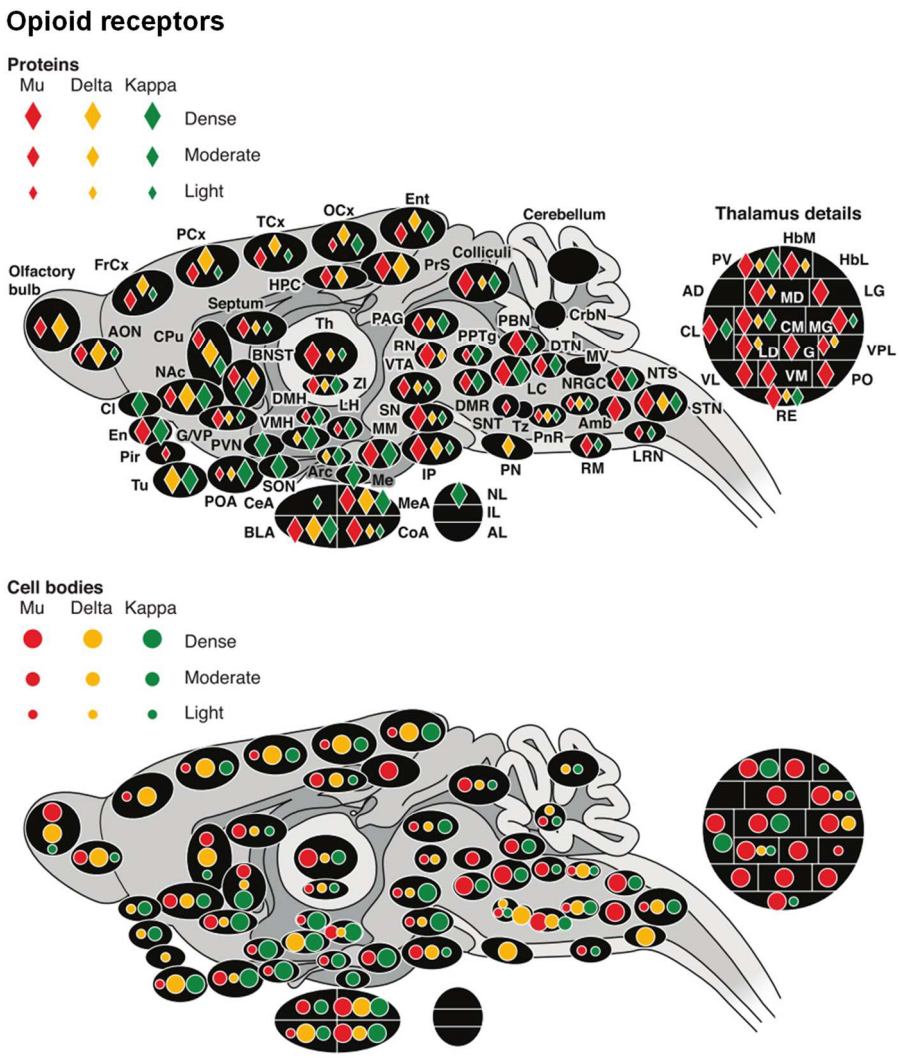

Figure 12. **Anatomy of the opioid receptors in the rodent brain (rat and mouse).** Amb, nucleus ambiguus; AD, anterodorsal thalamus; AL, anterior lobe pituitary; AON, anterior olfactory nucleus; Arc, arcuate nucleus, hypothalamus; BLA, basolateral nucleus, amygdala; BNST, bed nucleus of the stria terminalis; CeA, central nucleus, amygdala; Cl, claustrum; CL, centrolateral thalamus; CM, centromedial thalamus; CoA, cortical nucleus, amygdala; CPu, caudate putamen; CrbN, cerebellar nuclei; DMH, dorsomedial hypothalamus; DMR, dorsal and medial raphe´; DTN, dorsal tegmental nucleus; En, endopiriform cortex; Ent,

entorhinal cortex; FrCx, frontal cortex; G, nucleus gelatinosus, thalamus; G/VP, globus pallidus/ventral pallidum; HbL, lateral habenula; HbM, medial habenula; HPC, hippocampus; IL, intermediate lobe, pituitary; IP, interpeduncular nucleus; LC, locus coeruleus; LD, laterodorsal thalamus; LG, lateral geniculate, thalamus; LH, lateral hypothalamus; LRN, lateral reticular nucleus; MD, mediodorsal thalamus; Me, median eminence; MEA, median nucleus, amygdala; MG, medial geniculate; MM, medial mammillary nucleus; MV, medial vestibular nucleus; NAc, nucleus accumbens; NL, neuronal lobe, pituitary; NRGC, nucleus reticularis gigantocellularis; NTS, nucleus tractus solitarius; OCx, occipital cortex; PAG, periaqueductal gray; PCx, parietal cortex; Pir, piriform cortex; PN, pontine nucleus; PnR, pontine reticular; PO, posterior thalamus; POA, preoptic area; PPTg, pedunculopontine nucleus; PrS, presubiculum; PV, paraventricular thalamus; PVN, paraventricular hypothalamus; RE, reuniens thalamus; RN, red nucleus; RM, raphe´ magnus; SON, supraoptic nucleus; SN, substancia nigra; SNT, sensory trigeminal nucleus; STN, spinal trigeminal nucleus; TCx, temporal cortex; Th, thalamus; Tu, olfactory tubercle; Tz, trapezoid nucleus; VL, ventrolateral thalamus; VM, ventromedial thalamus; VMH, ventromedial hypothalamus; VPL, ventroposterolateral thalamus; VTA, ventral tegmental area; ZI, zona incerta (Le Merrer *et al*, 2009).

4.2 Endogenous opioid peptides

There are four major families of endogenous opioid ligands: β-endorphins, enkephalins, dynorphins, and nociceptin/orphanin FQ (Figure 13).

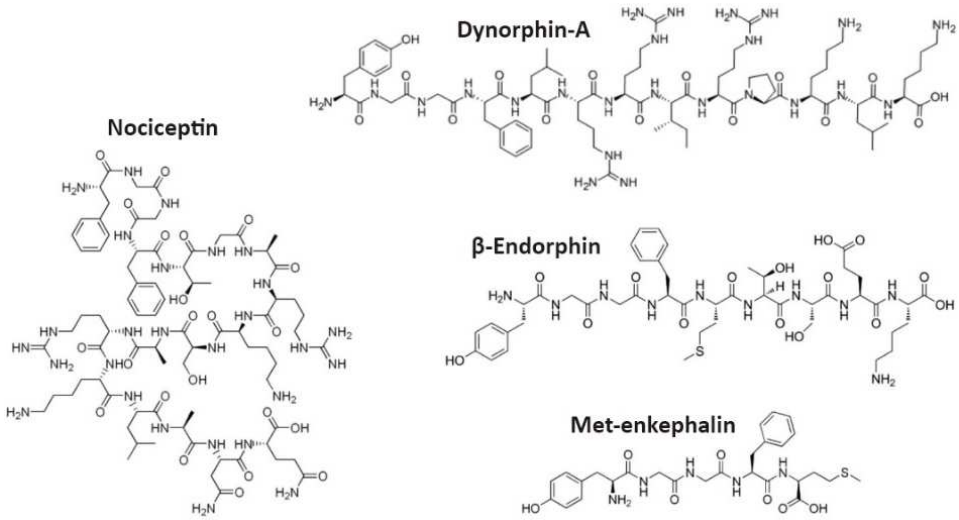

Figure 13. Endogenous opioid peptides. Chemical structures of the four main classes of opioid peptides: met-enkephalin, dynorphin-A, nociceptin, and β-endorphin (Corder *et al*, 2018).

The formation of the opioid peptides results from enzymatic splicing of precursor proteins, namely, proopiomelanocortin (POMC), preproenkephalin (PENK), prodynorphin (PDYN) and pronociceptin (PNOC), respectively (Corder *et al*, 2018). These proteins are characterized by repeatedly having certain amino acid sequences along their structure and generate several active peptides. Both POMC, PENK and PDYN contain one or more repetitions of met- or leu-enkephalin (Figure 14) (Flórez, 2007).

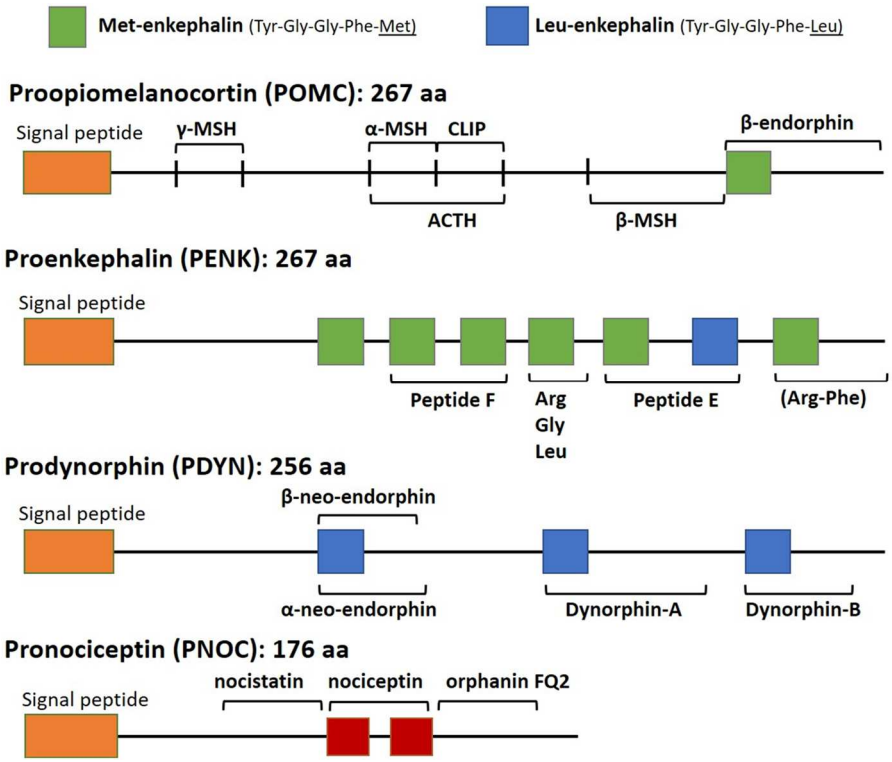

Figure 14. Precursor proteins of opioid peptides. ACTH, adrenocorticotropic hormone; CLIP, corticotropin-like intermediate peptide; MSH, melanocyte stimulating hormone (adapted from Flórez, 2007).

These opioid peptides along with their cognate receptors are widely expressed across the neuraxis and, in particular, pain pathways. The distribution of opioid peptide containing neuronal fibres and cell bodies has been assessed by immunohistochemistry, while *in situ* hybridization studies completed the mapping of opioid cell bodies (Le Merrer *et al*, 2009). Mismatches exist between the distribution of opioid peptide immunoreactivity and the localization of cell bodies. These discrepancies between peptide and cell body maps suggest that an important proportion of opioid peptides is released by projecting neurons (Le Merrer *et al*, 2009). Indeed, the opioid precursors are packaged into

dense core vesicles in the soma and transported down to axon terminals. Precursor proteins are cleaved during this process into opioid peptides (Corder *et al*, 2018). PENK-expressing cell bodies are the most abundant in the brain. PDYN cell bodies are also widespread, with a hot spot in the hypothalamus matching high KOR density. In contrast, POMC cell bodies are highly restricted and only detected in three regions of the brain: the arcuate nucleus of the hypothalamus, nucleus tractus solitarius (in the brain stem), and pituitary gland (anterior and intermediate lobe) (Le Merrer *et al*, 2009). The distribution of opioid precursors in the rodent brain is shown in Figure 15.

The endogenous ligands exhibit different affinities for each opioid receptor. β-endorphin acts on both MOR and DOR with similar affinity. Enkephalins act on DOR and MOR, with greater affinity for DOR. Dynorphins can activate KOR, MOR and DOR with a greater affinity for KOR (Table 8) (Kieffer, 1995; Meunier *et al*, 1995). Contrasting with the tight, spatially controlled synaptic transmission of small-molecule transmitters such as glutamate or dopamine, opioids are thought to rely on volumetric release into synaptic and extrasynaptic spaces and diffuse toward their receptors (Banghart and Sabatini, 2012; Chavkin, 2013; Duggan, 2000). Indeed, electron microscopy illustrates that most MOR are extrasynaptic, being hundreds of microns away from release sites (Glass *et al*, 2009; Svingos *et al*, 1996). This implies that opioid synapses may include a much broader area than typical fast transmitter synapses.

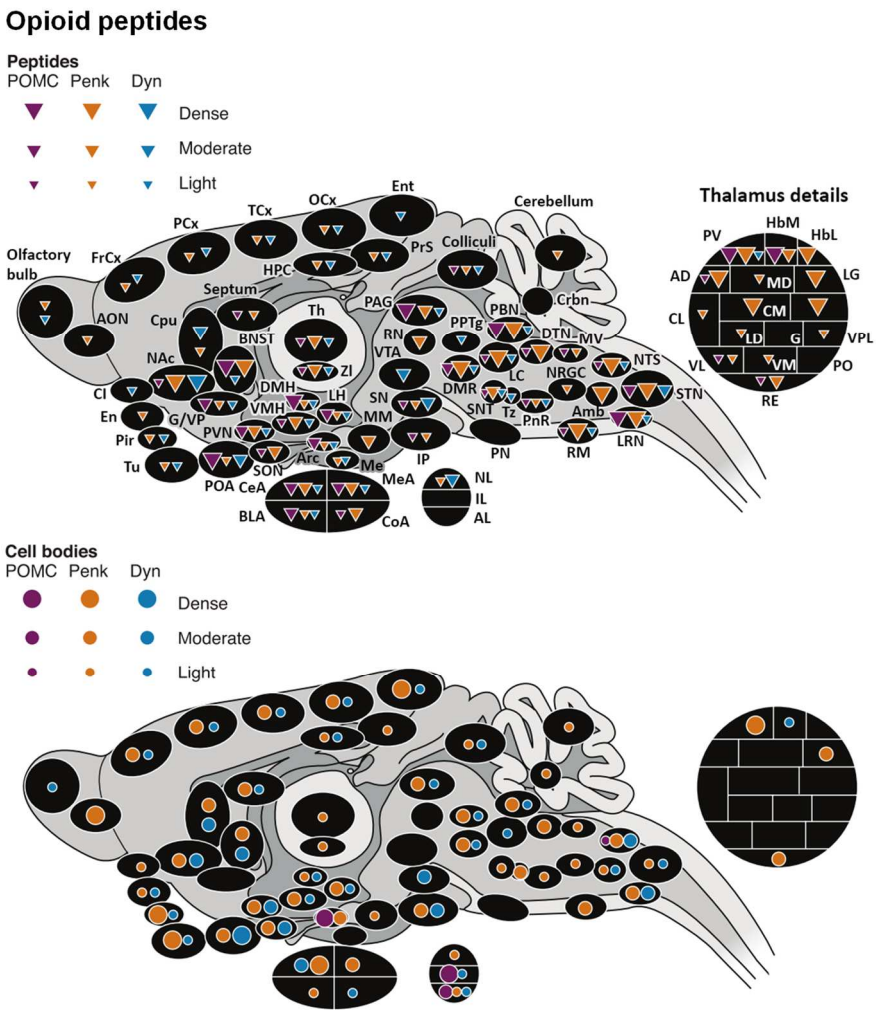

Figure 15. **Anatomy of the endogenous opioid peptides in the rodent brain (rat and mouse).** See abbreviations in Figure 10 caption (Le Merrer *et al*, 2009).

Table 8. Selectivity of opioid ligands for opioid receptors, represented from - (no selectivity) to +++ (high selectivity) (adapted from Kieffer, 1995; Meunier et al., 1995)

Endogenous ligands	MOR	DOR	KOR	NOR/ORL-1
β-endorphin	+++	+++	-	-
Enkephalins	+/++	+++	-	-
Dynorphins	++	+	+++	-
Nociceptin	-	-	-	+++

4.3 Enzymes involved in opioid peptides degradation

Opioid peptides catabolism results in the production of inactive metabolites. Once released into the synaptic cleft, opioid ligands are metabolized by two zinc metallopeptidases, the endopeptidase neprilysin and the aminopeptidase N, that catalyses the cleavage of peptide bonds on the N-terminal side of Tyr-Gly-Gly and Tyr residues, respectively (Figure 16) (Roques *et al*, 2012). The distribution of both enzymes coincides with the same brain areas of the opioid receptors expression. Aminopeptidase N is distributed throughout the cerebral cortex, the caudate, and moderately expressed in the hippocampus, and neprylisin distribution coincides with that of the MOR and DOR (de Gortari *et al*, 2007). *In situ* hybridization studies reported that neprylisin is mainly expressed in the hippocampus, cerebral cortex, caudate nucleus, substantia nigra and the nucleus accumbens, among others (Gaudoux *et al*, 1993).

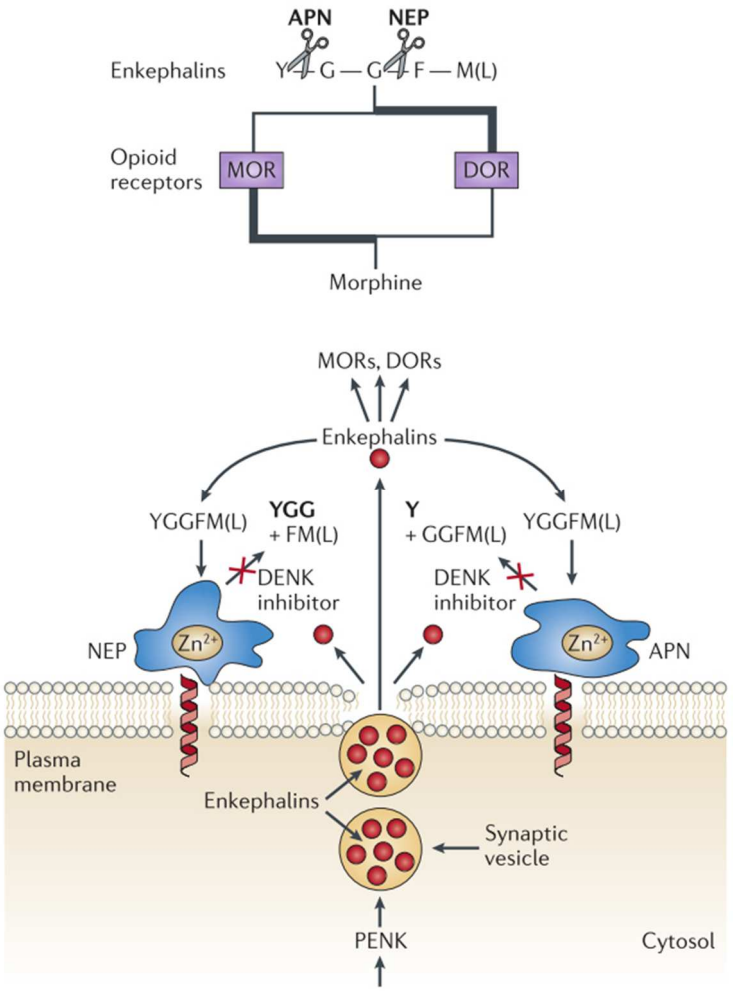

Figure 16. Schematic representation of the endogenous opioid catabolism. The arrows indicate the sites of the opioid peptide cleavage by aminopeptidase N (APN) and neprylisin (NEP) of the peptide bonds on the N-terminal side Tyr-Gly-Gly and Tyr, respectively. In this case, enkephalins are synthesized intracellularly from enzymatic processing of the gene-derived precursor preproenkephalin (PENK). Stored in large synaptic vesicles, they are released (under basal or phasic conditions) by a Ca^{2+}-dependent exocytosis mechanism. Outside the cells, enkephalins interact with opioid receptors, and their signal is interrupted by the concomitant action of NEP and APN that generate inactive metabolites (Roques *et al*, 2012)

4.4 Physiological functions of the endogenous opioid system

The broad role of the endogenous opioid system in the control of multiple physiological responses has been the subject of a vast number of investigations. The control of pain is probably the most well studied physiological function of the EOS. However, this system is involved in a wide range of functions related to behaviour, such as reward and addiction, stress and social status, learning and memory, mental and mood disorders, food intake, gastrointestinal transit, respiratory, cardiovascular and immunological functions, among others (Bodnar, 2017). For the aim of this Thesis, we will focus our attention on the role of the endogenous opioid system in pain, mood disorders and memory. The role of the endogenous opioid system inhibiting pain will be described in detail in the next section 4.5.

4.4.1 Role of the endogenous opioid system in mood disorders

Mood disorders are defined as a group of diagnoses where mood disturbance is the main underlying feature, and are a worldwide leading cause of disability recognized in the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-V) (American Psychiatric Association, 2013). The high density of endogenous opioid peptides and receptors in different limbic brain areas identifies this system as a crucial contributor in mood control. The implication of endogenous opioid system in the aetiology of mood disorders was reported some decades ago. Enkephalins and endorphins administration induced antidepressant-like effects (Kastin *et al*, 1978; Peppin and Raffa, 2015) and inhibitors of enkephalin metabolism also reduced anxiety levels and depressive-like responses (Jutkiewicz *et al*, 2006; Nieto *et al*, 2005; Tejedor-Real *et al*, 1993).

Pharmacological studies using selective MOR, DOR and KOR drugs allowed to characterize the distinct roles for each opioid receptor. Thus, acute pharmacological activation of MOR reduced depressive-like behaviours in some (Berrocoso *et al*, 2013; Berrocoso and Mico, 2009; Rojas-Corrales *et al*, 2002), but not all the preclinical studies (Zhang *et al*, 2006). In contrast, the preferential MOR-antagonist naloxone worsened depressive-like behaviour in the learned-helplessness paradigm in rats (Besson *et al*, 1996; Tejedor-Real *et al*, 1995). Clinical reports describe the effectiveness of the MOR agonists, oxycodone and oxymorphone, and the partial agonist buprenorphine, in patients with refractory major depression (Bodkin *et al*, 1995; Stoll and Rueter, 1999). In addition to this antidepressant effect of MOR agonists, a secondary mechanism has been suggested involving the serotoninergic system. Thereby, MOR activation in the dorsal raphe nucleus disinhibited serotonergic neurons, leading to the subsequent increased release of serotonin in forebrain projecting areas related with emotional integration, including the thalamus, nucleus accumbens, amygdala, frontal cortex, striatum, hypothalamus and ventral hippocampus (Tao *et al*, 1996; Tao and Auerbach, 1995). Tramadol is an atypical MOR agonist that also involves monoaminergic mechanism of action, by enhancing the extraneuronal concentration of noradrenaline and serotonin. It is widely used in clinical pain practice, especially for the treatment of neuropathic pain (Hollingshead *et al*, 2006). In addition to its well-known analgesic effect, some clinical and preclinical evidence has suggested that it elicits antidepressant-like effects (Rojas-Corrales *et al*, 1998, 2002; Shapira *et al*, 2001; Yalcin *et al*, 2007). Tramadol has also been used with positive effects in anxiety and anxiety-like disorders such as obsessive-compulsive disorders (Shapira *et al*, 1997). In addition, tramadol induces changes in the CNS similar to

those induced with conventional antidepressants (Berrocoso *et al*, 2009). Therefore, tramadol could be important in refractory cases of depression when pain is also present.

The anxiolytic- and antidepressant-like activities of DOR agonists are the most well-documented. DOR receptor activation by several selective peptidic and non-peptidic agonists consistently reduced anxiety and depressive-like behaviour in mice and rats across multiple behavioural paradigms (Broom *et al*, 2002; Naidu *et al*, 2007; Perrine *et al*, 2006; Saitoh *et al*, 2004; Vergura *et al*, 2008). The antidepressant-like effects were blocked by the selective DOR antagonist naltrindole, demonstrating that these behaviours were mediated by DOR (Torregrossa *et al*, 2006). Unfortunately, seizures have limited their therapeutic potential and clinical evidence is still lacking (Berrocoso *et al*, 2009). Nonetheless, a recent pilot study showed promising anxiolytic effects of the selective DOR agonist AZD2327 in patients with anxious major depressive disorder (Richards *et al*, 2016). The mechanism responsible for antidepressant-like effects induced by DOR agonists remains unknown. An increase in monoaminergic activity seems to participate in these behavioural responses produced by the activation of DOR. Preclinical findings suggest that DOR activation could restore serotoninergic dysfunction in depressive states (Jenny *et al*, 2008; Saitoh *et al*, 2008). Whether DOR agonists also increase dopaminergic pathway activity is not clear since results for and against this hypothesis have been reported (Jutkiewicz *et al*, 2004; Longoni *et al*, 1998; Spina *et al*, 1998). Interestingly, it has also been described that the effects of tricyclic antidepressants on neuropathic pain in mice require DOR stimulation (Peppin and Raffa, 2015). Therefore, all these findings suggest a mutual relationship between monoamine and DOR systems. The neurotrophic factor

hypothesis is another possible mechanism of action for antidepressant-like effects induced by DOR stimulation. Thereby, acute DOR agonist treatment increases BDNF expression in the frontal cortex and the hippocampus, similar to the effect of classic antidepressants (Torregrossa *et al*, 2004, 2006). BDNF plays an important role in the therapeutic actions of antidepressants by regulating neuronal survival, differentiation, and plasticity (Tardito *et al*, 2006). On the other hand, the anxiolytic effect of DOR may be exerted at the level of amygdala circuits, since microinjections of the delta selective agonist D-Pen2-D-Pen5-enkephalin into the amygdala reduced anxiety, and this effect was blocked by naltrindole (Randall-Thompson *et al*, 2010).

Preclinical data showed that systemic administration of KOR agonists induced anxiogenic and pro-depressant-like effects (Carlezon *et al*, 2006; Knoll *et al*, 2007; Mague *et al*, 2003). More interesting, central administration of KOR antagonists produced antidepressant- and anxiolytic-like behavioural effects in animal studies (Beardsley *et al*, 2005; Knoll *et al*, 2007; Shirayama *et al*, 2004; Zhang *et al*, 2007a). In humans, pharmacological activation of KOR produces dysphoria, anxiety and psychotomimetic effects (Pfeiffer *et al*, 1986). The neurobiological mechanisms by which KOR antagonists induce antidepressant-like effects and KOR agonists produce pro-depressant effects are not currently known. The blockade of KOR may produce, similar to MOR and DOR, an increase in monoaminergic signalling pathways. Indeed, some lines of evidence suggest that KOR agonists may reduce extracellular dopamine levels within the nucleus accumbens (Carlezon *et al*, 2006), which has been implicated in the pathophysiology of depressive conditions (Nestler and Carlezon, 2006). It was also hypothesized that KOR antagonists attenuate the behavioural effects of elevated cAMP response element-

binding (CREB) expression within the nucleus accumbens, most likely by blocking KOR that normally inhibit neurotransmitter release from mesolimbic dopaminergic neurons, contributing to an antidepressant-like effect (Pliakas *et al*, 2001). Activation of KOR in the locus coeruleus may diminish neural discharge evoked by engaging either glutamate or corticotropin-releasing factor inputs. This results in decreased noradrenergic innervations in forebrain areas, which could contribute to the pro-depressive effect (Kreibich *et al*, 2008).

In summary, DOR agonists and KOR antagonists have promising antidepressant potential. In contrast, data from MOR analysis appear more complex and the risk-benefit ratio of currently available MOR agonists as antidepressants remain difficult to evaluate, in addition to their inherent abuse liability (Lutz and Kieffer, 2013).

4.4.2 Role of the endogenous opioid system in cognition

The role of the endogenous opioid system in learning and memory is well documented. Systemic pharmacological activation of MOR has been reported to produce learning and memory impairments in rodents following acute (Castellano and Pavone, 1985; Stone *et al*, 1991) and chronic treatment (Sala *et al*, 1994; Spain and Newsom, 1991). This opioid-induced impairment can be modulated by opioid antagonists (Canli *et al*, 1990; Introini and Baratti, 1984). several lines of evidences suggest that molecular and synaptic plasticity changes in the hippocampus are also modulated by endogenous opioids (Dacher and Nugent, 2011). In agreement, clinical studies have shown that opioid addicts may have significant cognitive impairments with the duration of the addiction (Curran *et al*, 2001). The nucleus accumbens has been recognized for its role in motivational learning associated with goal-

directed actions and decision making that involves reward outcome (Da Cunha *et al*, 2012; Day *et al*, 2007). MOR are expressed in GABAergic medium-sized spiny neurons of the nucleus accumbens, suggesting that their contribution to these behaviours primarily involves effects on inhibitory input into the nucleus accumbens (Ma *et al*, 2012). Indeed, the MOR agonist DAMGO produced inhibition of spontaneous excitatory and inhibitory postsynaptic currents in medium-sized spiny neurons of the nucleus accumbens containing dopamine D_1 and D_2 receptors, leading to depolarization and enhanced intrinsic cell excitability (Ma *et al*, 2012).

DOR have also been shown to play an essential role in learning and memory processes. They are highly expressed in brain regions involved in cognitive functions, such as hippocampus, amygdala, striatum and other basal ganglia structures (Klenowski et al., 2015). The physiological effects of DOR within the hippocampus are well defined. DOR are mainly localized presynaptically in GABAergic interneurons that form afferent connections to glutamatergic pyramidal cells (Rezaï *et al*, 2012). Thus, DOR activation inhibits presynaptic neurotransmitter release and increases excitation of pyramidal cells in CA1, CA3 and dentate gyrus regions, leading to the facilitation of the LTP in the hippocampus (Klenowski *et al*, 2015). Strong evidence implicates the amygdala in incentive learning and motivational behaviours associated with the rewarding effects of addictive substances (Robbins and Everitt, 2002). DOR activity within the CeA contributes to learned associations that are formed during drug-context conditioning paradigms (Marinelli *et al*, 2009). Similar to MOR, DOR expressed in the nucleus accumbens contribute to motivational learning and processes that reinforce drug-seeking behaviour. Indeed, infusion of DOR agonists into the nucleus accumbens promotes cocaine seeking (Simmons and Self, 2009) and

feeding behaviour in rodents (Zhang *et al*, 2003; Zhang and Kelley, 2000). Conversely, the reduction in performance mediated by DOR antagonism was attributed to a deficit in predictive learning required to guide choice based on paired stimulus-reward outcomes (Klenowski *et al*, 2015). The expression of DOR within the rat nucleus accumbens is presynaptic (Cahill *et al*, 2001) and also postsynaptic in medium-sized spiny neurons and cholinergic interneurons (Bertran-Gonzalez *et al*, 2013). Preclinical evidence suggests that the effect of DOR on drug reward-related learning results from plastic changes affecting cholinergic interneurons synapses in the nucleus accumbens shell (Bertran-Gonzalez *et al*, 2013). The dorsal striatum and associated basal ganglia circuitry have key roles in motor and habit learning (Grahn *et al*, 2009; Graybiel, 2008; Lovinger, 2010). DOR-expressing neurons in the dorsal striatum also expressed D_1 receptors, indicating that these dopamine receptors may mediate DOR influence on these cognitive functions (Ambrose *et al*, 2006).

The involvement of KOR in memory function has been also reported. Both KOR and its endogenous ligand dynorphin are present in the hippocampus and amygdala (Schwarzer, 2009). Dynorphins modulate the information between the dentate gyrus and the CA3 region of the hippocampus decreasing excitatory glutamatergic signalling and therefore diminishing hippocampal activity (Bilkei-Gorzo *et al*, 2014). In addition, pharmacological KOR activation produces aversive emotional behaviours that contribute to the stress-induced learning and memory dysfunctions in mice (Carey *et al*, 2009), including reduced social memory (Bertran-Gonzalez *et al*, 2013). Interestingly, a human genetic study also revealed that subjects with a rare gene polymorphism associated with reduced PDYN expression is associated with a better episodic memory (Kölsch *et al*, 2009).

4.5 Role of the endogenous opioid system in pain

4.5.1 Acute nociception

The endogenous opioid system plays a crucial role modulating pain transmission at peripheral, spinal and supraspinal level (Corder *et al*, 2018). At the peripheral level, opioid peptides released by immune cells during inflammation locally inhibit pain transmission (Rittner *et al*, 2008). Initial studies suggested that the different types of opioid receptors, specially MOR and DOR, were co-expressed by the same class of DRG neurons. However, the emergence of novel techniques to investigate opioid receptor expression, particularly reporter mice expressing fluorescent opioid receptors and single-cell RNA sequencing has revealed that each opioid receptor is specifically distributed among different DRG neuron classes, implying that receptor classes preferentially control distinct types of pain and somatosensory modalities (Figure 17). Thus, DOR is enriched in myelinated mechanosensory neurons that project to the skin and that have been implicated in tactile hypersensitivity (allodynia) in the setting of chronic inflammatory or neuropathic pain (Bardoni *et al*, 2014; Scherrer *et al*, 2009; Usoskin *et al*, 2015). MOR in DRG are mainly expressed in unmyelinated peptidergic nociceptors that express substance P and TRPV1 (Chen and Pan, 2008; Ueda, 2006; Vetter *et al*, 2006). These neurons detect heat and chemical noxious stimuli in skin and internal organs. MOR in DRG can be targeted by peripherally restricted agonists (i.e., limited blood–brain barrier permeability) to produce analgesia without CNS-derived side effects (DeHaven-Hudkins and Dolle, 2004; Vadivelu *et al*, 2011), but these findings were not supported by studies using conditional knockout mice (see section 4.6.3) (Araldi *et al*, 2018; Corder *et al*, 2017). Animal studies provided evidence

that KOR in DRG may control visceral pain and proposed the use of peripherally acting KOR agonists for these pain modalities (Kivell and Prisinzano, 2010; Vanderah, 2010). The role of NOR in DRG is not completely understood, but the recent generation of a knock-in mice expressing NOR in fusion with the enhanced green fluorescent protein (NOR-eGFP) revealed a broad distribution of NOR in DRG neurons, including in unmyelinated peptidergic nociceptors, and in several populations of myelinated neurons that may include cutaneous mechanoreceptors and proprioceptors (Ozawa *et al*, 2015).

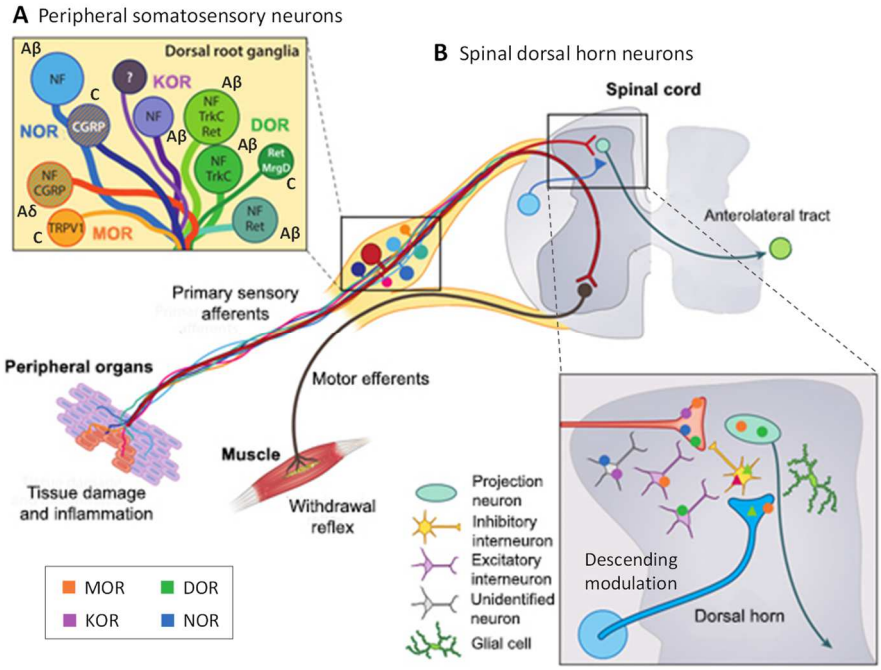

Figure 17. Opioid receptors distribution in DRG (A) and dorsal horn neurons (B). NF marking large-diameter DRG neurons with myelinated axons. Striped neurons coexpress different opioid receptor types. Abbreviations: CGRP, calcitonin gene-related peptide; DOR, delta opioid receptor; KOR, kappa opioid receptor; MOR, mu opioid receptor; MrgD, Mas-related G protein–coupled

receptor member D; NF, neurofilament; NOR, nociceptin opioid receptor; Ret, Ret proto-oncogene; TrkC, tropomyosin receptor kinase C; TRPV1, transient receptor potential vanilloid 1 (adapted from Corder *et al*, 2018).

At the spinal level, opioids control nociceptive responses by inhibiting the synapse between primary afferent nociceptive neurons and second-order neurons. The stimulation of presynaptic opioid receptors inhibits the release of neurotransmitters from nociceptors, whereas the activation of postsynaptic opioid receptors reduces the excitability of spinal cord dorsal horn projection neurons (Stein and Machelska, 2011). Opioid receptor are also distributed in distinct subpopulations of spinal cord dorsal horn neurons (Figure 17). MOR are expressed by nociceptive dorsal horn neurons, including excitatory interneurons and lamina I projection neurons (Aicher *et al*, 2000; Spike *et al*, 2002). DOR are expressed in somatostatin+ excitatory interneurons, which gate mechanosensory inputs (Duan *et al*, 2014), and in projection neurons, where it partially overlap with MOR (Wang *et al*, 2018). This co-expression suggests that these two receptors may cooperate postsynaptically in neurons receiving convergent inputs from segregated DOR+ and MOR+ afferents. Definitive identification of KOR distribution in specific neurons of the spinal cord dorsal horn circuits is still lacking, although the existence of responsive neurons to the KOR-selective agonist U50488H in the dorsal horn have been documented (Eckert and Light, 2002). NORs were shown to be expressed throughout laminae I–III dorsal horn neurons (Ozawa *et al*, 2015), but the precise identity of these neurons, as well as the endogenous source of nociceptin peptide that acts on these receptors remain to be established. Dynorphin and enkephalin are expressed by distinct classes of dorsal horn interneurons (Boyle *et al*, 2017; François and Scherrer, 2018) and under certain

conditions spinal dynorphins can have pronociceptive effects (Podvin *et al*, 2016).

At supraspinal level, the endogenous opioid system participates in descending inhibitory and facilitatory pathways by acting in the PAG and RVM (Fields, 2004; Ossipov *et al*, 2010). In the RVM, On and Off neurons have axons that reach the dorsal horn of the spinal cord to facilitate (On cells) or inhibit (Off cells) nociceptive transmission (Ossipov *et al*, 2010). Opioid receptor activation in the PAG produces the disinhibition of Off cells in the RVM, whereas On cells are directly inhibited by opioids in the RVM (Fields, 2004), both activities resulting in antinociceptive effects. At this level, the endogenous opioid system also increases the activity of noradrenergic neurons in the locus coeruleus, which inhibits synaptic transmission in the spinal cord. At supraspinal level, MOR also modulate pain processing by being recruited in nociceptive thalamic regions, including the ventral posterior nucleus and the intralaminar nuclei (Abdul Aziz *et al*, 2005; Pozza *et al*, 2010; Tamaddonfard and Erfanparast, 2017). Opioid receptors and peptides are expressed in limbic and cortical areas involved in affective processing of pain, as well as in the affective and rewarding aspects of pain analgesia (Cahill *et al*, 2013; Hummel *et al*, 2008; Kupers *et al*, 1991; Price *et al*, 1985). MOR signalling in the anterior cingulate cortex relieves pain affect (LaGraize *et al*, 2006; Navratilova *et al*, 2015). MOR system also acts on multiple cortical and subcortical sites to influence dopaminergic neurotransmission between the ventral tegmental area and nucleus accumbens to reduce pain aversion (Navratilova *et al*, 2012). MOR is expressed by GABAergic neurons of the central nucleus and intercalated cell masses of the amygdala, which represents a crucial node in affective brain circuits (Winters *et al*, 2017). Inhibition of these neurons by MOR stimulation may reduce aversive

behaviour and amygdala inhibitory input onto descending brainstem pain pathway responses (Han *et al*, 2015; Namburi *et al*, 2015). However, the precise aspects of the pain experience that are encoded in the nucleus accumbens and amygdala (salience, valence, motivation, analgesia), and the identity of MOR-expressing neurons that modulate pain in the anterior cingulate cortex, nucleus accumbens, amygdala, and ventral tegmental area, remain to be determined. KOR, DOR, and NOR also modulate pain supraspinally (Miaskowski *et al*, 1991; Yamamoto *et al*, 2001). KOR activation in the dorsal raphe nucleus mediates descending antinociception (Land *et al*, 2009; Zhao *et al*, 2007). The dynorphin–KOR system within the nucleus accumbens circuitry is known to modify the hedonic value of nociceptive events and shape motivational behaviours in response to painful experiences (Al-Hasani *et al*, 2015; Castro and Berridge, 2014). This system may also contribute to shaping pain-induced negative emotional disorders (Massaly *et al*, 2016). DOR and NOR are also distributed across the pain affect and descending control circuits, particularly in the anterior cingulate cortex and the amygdala (Goody *et al*, 2002; Ozawa *et al*, 2015; Scherrer *et al*, 2006; Toll *et al*, 2016), but how these opioid receptor populations modulate the affective dimensions of pain experience requires further clarification.

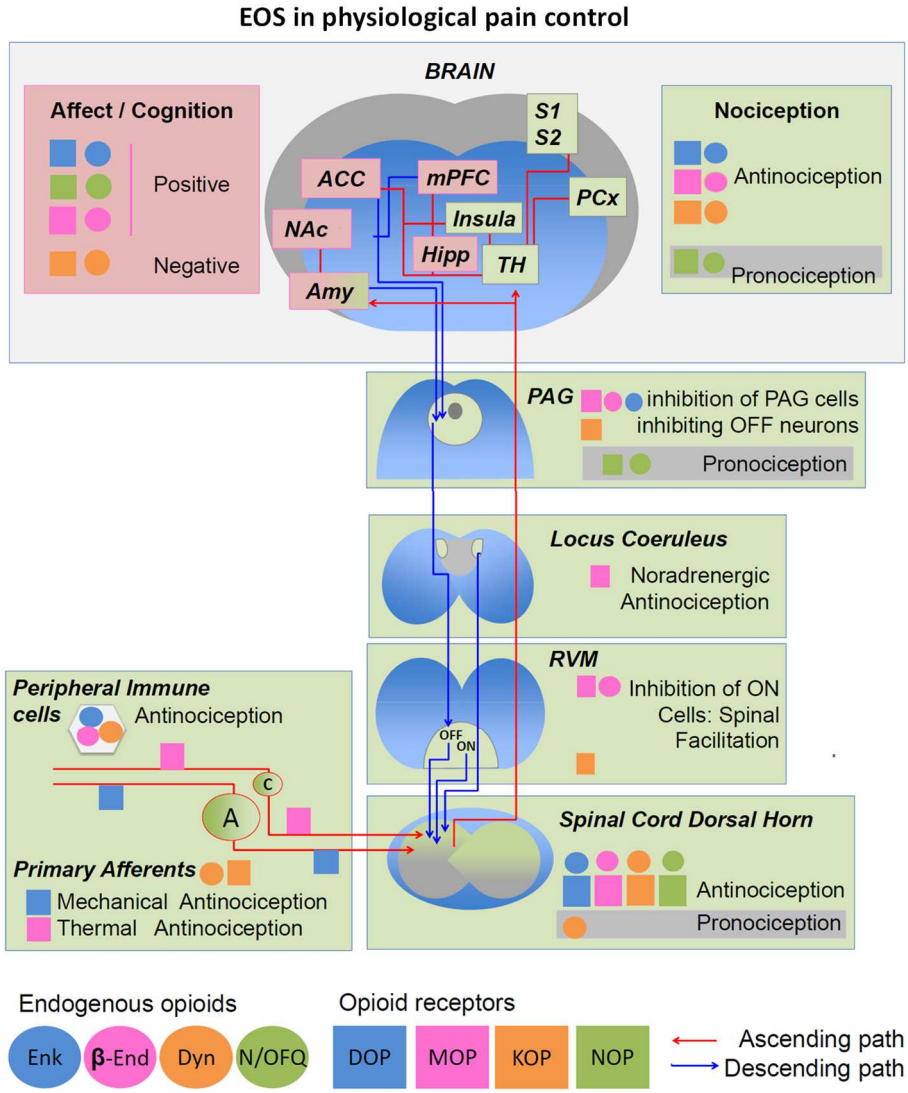

Figure 18. Schematic view of ascending and descending pain pathways, opioid locations and main effects in physiological conditions. A, A primary afferent fibres; ACC, anterior cingulate cortex; C, C primary afferent fibres; Enk, enkephalins; β-end, β-endorphin; Dyn, dynorphins; Hipp, hippocampus; mPFC, medial prefrontal cortex; NAcc, nucleus accumbens; NMDAR, OFF, off cells; ON, on cells; PAG, periaqueductal gray; PCx, parietal cortex; RVM, rostral ventromedial medulla; S1 and S2, primary and secondary somatosensory cortices; TH, thalamus (Maldonado *et al*, 2018).

When opioids are given for pain treatment, analgesia usually appears associated to a wide range of possible side effects including constipation, nausea, vomiting, somnolence, mental clouding, respiratory depression and dysphoria or euphoria (Galanter *et al*, 2014). Central opioid mechanisms can also lead to analgesic tolerance and hyperalgesia, and due to their reinforcement properties, opioids have important abuse liability as revealed by the opioid abuse epidemy currently affecting USA with dramatic consequences (Volkow and McLellan, 2016). Opioid epidemic began two decades ago with the promotion of a sustained-release oxycodone preparation, which was supposed to have reduced abuse liability according to the manufacturer (Van Zee, 2009). This claim was used to convince physicians to prescribe this oxycodone preparation for many years and the agent became one of the most prescribed opioids in USA (FindLaw, 2018). The high availability of oxycodone correlated with increased abuse, diversion, and addiction, and by 2004 oxycodon had become a leading drug of abuse in the United States (Cicero *et al*, 2005). In 2010, a new formulation of sustained-release oxycodone was developed using an abuse-deterrent formulation to be harder to crush and abuse (Hwang *et al*, 2015). Although this formulation did reduce the abuse capability of oxycodone, it pushed people toward other opioid drugs, including heroin (Cicero and Ellis, 2015). Oxycodone has now become a highly likeable analgesic by drug abusers, possibly more so than hydrocodone and morphine (Wightman *et al*, 2012). Thus, controlled drugs with potential for abuse and diversion can pose public health risks that may be more problematic than those of uncontrolled drugs when they are overpromoted and highly prescribed. Despite their severe side effects, the use of opioids in the management of severe

pain is considered the standard of care in most countries (Rosenblum *et al*, 2008).

4.5.2 Chronic pain

Chronic pain is not a symptom of a disorder but a pathological condition that results from maladaptive changes in the nociceptive transmission pathways. Alterations of the endogenous opioid system under such conditions may lead to different effects of opioid activity than in physiological pain. While moderate to severe acute pain can be efficiently treated with opioids, opioid treatment of chronic pain has many deleterious consequences for the patients. Blunted efficacy of opioids, tolerance, physical dependence, hyperalgesia and abuse liability undermine the efficiency of these treatments (Trang *et al*, 2015).

During chronic inflammatory pain, MOR have an important function limiting nociceptive inputs (Severino *et al*, 2018; Walwyn *et al*, 2016). Indeed, opioid analgesics continue to be the cornerstone for the management of moderate to severe nociceptive and inflammatory pain (Carroll *et al*, 2004). However, MOR agonists show low antinociceptive efficacy in animal models of neuropathic pain (Idänpään-Heikkilä *et al*, 1997; Kimura *et al*, 2014; Obara *et al*, 2004, 2007; Rashid *et al*, 2004). It has been even shown that subchronic treatment with systemic morphine, not only did not attenuate but may also aggravate cold and mechanical allodynia in nerve-injured mice (Roeckel et al., 2017). Preclinical studies with nerve-injured animals seem to indicate an effect of the route of administration on the analgesic activity of morphine. No decrease in morphine potency at the supraspinal level was reported, suggesting intact antinociceptive activity of supraspinal MOR (Bian *et al*, 1995; Lee *et al*, 1995). However, the lack of analgesic effect of intrathecal

morphine in such conditions suggests altered functionality of spinal MOR following nerve injury (Bian *et al*, 1999; Mao *et al*, 1995; Ossipov *et al*, 1995a, 1995b). The role of peripheral MOR is controversial since evidences for (Chung *et al*, 2012; Guan *et al*, 2008; Obara *et al*, 2004, 2007; Pertovaara and Wei, 2001) and against (Corder *et al*, 2017; Rashid *et al*, 2004; Weibel *et al*, 2013) the analgesic efficacy of peripheral MOR stimulation under neuropathic pain conditions have been reported. Furthermore, recent studies have shown that MOR in DRG are important contributors to two of the adverse side effects associated with chronic MOR agonist treatments, tolerance and opioid-induced hyperalgesia (Araldi *et al*, 2018; Corder *et al*, 2017; Tiwari *et al*, 2018), although these results are not supported by other studies (Roeckel *et al*, 2017; Weibel *et al*, 2013). However, preclinical data do not correlate well with clinical experience in neuropathic pain. Indeed, some severe neuropathic pain conditions are still treated by systemic MOR agonists, despite the important side effects, and intrathecal opioids have been shown to be effective in some intractable clinical cases of non-cancer neuropathic pain, alone or in combination with other drugs (Martin Paiz *et al*, 2015; Sadiq and Poopatana, 2007; Vigneri *et al*, 2016; Warner *et al*, 2018; Wu *et al*, 2013). Although peripheral opioid analgesia is of clinical relevance in inflammatory pain conditions, it is difficult to ascertain if peripheral MOR provide any contribution in opioid analgesia in neuropathic pain patients. However, long-term administration of systemic methyl-naltrexone, a peripherally acting MOR antagonist, to neuropathic pain patients was safe and effective against opioid-induced constipation without affecting analgesia (Webster *et al*, 2017; Webster and Israel, 2018), suggesting a minimal role of peripheral MOR in opioid analgesia under such pain conditions.

Many studies have examined the molecular mechanisms behind the decreased effectiveness of MOR agonists in neuropathic pain, focusing on spinal mechanisms. They include reduction in MOR expression in the spinal dorsal horn (Porreca *et al*, 1998), enhanced spinal release of dynorphin (Bian *et al*, 1999; Nichols *et al*, 1997) and cholecystokinin (Nichols *et al*, 1996; Zhang *et al*, 2000), increased NMDA activity and the subsequent intracellular activation of PKC and nitric oxide synthesis (Bian *et al*, 1999; Mao *et al*, 1995), upregulated expression of spinal $mGluR_1$ (Fundytus *et al*, 2001), and activation of tonic descending facilitation pathways from the brain (Vanderah *et al*, 2001). Alternatively, several peripheral dysregulations that operate after nerve lesion and may contribute to the reduction in systemic morphine potency have been also suggested. Drastic decrease of MOR expression in DRG neurons due to primary afferent fibres damage was reported years ago (Li *et al*, 1996; Obara *et al*, 2010; Ossipov *et al*, 1995b; Rashid *et al*, 2004; Zhang *et al*, 1998). More recently, epigenetic alterations have been suggested to be responsible for this down-regulation of MOR gene expression in the DRG and in the dorsal horn of the spinal cord, leading to limited morphine effectiveness (Rivat, 2016; Uchida *et al*, 2010, 2015; Zhang *et al*, 2016). Dysfunctional coupling of MOR to G protein in the DRG following nerve injury was also reported and related to the diminished analgesic efficacy of morphine in neuropathic pain (Obara *et al*, 2010). Interestingly, these pathophysiological mechanisms that underly pain hypersensitivity are triggered by both nerve injury and opioid treatment, which may modulate each other. Therefore, it would account for the low opioid analgesic efficacy in neuropathic pain conditions.

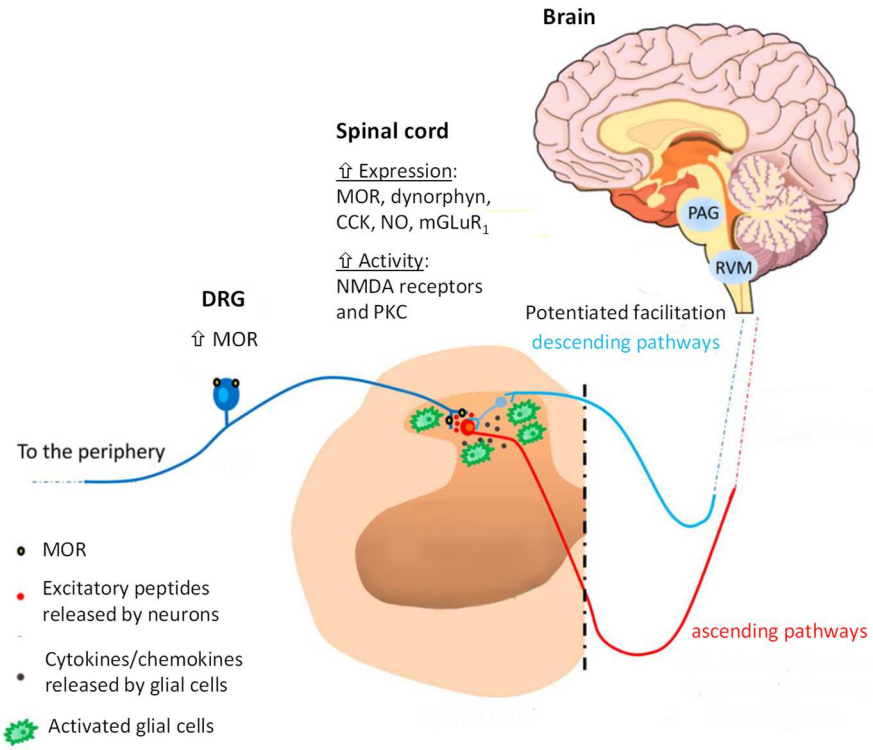

Figure 19. Peripheral and spinal molecular mechanisms that may account for the reduced effectiveness of MOR agonists in neuropathic pain. CCK, cholecystokinine; DRG, dorsal root ganglia; MOR, mu opioid receptor; $mGluR_1$, metabotropic glutamate receptor subtype 1 (adapted from Rivat and Ballantyne, 2016).

Additionally, chronic pain is accompanied by changes in plasticity in supraspinal pain related areas, such as the mesolimbic dopaminergic system. Inflammatory pain desensitizes MOR in the ventral tegmental area, promoting opioid consumption (Hipólito *et al*, 2015; Narita *et al*, 2005), and neuropathic pain is accompanied by decreased nucleus accumbens dopamine release, an effect that involves microglial activation in the ventral tegmental area (Taylor *et al*, 2015), as well as other negative regulators of dopamine transmission. These findings

suggest that chronic pain may reduce the involvement of MOR modulating the hedonic value of nociceptive inputs. In this line, it would be interesting to elucidate the contribution of MOR shaping pain-induced negative emotional disorders under chronic pain conditions.

On the other hand, several distinct chronic pain models are sensitive to DOR agonists, including inflammatory, neuropathic, cancer and diabetic pain, since DOR activation reduces hypersensitivity in heat, cold and mechanical modalities (Gavériaux-Ruff and Kieffer, 2011). Indeed, DOR-mediated antinociception seems to be more efficient under chronic pain than in acute pain conditions, and clinical trials should be performed to validate their translational potential to patients. However, the function of DOR modulating affective responses associated to neuropathic pain has not been thoroughly explored in animal models.

KOR activation produces analgesia (Cahill *et al*, 2014) and this receptor is involved in chronic pain states, including neuropathic and osteoarthritis pain. Pharmacological evidences demonstrate that selective KOR agonists attenuate mechanical allodynia and inflammation during osteoarthritis (Shen *et al*, 2005). Indeed, reduced KOR expression in osteoarthritis patients might be critical in the progression and maintenance of the osteoarthritis disease (Shen *et al*, 2005). Neuropathic pain is also sensitive to KOR-mediated analgesia although the antinociceptive effects appear to be weaker than that evoked by MOR or DOR agonists (Przewlocki and Przewlocka, 2005). Intraplantar injection of peripherally-selective KOR agonists induced antinociceptive effects in a rat model of neuropathic pain, indicating a peripheral component of this KOR-mediated analgesia during such conditions (Catheline *et al*, 1998; Walker *et al*, 1999). Accordingly, mechanical and thermal allodynia were

significantly enhanced after treatment with KOR antagonists norbinaltorphimine and 5'-guanidinonaltrindole (GNTI) in rodent models of neuropathic pain (Obara *et al*, 2003). However, enhanced expression and pronociceptive actions of spinal dynorphin have been reported following nerve injury (Lai *et al*, 2008; Podvin *et al*, 2016; Xu *et al*, 2004), suggesting that spinal dynorphin can act as an anti-opioid under such conditions, promoting the development of neuropathic pain.

Table 9. Role of opioid receptors in acute nociception and chronic pain based on pharmacological studies

Opioid receptor	Acute nociception	Chronic pain
MOR	↓ heat nociception ↓ chemical nociception	↓ inflammatory pain Low efficay in neuropathic pain
DOR	↓ mechanical nociception	↓ inflammatory pain ↓ neuropathic pain
KOR	↓ visceral nociception	↓ osteoarthitis pain ↓ neuropathic pain

4.6 Generation of knockout models of the endogenous opioid system

In addition to pharmacological studies, knockout mouse models represented excellent tools to better understand the physiological role of each component of the endogenous opioid system.

4.6.1 Usefulness of knockouts to clarify the role of the opioid system in mood disorders

The deletion of MOR in mice produced an anxiolytic-like effect in the elevated plus maze, but not in the light/dark box paradigm (Filliol *et al*, 2000; Yoo *et al*, 2004). MOR knockouts also showed decreased immobility time in the forced swimming test, suggesting that the blocking of MOR contributes to the establishment of antidepressant-like effects in mice (Filliol *et al*, 2000; Yoo *et al*, 2004). These results are in contrast with pharmacological findings (Besson *et al*, 1996; Tejedor-Real *et al*, 1995) and may indicate the possibility of a paradoxical depressant role of MOR in regulating emotional responses. Since the attenuation of conditioned suppression of motility, another model of depressive-like behaviour, observed in MOR knockouts was reversed by the DOR antagonist naltrindole (Filliol *et al*, 2000), a predominant tonic activation of DOR may be involved in the behavioural changes of MOR knockout mice. Furthermore, the phenotypic modifications were observed for males only, opening the possibility of sexual dimorphism in the activity of opioid receptors for these behaviours. Mice lacking β-endorphin did not show any alteration in levels of anxiety (Rubinstein *et al*, 1996; Trigo *et al*, 2009).

DOR deficient mice exhibited opposing affective responses than MOR knockout mice. DOR knockouts showed anxiogenic-like responses in both

elevated plus maze and light/dark box (Filliol *et al*, 2000), suggesting that the activity of DOR may contribute to diminish anxiety-like responses. This is also in accordance with the enhanced anxiety-like behaviour revealed in mice lacking preproenkephalin gene (König *et al*, 1996). The anxiolytic activity of DOR was proposed to be mediated by DOR in the amygdala, since conditional DLX5/6 DOR knockouts lacking DOR in forebrain areas, but not in the amygdala, did not replicate the anxiogenic phenotype of constitutive DOR knockouts (Chu Sin Chung *et al*, 2015). DOR knockouts also showed a pro-depressive phenotype as shown by increased immobility time in the forced swimming test (Filliol *et al*, 2000), suggesting that the blockade of DOR may contribute to the development of depressive-like behaviour. In this case, DOR knockout models and pharmacological studies using DOR selective drugs agreed to show a mood-enhancing activity of the enkephalin/DOR system. PENK suppression also enhanced aggressiveness and anxiety-like behaviour (Bilkei-Gorzo *et al*, 2004; König *et al*, 1996; Ragnauth *et al*, 2001), while depressive-like behaviour was normal in PENK-deficient mice under normal conditions (Bilkei-Gorzo *et al*, 2007).

Finally, the deletion of *Pdyn* gene increased anxiety-like responses, in contrast to the pharmacological data observed with KOR antagonists (Bilkei-Gorzo *et al*, 2008; Femenía *et al*, 2011). Conversely, KOR knockout mice did not display altered anxiety-like behaviours (Filliol *et al*, 2000; Simonin *et al*, 1998). Disruption of the gene coding for PDYN significantly reduced depressive-like behaviour (McLaughlin *et al*, 2003, 2006), supporting the above findings about the anti-depressant effect of KOR antagonists. However, KOR knockouts showed similar responses to those of wild-type mice in the forced swimming test, suggesting minor involvement of endogenous KOR activation modulating depressive states

(Filliol *et al*, 2000). These discrepancies between studies may be due to the use of different experimental paradigms and test conditions.

Taking together, findings from knockout models and pharmacological studies reveal that MOR, DOR and KOR exert highly distinct controls over mood-related processes as summarized in Table 10.

Table 10. Role of the opioid System in mood disorders based on pharmacological and genetical studies

	Opioid component	**Effect on emotional responses**
Pharmacological	MOR	Antidepressant-like effects??
	DOR	Anxiolytic & antidepressant-like effects
	KOR	Anxiogenic & pro-depressant-like effects
Genetical (knockout mouse models)	MOR	Anxiogenic- & pro-depressant-like effects
	DOR	Anxiolytic- & antidepressant-like effects
	KOR	No major role (unchanged anxiety & depressive-like behaviours)
	POMC	No major role in anxiety (unchanged behaviour)
	PENK	Anxiolytic & no major role on depression (unchanges behaviour)
	PDYN	Anxiolytic & pro-depressant-like effects

4.6.2 Usefulness of knockouts to clarify the role of the opioid system in cognitive functions

The lack of MOR decreased LTP in the dentate gyrus of the hippocampus (Matthies *et al*, 2000), suggesting the possibility that it may accompany a change in learning and memory. LTP deficits were correlated later with spatial memory impairment in MOR knockouts (Jang *et al*, 2003). Similarly, the impairment in the maintenance of LTP in mossy fibres in the CA3 area of the hippocampus was also associated with the impaired spatial learning observed in MOR null mutants (Jamot *et al*, 2003).

DOR knockouts showed impaired place conditioning in both appetitive and aversive conditions, indicating disrupted context-drug association (Le Merrer *et al*, 2011). This agree with a role of DOR facilitating context-drug association. According to pharmacological and electrophysiological studies, mice lacking DOR displayed impaired performance in two hippocampal-dependent tasks, including contextual and spatial learning, which suggests that DOR-mediated LTP in the hippocampus may be associated with the acquisition and consolidation of this form of declarative memory (Le Merrer *et al*, 2013). Alternatively, DOR knockouts showed facilitated striatum-dependent responses (Le Merrer *et al*, 2013). This study suggests that DOR activity tonically inhibits striatal function, and that DOR modulate learning and memory performance by regulating the hippocampal/striatum balance (Le Merrer *et al*, 2013).

In agreement with a negative impact of the PDYN/KOR system on memory function, genetic ablation of *Pdyn* gene enhanced social memory in mice (Bilkei-Gorzo et al., 2014). Spatial memory was unaffected in constitutive KOR knockout mice (Jamot *et al*, 2003).

Table 11. Opioid system knockout mice phenotypes in models of cognitive performance. Behavioural modifications are summarized for each receptor and PDYN constitutive KO mouse line.

Opioid component	Behavioural modifications
MOR	- Impaired spatial learning
DOR	- Disrupted context-drug association - Impaired performance in hippocampal-dependent tasks (contextual & spatial learning) - Facilitated striatum-dependent responses (including skill motor learning)
KOR	- Unaffected spatial memory
PDYN	- Increased social memory - Unchanged spatial memory

4.6.3 Usefulness of knockouts to clarify the role of the opioid system in pain

Acute pain

The acute nociceptive responses of mice lacking opioid receptors and opioid peptide precursors have been examined in several pain models. These evaluations revealed an antinociceptive opioid tone and distinct pattern of activities of each opioid receptor (Martin *et al*, 2003).

Baseline mechanical sensitivity was generally unaltered in constitutive MOR knockouts when assessing light touch sensitivity with von Frey hairs, although nociceptive responses to stronger mechanical stimuli were increased (Fuchs *et al*, 1999; Martin *et al*, 2003). MOR-deficient mice displayed increased nociceptive sensitivity to heat and in the early phase of the formalin test (Martin *et al*, 2003; Matthes *et al*, 1998). MOR suppression also disrupted analgesic effects of MOR agonists (Ide *et al*,

2006, 2008; Kögel *et al*, 2011; Pan *et al*, 2009). All these findings indicate that MOR influence responses to thermal, mechanical and chemical nociception. Conditional knockouts lacking MOR in Nav1.8+ primary afferent fibres displayed no behavioural alterations in baseline nociceptive thresholds, suggesting the lack of participation of peripheral MOR in acute antinociception (Corder *et al*, 2017; Weibel *et al*, 2013). On the other hand, POMC deficit induced no change (Fell *et al*, 2014; Gendron *et al*, 2007; Petraschka *et al*, 2007; Walwyn *et al*, 2016) or slight increase (Mogil *et al*, 2000; Trigo *et al*, 2009) in baseline heat and mechanical nociceptive thresholds.

In constitutive DOR knockouts, baseline nociceptive thresholds to somatic thermal or mechanical stimuli were not modified (Filliol *et al*, 2000; Gavériaux-Ruff *et al*, 2008; Nadal *et al*, 2006). However, DOR-deficient mice showed increased response in the late phase of the formalin test and in the tail pressure test, indicating a role of DOR modulating inflammatory pain and responses to certain mechanical nociceptive stimuli (Martin *et al*, 2003). Moreover, DOR Nav1.8 conditional knockouts revealed no behavioural alterations in acute nociception, indicating no role of this peripheral DOR population on acute nociception (Gaveriaux-Ruff *et al*, 2011). PENK-deficient mice showed increased supraspinal responses to nociceptive stimulation but unaltered reflex responses to heat or mechanical stimuli (Chen *et al*, 2008; Gendron *et al*, 2007; Kingery *et al*, 2001).

Total KOR knockouts displayed normal (Martin *et al*, 2003; Negrete *et al*, 2017; Simonin *et al*, 1998; Xu *et al*, 2004) or slightly enhanced (Gavériaux-Ruff *et al*, 2008; Martin *et al*, 2003) baseline sensitivity to mechanical and thermal stimuli. Inflammatory and nociceptive responses

were unchanged in the formalin test, whereas visceral pain was increased in the writhing test (Martin *et al*, 2003; Simonin *et al*, 1998). All these findings suggest the involvement of KOR in spinally mediated thermal nociception and chemical visceral pain. Accordingly to KOR knockouts, baseline sensitivities to heat and mechanical stimuli were not modified in constitutive PDYN knockouts (McLaughlin *et al*, 2003; Parikh *et al*, 2011; Wang *et al*, 2001; Zimmer *et al*, 2001), although certain experimental conditions have revealed increased sensitivity to mechanical (Walwyn *et al*, 2016) and heat stimuli (Wang *et al*, 2001).

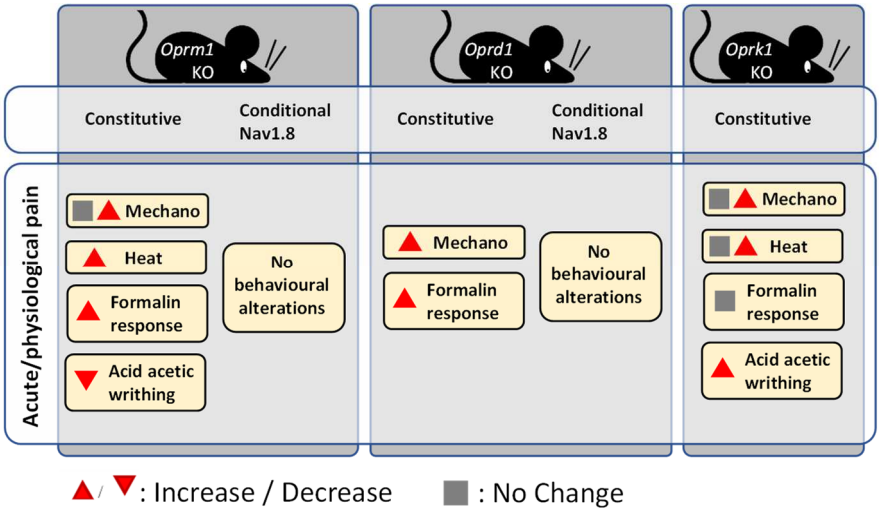

Figure 20. Summary of the results obtained from opioid receptor knockout mice in acute pain models. Heat, heat sensitivity; KO, knockout; Mechano, mechanical sensitivity (adapted from Maldonado et al., 2018).

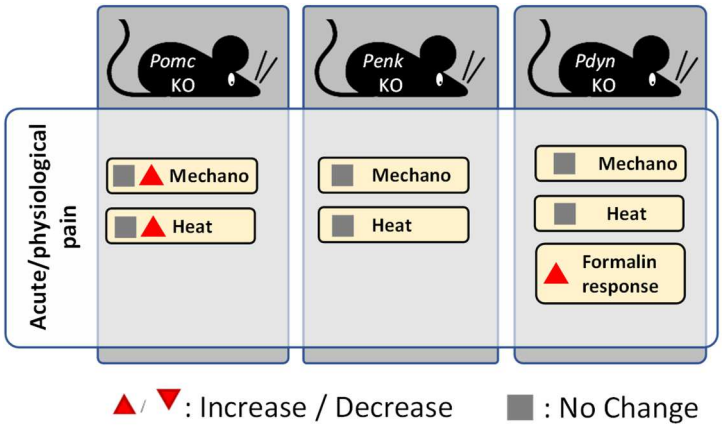

Figure 21. Summary of the results obtained from opioid precursors knockout mice in acute pain models. Heat, heat sensitivity; KO, knockout; Mechano, mechanical sensitivity (adapted from Maldonado et al., 2018).

Chronic pain

Knockout mice were certainly useful for the elucidation of the role of opioid receptors and opioid peptide precursors in chronic pain. In the case of constitutive MOR knockouts, partly conflicting results were obtained in different models of chronic inflammatory pain and neuropathic pain. Increased, no difference or decreased nociceptive behaviour have been reported depending on each specific study (Maldonado *et al*, 2018). Divergent results may indicate a complex role for MOR in the pathophysiology of chronic pain that could be explained by the multiplicity of MOR knockout models or also by methodological differences (Maldonado *et al*, 2018). Conditional knockouts with a selective deletion of MOR in Nav1.8+ nociceptors did not show altered hypersensitivity induced by CFA (Weibel *et al*, 2013). MOR in these fibres participated in the analgesic effects of classical MOR agonists during chronic inflammation induced by CFA, but not in basal conditions (Weibel

et al, 2013). However, another study using conditional knockouts lacking MOR in TRPV1+ nociceptors revealed that these peripheral MOR were not necessary for the antinociception resulting from systemic morphine (Corder *et al*, 2017). Instead, MOR in DRG were important contributors to two of the adverse side effects associated with chronic MOR agonist treatments, tolerance and opioid-induced hyperalgesia (Araldi *et al*, 2018; Corder *et al*, 2017). POMC knockouts displayed normal nociceptive sensitization to thermal and mechanical stimuli in inflammatory (Gendron *et al*, 2007; Walwyn *et al*, 2016) and peripheral neuropathic pain models (Labuz *et al*, 2016; Niikura *et al*, 2008a, 2008b; Petraschka *et al*, 2007). However, these mice retained sensitivity to analgesic effects of MOR agonists and showed decreased tolerance to MOR agonists during neuropathic pain. Therefore, β-endorphin activity would not be sufficient to modulate the nociceptive manifestations of chronic pain, but it could affect the functionality of opioid receptors.

In agreement with pharmacological studies, the use of genetic approaches confirmed that the role of DOR acquires more relevance in chronic pain conditions (Maldonado *et al*, 2018). Thus, constitutive DOR knockout mice showed enhanced mechanical and cold allodynia as well as heat hyperalgesia following CFA or after peripheral nerve injury (Gavériaux-Ruff *et al*, 2008; Nadal *et al*, 2006). These studies agreed on a protective function of DOR for the development of these chronic pain manifestations (Maldonado *et al*, 2018). It is not yet known if the heightened chronic pain sensitivity in constitutive DOR knockouts could be influenced by their inherent depressive and anxious phenotype (Filliol *et al*, 2000). Conditional DOR deletion in primary afferent fibres expressing Nav1.8 revealed the involvement of these receptors in chronic pain (Gaveriaux-Ruff et al., 2011). Indeed, these conditional mutants

showed unchanged heat hyperalgesia but increased mechanical allodynia after CFA and enhanced mechanical and cold hypersensitivity after PSNL (Gaveriaux-Ruff *et al*, 2011). As constitutive DOR knockouts showed increased sensitivity to heat and this increase was absent in the conditional lines, this result suggests that DOR in central structures modulate heat hypernociception, whereas DOR from peripheral neurons control mechanical and cold hypersensitivity. DOR in peripheral fibres also participated in the analgesic effects of SNC80 during both chronic inflammatory and neuropathic pain conditions (Gaveriaux-Ruff *et al*, 2011). On the other hand, no major modifications in heat and mechanical sensitization induced by chronic inflammatory or neuropathic pain were revealed after the constitutive genetic deletion of PENK (Labuz *et al*, 2016; Walwyn *et al*, 2016). This is in contrast to the changes on these chronic pain manifestations revealed in DOR knockouts. These controversial findings have suggested the possibility of ligand-independent opioid receptor constitutive activity in the control of nociceptive responses during chronic pain (Corder *et al*, 2013; Walwyn *et al*, 2016).

Removal of KOR favours a phenotype prone to nociceptive sensitization in chronic pain. These mutants showed increased heat sensitization after streptozotocin-induced diabetic neuropathy (Rutten *et al*, 2014) and enhanced heat and mechanical sensitivity as well as a contralateral mirror image sensitization after PSNL (Xu *et al*, 2004). Increased and contralateral sensitization was also reported in the CFA model of chronic inflammatory pain (Schepers *et al*, 2008), although another study did not show abnormal sensitivity in the same CFA model probably due to the high baseline sensitivity of the mice used (Gavériaux-Ruff *et al*, 2008). The model of monoiodoacetate-induced osteoarthritis pain also revealed

enhanced mechanical sensitivity in these mutants, although anxiety-like behaviour and cognitive impairment associated to these chronic pain manifestations were attenuated (Negrete *et al*, 2017). Another model of osteoarthritis showed enhanced joint damage in KOR knockouts (Wu *et al*, 2017), and increased formation of cancellous bone in these mutants (Baldock *et al*, 2012), suggesting that the effects of KOR deletion on chronic osteoarthritis pain could also be local. However, no histological modifications were found on the articular cartilage in KOR knockouts after monoiodoacetate (Negrete *et al*, 2017). Taking all the results together, KOR seem to have protective effects against nociceptive sensitization and detrimental effects promoting anxiety-like behaviour and cognitive impairment associated with chronic pain. PDYN knockouts have also been evaluated in different chronic pain models. In accordance to KOR knockouts, PDYN-deficient mice showed enhanced mechanical allodynia (Walwyn *et al*, 2016) or no change in mechanical and heat hypersensitivity after CFA (Gendron *et al*, 2007). Similarly, mice lacking PDYN showed increased sensitization to mechanical stimuli, but developed less anxiety-like behaviour during osteoarthritis pain induced by monoiodoacetate (Negrete *et al*, 2017), revealing the antinociceptive effects of KOR in chronic inflammatory pain models. However, PDYN knockouts showed decreased mechanical allodynia after chronic sciatic nerve constriction (Labuz *et al*, 2016) and decreased mechanical and heat hypersensitivity after spinal nerve ligation or PSNL (Wang *et al*, 2001; Xu *et al*, 2004), suggesting pronociceptive effects of dynorphins in neuropathic pain. Likewise, PDYN-deficient mice showed reduced KOR agonists-mediated analgesia in neuropathic pain conditions (Xu *et al*, 2004). Thus, dynorphins seem to play a complex role during chronic pain, dependent on the pain condition.

Despite the well-known influence of opioid receptors on mood disorders and cognitive functions, their roles in affective and cognitive manifestations of chronic pain states have not been examined in depth yet.

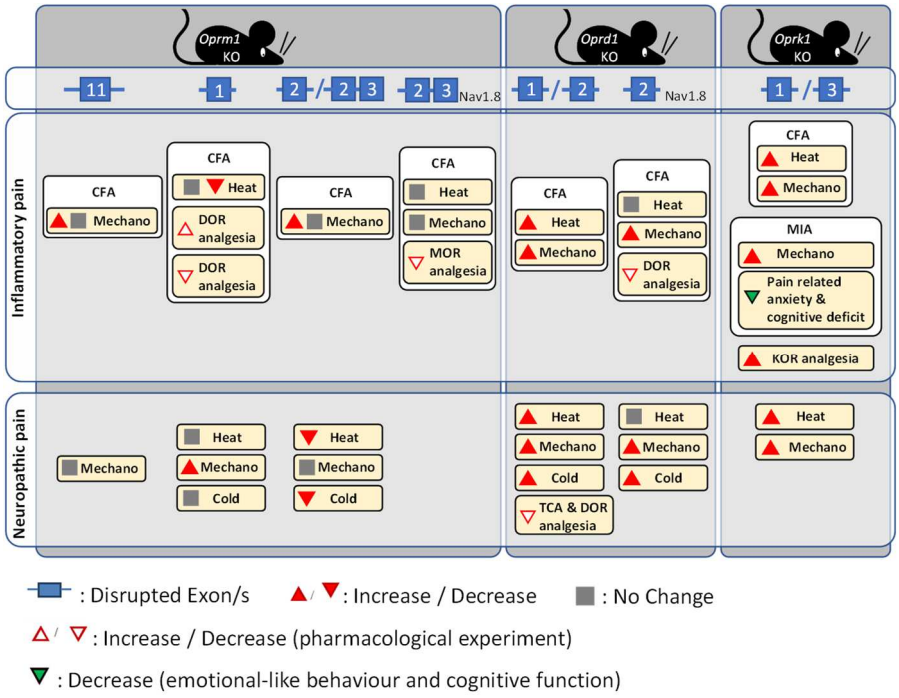

Figure 22. **Summary of the results obtained from opioid receptor knockout mice in chronic pain models.** Cold, cold sensitivity; DOR, delta opioid receptor; Heat, heat sensitivity; KO, knockout; KOR, kappa opioid receptor; Mechano, mechanical sensitivity; MIA, monoiodoacetate model; MOR, mu opioid receptor; TCA, triciclic antidepressants (adapted from Maldonado et al., 2018).

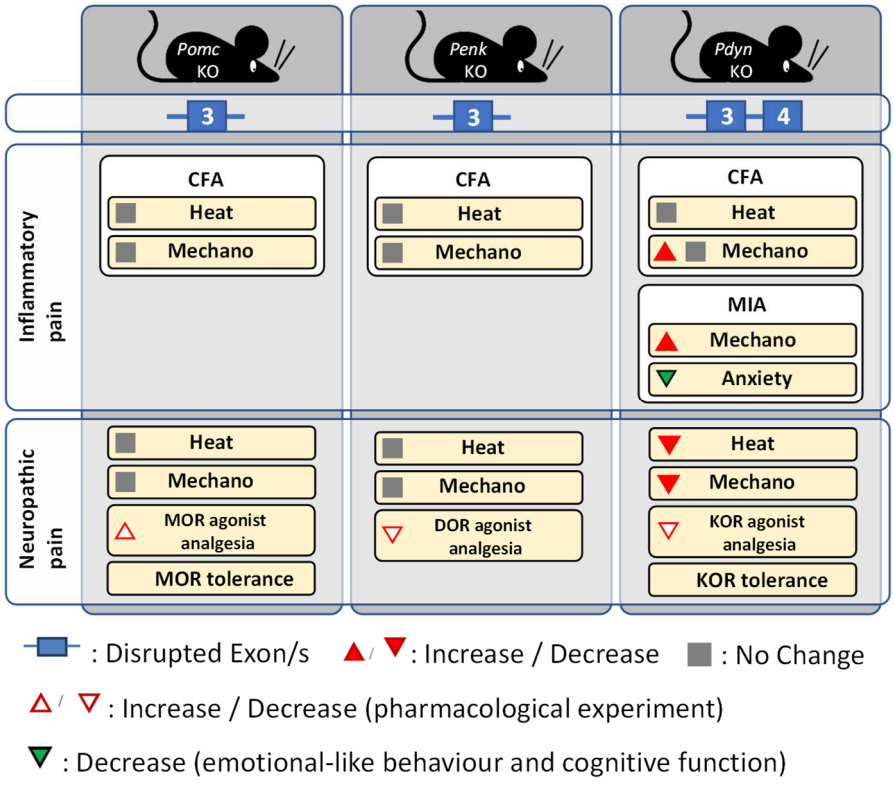

Figure 23. Summary of the results obtained from endogenous opioid peptide precursors knockout mice in chronic pain models. DOR, delta opioid receptor; Heat, heat sensitivity; KO, knockout; KOR, kappa opioid receptor; Mechano, mechanical sensitivity; MIA, monoiodoacetate model; MOR, mu opioid receptor (adapted from Maldonado et al., 2018).

OBJECTIVES

Objective 1

To evaluate the influence of sociability, anxiety-like and depressive-like behavioural traits on the nociceptive, emotional and cognitive manifestations of neuropathic pain, using an out-bred mouse line that resembles human genetic heterogeneity.

Study 1

Influence of behavioural traits on the inter-individual variability of nociceptive, emotional and cognitive manifestations of neuropathic pain

Objective 2

To investigate the participation of mu (MOR) and delta (DOR) opioid receptors expressed in specific central and peripheral neuronal populations modulating the different manifestations of neuropathic pain.

Study 2

Mu and delta opioid receptors play opposite nociceptive and behavioural roles on nerve-injured mice

The Annex includes a review related to the role of the opioid system in neuropathic pain, which has been published in European Journal of Pain.

Why mu-opioid agonists have less analgesic efficacy in neuropathic pain?

Martínez-Navarro M1, Maldonado R1, Baños JE1.

Eur J Pain. 2018 Oct 14. doi: 10.1002/ejp.1328.

MATERIALS & METHODS

Animals

In a first study, Swiss albino outbred male mice with an initial body weight between 20-22g (Charles River, Lyon, France) were used. In a second study, adult male and female mice (8-18 weeks old) of six different lines of genetically modified mice were used, all of them generated in the Institut Clinique de la Souris - Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch, France (IGBMC). Constitutive knockout lines of MOR (referred as CMV-MOR) (Weibel *et al*, 2013) or DOR (referred as CMV-DOR) (Gaveriaux-Ruff *et al*, 2011), and four different conditional knockout lines with specific deletion of MOR and DOR at peripheral and central sites. Two of them had the deletion of MOR or DOR restricted to Nav1.8+ primary afferent neurons and are referred to as Nav1.8-MOR and Nav1.8-DOR knockouts, respectively (Gaveriaux-Ruff *et al*, 2011; Weibel *et al*, 2013). The other two had a genetic inactivation of MOR or DOR in GABAergic interneurons of the forebrain and are identified thereafter as DLX5/6-MOR and DLX5/6-DOR knockouts (Charbogne *et al*, 2017; Chu Sin Chung *et al*, 2015). The Cre-negative littermates of conditional knockout mice for MOR and DOR were used as wildtype control groups (WT-MOR and WT-DOR).

In both studies mice were group-housed (2-4 animals) with free access to water and food. The housing conditions were maintained at 22 ± 1˚C and 55 ± 10% relative humidity in a controlled 12-12-hour light/dark cycle (light on between 8:00 A.M. and 8:00 P.M.). Animals were handled for 1 week before starting the experimental sequence. All experimental procedures and animal husbandry were conducted according to standard ethical guidelines (European Community Guidelines on the Care and Use of Laboratory Animals 86/609/EEC) and were approved by the local

ethical committee. All the experiments were performed under blinded conditions.

Experimental procedures

Study 1

Two hundred and fifty mice were exposed to locomotion, sociability, anxiety-like and depressive-like behavioural tests as indicated in Figure 24. Animals displaying high, intermediate and low social, anxious- and depressive-like responses were chosen for further experiments (see Results from study 1, 'Selection of the extreme phenotypes' for details of the selection procedure). The selected animals were homogeneously distributed in two experimental cohorts with representation of all the phenotypic groups. Spontaneous CeA neuronal activities were recorded in mice selected for each phenotype of the first cohort. Animals from the second cohort were exposed to a PSNL or sham surgery to induce neuropathic pain. Mechanical, heat and cold nociceptive responses were assessed under basal conditions (day -1) and on days 3, 6, 11, 16 and 21 after nerve injury using the von Frey, plantar and cold plate. Anhedonic state, anxiety like behaviour and cognitive performance were evaluated on day 10, 15 and 20 post-surgery, respectively, using different paradigms than in the initial screening step to reduce behavioural adaptation of the mice (Yalcin *et al*, 2011) (Figure 24). Finally, amygdala samples were freshly dissected at day 41 after neuropathic pain induction from animals used for the behavioural study. Transcriptional modifications in this area were examined.

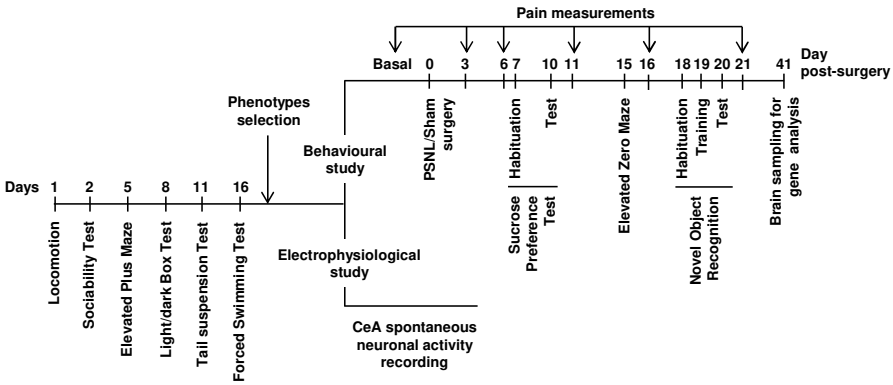

Figure 24. Experimental procedure (study 1). Extreme phenotypes selection for the assessment of electrophysiological correlates and for the evaluation of the nociceptive, affective and cognitive behaviours in mice exposed to neuropathic pain.

Study 2

The effects of genetic inactivation of MOR and DOR on neuropathic pain manifestations were evaluated using a battery of tests to assess nociception, anxiety-like behaviour, depressive-like behaviour and long-term memory, as previously reported (Liu and Chen, 2014; La Porta *et al*, 2016). Briefly, animals were habituated three times on alternative days to the environment of nociceptive tests (von Frey and plantar). PSNL or sham surgery were performed the day after the measurement of nociceptive baseline responses (day -1) and nociception was assessed again on days 5, 10, 16 and 21 after the surgery. Anxiety-like manifestations of neuropathic pain were evaluated at two different time points (days 11 and 23) using the elevated plus maze and the light/dark box. Depressive-like behaviour was assessed on day 13 with the forced swimming test and long-term memory was evaluated on day 20 after the surgery using the novel object recognition test (Figure 25).

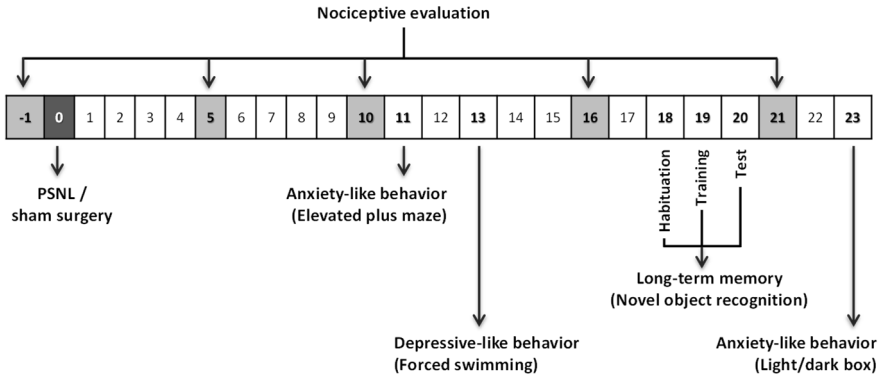

Figure 25. Experimental procedure (study 2). Baseline mechanical and heat nociceptive thresholds were measured the day before PSNL or sham surgery. Mechanical and heat nociception was assessed again on days 5, 10, 16 and 21 after the surgery. Affective behaviour and cognitive performance were evaluated under sham and neuropathic pain conditions. Anxiety-like behaviour was assessed at two different time points (days 11 and 23) using different paradigms. Depressive-like behaviour was assessed on day 13 and long-term memory was evaluated on day 20 after the surgery.

Neuropathic pain model

PSNL was performed as previously described (La Porta *et al*, 2016) to induce neuropathic pain. Briefly, mice were anaesthetized with isoflurane (induction 5% V/V, surgery 2% V/V; Virbac, Barcelona, Spain) and a tight ligature was created around 33-50% of the sciatic nerve ~1 cm proximal to the nerve trifurcation, using an 18 in (9-0) non-absorbable virgin silk suture (Alcon® Surgical Inc., Fort Worth, TX, USA). The rest of the nerve was left untouched. The muscle was stitched and the incision was closed with wound clips (AgnTho's, Lidingo, Sweden). Sham-operated control mice underwent the same surgical procedure but without manipulation nor ligation of the nerve.

Behavioural tests

Nociceptive behaviours

Mechanical allodynia, heat hyperalgesia and cold allodynia (only in the first study) were used as outcome measures of neuropathic pain, as previously reported (La Porta *et al*, 2016). Mice were tested in each paradigm at different time points (see experimental procedures), using the same sequence.

Mechanical allodynia was evaluated by measuring the hind paw withdrawal response to von Frey filaments stimulation, after 1h of habituation period. Animals were placed in Plexiglas cylinders (20 cm high, 9 cm diameter) on a grid surface through which the von Frey calibrated filaments (North Coast Medical, USA) were applied by following the up–down paradigm. The threshold of response was then calculated using the up–down Excel program provided by Dr A. Basbaum (University of California, San Francisco, CA), which applies a Dixon non-parametric test (Chaplan *et al*, 1994). Clear paw withdrawal, shaking, or licking was considered as a positive nociceptive response. Both hind paws were tested.

Heat hyperalgesia was evaluated by measuring the hind paw withdrawal latency in response to radiant heat with the Hargreaves plantar test apparatus (Ugo Basile, Italy). Mice were placed in Plexiglas cylinders (20 cm high, 9 cm diameter) positioned on a glass surface and habituated to the environment for 30 min before testing. The mean paw withdrawal latencies for the ipsilateral and contralateral hind paws were determined from the average of 3 separate trials, taken at 5-10 min intervals to avoid thermal sensitization (Hargreaves *et al*, 1988). A cut-off time of 20 s was used to prevent tissue damage. Both hind paws were tested.

Cold allodynia was assessed with the hot/cold plate analgesia meter (Columbus, USA). A glass cylinder (25 cm high, 20 cm diameter) was used to keep mice on the cold surface of the plate, which was maintained at 5±0.5º C. The number of each hind paw elevations, defined as clear paw lift without displacement, was recorded for 5 min. Walking/stepping movements were not considered. A score was calculated as the difference of number of elevations between ipsilateral and contralateral paws.

In the second study, for the comparative evaluation of MOR and DOR contributions to nociceptive sensitivity, we calculated the area under the curve (AUC) of the mechanical thresholds and withdrawal latencies of the time course after PSNL and sham surgery (days 5 to 21) and normalized them to their respective WT values.

Locomotion activity

Locomotor activity was evaluated as previously described (Martin *et al*, 2000) by using actimetry boxes (9 × 20 × 11 cm) (Imetronic, Lyon France) in a low luminosity room (5 lux), and with white noise. Each box contained two lines of photocells located 2 cm and 6 cm above the floor to measure horizontal and vertical movements, respectively. Mice were individually placed in the boxes and the number of activity counts was recorded for a period of 30 min.

Sociability behaviour

Sociability test was performed the day after the locomotor activity evaluation to determine the extreme phenotypes. A black Plexiglas V-maze was used with 15 cm bars of transparent Plexiglas placed at 6.5 cm

of the end of each arm that separate both sides, although allowing exploration (Panlab, Barcelona, Spain). The mouse was first habituated to the empty maze during 5 min. In a second step, sociability behaviour was evaluated during 5 min by placing one stranger animal in the maze, behind the Plexiglas bars. A sociability index was calculated as the difference between the time spent exploring either the stranger mouse or the empty space divided by the total exploration time, onwards considered as "social preference".

Anxiety-like behaviour

In the first study, the elevated plus maze and light/dark box tests were used to determine the extreme anxiety phenotypes, whereas the elevated zero maze was performed after sciatic nerve injury. The elevated plus maze and light/dark box tests were also used in the second study.

The elevated plus maze test was performed using a black Plexiglas apparatus with 2 open (45 lux) and 2 closed (5 lux) arms, set in cross from a neutral central square that was elevated 40 cm above the floor. The percentage of entries and time spent in the open arms were determined during 5 min, as previously reported (La Porta *et al*, 2015).

The light/dark box was carried out as previously described (Filliol *et al*, 2000). A Plexiglas box comprising a small dark compartment (10 lux) and a large lit compartment (500 lux) separated by a connecting 4 cm long tunnel was used. Floor lines separated the light compartment into three equal zones, from the tunnel to the opposite wall, designated as proximal, median and distal zones. The time spent in the lit compartment

as well as total and distal entries to the lit compartment were registered for 5 min.

The elevated zero maze was performed as previously described (Valverde *et al*, 2004), using a circular black Plexiglas apparatus with 2 open (100 lux) and 2 wall-enclosed sections (10 lux) elevated above the floor (50 cm). The percentage of time in open arms was measured during 5 min.

Depressive-like behaviour

In the first study, the tail suspension test and forced swimming test were used to determine the extreme depressive phenotypes, while the sucrose preference test was performed after sciatic nerve injury. In the second study, the forced swimming test was the paradigm used for depressive-like behaviour evaluation.

The tail suspension test was performed as previously described (Steru *et al*, 1985). Mice were suspended by their tails with tape, in such a position that escape or hold on to nearby surfaces were not allowed during 6 min. The immobility time was recorded during the last 4 min of the test, when mice show a sufficiently stable level of immobility.

The forced swimming test was performed in a narrow (17.5 x 12.5 cm) Plexiglas cylinder containing water to a depth of 15 cm (22 °C ± 0.2 °C) (Porsolt *et al*, 1977). Mice were subjected to a forced swimming during 6 min and the total duration of immobility, disregarding small maintenance movements, was measured during the last 4 min, when mice show a sufficiently stable level of immobility.

The sucrose preference test was performed using an extremely high sensitivity (0.02 g) monitoring system (Phecomp, Panlab, ES), recently

validated in our laboratory (Bura *et al*, 2013). Two-bottle choice procedure allows for a comparison between behavioural preference for sucrose solution (2%) in drinking water compared to water only. Three days before the test day, a 24 h session was performed to habituate the mice to the environment and the different drink solutions. During a test session of 24 h, preference is measured by volume of liquid consumed, which is then converted to a percent preference calculated as the ratio of the sucrose solution intake to total liquid intake x 100. Sucrose is a natural reinforcer and sucrose preference is attenuated by a diversity of chronic stressors, which is indicative of anhedonic-like state (i.e., inability to feel pleasure). Thus, sucrose preference test is useful to investigate anhedonia, a commonly-accepted symptom of depressive-like behaviour.

Cognitive evaluation

The novel object recognition test was performed as previously described (Puighermanal *et al*, 2009) in the same V-maze used for sociability behaviour evaluation without the transparent Plexiglas bars. Three phases of 9 min were performed on consecutive days. On day 1, mice were habituated to the empty maze. On the second day, mice were introduced in the maze where two identical objects were presented in the extremes of the maze. For the memory test, on third day, one of the familiar objects was replaced with a novel one, and the total time spent exploring each of the two objects (novel and familiar) was measured. Object exploration was defined as the orientation of the nose towards the object at a distance of less than 1 cm. A discrimination index (DI) was calculated as the difference between the time spent exploring either the novel (Tn) or familiar (Tf) object divided by the total time exploring both objects: (DI = (Tn − Tf)/(Tn + Tf)).

Electrophysiological procedures

Extracellular single-cell in vivo recordings were made from single neurons in the right CeA after the behavioural test used to select extreme phenotype mice. Parylene coated tungsten electrodes were applied (A-M Systems, USA) using the following stereotaxic coordinates (Franklin and Paxinos, 2008): 4.4 mm dorsoventral, 2.4 mm lateral and 1.06 mm caudal to bregma. The animals were anesthetized with isoflurane (1.5–1.7%) delivered in a gaseous mix of N2O (66%) and O2 (33%). Under anesthesia, animals were fixed in the stereotaxic device, the skull was exposed and the CeA coordinates found. A small craniotomy was performed and the dura mater taken, allowing access to the brain. Anesthesia was maintained with isoflurane (1.5–1.7%) delivered in a gaseous mix of N2O (66%) and O2 (33%) for the entire duration of the recordings. All the neurons found in the CeA that fired spontaneously for at least 20 min were recorded (2-5 neurons/animal). Besides spontaneous activity, neuronal firing evoked by von Frey filaments (0.008g, 1g, 4g, 8g, 15g, 26g and 60g), pinch, heat (48^{o}C) and cold (4^{o}C) applied to both paws as well as by pinch, heat (48^{o}C) and cold (4^{o}C) applied to the tail and both ears was recorded. Each stimulus was applied continuously during 5 seconds. Data was captured and analysed by a CED 1401 interface coupled to a Pentium computer with Spike 2 software (Cambridge Electronic Design; PSTH and rate functions). At the end of each experiment, after a lethal level of isoflurane had been delivered, the brains were extracted and sliced, the recording sites verified through the placement of the electrode and plotted on a standardized section from the mouse brain atlas (Franklin and Paxinos, 2008). All neurons included were located within the CeA.

RNA extraction and reverse transcription

At the end of the experimental procedures, mice were sacrificed and amygdala (study 1) or L3-L4 DRG (study 2) tissues were freshly dissected. The samples were rapidly placed in individual tubes with the tissue storage reagent RNAlater (Qiagen Inc., Valencia, CA, USA) and stored at −80°C until RNA isolation. Amygdala samples (study 1) were thawed at room temperature and homogenized in 1 ml Trizol reagent (Invitrogen, Carlsbad, CA, USA). RNA extraction was performed in accordance with the manufacturer's protocol. Isolation of RNA from DRG samples (study 2) was performed using the RNeasy Micro kit (Qiagen) according to the manufacturer's instructions. In both studies total RNA concentration was measured using a NanoDrop ND- 1000 Spectrophotometer (NanoDrop Technologies Inc., Montchanin, DE, USA). RNA quality was determined by chip-based capillary electrophoresis using an Agilent Bioanalyzer 2100 (Agilent, Palo Alto, CA, USA). Reverse transcription (RT) was performed using Omniscript reverse transcriptase (Qiagen Inc.) at 37°C for 60 min.

Quantitative real-time PCR analysis

The qRT-PCR reactions were performed using Assay-On-Demand TaqMan probes: Hprt1 Mm01545399_m1, Gadd45g Mm00442225_m1, Il6 Mm00446190_m1, Nr3c1 Mm00433832_m1, Pdyn Mm00457573_m1 and Tsc22d3 Mm00726417_s1 in the first study, and Hprt1 Mm01545399_m1, Oprd1 Mm01180757_m1, Oprm1 Mm01188089_m1, Tac1 Mm01166996_m1 in the second study (all from Applied Biosystems, Carlsbad, CA, USA) and were run on the CFX96 Touch Real-Time PCR machine (BioRad, Hercules, CA, USA). Each template was generated from an individual animal, and the amplification efficiency for each assay was determined by running a standard dilution curve. The expression of the

hypoxanthine guanine phosphoribosyltransferase 1 (Hprt1) transcript was quantified at a stable level between the experimental groups to control for variations in cDNA amounts. The cycle threshold values were calculated automatically by the CFX MANAGER v.2.1 software with default parameters. RNA abundance was calculated as $2^{-(Ct)}$. The transcript levels were normalized against the housekeeping gene, Hprt1, and interpreted using the comparative Ct method (study 1) or the comparative ΔΔCt method (Livak and Schmittgen, 2001) (study 2).

Tissue preparation for immunofluorescence

At the end of the experimental procedure of the study 2, some male mice from MOR genotypes (WT, CMV, Nav1.8 and DLX5/6, n=4-5/group) were deeply anesthetized by i.p. injection (0.2 ml/10 g of body weight) of a mixture of ketamine (100 mg/kg) and xylazine (20 mg/kg). Immediately after, intracardiac perfusion of 17ml of Na_2HPO_4/NaH_2PO_4/NaCl buffer (PBS) 0.1M, pH 7.4, 4°C, followed by perfusion of 30 ml 4% PFA in PBS 4°C, were delivered with a peristaltic pump at 15ml/minute. Spinal cords were quickly removed and postfixed for 4 hours in the same fixative solution at 4°C. Then spinal cords were rinsed 3 times for 5 minutes in PBS 0.1M before being cryoprotected in 30% sucrose in PBS and stored overnight at 4°C. After that, tissues were frozen at -80ºC in O.C.T. (Sakura, Finetek, Europe B.V., Alphen aan den Rijn). Spinal cords were cryosectioned coronally (20 µm) and sections were collected directly onto gelatine-coated slides in a 1:8 series, with slides stored at -20ºC until used.

Immunofluorescence

Staining was performed directly onto slides, with sections first air-dried at 37°C for 20 minutes and washed with PBS for 15 minutes. Sections were blocked for 2 hours in blocking buffer containing PBS, 0.3% triton and 5% normal goat serum (Vector Laboratories; Burlingame) for microglia analysis, or 5% normal donkey serum (Sigma-Aldrich; Saint Louis) for astrocyte analysis. Spinal cord sections were stained for microglia with rabbit IBA1antibody (1:500; Wako; Japan), and for astrocytes with rabbit GFAP antibody (1:500; DAKO; Glostrup), both diluted in their respective blocking buffer solutions, and incubated for 2 hours at room temperature. Sections were rinsed 3 times in PBS for 10 minutes and then incubated for 2 hours with Alexa Fluor 555 goat anti-rabbit antibody for microglia staining (1:1000; Abcam; Cambridge) and with Alexa Fluor 488 donkey anti-rabbit antibody for astrocytes (1:500; Thermo Fisher Scientific, Waltham) in the same buffer as the primary antibody. The sections were then washed 5 times in PBS for 10 minutes before cover-slipping with Mowiol mounting media (0.5 M Mowiol 40-88 [Sigma-Aldrich], 20% glycerol, 0.1 M Tris pH 8.5).

Image analysis

Images were acquired using a confocal microscope (Leica TCS Sp5 STED). Alexa Fluor 555 and 488 were excited with the 543-nm line of a green neon laser and the 488-nm line of an argon laser, respectively. Images of the ipsilateral dorsal horn were taken at different z levels (0.5-µm depth intervals) with an oil immersion lens (x40 objective; 1 zoom) from 4 different L3-L5 coronal spinal cord sections. Glial reactivity of superficial (I-II) and deep (IV-V) laminae of the dorsal horn was analysed separately in 16 to 20 images from 4 to 5 animals (4 images per animal) for each

experimental group. All image analyses were performed by an experienced observer blind to the experimental conditions.

Microglial reactivity: Perimeter measurements of the microglial soma were made by applying the Z-projection of 35 images taken at consecutive z levels and using the 'Freehand selections' tool from ImageJ software (National Institutes of Health, Bethesda, MD, USA). Mean perimeter of superficial and deep fields from each image were averaged for each animal.

Astrocytic reactivity. The number of astrocytic cells was quantified by densitometry in the most representative image at one z level for each section. Densitometry measurements were made using the ImageJ software, by applying the auto-threshold function to the images and measuring the percentage of immunopositive area. Densitometry measurements were averaged for each animal. Data were plotted as percentage immunoreactive area of superficial and deep laminae.

Statistical analysis

Study 1

All data are presented as mean ± SEM. Statistical analyses were performed using the Statistica 6.0 software (StatSoft, Tulsa OK, USA). For behavioural studies one or two-way ANOVA were performed followed by Bonferroni post hoc analysis. Electrophysiological data were analysed with a one-way ANOVA followed by Dunn's multiple comparison test. RT-qPCR data were analysed for PSNL and phenotype differences with one-way ANOVA followed by Bonferroni post hoc test. Correlation analyses between the behavioural traits and the neuropathic pain manifestations as well as between gene expression and the behavioural traits were

performed with the IBM SPSS 19 (SPSS Inc., Chicago, IL, USA) software. A probability of 0.05 or less was considered statistically significant. Detailed statistical analyses are presented in Supporting Tables S1-S3.

Study 2

Non-parametric analysis was used for all behavioural and molecular studies, since most of the groups did not follow normal distribution. The time course of nociceptive thresholds was analysed using a linear mixed model with three factor (surgery, genotype, time) or four (same three plus sex) and their interactions. A random intercept was considered, but random effects were not included. For the covariance structure of the repeated measures, a diagonal matrix was chosen. Bonferroni post hoc analysis was performed when pertinent. Baseline nociceptive thresholds, the AUC of the post-surgery nociceptive thresholds, the affective and cognitive behavioural measures, data from qPCR and immunofluorescence outcomes were analysed with a Kruskal-Wallis followed by U Mann Whitney with Bonferroni adjustment. A probability of 0.05 or less was considered statistically significant. IBM SPSS 19 (SPSS Inc., Chicago, IL, USA) was used to analyze the data. Detailed statistical analyses are presented in Supporting Tables S4-S12.

RESULTS

Martínez-Navarro M, Lara-Mayorga IM, Negrete R, Bilecki W, Wawrzczak-Bargieła A, Gonçalves L, et al. Influence of behavioral traits in the inter-individual variability of nociceptive, emotional and cognitive manifestations of neuropathic pain. Neuropharmacology. 2019 Apr 1;148:291–304. DOI: 10.1016/j.neuropharm.2019.01.012

Martínez-Navarro M, Cabañero D, Wawrzczak-Bargiela A, Robe A, Gavériaux-Ruff C, Kieffer BL, et al. Mu and delta opioid receptors play opposite nociceptive and behavioural roles on nerve-injured mice. Br J Pharmacol. 2020 Mar 1;177(5):1187–205. DOI: 10.1111/bph.14911

DISCUSSION

Our first study reveals that some specific behavioural traits may influence spontaneous and evoked CeA neuronal activity and basal nociceptive responses, and seem to be crucial for the nociceptive, emotional and cognitive manifestations of neuropathic pain. They also show that these behavioural traits may be linked to gene expression changes in the amygdala.

The amygdala is a critical integrator for affective processing. Indeed, alterations in amygdala activation have been found in a variety of neuropsychiatric disorders, including autism and social phobia (Wellman *et al*, 2016). Both amygdala hyperactivity and hypoactivity have been associated with altered social processing (Becker *et al*, 2012; Sladky *et al*, 2012), which have placed the amygdala at the centre of the social brain. Our results revealed a direct association between spontaneous and evoked CeA activity and sociability behaviour. Indeed, higher CeA activity was observed in high sociable than in low sociable mice. In agreement with a prosocial role of the amygdala, many previous studies reported loss of social interactions following permanent damage to the amygdala in nonhuman primates. These deficits in social behaviour included loss of social status, decreased affiliative interactions, and decreased response to threats following amygdala ablation (Kalin *et al*, 2004; Meunier and Bachevalier, 2002), and less severe behavioural alterations after more circumscribed excitotoxic amygdala lesions (Machado *et al*, 2008; Machado and Bachevalier, 2006). In our experimental settings, CeA function was unrelated to the anxiety- and depressive-like traits. Several studies agreed that the CeA has a crucial role in fear, but not in the control of anxiety- and depressive-like behaviour (Davis *et al*, 1997, 2010).

CeA neuronal hyperactivity has also been reported under pain conditions (Gonçalves and Dickenson, 2012). The maintained activation of CeA due to sustained nociceptive input may trigger anxious and depressive alterations associated to chronic pain (Gonçalves and Dickenson, 2012). Thus, we hypothesized that the CeA might be the brain area that allows for a bridge between the nociceptive and affective-motivational dimensions of neuropathic pain. Therefore, it would be of interest to evaluate amygdala function under neuropathic pain conditions. Unfortunately, there were not enough animals after selection of extreme phenotype mice to perform electrophysiological recordings in naïve, neuropathic and sham conditions. Since the results in the pain model cannot be interpreted without the baseline recording in naïve conditions, we decided to perform recordings in the absence of chronic pain.

We revealed that low sociability was associated to enhanced mechanical sensitivity (sham conditions), whereas low depression trait increased responding to heat and cold stimulation in sham mice. Therefore, low sociability and low depression phenotypes could represent vulnerability factors to enhance nociceptive responses. Decreased pain sensitivity was previously demonstrated by social interaction with conspecifics in rodents (D'Amato and Pavone, 2012). Greater social support was associated with lower nociceptive manifestations to painful experimental stimuli (Montoya *et al*, 2004), and social relationships were suggested to promote pain-specific resilience in humans (Sturgeon and Zautra, 2016). Therefore, our results also suggest that individuals prone to social interaction may engage neural networks associated with adaptive responses to pain, leading to decreased pain perception. In agreement with our findings, depressive-like behaviour was previously shown to decreased the perceived intensity of painful stimulation in rats (Shi *et al*,

2010a), and individuals with depressive disorders showed decreased sensitivity to noxious stimulation (Bär *et al*, 2006; Schwier *et al*, 2010). One interpretation for this unexpected result could be that the generalized emotional suffering of depressed individuals may become their baseline threshold of discomfort. Thus, normally noxious stimuli could go unnoticed for them compared to non-depressed individuals.

Our behavioural results also indicate that sociability, anxiety and depression traits modulate nociceptive manifestations after PSNL. Low sociability trait was associated to enhanced mechanical and cold allodynia. To our knowledge, the influence of social behaviour in chronic pain has only been addressed in one study of a neuroma rat model (Raber and Devor, 2002). The authors investigated how pain can be transferred by emotional contagion from one rat in pain after nerve injury to another. However, we specifically evaluated how pain behaviour of a nerve-injured mouse was affected by the ability of the mouse to interact with conspecifics. Our results suggest high sociability as an attenuating factor of chronic pain hypersensitivity. Clinical studies revealed that social factors may improve coping responses and overall function in chronic pain (Sturgeon and Zautra, 2016). It was also reported that social support protects patients against pain-related exacerbations in negative mood (Onoda *et al*, 2009). Thus, we can hypothesise that social relationships may provide chronic pain patients with a way to alleviate their current situation, and the feeling of company may reduce catastrophising.

We have demonstrated that anxiety-like behaviour has a modulatory effect on nociception after PSNL that depends on the modality of the stimuli. A positive correlation between anxiety trait and mechanical

allodynia was revealed. However, mice displaying low anxiety-like behaviour showed higher cold sensitivity. Different noxious sensory modalities are transduced by distinct nociceptive primary fibres. Therefore, anxiety could have a particular impact in specific sensory pathways. In agreement, opposite effects of the anxiety trait depending on the nociceptive modality were previously reported in animals. Indeed, high anxiety increased mechanical hypersensitivity in neuropathic rats (Roeska *et al*, 2009), but decreased thermal pain sensitivity (Jochum *et al*, 2007). However, a negative influence of anxiety on pain perception was consistently reported in humans. In clinical studies, both experimentally induced-anxiety (Rhudy and Meagher, 2000b, 2003) and high anxiety sensitivity (Keogh and Mansoor, 2001) increased nociception. In agreement, amplified pain intensity was reported in patients with generalized anxiety or post-traumatic stress disorder (Barlow *et al*, 1996; Defrin *et al*, 2008). A possible explanation would be that the alertness characteristic of anxious people may lead them to pay enhanced attention to any stimulus, including noxious ones, thus amplifying pain intensity.

Depression trait negatively correlated with mechanical allodynia and similarly, mice with low depressive-like behaviour also showed enhanced cold allodynia in our experimental conditions. The consistent influence of low depression phenotype enhancing different pain modalities suggests that depressive trait is not directly related to pain severity. In agreement, decreased mechanical allodynia was previously reported in neuropathic rats with depressive-like behaviour (Shi *et al*, 2010a). Pain is among the most common physical symptoms in patients with depression, and a common complaint reported to specialists (Leo, 2005). However, patients with depressive disorders are often less sensitive to experimental pain.

Indeed, decreased sensitivity to thermal and electrical pain was reported in patients with depressive disorders (Bär et al., 2006). Similarly, patients with major depressive disorder also showed decreased sensitivity for cold pain (Schwier et al., 2010). Although none of these studies evaluated the role of depression in chronic pain, our findings suggest that depression may also decrease neuropathic pain-induced hypersensitivity. Avoidance of noxious stimulation is considered a motivation-driven behaviour. Therefore, the inhibitory effect of depression on the stimulus-evoked pain, may be related to the loss of motivation, a key symptom of depression reported in neuropathic mice (La Porta *et al*, 2016).

We further evaluated the influence of behavioural traits on emotional and cognitive dimensions after PSNL. We first confirmed different behavioural outcomes used in our laboratory as reliable measurements of emotional and cognitive manifestations of neuropathic pain. The post-surgery evaluation of emotional behaviours revealed the stability of previously selected extreme phenotypes. The retention of extreme anxiety traits in sham-operated mice 3 weeks after phenotyping demonstrated that the defined extreme phenotypes referred to actually extreme behavioural traits. The lack of consistency between the extreme depression phenotypes and the responses in sham-operated mice may be related to the different behavioural responses evaluated in each paradigm. The immobility measured in the forced swimming and tail suspension tests reflects a behavioural despair, directly related to the reduced motivation to maintain effort in an inescapable situation, whereas the sucrose preference test includes different components of the reward processing that are related to the pleasure cycle (Thomsen, 2015). We found that high sociable mice developed enhanced anxiety and cognitive manifestations of neuropathic pain, while low sociable

mice developed more intense nociceptive hypersensitivity. In contrast to the enhancer effect of low depression trait on nociceptive manifestations, high depressive neuropathic mice were the most anxious and had the worst memory index. Anxiety trait modulated emotional and cognitive neuropathic pain manifestations in the same direction as mechanical nociception, since mice with high anxiety prior to the lesion were the most anxious and showed the most severe memory impairment after PSNL. These results indicate that high sociability, high anxiety and high depression play a crucial role in anxiety manifestations related to neuropathic pain, while high anxiety and high depression are also crucial factors for neuropathy-induced cognitive impairment. Collectively, our findings show that certain behavioural traits in mice, which can be translated into human personality traits, are crucial factors in modulating sensory processes and affective and cognitive comorbidities of neuropathic pain that do not need to be proportional to allodynia and hyperalgesia. These findings support once more the importance of evaluating not only simple nociceptive endpoints, but also complex behavioural manifestations of pain in animal models of neuropathies.

We also revealed that extreme sociability, anxiety and depression traits influence gene expression in the amygdala in the absence of pain. *Pdyn* expression correlated negatively with sociability and positively with anxiety trait. In agreement, several studies have shown that low sociability is related to higher levels of anxiety (Kudryavtseva *et al*, 2004; Tõnissaar *et al*, 2008). PDYN deletion and blockade of KOR enhanced social memory (Bilkei-Gorzo *et al*, 2014). The PDYN system may play a role in anxiety (Knoll *et al*, 2011), but currently available data do not provide a consistent picture of the PDYN functions in anxiety. Consistent with our results, PDYN deletion and KOR blockade decreased anxiety in

mice, and treatment of PDYN knockouts with a KOR agonist reversed their anxiolytic phenotype (Wittmann *et al*, 2009). However, increased anxiety-like behaviours was also observed in PDYN knockouts (Femenía *et al*, 2011). Considering the role of the dynorphin system in limbic areas in driving dysphoric and aversive behaviour (Palmisano *et al*, 2018), it seems reasonable that high *Pdyn* expression in the amygdala could facilitate the development of social disability and anxiety disorders.

The depression trait was negatively correlated with *Nr3c1* levels in the amygdala. This observation agrees with the association of glucocorticoid receptor with depressive disorders. Thus, high levels of *Nr3c1* promoter methylation have been associated with major depressive disorder (Nantharat *et al*, 2015). As DNA methylation usually represses gene transcription, our results support the hypothesis that decreased *Nr3c1* receptor level could be an indicative factor for depressive-like behaviour.

Gadd45 expression in the amygdala showed a positive correlation with depression trait. GADD45 protein is considered a molecular player for active DNA demethylation under stressful situations, which may suggest that GADD45 is inducing stable changes in amygdala gene expression, neural circuit function, and ultimately behaviour in mice with depression. Indeed, aberrant epigenetic regulation induced by environmental factors and subsequent transcriptional dysregulation is a unifying topic in psychiatric disorders, including depression (Bagot *et al*, 2014).

Changes in the amygdala gene expression profiles were also observed after PSNL. The up-regulation of spinal dynorphin and its precursor (PDYN) expression is a common critical feature in neuropathic pain, and seems to be required for the maintenance phase rather than for its initiation (Laughlin *et al*, 2001; Wang *et al*, 2001). In this regard, *Pdyn*

knockout mice showed similar pain threshold than WT mice immediately after spinal nerve ligation. However, *Pdyn* knockout animals returned to nociceptive baseline values only 10 days after the nerve injury, suggesting that dynorphin could play a pronociceptive role through the maintenance of central sensitization (Wang *et al*, 2001). Here, we show that *Pdyn* up-regulation after PSNL also takes place in the amygdala, in agreement with a recent study showing alterations of the dynorphin system in the corticostriatal circuitry (Palmisano *et al*, 2018). Specifically, marked increase of *Pdyn* mRNA was observed in the anterior cingulate and prefrontal cortices, in parallel with enhanced *Bdnf* mRNA expression in the same cortical regions (Palmisano *et al*, 2018). The relationship between dynorphin and BDNF (Kim *et al*, 2000), and the role of this neurotrophin in chronic pain-related neuroplasticity had previously been reported (Smith, 2014; Vanelderen *et al*, 2010). These findings together with ours indicate that the dynorphin system undergoes alterations during neuropathic pain involving limbic areas, which could contribute to the negative affective component of pain. Moreover, parallel increases in *Pdyn* and *Bdnf* mRNA at cortical level suggest the possible occurrence of interactions between these systems in neuropathic pain maladaptive neuroplasticity (Palmisano *et al*, 2018).

Increased *Gadd45* expression in the spinal cord and the DRG have also been reported during neuropathic pain (Lacroix-Fralish *et al*, 2011; Perkins *et al*, 2014). Our results revealed that these changes can be spread to more distant brain areas, since we show for the first time upregulated *Gadd45* mRNA expression in the amygdala of nerve-injured mice. *Gadd45* expression is induced after ischemic damage and neurodegenerative processes with anti-apoptotic properties (Chen *et al*, 1998; Torp *et al*, 1998). Therefore, we speculate that *Gadd45* could be

induced after PSNL to maintain genomic stability. *Npas4* is an early-response transcription factor that represents a homeostatic switch regulating excitatory-inhibitory neural balance (Spiegel *et al*, 2014). The increase in *Npas4* following PSNL indicates an amygdala overactivation, which may contribute to the nociceptive and emotional manifestations of neuropathic pain. Our results also revealed the up-regulation of *Tsc22d3* in the amygdala after PSNL. *Tsc22d3* encodes a glucocorticoid-induced leucine zipper protein that functions as transcriptional regulator. The reason for including this gene in the analysis of gene expression profiles in the amygdala was based on a potential relationship with the affective behavioural traits. However, no evidence linking this gene with chronic pain has been reported so far. Thus, further studies should be performed to elucidate the biological meaning for the enhanced expression of this transcriptional regulator during neuropathic pain.

Neuropathic pain-induced gene expression changes in the amygdala varied in line with the behavioural traits. *Pdyn* expression showed a negative and a positive correlation with sociability and anxiety traits, respectively, also under conditions of neuropathic pain. Interestingly, the groups with higher *Pdyn* levels (low sociability and high anxiety) also showed enhanced nociceptive manifestations of neuropathic pain and/or enhanced related comorbidities. These findings suggest a role of amygdala *Pdyn* in aggravating neuropathic pain syndrome, in agreement with a recent publication (Palmisano *et al*, 2018).

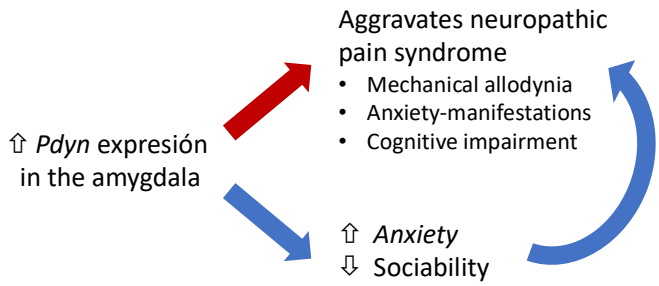

Figure 42. Influence of *Pdyn* expression profiles in the amygdala modulating behavioural traits and neuropathic pain syndrome.

We previously suggested that *Gadd45* expression could be induced after PSNL to restrain the stress response resulting from the lesion. In agreement with this hypothesis, the induction of *Gadd45* expression following PSNL was almost restricted to mice with low anxiety- and low depressive-like behaviour, the experimental groups showing milder emotional and cognitive manifestations of neuropathic pain. Therefore, *Gadd45* induction in the amygdala after PSNL may play a protective role against emotional and cognitive chronic pain manifestations, probably by promoting genomic stability and protecting neurons from apoptosis. The role of Gadd45 in chronic pain has previously been unexplored.

Although PSNL did not globally modify *Il6* expression in the amygdala, it differentially modulated *Il6* expression depending on anxiety and depression traits. Thus, *Il6* transcript levels showed under neuropathic pain conditions a positive and a negative correlation with anxiety and depression traits, respectively. Higher levels of *Il6* were observed in nerve-injured mice with high anxiety and low depression traits, both showing enhanced nociceptive hypersensitivity. These results agree with previous data demonstrating lower *Il6* expression in the amygdala of depressed-like compared to non-depressed-like nerve-injured rats (Burke

et al, 2013). Considering this interleukin as an inflammatory marker, these findings suggest that neuroinflammatory processes in the amygdala may contribute to enhance neuropathic pain, what may be favoured by anxiety and attenuated by depression. In agreement, various pathological pain models have recently shown elevated expression levels of IL-6 and its receptor in the spinal cord and dorsal root ganglia (Zhou *et al*, 2016). Additionally, the administration of IL6 has been reported to cause mechanical allodynia and thermal hyperalgesia, and an intrathecal injection of anti-IL-6 neutralizing antibody alleviated these pain-related behaviours (Murakami *et al*, 2013). In the present study, we demonstrate that changes in inflammatory gene expression following nerve injury may also take place at supraspinal pain-related structures, such as the amygdala. Further determination of protein level expression would strengthen the biological meaning of the observed transcriptional changes.

Our second study provides a comprehensive assessment of MOR and DOR contributions to nociceptive, emotional and cognitive consequences of neuropathic pain, which operate at the level of either peripheral nociceptors, central GABAergic forebrain neurons, or throughout the entire nervous system. Behavioural results are summarised in Table 17. Most strikingly, this work reveals detrimental effects of MOR activity and protective effects of DOR in a mouse model of neuropathic pain. Unexpectedly, constitutive deletion of MOR prevented mechanical hypersensitivity of nerve-injured mice, regardless of their gender. MOR-induced pronociception was not due to Nav1.8+ fibres or to GABAergic forebrain neurons, since conditional MOR knockouts in these locations

and WT mice showed similar mechanical sensitivity. The pain-sensitizing effects of MOR are in contrast with the analgesic effects of exogenous MOR opioid agonists and with the antinociceptive effect of MOR described in models of inflammatory pain (Corder *et al*, 2013; Severino *et al*, 2018; Walwyn *et al*, 2016). Indeed, a recent work investigating inflammatory pain showed increased mechanosensitivity in conditional Nav1.8-MOR knockouts (Severino *et al*, 2018). Opposing to these results, unaltered nociception was previously observed in these conditional Nav1.8-MOR knockouts after CFA injection, although they showed decreased systemic morphine, fentanyl and loperamide-induced analgesia under these conditions (Weibel *et al*, 2013). However, previous studies have described attenuated nociceptive manifestations of nerve-injured full MOR knockout mice, in agreement with our results (Kögel *et al*, 2011; Maldonado, 2016), suggesting pronociceptive activity of MOR under neuropathic pain conditions. Unchanged or increased pain sensitization in different MOR knockout lines and animal models of neuropathic pain have also been reported (Bohren *et al*, 2010; Mansikka *et al*, 2004; Roeckel *et al*, 2017; Wieskopf *et al*, 2014). These divergent results could be explained by the heterogeneous genetical constructs used to generate the full MOR knockouts (targeting exon 2, exons 2 and 3, exon 1 or exon 11) and the different neuropathic pain models used in each study. These conflicting findings may also suggest a complex role for MOR in the pathophysiology of chronic neuropathic pain.

Table 17. Summary of the behavioural results obtained in each knockout line, compared to WT group.

Knockout line	Nociceptive behavior								Affective behavior								Cognitive behavior			
	Mechanical nociception				Heat nociception				Anxiety				Depression				Long-term memory			
	Naïve/sham conditions		Neuropathic pain		Naïve/sham conditions		Neuropathic pain		Sham conditions		Neuropathic pain		Sham conditions		Neuropathic pain		Sham conditions		Neuropathic pain	
CMV MOR	♂♀	NC	♂♀	⇩⇩	♂♀	⇧	♂♀	⇧⇧	♂	NC/⇧	♂	NC/⇩	♂♀	⇧	♂♀	NC	♂	NC	♂	NC
									♀	⇧	♀	NC								
Nav MOR	♂♀	NC	♂♀	NC	♂♀	NC	♂♀	NC	♂♀	NC	♂♀	NC	♂♀	NC	♂♀	NC	♂	NC	♂	NC
DLX MOR	♂♀	NC	♂♀	NC	♂♀	NC	♂	⇧	♂	NC	♂♀	⇩	♂♀	NC	♂♀	NC	♂	NC	♂	NC
							♀	NC	♀	⇩										
CMV DOR	♂	⇧	♂♀	⇧⇧	♂♀	NC	♂♀	NC	♂♀	⇧	♂♀	NC	♂♀	⇧⇧	♂♀	⇧⇧	♂	⇩⇩	♂	NC
	♀	NC																		
Nav DOR	♂	⇧	♂♀	⇧	♂♀	NC	♂♀	NC	♂♀	NC	♂♀	NC	♂♀	NC	♂♀	NC	♂	NC	♂	NC
	♀	NC																		
DLX DOR	♂♀	NC	♂	⇧	♂♀	NC	♂♀	NC	♂♀	NC	♂♀	NC	♂♀	⇧	♂♀	⇧	♂	⇩	♂	NC
			♀	NC																

⇧/⇩: increase/decrease; NC: no change

On the other hand, repeated MOR stimulation has been associated with opioid-induced hyperalgesia and reduced opioid-antinociception in models of inflammatory and neuropathic pain (Roeckel *et al*, 2016). Indeed, full MOR knockout mice failed to develop hyperalgesia after repeated morphine treatment (Corder *et al*, 2017; Roeckel *et al*, 2017), suggesting MOR implication in this morphine-induced detrimental effect. Our results with the Nav1.8-MOR knockout mice show lack of pronociceptive effects of MOR expressed in primary afferents, in contrast to the involvement of peripheral MOR in opioid-induced pronociception previously described (Corder *et al*, 2017). These discrepancies may be attributed to the different conditions of neuropathic pain instead of repeated opioid administration. Potentiation of C-fibre synapses and descending facilitation (Dogrul *et al*, 2009; Heinl *et al*, 2011), coupling of MOR to excitatory G proteins (Tsai *et al*, 2009; Wang *et al*, 2005) or recruitment of spinal glutamate receptors (Cabañero *et al*, 2013) are some of the mechanisms triggered by MOR activation that may contribute to the excitatory effects of MOR in mice with chronic neuropathic pain (Martínez-Navarro *et al*, 2018). Furthermore, it is clear that MOR activity mediates additional deleterious effects of opioid drugs including addiction, respiratory depression or constipation (Corder *et al*, 2018). Thus, our data show a maladaptive pronociceptive effect of MOR in conditions of chronic neuropathic pain.

Nerve-injured constitutive MOR knockout mice showed a 2.6-fold increase of *Oprd1* mRNA in the affected DRG when compared to nerve-injured WT mice. DOR is expressed in myelinated and subsets of unmyelinated primary afferents, where it inhibits mechanical tactile and nociceptive transmission (Bardoni *et al*, 2014; François and Scherrer, 2018; Usoskin *et al*, 2015). Data from conditional knockout mice showed

that *Oprd1* overexpression was not due to the absence of MOR in Nav1.8+ fibres. From our results, we cannot conclude whether this upregulation is due to molecular, synaptic, or network-level adaptations resulting from constitutive MOR deletion. While MOR and DOR are often expressed in different subsets of peripheral neurons, a percentage of myelinated neurons positive for the calcitonin gene related peptide co-express both opioid receptors (Bardoni *et al*, 2014; François and Scherrer, 2018; Wang *et al*, 2010b). It is unknown whether MOR and DOR co-expression could be different in conditions of peripheral nerve injury or whether spinal MOR-expressing neurons could modulate DOR+ primary afferent fibres. The upregulated DOR expression in primary afferents sensitive to mechanical stimuli may contribute to limit nerve injury-induced mechanical allodynia.

Our study confirms that microgliosis develops following nerve injury as previously shown (Denk *et al*, 2016; Gu *et al*, 2016; Guan *et al*, 2016). *Oprd1* overexpression in DRG of CMV-MOR knockouts concurred with absence of neuropathic microgliosis in deep laminae of spinal cord and lack of astrocytosis in superficial and deep laminae, suggesting a role of MOR promoting PSNL-induced gliosis. Since DOR+ myelinated nociceptors project widely from spinal laminae I to V (Bardoni *et al*, 2014; Woodbury and Koerber, 2003), it is likely that the enhanced *Oprd1* expression prevented the neuropathic gliosis. In agreement with a participation of MOR favouring glial reactivity in the spinal cord, previous studies have revealed that intrathecal or subcutaneous ultra-low doses of MOR antagonists alleviated neuropathic pain by diminishing glial activation and neuroinflammation (Rivat and Ballantyne, 2016; Roeckel *et al*, 2016). The absence of neuropathic gliosis in the CMV-MOR knockouts is in contrast with the results obtained in a recent study

showing unaltered morphine-induced gliosis in the full MOR knockout mice (Corder *et al*, 2017). This difference could be due to the distinct triggers involved (neuropathic pain vs morphine). Our results show similar glial reactivity in WT and Nav1.8-MOR knockout mice and therefore, do not support a participation of peripheral MOR in microgliosis associated with neuropathic pain.

Constitutive deletion of MOR also increased sensitivity to heat before and after the nerve injury, in agreement with previous literature describing a primary role of MOR limiting heat sensitivity (Matthes *et al*, 1996; Scherrer *et al*, 2009). These studies showed a preferential role of MOR influencing acute heat and chemical nociception rather than responses to mechanical stimulation (Martin *et al*, 2003; Scherrer *et al*, 2009). In the present study, increased thermosensation was partly replicated when MOR was removed from GABAergic forebrain neurons of male mice. This male-specific exacerbated sensitivity to heat was restricted to the neuropathic condition, since naïve DLX5/6 male mice did not show significant nociceptive enhancement in our settings or in previous nociceptive assays (Charbogne *et al*, 2017). Increased heat sensitivity was unrelated to the absence of MOR in Nav1.8+ fibres, which is consistent with previous studies describing absence of peripheral involvement of MOR in acute heat perception (Corder *et al*, 2017; Weibel *et al*, 2013). However, the enhanced thermal hyperalgesia involved an overexpression of *Tac1* in the DRG of nerve-injured constitutive mutants that was absent in Nav1.8 or DLX5/6-MOR knockouts. *Tac1* gene encodes the precursor of the excitatory neuropeptide substance P, which is restricted to peptidergic unmyelinated fibres that respond to heat. Hence, this would be consistent with the exacerbated thermonociception. Since most of Substance P+ fibres express Nav1.8

(Usoskin *et al*, 2015) and Nav1.8-MOR knockouts did not show *Tac1* overexpression, this molecular change may be attributed to spinal MOR-related circuits that modulate primary afferent activity. In this line, recent research investigating MOR and DOR thorough the CNS suggest reciprocal modulation of MOR and DOR expressed in different cell types (Wang *et al*, 2018). Interestingly, *Tac1* overexpression did not produce further gliosis in superficial or deep spinal laminae, suggesting absence of glial involvement on the heat hyperalgesia of CMV-MOR constitutive knockouts.

Mice lacking MOR systemically and subjected to the sham surgery showed responses of negative affect in the forced swimming and the light/dark box tests. This is in contrast with the decreased anxiety- and depressive-like behaviour previously described in naïve MOR knockout mice (Filliol *et al*, 2000), which attributed anxiogenic and pro-depressive properties to MOR. In contrast to these effects, other studies showed effects of MOR inhibiting affective responses during inflammatory pain and other stressful conditions (Corder *et al*, 2013; Ghozland *et al*, 2005; Taylor and Corder, 2014). Since our experimental settings involve an inflammatory response associated to the resolution of the surgical incision in the mid-thigh, it is likely that central MOR could mask the affective consequences of inflammatory pain in sham-operated WT mice. In contrast, sham-operated mice lacking MOR constitutively lack this protective mechanism and develop the increased affective responses after the sham surgery. The induction of the neuropathic condition showed a different participation of MOR on the affective responses to pathological neuropathic pain. Both constitutive and conditional DLX5/6-MOR knockouts showed decreased anxiety-like responses to the neuropathic injury, suggesting anxiogenic functions of MOR in this

maladaptive chronic pain condition. Previous studies suggested participation of MOR from amygdalar and striatal regions on the affective component of pain (Martikainen *et al*, 2015; Wang *et al*, 2018), however our data adds information on the consequences of MOR activity in a specific GABAergic population involved in the affective manifestations of pathological pain.

The effects of DOR on nociceptive sensitivity of nerve-injured mice were to some extent opposite to the effects of MOR. As previously reported, constitutive deletion of DOR increased mechanical hypersensitivity in nerve injured male and female mice, whereas it had no overt effects on basal nociception or heat sensitivity after the nerve injury (Bardoni *et al*, 2014; Martin *et al*, 2003; Nadal *et al*, 2006). This effect restricted to mechanosensation is in agreement with a predominant distribution of DOR in fibres sensitive to touch and a relative absence in fibres sensitive to heat (Bardoni *et al*, 2014). According to our nociceptive data, similar distribution could be expected during neuropathic conditions. Nav1.8 and DLX5/6-DOR knockouts showed partial increases in mechanosensitivity, being these effects restricted to males. Peripheral DOR involvement on mechanical nociception was previously described in models of chronic inflammatory and neuropathic pain (Gaveriaux-Ruff *et al*, 2011). Thus, Nav1.8-MOR knockouts displayed enhanced mechanical allodynia and unaltered heat hyperalgesia induced by both CFA and PSNL (Gaveriaux-Ruff *et al*, 2011). The analgesic effects of the DOR agonist SNC80 administered either systemically or into the injured paw were absent in these conditional knockout mice, suggesting the involvement of peripheral DOR in opioid-mediated analgesia under these chronic pain conditions (Gaveriaux-Ruff *et al*, 2011). However, a contribution of DOR

from GABAergic forebrain neurons on mechanical nociception during neuropathic pain was not described before.

Interestingly, constitutive CMV-DOR knockouts showed increased affective and cognitive impairment in sham conditions and enhanced depressive-like manifestations of neuropathic pain. Anxiolytic and antidepressant effects of DOR activation in the absence of pain have consistently been reported (Pradhan *et al*, 2011), and our results now provide further evidence of the role of DOR attenuating depressive-like behaviour associated with chronic neuropathic pain. The memory deficit of sham-operated CMV-MOR knockouts is consistent with the previously reported impaired performance of full DOR knockout mice in hippocampal-dependent tasks (Le Merrer *et al*, 2013). Our findings suggest the participation of forebrain DOR in depressive-like behaviour and memory performance, but not in anxiety-like behaviour. Striatum and olfactory bulb are the areas where DLX5/6-DOR knockouts show major DOR protein deletion. Considering the association between these two structures and depressive disorders (Minami *et al*, 2017; Takamura *et al*, 2017; Zhu *et al*, 2017), it is reasonable to postulate that DOR activity in GABAergic neurons of the striatum and olfactory bulb may modulate depressive-like behaviour. Since the novel object recognition test evaluates hippocampal-dependent learning, we can assume that DOR expressed in GABAergic interneurons in the hippocampus may be responsible for the pro-cognitive effects of DOR. The partial amnesic phenotype observed in the DLX5/6-DOR knockouts may be due to the partial deletion (-56%) of DOR in the hippocampus in these mice (Chu Sin Chung *et al*, 2015). Our results also agree with the lack of involvement of DOR in the olfactory bulb and striatum in the anxiolytic role of DOR (Chu Sin Chung *et al*, 2015). Several lines of evidence support the influence of

negative affect increasing mechanical nociception (Burke *et al*, 2013; Roeska *et al*, 2009; Zeng *et al*, 2008). Thus, our findings suggest that the altered emotional responses shown by CMV-DOR knockout mice could have an impact increasing mechanical sensitivity after the nerve injury. Overall, our results with DOR knockout mice depict DOR as a component of the endogenous opioid system that protects against nociceptive and affective dimensions of chronic neuropathic pain.

In terms of sex-differences, a slightly higher mechanical and heat sensitivity in females compared to males was observed in particular experimental measurements. These results agree with previous studies showing sex-differences in pain responses, with females displaying greater sensitivities (Bartley and Fillingim, 2013; Riley *et al*, 1998). Interestingly, a sex-dependent involvement of MOR and DOR in the modulation of acute mechanical transmission was also observed. Total deletion of MOR induced a slight attenuation of mechanical nociception only to uninjured females, but no differences were revealed in males. Similarly, constitutive deletion of DOR enhanced mechanical nociception in uninjured males, but not in females. These results suggest a differential role of the endogenous opioid tone acting on MOR and DOR in male and female mice under physiological conditions. Both pharmacokinetic and pharmacodynamic factors have been reported to participate in sex-differences in opioid analgesia in animals and humans (Craft, 2003; Doyle and Murphy, 2017). In rodents, MOR agonists were more potent or effective in males than females, but in humans, opioids with MOR agonist activity were often more potent or effective in females than males (Craft, 2003). Whether these discrepancies are due to a true genetic difference or due to the widely variant analgesic testing procedures used in rodents versus humans is not yet known. Little

evidence exists for sex differences in analgesia produced by DOR agonists. One study reported greater DOR-mediated analgesia in males than in females (Bartok and Craft, 1997), while other two revealed no differences (Craft *et al*, 2001; Kepler *et al*, 1991). Although male and female mice achieve similar levels of pain hypersensitivity after nerve injury, sexual dimorphism in the underlying mechanisms leading to chronic pain can exist (Sorge *et al*, 2015). Further investigation of sexual dimorphism in molecular mechanisms underlying pronociceptive effects of MOR under neuropathic pain conditions would be of great interest in the future.

This work describes participation of MOR and DOR on the behavioural phenotypes triggered by persistent nerve damage. MOR showed unexpected detrimental effects including heightened mechanical sensitivity and increased affective responses to neuropathic pain, whereas DOR demonstrated opposing roles limiting both mechanical pain and emotional impairments. The absence of phenotypes in peripheral MOR knockouts suggests participation of central MOR on the increased nociceptive responses associated to the neuropathic injury. One of the mechanisms involved is the inhibition of DOR and *Tac1* expression in primary afferent neurons, which may occur through spinal MOR-related circuits. However, the precise cellular entity involved in these pain-sensitizing effects of MOR remains to be elucidated. The anxiogenic function of MOR during neuropathic pain could be located in a population of GABAergic neurons of the forebrain, the same cell type in which DOR have opposite effects limiting depressive-like behaviour. These results contribute to explain the renowned lack of efficacy of MOR opioid agonists for the treatment of chronic neuropathic pain (Bian *et al*, 1999; Huffman *et al*, 2017; Kimura *et al*, 2014; Mao *et al*, 1995; Rashid *et*

al, 2004; Wegert *et al*, 1997) and suggest the need for searching alternatives to MOR opioids. The identification of neuropathic pain as a pathological entity aggravated through MOR activity could be relevant to mitigate the use of opioid drugs and to limit the clinical damage associated with the current opioid crisis (Volkow and McLellan, 2016). The protective function revealed in this study of DOR minimizing the nociceptive and affective consequences of neuropathic pain underlines the potential interest of DOR agonists for neuropathic pain treatment.

CONCLUSIONS

The main conclusions of the work presented in this Thesis can be summarized as follows:

1. Social and emotional behavioural traits contribute to the inter-individual variability of the neuropathic pain manifestations in mice, which suggest the potential benefit of efficient personalized treatments for chronic pain.
2. Indeed, anxiety trait might be a vulnerability factor to develop enhanced nociceptive, affective and cognitive manifestations of neuropathic pain.
3. Sociability and depressive traits may decrease neuropathic pain-induced hypersensitivity, whereas they increase vulnerability to affective and cognitive manifestations of neuropathic pain.
4. Nociceptive hypersensitivity does not seem to be proportional to affective and cognitive manifestations of neuropathic pain, which supports the importance of evaluating the different behavioural manifestations associated to pain in animal models of neuropathies.
5. The impact of behavioural traits on pain manifestations may be partially due to the modulation of gene expression in the amygdala.
6. *Pdyn* and *Il6* expression in the amygdala may be involved in aggravating neuropathic pain syndrome, while *Gadd45* may play a protective role against emotional and cognitive chronic pain manifestations, probably by promoting genomic stability and protecting neurons from apoptosis.
7. We confirm a primary role of MOR limiting heat sensitivity in a mouse model of neuropathic pain, which may be attributed to spinal MOR-related circuits that modulate substance P neurotransmission from peptidergic unmyelinated afferent fibres.

8. However, MOR activity may induce detrimental effects under neuropathic pain conditions including heightened mechanical sensitivity and increased affective responses to neuropathic pain.
9. MOR-induced modulation of DOR expression and function in the DRG, and direct or indirect promotion of microgliosis and astrogliosis in the spinal cord dorsal horn may underly pain sensitizing effects of MOR.
10. MOR from Nav1.8+ primary afferent fibres do not seem involved in basal nociception or in nerve injury-induced hypersensitivity, while the anxiogenic function of MOR during neuropathic pain could be mediated by a population of GABAergic neurons of the forebrain.
11. Conversely, our results depict DOR as a component of the endogenous opioid system that protects against nociceptive and affective dimensions of chronic neuropathic pain, which underlines the potential interest of DOR agonists for neuropathic pain treatment.
12. Both DOR from Nav1.8+ primary afferent fibres and forebrain GABAergic neurons contribute to limit the mechanical allodynic manifestations, while DOR in forebrain GABAergic neurons are involved in limiting the depressive-like manifestations associated to neuropathic pain.
13. These results contribute to explain the renowned lack of efficacy of MOR opioid agonists for the treatment of chronic neuropathic pain and suggest the need for searching alternatives to MOR opioids for these pain conditions.
14. The identification of neuropathic pain as a pathological entity aggravated through MOR activity could be relevant to mitigate the use of opioid drugs and to limit the clinical damage associated with the current opioid crisis.

REFERENCES

Aasvang EK, Brandsborg B, Christensen B, Jensen TS, Kehlet H (2008). Neurophysiological characterization of postherniotomy pain. *Pain* **137**: 173–81.

Ab Aziz CB, Ahmad AH (2006). The role of the thalamus in modulating pain. *Malays J Med Sci* **13**: 11–8.

Abbott CA, Malik RA, Ross ERE van, Kulkarni J, Boulton AJM (2011). Prevalence and characteristics of painful diabetic neuropathy in a large community-based diabetic population in the U.K. *Diabetes Care* **34**: 2220–2224.

Abdul Aziz AA, Finn DP, Mason R, Chapman V (2005). Comparison of responses of ventral posterolateral and posterior complex thalamic neurons in naive rats and rats with hindpaw inflammation: mu-opioid receptor mediated inhibitions. *Neuropharmacology* **48**: 607–16.

Acuña C (2008). Duloxetine for the treatment of fibromyalgia. *Drugs of Today* **44**: 725.

Aicher SA, Punnoose A, Goldberg A (2000). mu-Opioid receptors often colocalize with the substance P receptor (NK1) in the trigeminal dorsal horn. *J Neurosci* **20**: 4345–54.

Aira Z, Buesa I, Gallego M, Garcia del Cano G, Mendiable N, Mingo J, *et al* (2012). Time-Dependent Cross Talk between Spinal Serotonin 5-HT2A Receptor and mGluR1 Subserves Spinal Hyperexcitability and Neuropathic Pain after Nerve Injury. *J Neurosci* **32**: 13568–13581.

Al-Hasani R, Bruchas MR (2011). Molecular mechanisms of opioid receptor-dependent signaling and behavior. *Anesthesiology* **115**: 1363–81.

Al-Hasani R, McCall JG, Shin G, Gomez AM, Schmitz GP, Bernardi JM, *et al* (2015). Distinct subpopulations of nucleus accumbens dynorphin neurons drive aversion and reward. *Neuron* **87**: 1063–77.

Aliaga L, Baños J-E, Barutell C de, Molet J, Rofríguez de la Serna A (Publicaciones Permanyer: Barcelona, 2002). *Tratamiento del dolor : teoría y práctica*. .

Ambrose LM, Gallagher SM, Unterwald EM, Bockstaele EJ Van (2006). Dopamine-D1 and δ-opioid receptors co-exist in rat striatal neurons. *Neurosci Lett* **399**: 191–196.

American Psychiatric Association (American Psychiatric Association: 2013). *Diagnostic and Statistical Manual of Mental Disorders*. .

Andersen G, Vestergaard K, Ingeman-Nielsen M, Jensen TS (1995). Incidence of central post-stroke pain. *Pain* **61**: 187–93.

Andre J, Zeau B, Pohl M, Cesselin F, Benoliel J-J, Becker C (2005). Involvement of cholecystokininergic systems in anxiety-induced hyperalgesia in male rats: behavioral and biochemical studies. *J Neurosci* **25**: 7896–904.

Apkarian a. V, Sosa Y, Krauss BR, Thomas PS, Fredrickson BE, Levy RE, *et al* (2004). Chronic pain patients are impaired on an emotional decision-making task. *Pain* **108**: 129–136.

Apkarian AV, Baliki MN, Farmer MA (2013). Predicting transition to chronic pain. *Curr Opin Neurol* **26**: 360–7.

Araldi D, Khomula E V, Ferrari LF, Levine JD (2018). Fentanyl Induces Rapid Onset Hyperalgesic Priming: Type I at Peripheral and Type II at Central Nociceptor Terminals. *J Neurosci* **38**: 2226–2245.

Arntz A, Dreessen L, Jong P De (1994). The influence of anxiety on pain: attentional and attributional mediators. *Pain* **56**: 307–14.

Asghari A, Nicholas MK (2006). Personality and pain-related beliefs/coping strategies: a prospective study. *Clin J Pain* **22**: 10–8.

Asmundson GJ, Norton GR (1995). Anxiety sensitivity in patients with physically unexplained chronic back pain: a preliminary report. *Behav Res Ther* **33**: 771–7.

Attal N, Cruccu G, Baron R, Haanpää M, Hansson P, Jensen TS, *et al* (2010). EFNS guidelines on the pharmacological treatment of neuropathic pain: 2010 revision. *Eur J Neurol* **17**: 1113-e88.

Attal N, Fermanian C, Fermanian J, Lanteri-Minet M, Alchaar H, Bouhassira D (2008). Neuropathic pain: Are there distinct subtypes depending on the aetiology or anatomical lesion? *Pain* **138**: 343–353.

Attal N, Lanteri-Minet M, Laurent B, Fermanian J, Bouhassira D (2011). The specific disease burden of neuropathic pain: Results of a French nationwide survey. *Pain* **152**: 2836–2843.

Austin PJ, Moalem-Taylor G (2010). The neuro-immune balance in neuropathic pain: Involvement of inflammatory immune cells, immune-like glial cells and cytokines. *J Neuroimmunol* **229**: 26–50.

Bagot RC, Labonté B, Peña CJ, Nestler EJ (2014). Epigenetic signaling in psychiatric disorders: stress and depression. *Dialogues Clin Neurosci* **16**: 281–95.

Baldock PA, Driessler F, Lin S, Wong IPL, Shi Y, Yulyaningsih E, *et al* (2012). The endogenous opioid dynorphin is required for normal bone homeostasis in mice. *Neuropeptides* **46**: 383–94.

Banghart MR, Sabatini BL (2012). Photoactivatable neuropeptides for spatiotemporally precise delivery of opioids in neural tissue. *Neuron* **73**: 249–59.

Bannister K, Lee YS, Goncalves L, Porreca F, Lai J, Dickenson AH (2014). Neuropathic

plasticity in the opioid and non-opioid actions of dynorphin A fragments and their interactions with bradykinin B2 receptors on neuronal activity in the rat spinal cord. *Neuropharmacology* **85**: 375–83.

Bär K-J, Brehm S, Boettger MK, Boettger S, Wagner G, Sauer H (2005). Pain perception in major depression depends on pain modality. *Pain* **117**: 97–103.

Bär K-J, Brehm S, Boettger MK, Wagner G, Boettger S, Sauer H (2006). Decreased sensitivity to experimental pain in adjustment disorder. *Eur J Pain* **10**: 467–71.

Bardoni R, Tawfik VL, Wang D, François A, Solorzano C, Shuster S a., *et al* (2014). Delta opioid receptors presynaptically regulate cutaneous mechanosensory neuron input to the spinal cord dorsal horn. *Neuron* **81**: 1312–1327.

Barlow DH, Chorpita BF, Turovsky J (1996). Fear, panic, anxiety, and disorders of emotion. *Nebr Symp Motiv* **43**: 251–328.

Baron R, Binder A, Wasner G (2010). Neuropathic pain: Diagnosis, pathophysiological mechanisms, and treatment. *Lancet Neurol* **9**: 807–819.

Baron R, Schattschneider J, Binder A, Siebrecht D, Wasner G (2002). Relation between sympathetic vasoconstrictor activity and pain and hyperalgesia in complex regional pain syndromes: a case-control study. *Lancet (London, England)* **359**: 1655–60.

Bartley EJ, Fillingim RB (2013). Sex differences in pain: A brief review of clinical and experimental findings. *Br J Anaesth* **111**: 52–58.

Bartok RE, Craft RM (1997). Sex differences in opioid antinociception. *J Pharmacol Exp Ther* **282**: 769–78.

Basbaum AI, Bautista DM, Scherrer G, Julius D (2009). Cellular and molecular mechanisms of pain. *Cell* **139**: 267–84.

Basbaum AI, Fields HL (1984). Endogenous Pain Control Systems: Brainstem Spinal Pathways and Endorphin Circuitry. *Annu Rev Neurosci* **7**: 309–338.

Basso L, Altier C (2017). Transient Receptor Potential Channels in neuropathic pain. *Curr Opin Pharmacol* **32**: 9–15.

Battistin L, Varotto M, Berlese G, Roman G (1984). Effects of some anticonvulsant drugs on brain GABA level and GAD and GABA-T activities. *Neurochem Res* **9**: 225–31.

Beardsley PM, Howard JL, Shelton KL, Carroll FI (2005). Differential effects of the novel kappa opioid receptor antagonist, JDTic, on reinstatement of cocaine-seeking induced by footshock stressors vs cocaine primes and its antidepressant-like effects in rats. *Psychopharmacology (Berl)* **183**: 118–26.

Becker A, Grecksch G, Schröder H (2006). Pain sensitivity is altered in animals after

subchronic ketamine treatment. *Psychopharmacology (Berl)* **189**: 237–247.

Becker B, Mihov Y, Scheele D, Kendrick KM, Feinstein JS, Matusch A, *et al* (2012). Fear Processing and Social Networking in the Absence of a Functional Amygdala. *Biol Psychiatry* **72**: 70–77.

Benarroch EE (2010). Central neuron-glia interactions and neuropathic pain: overview of recent concepts and clinical implications. *Neurology* **75**: 273–8.

Benbouzid M, Gavériaux-Ruff C, Yalcin I, Waltisperger E, Tessier L-H, Muller A, *et al* (2008a). Delta-opioid receptors are critical for tricyclic antidepressant treatment of neuropathic allodynia. *Biol Psychiatry* **63**: 633–6.

Benbouzid M, Pallage V, Rajalu M, Waltisperger E, Doridot S, Poisbeau P, *et al* (2008b). Sciatic nerve cuffing in mice: A model of sustained neuropathic pain. *Eur J Pain* **12**: 591–599.

Bennett MI, Rayment C, Hjermstad M, Aass N, Caraceni A, Kaasa S (2012). Prevalence and aetiology of neuropathic pain in cancer patients: A systematic review. *Pain* **153**: 359–365.

Berman D, Rodin BE (1982). The influence of housing condition on autotomy following dorsal rhizotomy in rats. *Pain* **13**: 307–11.

Berrocoso E, Ikeda K, Sora I, Uhl GR, Sánchez-Blázquez P, Mico JA (2013). Active behaviours produced by antidepressants and opioids in the mouse tail suspension test. *Int J Neuropsychopharmacol* **16**: 151–62.

Berrocoso E, Mico J-A (2009). Cooperative opioid and serotonergic mechanisms generate superior antidepressant-like effects in a mice model of depression. *Int J Neuropsychopharmacol* **12**: 1033–44.

Berrocoso E, Sanchez-Blazquez P, Garzon J, Mico J (2009). Opiates as Antidepressants. *Curr Pharm Des* **15**: 1612–1622.

Bertran-Gonzalez J, Laurent V, Chieng BC, Christie MJ, Balleine BW (2013). Learning-related translocation of δ-opioid receptors on ventral striatal cholinergic interneurons mediates choice between goal-directed actions. *J Neurosci* **33**: 16060–71.

Besson A, Privat AM, Eschalier A, Fialip J (1996). Effects of morphine, naloxone and their interaction in the learned-helplessness paradigm in rats. *Psychopharmacology (Berl)* **123**: 71–8.

Bian D, Nichols ML, Ossipov MH, Lai J, Porreca F (1995). Characterization of the antiallodynic efficacy of morphine in a model of neuropathic pain in rats.

Neuroreport **6**: 1981–4.

Bian D, Ossipov MH, Ibrahim M, Raffa RB, Tallarida RJ, Philip T, *et al* (1999). Loss of antiallodynic and antinociceptive spinalrsupraspinal morphine synergy in nerve-injured rats: restoration by MK-801 or dynorphin antiserum. *Brain Res* **831**: 55–63.

Biessels GJ, Kamal A, Urban IJ, Spruijt BM, Erkelens DW, Gispen WH (1998). Water maze learning and hippocampal synaptic plasticity in streptozotocin-diabetic rats: effects of insulin treatment. *Brain Res* **800**: 125–35.

Bigliardi PL, Bigliardi-Qi M (2014). *Peripheral Opioids*. *Itch Mech Treat* .

Bilkei-Gorzo A, Mauer D, Michel K, Zimmer A (2014). Dynorphins regulate the strength of social memory. *Neuropharmacology* **77**: 406–413.

Bilkei-Gorzo A, Michel K, Noble F, Roques BP, Zimmer A (2007). Preproenkephalin knockout mice show no depression-related phenotype. *Neuropsychopharmacology* **32**: 2330–7.

Bilkei-Gorzo A, Racz I, Michel K, Mauer D, Zimmer A, Klingmüller D, *et al* (2008). Control of hormonal stress reactivity by the endogenous opioid system. *Psychoneuroendocrinology* **33**: 425–436.

Bilkei-Gorzo A, Racz I, Michel K, Zimmer A, Klingmüller D, Zimmer A (2004). Behavioral phenotype of pre-proenkephalin-deficient mice on diverse congenic backgrounds. *Psychopharmacology (Berl)* **176**: 343–52.

Binder W, Mousa SA, Sitte N, Kaiser M, Stein C, Schafer M (2004). Sympathetic activation triggers endogenous opioid release and analgesia within peripheral inflamed tissue. *Eur J Neurosci* **20**: 92–100.

Birklein F, Weber M, Ernst M, Riedl B, Neundörfer B, Handwerker HO (2000). Experimental tissue acidosis leads to increased pain in complex regional pain syndrome (CRPS). *Pain* **87**: 227–34.

Bodkin JA, Zornberg GL, Lukas SE, Cole JO (1995). Buprenorphine treatment of refractory depression. *J Clin Psychopharmacol* **15**: 49–57.

Bodnar RJ (2017). Endogenous Opiates and Behavior: 2015. *Peptides* **88**: 126–188.

Bodnar RJ, Kelly DD, Brutus M, Glusman M (1980). Stress-induced analgesia: neural and hormonal determinants. *Neurosci Biobehav Rev* **4**: 87–100.

Böhm C, Newrzella D, Herberger S, Schramm N, Eisenhardt G, Schenk V, *et al* (2006). Effects of antidepressant treatment on gene expression profile in mouse brain: cell type-specific transcription profiling using laser microdissection and microarray analysis. *J Neurochem* **97 Suppl 1**: 44–9.

Bohn LM, Lefkowitz RJ, Gainetdinov RR, Peppel K, Caron MG, Lin FT (1999). Enhanced morphine analgesia in mice lacking beta-arrestin 2. *Science* **286**: 2495–8.

Bohren Y, Karavelic D, Tessier L-H, Yalcin I, Gavériaux-Ruff C, Kieffer BL, *et al* (2010). Mu-opioid receptors are not necessary for nortriptyline treatment of neuropathic allodynia. *Eur J Pain* **14**: 700–704.

Botvinick M, Jha AP, Bylsma LM, Fabian SA, Solomon PE, Prkachin KM (2005). Viewing facial expressions of pain engages cortical areas involved in the direct experience of pain. *Neuroimage* **25**: 312–9.

Bouhassira D, Lantéri-Minet M, Attal N, Laurent B, Touboul C (2008). Prevalence of chronic pain with neuropathic characteristics in the general population. *Pain* **136**: 380–7.

Boyle KA, Gutierrez-Mecinas M, Polgár E, Mooney N, O'Connor E, Furuta T, *et al* (2017). A quantitative study of neurochemically defined populations of inhibitory interneurons in the superficial dorsal horn of the mouse spinal cord. *Neuroscience* **363**: 120–133.

Bravo L, Mico JA, Rey-Brea R, Pérez-Nievas B, Leza JC, Berrocoso E (2012). Depressive-like states heighten the aversion to painful stimuli in a rat model of comorbid chronic pain and depression. *Anesthesiology* **117**: 613–25.

Breivik H, Eisenberg E, O'Brien T, OPENMinds (2013). The individual and societal burden of chronic pain in Europe: the case for strategic prioritisation and action to improve knowledge and availability of appropriate care. *BMC Public Health* **13**: 1229.

Bridges D, Thompson SW, Rice AS (2001). Mechanisms of neuropathic pain. *Br J Anaesth* **87**: 12–26.

Broom DC, Jutkiewicz EM, Folk JE, Traynor JR, Rice KC, Woods JH (2002). Nonpeptidic delta-opioid receptor agonists reduce immobility in the forced swim assay in rats. *Neuropsychopharmacology* **26**: 744–55.

Brown JL, Sheffield D, Leary MR, Robinson ME (2003). Social support and experimental pain. *Psychosom Med* **65**: 276–83.

Brüggemann J, Galhardo V, Apkarian a V (2001). Immediate reorganization of the rat somatosensory thalamus after partial ligation of sciatic nerve. *J Pain* **2**: 220–228.

Bunzow JR, Saez C, Mortrud M, Bouvier C, Williams JT, Low M, *et al* (1994). Molecular cloning and tissue distribution of a putative member of the rat opioid receptor gene family that is not a mu, delta or kappa opioid receptor type. *FEBS Lett* **347**: 284–8.

Bura AS, Guegan T, Zamanillo D, Vela JM, Maldonado R (2013). Operant self-administration of a sigma ligand improves nociceptive and emotional manifestations of neuropathic pain. *Eur J Pain* **17**: 832–43.

Burke NN, Geoghegan E, Kerr DM, Moriarty O, Finn DP, Roche M (2013). Altered neuropathic pain behaviour in a rat model of depression is associated with changes in inflammatory gene expression in the amygdala. *Genes Brain Behav* **12**: 705–13.

Burke NN, Hayes E, Calpin P, Kerr DM, Moriarty O, Finn DP, *et al* (2010). Enhanced nociceptive responding in two rat models of depression is associated with alterations in monoamine levels in discrete brain regions. *Neuroscience* **171**: 1300–13.

Burns TL, Ineck JR (2006). Cannabinoid Analgesia as a Potential New Therapeutic Option in the Treatment of Chronic Pain. *Ann Pharmacother* **40**: 251–260.

Butler S, Jonzon B, Branting-Ekenbäck C, Wadell C, Farahmand B (2013). Predictors of severe pain in a cohort of 5271 individuals with self-reported neuropathic pain. *Pain* **154**: 141–146.

Cabañero D, Baker A, Zhou S, Hargett GL, Irie T, Xia Y, *et al* (2013). Pain after discontinuation of morphine treatment is associated with synaptic increase of GluA4-containing AMPAR in the dorsal horn of the spinal cord. *Neuropsychopharmacology* **38**: 1472–84.

Cahill CM, McClellan KA, Morinville A, Hoffert C, Hubatsch D, O'Donnell D, *et al* (2001). Immunohistochemical distribution of delta opioid receptors in the rat central nervous system: evidence for somatodendritic labeling and antigen-specific cellular compartmentalization. *J Comp Neurol* **440**: 65–84.

Cahill CM, Taylor AMW, Cook C, Ong E, MorÃ³n JA, Evans CJ (2014). Does the kappa opioid receptor system contribute to pain aversion? *Front Pharmacol* **5**: 1–15.

Cahill CM, Xue L, Grenier P, Magnussen C, Lecour S, Olmstead MC (2013). Changes in morphine reward in a model of neuropathic pain. *Behav Pharmacol* **24**: 207–13.

Canli T, Cook RG, Miczek KA (1990). Opiate antagonists enhance the working memory of rats in the radial maze. *Pharmacol Biochem Behav* **36**: 521–5.

Cao L, DeLeo JA (2008). CNS-infiltrating CD4+ T lymphocytes contribute to murine spinal nerve transection-induced neuropathic pain. *Eur J Immunol* **38**: 448–458.

Capuano A, Corato A De, Lisi L, Tringali G, Navarra P, Russo C Dello (2009). Proinflammatory-activated trigeminal satellite cells promote neuronal sensitization: relevance for migraine pathology. *Mol Pain* **5**: 43.

Carey AN, Lyons AM, Shay CF, Dunton O, McLaughlin JP (2009). Endogenous Opioid Activation Mediates Stress-Induced Deficits in Learning and Memory. *J Neurosci* **29**: 4293–4300.

Carlezon WA, Béguin C, DiNieri JA, Baumann MH, Richards MR, Todtenkopf MS, *et al* (2006). Depressive-like effects of the kappa-opioid receptor agonist salvinorin A on behavior and neurochemistry in rats. *J Pharmacol Exp Ther* **316**: 440–7.

Carroll IR, Angst MS, Clark JD (2004). Management of perioperative pain in patients chronically consuming opioids. *Reg Anesth Pain Med* **29**: 576–91.

Castellano C, Pavone F (1985). Dose- and strain-dependent effects of dermorphin and [D-Ala2-D-Leu5]enkephalin on passive avoidance behavior in mice. *Behav Neurosci* **99**: 1120–7.

Castro DC, Berridge KC (2014). Opioid hedonic hotspot in nucleus accumbens shell: mu, delta, and kappa maps for enhancement of sweetness "liking" and "wanting". *J Neurosci* **34**: 4239–50.

Catheline G, Guilbaud G, Kayser V (1998). Peripheral component in the enhanced antinociceptive effect of systemic U-69,593, a kappa-opioid receptor agonist in mononeuropathic rats. *Eur J Pharmacol* **357**: 171–8.

Cavanaugh DJ, Lee H, Lo L, Shields SD, Zylka MJ, Basbaum AI, *et al* (2009). Distinct subsets of unmyelinated primary sensory fibers mediate behavioral responses to noxious thermal and mechanical stimuli. *Proc Natl Acad Sci* **106**: 9075–9080.

Cervero F (1991). Mechanisms of acute visceral pain. *Br Med Bull* **47**: 549–60.

Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL (1994). Quantitative assessment of tactile allodynia in the rat paw. *J Neurosci Methods* **53**: 55–63.

Charbogne P, Gardon O, Martín-García E, Keyworth HL, Matsui A, Mechling AE, *et al* (2017). Mu Opioid Receptors in Gamma-Aminobutyric Acidergic Forebrain Neurons Moderate Motivation for Heroin and Palatable Food. *Biol Psychiatry* **81**: 778–788.

Chavkin C (2013). Dynorphin--still an extraordinarily potent opioid peptide. *Mol Pharmacol* **83**: 729–36.

Chen J, Uchimura K, Stetler RA, Zhu RL, Nakayama M, Jin K, *et al* (1998). Transient global ischemia triggers expression of the DNA damage-inducible gene GADD45 in the rat brain. *J Cereb Blood Flow Metab* **18**: 646–57.

Chen S-R, Pan H-L (2008). Removing TRPV1-expressing primary afferent neurons potentiates the spinal analgesic effect of delta-opioid agonists on mechano-nociception. *Neuropharmacology* **55**: 215–22.

Chen T-C, Cheng Y-Y, Sun W-Z, Shyu B-C (2008). Differential regulation of morphine antinociceptive effects by endogenous enkephalinergic system in the forebrain of mice. *Mol Pain* **4**: 41.

Chiu YH, Silman AJ, Macfarlane GJ, Ray D, Gupta A, Dickens C, *et al* (2005). Poor sleep and depression are independently associated with a reduced pain threshold. Results of a population based study. *Pain* **115**: 316–321.

Choucair-Jaafar N, Salvat E, Freund-Mercier M-J, Barrot M (2014). The antiallodynic action of nortriptyline and terbutaline is mediated by β2 adrenoceptors and δ opioid receptors in the ob/ob model of diabetic polyneuropathy. *Brain Res* **1546**: 18–26.

Chu Sin Chung P, Keyworth HL, Martin-Garcia E, Charbogne P, Darcq E, Bailey A, *et al* (2015). A novel anxiogenic role for the delta opioid receptor expressed in GABAergic forebrain neurons. *Biol Psychiatry* **77**: 404–15.

Chung C, Carteret AF, McKelvy AD, Ringkamp M, Yang F, Hartke T V, *et al* (2012). Analgesic properties of loperamide differ following systemic and local administration to rats after spinal nerve injury. *Eur J Pain* **16**: 1021–32.

Cicero TJ, Ellis MS (2015). Abuse-Deterrent Formulations and the Prescription Opioid Abuse Epidemic in the United States: Lessons Learned From OxyContin. *JAMA psychiatry* **72**: 424–30.

Cicero TJ, Inciardi JA, Muñoz A (2005). Trends in Abuse of OxyContin® and Other Opioid Analgesics in the United States: 2002-2004. *J Pain* **6**: 662–672.

Collingridge GL, Peineau S, Howland JG, Wang YT (2010). Long-term depression in the CNS. *Nat Rev Neurosci* **11**: 459–473.

Corder G, Castro DC, Bruchas MR, Scherrer G (2018). Endogenous and Exogenous Opioids in Pain. *Annu Rev Neurosci* **41**: 453–473.

Corder G, Doolen S, Donahue RR, Winter MK, Jutras BL, He Y, *et al* (2013). Constitutive μ-opioid receptor activity leads to long-term endogenous analgesia and dependence. *Science* **341**: 1394–9.

Corder GF, Tawfik VL, Wang D, Sypek E, Low S, Dickenson J, *et al* (2017). Loss of μ-opioid receptor signaling in nociceptors, and not spinal microglia, abrogates morphine tolerance without disrupting analgesic efficacy. *Nat Med* **23**: 164–173.

Costigan M, Scholz J, Woolf CJ (2009). Neuropathic Pain: A Maladaptive Response of the Nervous System to Damage. *Annu Rev Neurosci* **32**: 1–32.

Coudereau JP, Monier C, Bourre JM, Frances H (1997). Effect of isolation on pain threshold and on different effects of morphine. *Prog Neuropsychopharmacol Biol*

Psychiatry **21**: 997–1018.

Cousins MJ, Giamberardino MA, Jamison RN, Mcgrath PA, Rajagopal MR, Smith MT, *et al* (2010). Upcoming Issues Diagnosis and Classifi cation of Neuropathic Pain Epidemiology and Impact of Neuropathic Pain The Nature and Management of Neuropathic Pain. *PAIN Clin Updat* **18**: .

Cox BM, Goldstein A, Hi CH (1976). Opioid activity of a peptide, beta-lipotropin-(61-91), derived from beta-lipotropin. *Proc Natl Acad Sci U S A* **73**: 1821–3.

Craft RM (2003). Sex differences in opioid analgesia: "from mouse to man". *Clin J Pain* **19**: 175–86.

Craft RM, Tseng AH, McNiel DM, Furness MS, Rice KC (2001). Receptor-selective antagonism of opioid antinociception in female versus male rats. *Behav Pharmacol* **12**: 591–602.

Craig KD, Best H, Ward LM (1975). Social modeling influences on psychophysical judgments of electrical stimulation. *J Abnorm Psychol* **84**: 366–73.

Craig KD, Weiss SM (1971). Vicarious influences on pain-threshold determinations. *J Pers Soc Psychol* **19**: 53–9.

Cui JG, O'Connor WT, Ungerstedt U, Linderoth B, Meyerson BA (1997). Spinal cord stimulation attenuates augmented dorsal horn release of excitatory amino acids in mononeuropathy via a GABAergic mechanism. *Pain* **73**: 87–95.

Cunha C Da, Gomez-A A, Blaha CD (2012). The role of the basal ganglia in motivated behavior. *Rev Neurosci* **23**: 747–67.

Cunha TM, Dias QM (2009). Glial modulation of pain: a step beyond. *J Neurosci* **29**: 3340–2.

Curran H V, Kleckham J, Bearn J, Strang J, Wanigaratne S (2001). Effects of methadone on cognition, mood and craving in detoxifying opiate addicts: a dose-response study. *Psychopharmacology (Berl)* **154**: 153–60.

D'Amato FR (1998). Kin interaction enhances morphine analgesia in male mice. *Behav Pharmacol* **9**: 369–73.

D'Amato FR, Pavone F (2012). Modulation of nociception by social factors in rodents: contribution of the opioid system. *Psychopharmacology (Berl)* **224**: 189–200.

D'Mello R, Dickenson AH (2008). Spinal cord mechanisms of pain. *Br J Anaesth* **101**: 8–16.

Dacher M, Nugent FS (2011). Opiates and plasticity. *Neuropharmacology* **61**: 1088–1096.

Davis M, Walker DL, Lee Y (1997). Amygdala and bed nucleus of the stria terminalis: differential roles in fear and anxiety measured with the acoustic startle reflex.

Philos Trans R Soc Lond B Biol Sci **352**: 1675–87.

Davis M, Walker DL, Miles L, Grillon C (2010). Phasic vs sustained fear in rats and humans: role of the extended amygdala in fear vs anxiety. *Neuropsychopharmacology* **35**: 105–35.

Day JJ, Roitman MF, Wightman RM, Carelli RM (2007). Associative learning mediates dynamic shifts in dopamine signaling in the nucleus accumbens. *Nat Neurosci* **10**: 1020–8.

Defrin R, Ginzburg K, Solomon Z, Polad E, Bloch M, Govezensky M, *et al* (2008). Quantitative testing of pain perception in subjects with PTSD--implications for the mechanism of the coexistence between PTSD and chronic pain. *Pain* **138**: 450–9.

DeHaven-Hudkins DL, Dolle RE (2004). Peripherally restricted opioid agonists as novel analgesic agents. *Curr Pharm Des* **10**: 743–57.

Deleo JA, Tanga FY, Tawfik VL (2004). Neuroimmune Activation and Neuroinflammation in Chronic Pain and Opioid Tolerance/Hyperalgesia. *Neurosci* **10**: 40–52.

Denk F, Crow M, Didangelos A, Lopes DM, McMahon SB (2016). Persistent Alterations in Microglial Enhancers in a Model of Chronic Pain. *Cell Rep* **15**: 1771–81.

Dick B, Eccleston C, Crombez G (2002). Attentional functioning in fibromyalgia, rheumatoid arthritis, and musculoskeletal pain patients. *Arthritis Rheum* **47**: 639–44.

Dick IE, Brochu RM, Purohit Y, Kaczorowski GJ, Martin WJ, Priest BT (2007). Sodium Channel Blockade May Contribute to the Analgesic Efficacy of Antidepressants. *J Pain* **8**: 315–324.

Dimitrov EL, Tsuda MC, Cameron HA, Usdin TB (2014). Anxiety- and depression-like behavior and impaired neurogenesis evoked by peripheral neuropathy persist following resolution of prolonged tactile hypersensitivity. *J Neurosci* **34**: 12304–12.

Dogrul A, Ossipov MH, Porreca F (2009). Differential mediation of descending pain facilitation and inhibition by spinal 5HT-3 and 5HT-7 receptors. *Brain Res* **1280**: 52–59.

Doyle HH, Murphy AZ (2017). Sex-dependent influences of morphine and its metabolites on pain sensitivity in the rat. *Physiol Behav* doi:10.1016/j.physbeh.2017.11.030.

Drew LJ, Harris J, Millns PJ, Kendall DA, Chapman V (2000). Activation of spinal cannabinoid 1 receptors inhibits C-fibre driven hyperexcitable neuronal responses and increases [35S]GTPgammaS binding in the dorsal horn of the spinal cord of noninflamed and inflamed rats. *Eur J Neurosci* **12**: 2079–86.

Duan B, Cheng L, Bourane S, Britz O, Padilla C, Garcia-Campmany L, *et al* (2014). Identification of spinal circuits transmitting and gating mechanical pain. *Cell* **159**: 1417–1432.

Dubin AE, Patapoutian A (2010). Nociceptors: the sensors of the pain pathway. *J Clin Invest* **120**: 3760–3772.

Duggan AW (2000). Neuropeptide spread in the brain and spinal cord. *Prog Brain Res* **125**: 369–380.

Eckert WA, Light AR (2002). Hyperpolarization of substantia gelatinosa neurons evoked by mu-, kappa-, delta 1-, and delta 2-selective opioids. *J Pain* **3**: 115–25.

Eichel K, Jullié D, Zastrow M von (2016). β-Arrestin drives MAP kinase signalling from clathrin-coated structures after GPCR dissociation. *Nat Cell Biol* **18**: 303–10.

Ellis A, Bennett DLH (2013). Neuroinflammation and the generation of neuropathic pain. *Br J Anaesth* **111**: 26–37.

Epley SW (1974). Reduction of the behavioral effects of aversive stimulation by the presence of companions. *Psychol Bull* **81**: 271–83.

Etkin A, Wager TD (2007). Functional Neuroimaging of Anxiety: A Meta-Analysis of Emotional Processing in PTSD, Social Anxiety Disorder, and Specific Phobia. *Am J Psychiatry* **164**: 1476–1488.

Evans S, Fishman B, Spielman L, Haley A (2003). Randomized trial of cognitive behavior therapy versus supportive psychotherapy for HIV-related peripheral neuropathic pain. *Psychosomatics* **44**: 44–50.

Fell GL, Robinson KC, Mao J, Woolf CJ, Fisher DE (2014). Skin β-endorphin mediates addiction to UV light. *Cell* **157**: 1527–34.

Femenía T, Pérez-Rial S, Urigüen L, Manzanares J (2011). Prodynorphin gene deletion increased anxiety-like behaviours, impaired the anxiolytic effect of bromazepam and altered GABA $_{A}$ receptor subunits gene expression in the amygdala. *J Psychopharmacol* **25**: 87–96.

Fields H (2004). State-dependent opioid control of pain. *Nat Rev Neurosci* **5**: 565–575.

Fields HL, Bry J, Hentall I, Zorman G (1983). The activity of neurons in the rostral medulla of the rat during withdrawal from noxious heat. *J Neurosci* **3**: 2545–52.

Fields HL, Heinricher MM (1985). Anatomy and physiology of a nociceptive modulatory system. *Philos Trans R Soc Lond B Biol Sci* **308**: 361–74.

Fields HL, Heinricher MM, Mason P (1991). NEUROTRANSMITTERS IN NOCICEPTIVE MODULATORY CIRCUITS. *Annu Rev Neurosci* **14**: 219–45.

Filliol D, Ghozland S, Chluba J, Martin M, Matthes HWD, Befort K, *et al* (2000). Mice deficient for δ - and μ -opioid receptors exhibit opposing alterations of emotional responses. **25**: 195–200.

FindLaw (2018). Purdue pharma v. Kentucky. at <https://caselaw.findlaw.com/us-2nd-circuit/1619840.html>.

Finnerup N, Johannesen I, Sindrup S, Bach F, Jensen T (2001). Pain and dysesthesia in patients with spinal cord injury: A postal survey. *Spinal Cord* **39**: 256–262.

Finnerup NB, Attal N, Haroutounian S, McNicol E, Baron R, Dworkin RH, *et al* (2015). Pharmacotherapy for neuropathic pain in adults: a systematic review and meta-analysis. *Lancet Neurol* **14**: 162–173.

Flor H, Elbert T, Knecht S, Wienbruch C, Pantev C, Birbaumers N, *et al* (1995). Phantom-limb pain as a perceptual correlate of cortical reorganization following arm amputation. *Nature* **375**: 482–484.

Floresco SB, Ghods-Sharifi S (2006). Amygdala-Prefrontal Cortical Circuitry Regulates Effort-Based Decision Making. *Cereb Cortex* **17**: 251–260.

Flórez J (Ars Medica: Barcelona, 2007). *El Tratamiento farmacologico del dolor*. .

François A, Scherrer G (2018). Delta Opioid Receptor Expression and Function in Primary Afferent Somatosensory Neurons. *Handb Exp Pharmacol* **247**: 87–114.

Franklin KBJ, Paxinos G (Academic Press: Boston, 2008). *The mouse brain in stereotaxic coordinates*. .

Frettlöh J, Hüppe M, Maier C (2006). Severity and specificity of neglect-like symptoms in patients with complex regional pain syndrome (CRPS) compared to chronic limb pain of other origins. *Pain* **124**: 184–9.

Fuchs PN, Roza C, Sora I, Uhl G, Raja SN (1999). Characterization of mechanical withdrawal responses and effects of mu-, delta- and kappa-opioid agonists in normal and mu-opioid receptor knockout mice. *Brain Res* **821**: 480–6.

Fundytus ME, Yashpal K, Chabot JG, Osborne MG, Lefebvre CD, Dray A, *et al* (2001). Knockdown of spinal metabotropic glutamate receptor 1 (mGluR(1)) alleviates pain and restores opioid efficacy after nerve injury in rats. *Br J Pharmacol* **132**: 354–67.

Galanter M, Kleber HD, Brady KT (American Psychiatric Publishing: 2014). *The American Psychiatric Publishing Textbook of Substance Abuse Treatment*. .

Galil K, Choo PW, Donahue JG, Platt R (1997). The sequelae of herpes zoster. *Arch Intern Med* **157**: 1209–13.

Gardell LR, Wang R, Burgess SE, Ossipov MH, Vanderah TW, Malan TP, *et al* (2002).

Sustained morphine exposure induces a spinal dynorphin-dependent enhancement of excitatory transmitter release from primary afferent fibers. *J Neurosci* **22**: 6747–55.

Gaudoux F, Boileau G, Crine P (1993). Localization of neprilysin (EC 3.4.24.11) mRNA in rat brain by in situ hybridization. *J Neurosci Res* **34**: 426–433.

Gavériaux-Ruff C, Karchewski L a., Hever X, Matifas A, Kieffer BL (2008). Inflammatory pain is enhanced in delta opioid receptor-knockout mice. *Eur J Neurosci* **27**: 2558–2567.

Gavériaux-Ruff C, Kieffer BL (2011). Delta opioid receptor analgesia: recent contributions from pharmacology and molecular approaches. *Behav Pharmacol* **22**: 405–414.

Gaveriaux-Ruff C, Nozaki C, Nadal X, Hever XC, Weibel R, Matifas A, *et al* (2011). Genetic ablation of delta opioid receptors in nociceptive sensory neurons increases chronic pain and abolishes opioid analgesia. *Pain* **152**: 1238–48.

Gendron L, Pintar JE, Chavkin C (2007). Essential role of mu opioid receptor in the regulation of delta opioid receptor-mediated antihyperalgesia. *Neuroscience* **150**: 807–17.

Ghoname EA, White PF, Ahmed HE, Hamza MA, Craig WF, Noe CE (1999). Percutaneous electrical nerve stimulation: an alternative to TENS in the management of sciatica. *Pain* **83**: 193–9.

Ghozland S, Chu K, Kieffer BL, Roberts AJ (2005). Lack of stimulant and anxiolytic-like effects of ethanol and accelerated development of ethanol dependence in mu-opioid receptor knockout mice. *Neuropharmacology* **49**: 493–501.

Gilron I, Watson CPN, Cahill CM, Moulin DE (2006). Neuropathic pain: a practical guide for the clinician. *CMAJ* **175**: 265–75.

Glass MJ, Vanyo L, Quimson L, Pickel VM (2009). Ultrastructural relationship between N-methyl-D-aspartate-NR1 receptor subunit and mu-opioid receptor in the mouse central nucleus of the amygdala. *Neuroscience* **163**: 857–67.

Goldstein A, Lowney LI, Pal BK (1971). Stereospecific and nonspecific interactions of the morphine congener levorphanol in subcellular fractions of mouse brain. *Proc Natl Acad Sci U S A* **68**: 1742–7.

Goldstein A, Tachibana S, Lowney LI, Hunkapiller M, Hood L (1979). Dynorphin-(1-13), an extraordinarily potent opioid peptide. *Proc Natl Acad Sci U S A* **76**: 6666–70.

Gonçalves L, Dickenson AH (2012). Asymmetric time-dependent activation of right central amygdala neurones in rats with peripheral neuropathy and pregabalin modulation.

Eur J Neurosci **36**: 3204–3213.

Gonçalves L, Silva R, Pinto-Ribeiro F, Pêgo JM, Bessa JM, Pertovaara A, *et al* (2008). Neuropathic pain is associated with depressive behaviour and induces neuroplasticity in the amygdala of the rat. *Exp Neurol* **213**: 48–56.

Goody RJ, Oakley SM, Filliol D, Kieffer BL, Kitchen I (2002). Quantitative autoradiographic mapping of opioid receptors in the brain of delta-opioid receptor gene knockout mice. *Brain Res* **945**: 9–19.

Gortari P de, Vargas MA, Martínez A, García-Vázquez AI, Uribe RM, Chávez-Gutiérrez L, *et al* (2007). Stage-specific Modulation of Neprilysin and Aminopeptidase N in the Limbic System During Kindling Progression. *J Mol Neurosci* **33**: 252–261.

Grahn JA, Parkinson JA, Owen AM (2009). The role of the basal ganglia in learning and memory: Neuropsychological studies. *Behav Brain Res* **199**: 53–60.

Granier S, Manglik A, Kruse AC, Kobilka TS, Thian FS, Weis WI, *et al* (2012). Structure of the δ-opioid receptor bound to naltrindole. *Nature* **485**: 400–4.

Graybiel AM (2008). Habits, rituals, and the evaluative brain. *Annu Rev Neurosci* **31**: 359–87.

Gu N, Peng J, Murugan M, Wang X, Eyo UB, Sun D, *et al* (2016). Spinal Microgliosis Due to Resident Microglial Proliferation Is Required for Pain Hypersensitivity after Peripheral Nerve Injury. *Cell Rep* **16**: 605–14.

Guan Y, Johanek LM, Hartke T V, Shim B, Tao Y-X, Ringkamp M, *et al* (2008). Peripherally acting mu-opioid receptor agonist attenuates neuropathic pain in rats after L5 spinal nerve injury. *Pain* **138**: 318–29.

Guan Y, Wacnik PW, Yang F, Carteret AF, Chung C-Y, Meyer RA, *et al* (2010). Spinal cord stimulation-induced analgesia: electrical stimulation of dorsal column and dorsal roots attenuates dorsal horn neuronal excitability in neuropathic rats. *Anesthesiology* **113**: 1392–405.

Guan Z, Kuhn JA, Wang X, Colquitt B, Solorzano C, Vaman S, *et al* (2016). Injured sensory neuron-derived CSF1 induces microglial proliferation and DAP12-dependent pain. *Nat Neurosci* **19**: 94–101.

Hamon M, Gozlan H, Bourgoin S, Benoliel JJ, Mauborgne A, Taquet H, *et al* (1987). Opioid receptors and neuropeptides in the CNS in rats treated chronically with amoxapine or amitriptyline. *Neuropharmacology* **26**: 531–539.

Han JS, Neugebauer V (2004). Synaptic plasticity in the amygdala in a visceral pain model in rats. *Neurosci Lett* **361**: 254–257.

Han S, Soleiman MT, Soden ME, Zweifel LS, Palmiter RD (2015). Elucidating an Affective Pain Circuit that Creates a Threat Memory. *Cell* **162**: 363–374.

Hargreaves K, Dubner R, Brown F, Flores C, Joris J (1988). A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. *Pain* **32**: 77–88.

Hasnie FS, Wallace VCJ, Hefner K, Holmes A, Rice ASC (2007). Mechanical and cold hypersensitivity in nerve-injured C57BL/6J mice is not associated with fear-avoidance- and depression-related behaviour. *Br J Anaesth* **98**: 816–22.

Hatfield E, Cacioppo JT, Rapson RL (1993). Emotional Contagion. *Curr Dir Psychol Sci* **2**: 96–100.

Heinl C, Drdla-Schutting R, Xanthos DN, Sandkühler J (2011). Distinct mechanisms underlying pronociceptive effects of opioids. *J Neurosci* **31**: 16748–56.

Hipólito L, Wilson-Poe A, Campos-Jurado Y, Zhong E, Gonzalez-Romero J, Virag L, *et al* (2015). Inflammatory Pain Promotes Increased Opioid Self-Administration: Role of Dysregulated Ventral Tegmental Area μ Opioid Receptors. *J Neurosci* **35**: 12217–31.

Hoggart B, Ratcliffe S, Ehler E, Simpson KH, Hovorka J, Lejčko J, *et al* (2015). A multicentre, open-label, follow-on study to assess the long-term maintenance of effect, tolerance and safety of THC/CBD oromucosal spray in the management of neuropathic pain. *J Neurol* **262**: 27–40.

Hohmann AG, Herkenham M (1999). Localization of central cannabinoid CB1 receptor messenger RNA in neuronal subpopulations of rat dorsal root ganglia: a double-label in situ hybridization study. *Neuroscience* **90**: 923–31.

Hollingshead J, Dühmke RM, Cornblath DR (2006). Tramadol for neuropathic pain. *Cochrane database Syst Rev* CD003726.

Hong S, Morrow TJ, Paulson PE, Isom LL, Wiley JW (2004). Early painful diabetic neuropathy is associated with differential changes in tetrodotoxin-sensitive and -resistant sodium channels in dorsal root ganglion neurons in the rat. *J Biol Chem* **279**: 29341–50.

Hong S, Wiley JW (2005). Early painful diabetic neuropathy is associated with differential changes in the expression and function of vanilloid receptor 1. *J Biol Chem* **280**: 618–27.

Hsieh JC, Belfrage M, Stone-Elander S, Hansson P, Ingvar M (1995). Central representation of chronic ongoing neuropathic pain studied by positron emission tomography. *Pain* **63**: 225–36.

Hu P, Bembrick AL, Keay KA, Mclachlan EM (2007). Immune cell involvement in dorsal

root ganglia and spinal cord after chronic constriction or transection of the rat sciatic nerve. *Brain Behav Immun* **21**: 599–616.

Hu P, McLachlan EM (2002). Macrophage and lymphocyte invasion of dorsal root ganglia after peripheral nerve lesions in the rat. *Neuroscience* **112**: 23–38.

Hucho T, Levine JD (2007). Signaling pathways in sensitization: toward a nociceptor cell biology. *Neuron* **55**: 365–76.

Huffman KL, Rush TE, Fan Y, Sweis GW, Vij B, Covington EC, *et al* (2017). Sustained improvements in pain, mood, function and opioid use post interdisciplinary pain rehabilitation in patients weaned from high and low dose chronic opioid therapy. *Pain* **158**: 1380–1394.

Hughes J, Smith TW, Kosterlitz HW, Fothergill LA, Morgan BA, Morris HR (1975). Identification of two related pentapeptides from the brain with potent opiate agonist activity. *Nature* **258**: 577–80.

Hummel M, Lu P, Cummons TA, Whiteside GT (2008). The persistence of a long-term negative affective state following the induction of either acute or chronic pain. *Pain* **140**: 436–445.

Hwang CS, Chang H-Y, Alexander GC (2015). Impact of abuse-deterrent OxyContin on prescription opioid utilization. *Pharmacoepidemiol Drug Saf* **24**: 197–204.

Iadarola MJ, Max MB, Berman KF, Byas-Smith MG, Coghill RC, Gracely RH, *et al* (1995). Unilateral decrease in thalamic activity observed with positron emission tomography in patients with chronic neuropathic pain. *Pain* **63**: 55–64.

Ibrahim MM, Porreca F, Lai J, Albrecht PJ, Rice FL, Khodorova A, *et al* (2005). CB2 cannabinoid receptor activation produces antinociception by stimulating peripheral release of endogenous opioids. *Proc Natl Acad Sci U S A* **102**: 3093–8.

Idänpään-Heikkilä JJ, Guilbaud G, Kayser V (1997). Prevention of tolerance to the antinociceptive effects of systemic morphine by a selective cholecystokinin-B receptor antagonist in a rat model of peripheral neuropathy. *J Pharmacol Exp Ther* **282**: 1366–72.

Ide S, Minami M, Ishihara K, Uhl GR, Satoh M, Sora I, *et al* (2008). Abolished thermal and mechanical antinociception but retained visceral chemical antinociception induced by butorphanol in μ-opioid receptor knockout mice. *Neuropharmacology* **54**: 1182–1188.

Ide S, Minami M, Ishihara K, Uhl GR, Sora I, Ikeda K (2006). Mu opioid receptor-dependent and independent components in effects of tramadol.

Neuropharmacology **51**: 651–658.

Ikeda R, Takahashi Y, Inoue K, Kato F (2007). NMDA receptor-independent synaptic plasticity in the central amygdala in the rat model of neuropathic pain. *Pain* **127**: 161–72.

Inoue M, Rashid MH, Fujita R, Contos JJA, Chun J, Ueda H (2004). Initiation of neuropathic pain requires lysophosphatidic acid receptor signaling. *Nat Med* **10**: 712–718.

Introini IB, Baratti CM (1984). The impairment of retention induced by beta-endorphin in mice may be mediated by a reduction of central cholinergic activity. *Behav Neural Biol* **41**: 152–63.

Irannejad R, Tomshine JC, Tomshine JR, Chevalier M, Mahoney JP, Steyaert J, *et al* (2013). Conformational biosensors reveal GPCR signalling from endosomes. *Nature* **495**: 534–8.

Jackson T, Huang X, Chen H, Phillips H (2009). Effects of threatening information on interpersonal responses to pain. *Eur J Pain* **13**: 431–8.

Jamot L, Matthes HWD, Simonin F, Kieffer BL, Roder JC (2003). Differential involvement of the mu and kappa opioid receptors in spatial learning. *Genes Brain Behav* **2**: 80–92.

Jang C-G, Lee S-Y, Yoo J-H, Yan J-J, Song D-K, Loh HH, *et al* (2003). Impaired water maze learning performance in μ-opioid receptor knockout mice. *Mol Brain Res* **117**: 68–72.

Janssen SA, Arntz A (1996). Anxiety and pain: attentional and endorphinergic influences. *Pain* **66**: 145–50.

Jenny M, Winkler C, Spetea M, Schennach H, Schmidhammer H, Fuchs D (2008). Non-peptidic delta-opioid receptor antagonists suppress mitogen-induced tryptophan degradation in peripheral blood mononuclear cells in vitro. *Immunol Lett* **118**: 82–7.

Ji R-R, Kohno T, Moore KA, Woolf CJ (2003). Central sensitization and LTP: do pain and memory share similar mechanisms? *Trends Neurosci* **26**: 696–705.

Jin S-X, Zhuang Z-Y, Woolf CJ, Ji R-R (2003). p38 Mitogen-Activated Protein Kinase Is Activated after a Spinal Nerve Ligation in Spinal Cord Microglia and Dorsal Root Ganglion Neurons and Contributes to the Generation of Neuropathic Pain. *J Neurosci* **23**: .

Jochum T, Boettger MK, Wigger A, Beiderbeck D, Neumann ID, Landgraf R, *et al* (2007). Decreased sensitivity to thermal pain in rats bred for high anxiety-related behaviour is attenuated by citalopram or diazepam treatment. *Behav Brain Res*

183: 18–24.

Julius D, Basbaum AI (2001). Molecular mechanisms of nociception. *Nature* **413**: 203–210.

Jutkiewicz EM, Eller EB, Folk JE, Rice KC, Traynor JR, Woods JH (2004). Delta-opioid agonists: differential efficacy and potency of SNC80, its 3-OH (SNC86) and 3-desoxy (SNC162) derivatives in Sprague-Dawley rats. *J Pharmacol Exp Ther* **309**: 173–81.

Jutkiewicz EM, Torregrossa MM, Sobczyk-Kojiro K, Mosberg HI, Folk JE, Rice KC, *et al* (2006). Behavioral and neurobiological effects of the enkephalinase inhibitor RB101 relative to its antidepressant effects. *Eur J Pharmacol* **531**: 151–9.

Kalin NH, Shelton SE, Davidson RJ (2004). The Role of the Central Nucleus of the Amygdala in Mediating Fear and Anxiety in the Primate. *J Neurosci* **24**: 5506–5515.

Kalso E, Edwards JE, Moore RA, McQuay HJ (2004). Opioids in chronic non-cancer pain: systematic review of efficacy and safety. *Pain* **112**: 372–80.

Kamal A, Biessels GJ, Duis SE, Gispen WH (2000). Learning and hippocampal synaptic plasticity in streptozotocin-diabetic rats: interaction of diabetes and ageing. *Diabetologia* **43**: 500–6.

Kastin AJ, Scollan EL, Ehrensing RH, Schally A V, Coy DH (1978). Enkephalin and other peptides reduce passiveness. *Pharmacol Biochem Behav* **9**: 515–9.

Katsura H, Obata K, Mizushima T, Sakurai J, Kobayashi K, Yamanaka H, *et al* (2006). Activation of Src-Family Kinases in Spinal Microglia Contributes to Mechanical Hypersensitivity after Nerve Injury. *J Neurosci* **26**: .

Keating SCJ, Thomas AA, Flecknell PA, Leach MC (2012). Evaluation of EMLA cream for preventing pain during tattooing of rabbits: changes in physiological, behavioural and facial expression responses. *PLoS One* **7**: e44437.

Kehlet H, Jensen TS, Woolf CJ (2006). Persistent postsurgical pain: risk factors and prevention. *Lancet* **367**: 1618–1625.

Keogh E, Mansoor L (2001). Investigating the effects of anxiety sensitivity and coping on the perception of cold pressor pain in healthy women. *Eur J Pain* **5**: 11–22.

Kepler KL, Standifer KM, Paul D, Kest B, Pasternak GW, Bodnar RJ (1991). Gender effects and central opioid analgesia. *Pain* **45**: 87–94.

Kieffer BL (1995). Recent advances in molecular recognition and signal transduction of active peptides: Receptors for opioid peptides. *Cell Mol Neurobiol* **15**: 615–635.

Kieffer BL (1999). Opioids: first lessons from knockout mice. *Trends Pharmacol Sci* **20**: 19–26.

Kim H, Chen L, Lim G, Sung B, Wang S, McCabe MF, *et al* (2012). Brain indoleamine 2,3-

dioxygenase contributes to the comorbidity of pain and depression. *J Clin Invest* **122**: 2940–54.

Kim HS, Lee SJ, Kim DS, Cho HJ (2000). Effects of brain-derived neurotrophic factor and neurotrophin-3 on expression of mRNAs encoding c-Fos, neuropeptides and glutamic acid decarboxylase in cultured spinal neurons. *Neuroreport* **11**: 3873–6.

Kimura M, Obata H, Saito S (2014). Peripheral Nerve Injury Reduces Analgesic Effectsof Systemic Morphine via Spinal 5-Hydroxytryptamine 3 Receptors. *Anesthesiology* **121**: 362–371.

Kingery WS, Sawamura S, Agashe GS, Davies MF, Clark JD, Zimmer A (2001). Enkephalin release and opioid receptor activation does not mediate the antinociceptive or sedative/hypnotic effects of nitrous oxide. *Eur J Pharmacol* **427**: 27–35.

Kivell B, Prisinzano TE (2010). Kappa opioids and the modulation of pain. *Psychopharmacology (Berl)* **210**: 109–19.

Kiyokawa Y, Kikusui T, Takeuchi Y, Mori Y (2004). Partner's stress status influences social buffering effects in rats. *Behav Neurosci* **118**: 798–804.

Klenowski P, Morgan M, Bartlett SE (2015). The role of δ-opioid receptors in learning and memory underlying the development of addiction. *Br J Pharmacol* **172**: 297–310.

Knoll AT, Carlezon WA (2010). Dynorphin, stress, and depression. *Brain Res* **1314**: 56–73.

Knoll AT, Meloni EG, Thomas JB, Carroll FI, Carlezon WA (2007). Anxiolytic-like effects of kappa-opioid receptor antagonists in models of unlearned and learned fear in rats. *J Pharmacol Exp Ther* **323**: 838–45.

Knoll AT, Muschamp JW, Sillivan SE, Ferguson D, Dietz DM, Meloni EG, *et al* (2011). Kappa Opioid Receptor Signaling in the Basolateral Amygdala Regulates Conditioned Fear and Anxiety in Rats. *Biol Psychiatry* **70**: 425–433.

Kodama D, Ono H, Tanabe M (2007). Altered hippocampal long-term potentiation after peripheral nerve injury in mice. *Eur J Pharmacol* **574**: 127–32.

Kodama D, Ono H, Tanabe M (2011). Increased hippocampal glycine uptake and cognitive dysfunction after peripheral nerve injury. *Pain* **152**: 809–17.

Koetzner L, Hua X-Y, Lai J, Porreca F, Yaksh T (2004). Nonopioid actions of intrathecal dynorphin evoke spinal excitatory amino acid and prostaglandin E2 release mediated by cyclooxygenase-1 and -2. *J Neurosci* **24**: 1451–8.

Kögel B, Vry J De, Tzschentke TM, Christoph T (2011). The antinociceptive and antihyperalgesic effect of tapentadol is partially retained in OPRM1 (μ-opioid receptor) knockout mice. *Neurosci Lett* **491**: 104–7.

Kölsch H, Wagner M, Bilkei-Gorzó A, Toliat MR, Pentzek M, Fuchs A, *et al* (2009). Gene polymorphisms in prodynorphin (PDYN) are associated with episodic memory in the elderly. *J Neural Transm* **116**: 897–903.

König M, Zimmer AM, Steiner H, Holmes P V, Crawley JN, Brownstein MJ, *et al* (1996). Pain responses, anxiety and aggression in mice deficient in pre-proenkephalin. *Nature* **383**: 535–8.

Koob GF (2009). Brain stress systems in the amygdala and addiction. *Brain Res* **1293**: 61–75.

Krahé C, Springer A, Weinman JA, Fotopoulou A (2013). The social modulation of pain: others as predictive signals of salience - a systematic review. *Front Hum Neurosci* **7**: 386.

Kreibich A, Reyes BAS, Curtis AL, Ecke L, Chavkin C, Bockstaele EJ Van, *et al* (2008). Presynaptic inhibition of diverse afferents to the locus ceruleus by kappa-opiate receptors: a novel mechanism for regulating the central norepinephrine system. *J Neurosci* **28**: 6516–25.

Kremer M, Salvat E, Muller A, Yalcin I, Barrot M (2016). Antidepressants and gabapentinoids in neuropathic pain: Mechanistic insights. *Neuroscience* **338**: 183–206.

Kroenke K, Wu J, Bair MJ, Krebs EE, Damush TM, Tu W (2011). Reciprocal relationship between pain and depression: a 12-month longitudinal analysis in primary care. *J Pain* **12**: 964–73.

Kudryavtseva NN, Gerrits MAFM, Avgustinovich DF, Tenditnik M V., Ree JM Van (2004). Modulation of anxiety-related behaviors by μ- and κ-opioid receptor agonists depends on the social status of mice. *Peptides* **25**: 1355–1363.

Kumar A, Kaur H, Singh A (2018). Neuropathic Pain models caused by damage to central or peripheral nervous system. *Pharmacol Reports* **70**: 206–216.

Kumar D, Marshall HJ (1997). Diabetic peripheral neuropathy: amelioration of pain with transcutaneous electrostimulation. *Diabetes Care* **20**: 1702–5.

Kupers RC, Konings H, Adriaensen H, Gybels JM (1991). Morphine differentially affects the sensory and affective pain ratings in neurogenic and idiopathic forms of pain. *Pain* **47**: 5–12.

Kurvers HA, Jacobs MJ, Beuk RJ, Wildenberg FA Van den, Kitslaar PJ, Slaaf DW, *et al* (1995). Reflex sympathetic dystrophy: evolution of microcirculatory disturbances in time. *Pain* **60**: 333–40.

Labombarda F, Coronel MF, Villar MJ, Nicola AF De, González SL (2008). Neuropathic pain and temporal expression of preprodynorphin, protein kinase C and N-methyl-d-aspartate receptor subunits after spinal cord injury. *Neurosci Lett* **447**: 115–119.

Labuz D, Celik MÖ, Zimmer A, Machelska H (2016). Distinct roles of exogenous opioid agonists and endogenous opioid peptides in the peripheral control of neuropathy-triggered heat pain. *Sci Rep* **6**: 32799.

Lacroix-Fralish ML, Austin JS, Zheng FY, Levitin DJ, Mogil JS (2011). Patterns of pain: Meta-analysis of microarray studies of pain. *Pain* **152**: 1888–1898.

LaGraize SC, Borzan J, Peng YB, Fuchs PN (2006). Selective regulation of pain affect following activation of the opioid anterior cingulate cortex system. *Exp Neurol* **197**: 22–30.

Lai J, Luo M, Chen Q, Porreca F (2008). Pronociceptive actions of dynorphin via bradykinin receptors. *Neurosci Lett* **437**: 175–9.

Land BB, Bruchas MR, Schattauer S, Giardino WJ, Aita M, Messinger D, *et al* (2009). Activation of the kappa opioid receptor in the dorsal raphe nucleus mediates the aversive effects of stress and reinstates drug seeking. *Proc Natl Acad Sci U S A* **106**: 19168–73.

Langford DJ, Bailey AL, Chanda ML, Clarke SE, Drummond TE, Echols S, *et al* (2010a). Coding of facial expressions of pain in the laboratory mouse. *Nat Methods* **7**: 447–9.

Langford DJ, Crager SE, Shehzad Z, Smith SB, Sotocinal SG, Levenstadt JS, *et al* (2006). Social Modulation of Pain as Evidence for Empathy in Mice. *Science (80-)* **312**: 1967–1970.

Langford DJ, Tuttle AH, Brown K, Deschenes S, Fischer DB, Mutso A, *et al* (2010b). Social approach to pain in laboratory mice. *Soc Neurosci* **5**: 163–170.

Langley PC, Litsenburg C Van, Cappelleri JC, Carroll D (2013). The burden associated with neuropathic pain in Western Europe. *J Med Econ* **16**: 85–95.

Laughlin TM, Larson AA, Wilcox GL (2001). Mechanisms of induction of persistent nociception by dynorphin. *J Pharmacol Exp Ther* **299**: 6–11.

Law PY, Wong YH, Loh HH (2000). Molecular mechanisms and regulation of opioid receptor signaling. *Annu Rev Pharmacol Toxicol* **40**: 389–430.

Ledent C, Valverde O, Cossu G, Petitet F, Aubert JF, Beslot F, *et al* (1999). Unresponsiveness to cannabinoids and reduced addictive effects of opiates in CB1 receptor knockout mice. *Science* **283**: 401–4.

Lee YW, Chaplan SR, Yaksh TL (1995). Systemic and supraspinal, but not spinal, opiates suppress allodynia in a rat neuropathic pain model. *Neurosci Lett* **199**: 111–4.

Leite-Almeida H, Almeida-Torres L, Mesquita AR, Pertovaara A, Sousa N, Cerqueira JJ, *et al* (2009). The impact of age on emotional and cognitive behaviours triggered by experimental neuropathy in rats. *Pain* **144**: 57–65.

Li JL, Kaneko T, Mizuno N (1996). Effects of peripheral nerve ligation on expression of mu-opioid receptor in sensory ganglion neurons: an immunohistochemical study in dorsal root and nodose ganglion neurons of the rat. *Neurosci Lett* **214**: 91–4.

Li X-Y, Ko H-G, Chen T, Descalzi G, Koga K, Wang H, *et al* (2010). Alleviating neuropathic pain hypersensitivity by inhibiting PKMzeta in the anterior cingulate cortex. *Science* **330**: 1400–4.

Liu M-G, Chen J (2009). Roles of the hippocampal formation in pain information processing. *Neurosci Bull* **25**: 237–66.

Liu M-G, Chen J (2014). Preclinical research on pain comorbidity with affective disorders and cognitive deficits: Challenges and perspectives. *Prog Neurobiol* **116**: 13–32.

Liu M-G, Kang SJ, Shi T-Y, Koga K, Zhang M-M, Collingridge GL, *et al* (2013). Long-term potentiation of synaptic transmission in the adult mouse insular cortex: multielectrode array recordings. *J Neurophysiol* **110**: 505–21.

Livak KJ, Schmittgen TD (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* **25**: 402–8.

Longoni R, Cadoni C, Mulas A, Chiara G Di, Spina L (1998). Dopamine-dependent behavioural stimulation by non-peptide delta opioids BW373U86 and SNC 80: 2. Place-preference and brain microdialysis studies in rats. *Behav Pharmacol* **9**: 9–14.

López de Armentia M, Sah P (2007). Bidirectional synaptic plasticity at nociceptive afferents in the rat central amygdala. *J Physiol* **581**: 961–70.

Lord JA, Waterfield AA, Hughes J, Kosterlitz HW (1977). Endogenous opioid peptides: multiple agonists and receptors. *Nature* **267**: 495–9.

Lovinger DM (2010). Neurotransmitter roles in synaptic modulation, plasticity and learning in the dorsal striatum. *Neuropharmacology* **58**: 951–61.

Low LA, Millecamps M, Seminowicz DA, Naso L, Thompson SJ, Stone LS, *et al* (2012). Nerve injury causes long-term attentional deficits in rats. *Neurosci Lett* **529**: 103–107.

Lu Y-F, Wang Y, He Y, Zhang F-K, He T, Wang R-R, *et al* (2014). Spatial and temporal plasticity of synaptic organization in anterior cingulate cortex following peripheral

inflammatory pain: multi-electrode array recordings in rats. *Neurosci Bull* **30**: 1–20.

Luo ZD, Chaplan SR, Higuera ES, Sorkin LS, Stauderman KA, Williams ME, *et al* (2001). Upregulation of dorsal root ganglion (alpha)2(delta) calcium channel subunit and its correlation with allodynia in spinal nerve-injured rats. *J Neurosci* **21**: 1868–75.

Lutz P-E, Kieffer BL (2013). Opioid receptors: distinct roles in mood disorders. *Trends Neurosci* **36**: 195–206.

Ma Y-Y, Cepeda C, Chatta P, Franklin L, Evans CJ, Levine MS (2012). Regional and cell-type-specific effects of DAMGO on striatal D1 and D2 dopamine receptor-expressing medium-sized spiny neurons. *ASN Neuro* **4**: .

Machado CJ, Bachevalier J (2006). The impact of selective amygdala, orbital frontal cortex, or hippocampal formation lesions on established social relationships in rhesus monkeys (Macaca mulatta). *Behav Neurosci* **120**: 761–786.

Machado CJ, Emery NJ, Capitanio JP, Mason WA, Mendoza SP, Amaral DG (2008). Bilateral neurotoxic amygdala lesions in rhesus monkeys (Macaca mulatta): consistent pattern of behavior across different social contexts. *Behav Neurosci* **122**: 251–66.

Mague SD, Pliakas AM, Todtenkopf MS, Tomasiewicz HC, Zhang Y, Stevens WC, *et al* (2003). Antidepressant-Like Effects of kappa -Opioid Receptor Antagonists in the Forced Swim Test in Rats. *J Pharmacol Exp Ther* **305**: 323–330.

Maihöfner C, Handwerker HO, Neundörfer B, Birklein F (2003). Patterns of cortical reorganization in complex regional pain syndrome. *Neurology* **61**: 1707–15.

Maldonado R, Baños JE, Cabañero D (2016). The endocannabinoid system and neuropathic pain. *Pain* **157 Suppl**: S23-32.

Maldonado R (2016). Opioid receptors and drug-seeking behaviour. In INRC Meeting, (Bath, United Kingdom), p 12.

Maldonado R, Baños JE, Cabañero D (2018). Usefulness of knockout mice to clarify the role of the opioid system in chronic pain. *Br J Pharmacol* **175**: 2791–2808.

Manglik A, Kruse AC, Kobilka TS, Thian FS, Mathiesen JM, Sunahara RK, *et al* (2012). Crystal structure of the μ-opioid receptor bound to a morphinan antagonist. *Nature* **485**: 321–6.

Manning BH, Martin WJ, Meng ID (2003). The rodent amygdala contributes to the production of cannabinoid-induced antinociception. *Neuroscience* **120**: 1157–70.

Mansikka H, Zhao C, Sheth RN, Sora I, Uhl G, Raja SN (2004). Nerve Injury Induces a Tonic Bilateral μ-Opioid Receptor–mediated Inhibitory Effect on Mechanical Allodynia in

Mice. *J Am Soc Anesthesiol* **100**: 912–921.

Mansour A, Fox CA, Burke S, Meng F, Thompson RC, Akil H, *et al* (1994). Mu, delta, and kappa opioid receptor mRNA expression in the rat CNS: an in situ hybridization study. *J Comp Neurol* **350**: 412–38.

Mansour A, Khachaturian H, Lewis ME, Akil H, Watson SJ (1988). Anatomy of CNS opioid receptors. *Trends Neurosci* **11**: 308–14.

Mao J, Price DD, Mayer DJ (1995). Experimental mononeuropathy reduces the antinociceptive effects of morphine: implications for common intracellular mechanisms involved in morphine tolerance and neuropathic pain. *Pain* **61**: 353–364.

Marchand F, Ardid D, Chapuy E, Alloui A, Jourdan D, Eschalier A (2003). Evidence for an involvement of supraspinal delta- and spinal mu-opioid receptors in the antihyperalgesic effect of chronically administered clomipramine in mononeuropathic rats. *J Pharmacol Exp Ther* **307**: 268–74.

Marinelli PW, Funk D, Harding S, Li Z, Juzytsch W, Lê AD (2009). Roles of opioid receptor subtypes in mediating alcohol-seeking induced by discrete cues and context. *Eur J Neurosci* **30**: 671–8.

Marinelli S, Vaughan CW, Schnell S a, Wessendorf MW, Christie MJ (2002). Rostral ventromedial medulla neurons that project to the spinal cord express multiple opioid receptor phenotypes. *J Neurosci* **22**: 10847–10855.

Martikainen IK, Nuechterlein EB, Peciña M, Love TM, Cummiford CM, Green CR, *et al* (2015). Chronic Back Pain Is Associated with Alterations in Dopamine Neurotransmission in the Ventral Striatum. *J Neurosci* **35**: 9957–65.

Martin LJ, Tuttle AH, Mogil JS (2014). The Interaction Between Pain and Social Behavior in Humans and Rodents. *Curr Top Behav Neurosci* **20**: 233–250.

Martin M, Ledent C, Parmentier M, Maldonado R, Valverde O (2000). Cocaine, but not morphine, induces conditioned place preference and sensitization to locomotor responses in CB1 knockout mice. *Eur J Neurosci* **12**: 4038–46.

Martin M, Matifas A, Maldonado R, Kieffer BL (2003). Acute antinociceptive responses in single and combinatorial opioid receptor knockout mice: Distinct mu, delta and kappa tones. *Eur J Neurosci* **17**: 701–708.

Martin WJ, Coffin PO, Attias E, Balinsky M, Tsou K, Walker JM (1999). Anatomical basis for cannabinoid-induced antinociception as revealed by intracerebral microinjections. *Brain Res* **822**: 237–42.

Martin WR, Eades CG, Thompson JA, Huppler RE, Gilbert PE (1976). The effects of morphine- and nalorphine- like drugs in the nondependent and morphine-dependent chronic spinal dog. *J Pharmacol Exp Ther* **197**: .

Martin Paiz JA, Paiz M, Rosa CP, Risso R, Monaco B, Duarte KP, *et al* (2015). Intrathecal Morphine Therapy for Chronic Non-malignant Pain Using a Constant Flow Infusion System. *J Pain Reli* **4**: 1–5.

Martínez-Navarro M, Maldonado R, Baños J-E (2018). Why mu-opioid agonists have less analgesic efficacy in neuropathic pain? *Eur J Pain* doi:10.1002/ejp.1328.

Mason L, Moore RA, Derry S, Edwards JE, McQuay HJ (2004). Systematic review of topical capsaicin for the treatment of chronic pain. *BMJ* **328**: 991.

Massaly N, Morón JA, Al-Hasani R (2016). A Trigger for Opioid Misuse: Chronic Pain and Stress Dysregulate the Mesolimbic Pathway and Kappa Opioid System. *Front Neurosci* **10**: 480.

Matthes HW, Maldonado R, Simonin F, Valverde O, Slowe S, Kitchen I, *et al* (1996). Loss of morphine-induced analgesia, reward effect and withdrawal symptoms in mice lacking the mu-opioid-receptor gene. *Nature* **383**: 819–823.

Matthes HW, Smadja C, Valverde O, Vonesch JL, Foutz a S, Boudinot E, *et al* (1998). Activity of the delta-opioid receptor is partially reduced, whereas activity of the kappa-receptor is maintained in mice lacking the mu-receptor. *J Neurosci* **18**: 7285–7295.

Matthies H, Schroeder H, Becker A, Loh H, Höllt V, Krug M (2000). Lack of expression of long-term potentiation in the dentate gyrus but not in the CA1 region of the hippocampus of mu-opioid receptor-deficient mice. *Neuropharmacology* **39**: 952–60.

McClane TK, Martin WR (1967). Effects of morphine, nalorphine, cyclazocine, and naloxone on the flexor reflex. *Int J Neuropharmacol* **6**: 89–98.

McCleane G (2008). Antidepressants as analgesics. *CNS Drugs* **22**: 139–56.

McLaughlin JP, Land BB, Li S, Pintar JE, Chavkin C (2006). Prior activation of kappa opioid receptors by U50,488 mimics repeated forced swim stress to potentiate cocaine place preference conditioning. *Neuropsychopharmacology* **31**: 787–94.

McLaughlin JP, Marton-Popovici M, Chavkin C (2003). Kappa opioid receptor antagonism and prodynorphin gene disruption block stress-induced behavioral responses. *J Neurosci* **23**: 5674–83.

McWilliams LA, Cox BJ, Enns MW (2003). Mood and anxiety disorders associated with

chronic pain: an examination in a nationally representative sample. *Pain* **106**: 127–33.

Meagher MW, Arnau RC, Rhudy JL (2001). Pain and emotion: effects of affective picture modulation. *Psychosom Med* **63**: 79–90.

Melzack R, Wall PD (1965). Pain mechanisms: a new theory. *Science* **150**: 971–9.

Meng ID, Manning BH, Martin WJ, Fields HL (1998). An analgesia circuit activated by cannabinoids. *Nature* **395**: 381–3.

Merrer J Le, Becker J a J, Befort K, Kieffer BL (2009). Reward processing by the opioid system in the brain. *Physiol Rev* **89**: 1379–1412.

Merrer J Le, Plaza-Zabala A, Boca C Del, Matifas A, Maldonado R, Kieffer BL (2011). Deletion of the Opioid Receptor Gene Impairs Place Conditioning But Preserves Morphine Reinforcement. *BPS* **69**: 700–703.

Merrer J Le, Rezai X, Scherrer G, Becker JAJ, Kieffer BL (2013). Impaired hippocampus-dependent and facilitated striatum-dependent behaviors in mice lacking the δ opioid receptor. *Neuropsychopharmacology* **38**: 1050–9.

Merskey H, Bogduk N (IASP Press: Seattle, 1994). *Classification of chronic pain*. .

Meunier JC, Mollereau C, Toll L, Suaudeau C, Moisand C, Alvinerie P, *et al* (1995). Isolation and structure of the endogenous agonist of opioid receptor-like ORL1 receptor. *Nature* **377**: 532–5.

Meunier M, Bachevalier J (2002). Comparison of emotional responses in monkeys with rhinal cortex or amygdala lesions. *Emotion* **2**: 147–61.

Miao FJ, Janig W, Levine J (1996). Role of sympathetic postganglionic neurons in synovial plasma extravasation induced by bradykinin. *J Neurophysiol* **75**: 715–724.

Miaskowski C, Taiwo YO, Levine JD (1991). Contribution of supraspinal mu- and delta-opioid receptors to antinociception in the rat. *Eur J Pharmacol* **205**: 247–52.

Micó JA, Ardid D, Berrocoso E, Eschalier A (2006). Antidepressants and pain. *Trends Pharmacol Sci* **27**: 348–354.

Minami S, Satoyoshi H, Ide S, Inoue T, Yoshioka M, Minami M (2017). Suppression of reward-induced dopamine release in the nucleus accumbens in animal models of depression: Differential responses to drug treatment. *Neurosci Lett* **650**: 72–76.

Misawa S (2012). [Pathophysiology of neuropathic pain: Na^+ channel and hyperexcitability of primary afferents]. *Brain Nerve* **64**: 1249–53.

Mogil JS (2012). Pain genetics: Past, present and future. *Trends Genet* **28**: 258–266.

Mogil JS, Grisel JE, Hayward MD, Bales JR, Rubinstein M, Belknap JK, *et al* (2000).

Disparate spinal and supraspinal opioid antinociceptive responses in beta-endorphin-deficient mutant mice. *Neuroscience* **101**: 709–17.

Mollereau C, Parmentier M, Mailleux P, Butour JL, Moisand C, Chalon P, *et al* (1994). ORL1, a novel member of the opioid receptor family. Cloning, functional expression and localization. *FEBS Lett* **341**: 33–8.

Montoya P, Larbig W, Braun C, Preissl H, Birbaumer N (2004). Influence of social support and emotional context on pain processing and magnetic brain responses in fibromyalgia. *Arthritis Rheum* **50**: 4035–44.

Mor D, Bembrick AL, Austin PJ, Wyllie PM, Creber NJ, Denyer GS, *et al* (2010). Anatomically specific patterns of glial activation in the periaqueductal gray of the sub-population of rats showing pain and disability following chronic constriction injury of the sciatic nerve. *Neuroscience* **166**: 1167–84.

Moriarty O, Gorman CL, Mcgowan F, Ford GK, Roche M, Thompson K, *et al* (2016). Impaired recognition memory and cognitive flexibility in the rat L5–L6 spinal nerve ligation model of neuropathic pain. *Scand J Pain* **10**: 61–73.

Moriarty O, McGuire BE, Finn DP (2011). The effect of pain on cognitive function: A review of clinical and preclinical research. *Prog Neurobiol* **93**: 385–404.

Morin N, Owolabi SA, Harty MW, Papa EF, Tracy TF, Shaw SK, *et al* (2007). Neutrophils invade lumbar dorsal root ganglia after chronic constriction injury of the sciatic nerve. *J Neuroimmunol* **184**: 164–71.

Morrison I, Lloyd D, Pellegrino G di, Roberts N (2004). Vicarious responses to pain in anterior cingulate cortex: is empathy a multisensory issue? *Cogn Affect Behav Neurosci* **4**: 270–8.

Moseley GL (2004). Graded motor imagery is effective for long-standing complex regional pain syndrome: a randomised controlled trial. *Pain* **108**: 192–8.

Muñoz M, Esteve R (2005). Reports of memory functioning by patients with chronic pain. *Clin J Pain* **21**: 287–91.

Murakami T, Kanchiku T, Suzuki H, Imajo Y, Yoshida Y, Nomura H, *et al* (2013). Anti-interleukin-6 receptor antibody reduces neuropathic pain following spinal cord injury in mice. *Exp Ther Med* **6**: 1194–1198.

Mutso a. a., Radzicki D, Baliki MN, Huang L, Banisadr G, Centeno M V., *et al* (2012). Abnormalities in Hippocampal Functioning with Persistent Pain. *J Neurosci* **32**: 5747–5756.

Nadal X, Baños J-E, Kieffer BL, Maldonado R (2006). Neuropathic pain is enhanced in

delta-opioid receptor knockout mice. *Eur J Neurosci* **23**: 830–834.

Nadal X, Porta C La, Bura SA, Maldonado R (2013). Involvement of the opioid and cannabinoid systems in pain control: New insights from knockout studies. *Eur J Pharmacol* **716**: 142–157.

Nagakura Y, Oe T, Aoki T, Matsuoka N (2009). Biogenic amine depletion causes chronic muscular pain and tactile allodynia accompanied by depression: A putative animal model of fibromyalgia. *Pain* **146**: 26–33.

Naidu PS, Lichtman AH, Archer CC, May EL, Harris LS, Aceto MD (2007). NIH 11082 produces anti-depressant-like activity in the mouse tail-suspension test through a delta-opioid receptor mechanism of action. *Eur J Pharmacol* **566**: 132–6.

Namburi P, Beyeler A, Yorozu S, Calhoon GG, Halbert SA, Wichmann R, *et al* (2015). A circuit mechanism for differentiating positive and negative associations. *Nature* **520**: 675–8.

Nantharat M, Wanitchanon T, Amesbutr M, Tammachote R, Praphanphoj V (2015). Glucocorticoid receptor gene (NR3C1) promoter is hypermethylated in Thai females with major depressive disorder. *Genet Mol Res* **14**: 19071–19079.

Narita M, Kaneko C, Miyoshi K, Nagumo Y, Kuzumaki N, Nakajima M, *et al* (2006a). Chronic pain induces anxiety with concomitant changes in opioidergic function in the amygdala. *Neuropsychopharmacology* **31**: 739–50.

Narita M, Kishimoto Y, Ise Y, Yajima Y, Misawa K, Suzuki T (2005). Direct evidence for the involvement of the mesolimbic kappa-opioid system in the morphine-induced rewarding effect under an inflammatory pain-like state. *Neuropsychopharmacology* **30**: 111–8.

Narita M, Kuzumaki N, Narita M, Kaneko C, Hareyama N, Miyatake M, *et al* (2006b). Chronic pain-induced emotional dysfunction is associated with astrogliosis due to cortical delta-opioid receptor dysfunction. *J Neurochem* **97**: 1369–78.

Navratilova E, Xie JY, Meske D, Qu C, Morimura K, Okun A, *et al* (2015). Endogenous opioid activity in the anterior cingulate cortex is required for relief of pain. *J Neurosci* **35**: 7264–71.

Navratilova E, Xie JY, Okun A, Qu C, Eyde N, Ci S, *et al* (2012). Pain relief produces negative reinforcement through activation of mesolimbic reward-valuation circuitry. *Proc Natl Acad Sci U S A* **109**: 20709–13.

Neal CR, Mansour A, Reinscheid R, Nothacker HP, Civelli O, Akil H, *et al* (1999). Opioid receptor-like (ORL1) receptor distribution in the rat central nervous system:

comparison of ORL1 receptor mRNA expression with (125)I-[(14)Tyr]-orphanin FQ binding. *J Comp Neurol* **412**: 563–605.

Negrete R, García Gutiérrez MS, Manzanares J, Maldonado R (2017). Involvement of the dynorphin/KOR system on the nociceptive, emotional and cognitive manifestations of joint pain in mice. *Neuropharmacology* **116**: 315–327.

Nestler EJ, Carlezon WA (2006). The Mesolimbic Dopamine Reward Circuit in Depression. *Biol Psychiatry* **59**: 1151–1159.

Neugebauer V (2006). Chapter 11 Subcortical processing of nociceptive information: basal ganglia and amygdala. *Handb Clin Neurol* **81**: 141–158.

Neugebauer V, Galhardo V, Maione S, Mackey SC (2009). Forebrain pain mechanisms. *Brain Res Rev* **60**: 226–42.

Neugebauer V, Li W, Bird GC, Bhave G, Gereau RW (2003). Synaptic plasticity in the amygdala in a model of arthritic pain: differential roles of metabotropic glutamate receptors 1 and 5. *J Neurosci* **23**: 52–63.

Neugebauer V, Li W, Bird GC, Han JS (2004). The amygdala and persistent pain. *Neuroscientist* **10**: 221–234.

Neves G, Cooke SF, Bliss TVP (2008). Synaptic plasticity, memory and the hippocampus: a neural network approach to causality. *Nat Rev Neurosci* **9**: 65–75.

Nichols ML, Bian D, Ossipov MH, Malan TP, Porreca F (1996). Antiallodynic effects of a CCKB antagonist in rats with nerve ligation injury: role of endogenous enkephalins. *Neurosci Lett* **215**: 161–4.

Nichols ML, Lopez Y, Ossipov MH, Bian D, Porreca F (1997). Enhancement of the antiallodynic and antinociceptive efficacy of spinal morphine by antisera to dynorphin A (1-13) or MK-801 in a nerve-ligation model of peripheral neuropathy. *Pain* **69**: 317–22.

Nickel FT, Seifert F, Lanz S, Maihöfner C (2012). Mechanisms of neuropathic pain. *Eur Neuropsychopharmacol* **22**: 81–91.

Nicolson SE, Caplan JP, Williams DE, Stern TA (2009). Comorbid Pain, Depression, and Anxiety. *Harv Rev Psychiatry* **17**: 407–420.

Nieto MM, Guen SLE, Kieffer BL, Roques BP, Noble F (2005). Physiological control of emotion-related behaviors by endogenous enkephalins involves essentially the delta opioid receptors. *Neuroscience* **135**: 305–13.

Niikura K, Narita M, Narita M, Nakamura A, Okutsu D, Ozeki A, *et al* (2008a). Direct evidence for the involvement of endogenous β-endorphin in the suppression of the

morphine-induced rewarding effect under a neuropathic pain-like state. *Neurosci Lett* **435**: 257–262.

Niikura K, Narita M, Okutsu D, Tsurukawa Y, Nanjo K, Kurahashi K, *et al* (2008b). Implication of endogenous beta-endorphin in the inhibition of the morphine-induced rewarding effect by the direct activation of spinal protein kinase C in mice. *Neurosci Lett* **433**: 54–8.

Obara I, Gunduz Cinar O, Starowicz K, Benyhe S, Borsodi A, Przewlocka B (2010). Agonist-dependent attenuation of mu-opioid receptor-mediated G-protein activation in the dorsal root ganglia of neuropathic rats. *J Neural Transm* **117**: 421–9.

Obara I, Makuch W, Spetea M, Schütz J, Schmidhammer H, Przewlocki R, *et al* (2007). Local peripheral antinociceptive effects of 14-O-methyloxymorphone derivatives in inflammatory and neuropathic pain in the rat. *Eur J Pharmacol* **558**: 60–67.

Obara I, Mika J, Schafer MK-H, Przewlocka B (2003). Antagonists of the kappa-opioid receptor enhance allodynia in rats and mice after sciatic nerve ligation. *Br J Pharmacol* **140**: 538–46.

Obara I, Przewlocki R, Przewlocka B (2004). Local peripheral effects of μ-opioid receptor agonists in neuropathic pain in rats. *Neurosci Lett* **360**: 85–89.

Onoda K, Okamoto Y, Nakashima K, Nittono H, Ura M, Yamawaki S (2009). Decreased ventral anterior cingulate cortex activity is associated with reduced social pain during emotional support. *Soc Neurosci* **4**: 443–54.

Ossipov MH, Dussor GO, Porreca F, Eisenach J, Wilcox G, Riley J (2010). Central modulation of pain. *J Clin Invest* **120**: 3779–87.

Ossipov MH, Lai J, Malan TP, Porreca F (2000). Spinal and supraspinal mechanisms of neuropathic pain. *Ann N Y Acad Sci* **909**: 12–24.

Ossipov MH, Lopez Y, Nichols ML, Bian D, Porreca F (1995a). Inhibition by spinal morphine of the tail-flick response is attenuated in rats with nerve ligation injury. *Neurosci Lett* **199**: 83–6.

Ossipov MH, Lopez Y, Nichols ML, Bian D, Porreca F (1995b). The loss of antinociceptive efficacy of spinal morphine in rats with nerve ligation injury is prevented by reducing spinal afferent drive. *Neurosci Lett* **199**: 87–90.

Österberg A, Boivie J, Thuomas K-Å (2005). Central pain in multiple sclerosis - prevalence and clinical characteristics. *Eur J Pain* **9**: 531–531.

Ozawa A, Brunori G, Mercatelli D, Wu J, Cippitelli A, Zou B, *et al* (2015). Knock-In Mice with NOP-eGFP Receptors Identify Receptor Cellular and Regional Localization. *J*

Neurosci **35**: 11682–93.

Palmisano M, Caputi FF, Mercatelli D, Romualdi P, Candeletti S (2018). Dynorphinergic system alterations in the corticostriatal circuitry of neuropathic mice support its role in the negative affective component of pain. *Genes Brain Behav* e12467doi:10.1111/gbb.12467.

Pan H-L, Wu Z-Z, Zhou H-Y, Chen S-R, Zhang H-M, Li D-P (2008). Modulation of pain transmission by G-protein-coupled receptors. *Pharmacol Ther* **117**: 141–61.

Pan Y-X, Xu J, Xu M, Rossi GC, Matulonis JE, Pasternak GW (2009). Involvement of exon 11-associated variants of the mu opioid receptor MOR-1 in heroin, but not morphine, actions. *Proc Natl Acad Sci U S A* **106**: 4917–22.

Parikh D, Hamid A, Friedman TC, Nguyen K, Tseng A, Marquez P, *et al* (2011). Stress-induced analgesia and endogenous opioid peptides: The importance of stress duration. *Eur J Pharmacol* **650**: 563–567.

Patel R, Dickenson AH (2016). Mechanisms of the gabapentinoids and α 2 δ-1 calcium channel subunit in neuropathic pain. *Pharmacol Res Perspect* **4**: e00205.

Paulson PE, Casey KL, Morrow TJ (2002). Long-term changes in behavior and regional cerebral blood flow associated with painful peripheral mononeuropathy in the rat. *Pain* **95**: 31–40.

Peppin JF, Raffa RB (2015). Delta opioid agonists: a concise update on potential therapeutic applications. *J Clin Pharm Ther* **40**: 155–166.

Perkins JR, Antunes-Martins A, Calvo M, Grist J, Rust W, Schmid R, *et al* (2014). A comparison of RNA-seq and exon arrays for whole genome transcription profiling of the L5 spinal nerve transection model of neuropathic pain in the rat. *Mol Pain* **10**: 7.

Perrine S a, Hoshaw B a, Unterwald EM (2006). Delta opioid receptor ligands modulate anxiety-like behaviors in the rat. *Br J Pharmacol* **147**: 864–72.

Pert CB, Snyder SH (1973). Opiate receptor: demonstration in nervous tissue. *Science* **179**: 1011–4.

Pertovaara A, Wei H (2001). Peripheral effects of morphine in neuropathic rats: role of sympathetic postganglionic nerve fibers. *Eur J Pharmacol* **429**: 139–145.

Petraschka M, Li S, Gilbert TL, Westenbroek RE, Bruchas MR, Schreiber S, *et al* (2007). The absence of endogenous beta-endorphin selectively blocks phosphorylation and desensitization of mu opioid receptors following partial sciatic nerve ligation. *Neuroscience* **146**: 1795–807.

Petrovic P, Ingvar M, Stone-Elander S, Petersson KM, Hansson P (1999). A PET activation study of dynamic mechanical allodynia in patients with mononeuropathy. *Pain* **83**: 459–70.

Pfeiffer A, Brantl V, Herz A, Emrich HM (1986). Psychotomimesis mediated by kappa opiate receptors. *Science* **233**: 774–6.

Phelps EA, LeDoux JE (2005). Contributions of the Amygdala to Emotion Processing: From Animal Models to Human Behavior. *Neuron* **48**: 175–187.

Pilcher CWT, Browne J (1982). Environmental crowding modifies responding to noxious stimuli and the effects of μ- and k-agonists. *Life Sci* **31**: 1213–1216.

Pliakas AM, Carlson RR, Neve RL, Konradi C, Nestler EJ, Carlezon WA, *et al* (2001). Altered responsiveness to cocaine and increased immobility in the forced swim test associated with elevated cAMP response element-binding protein expression in nucleus accumbens. *J Neurosci* **21**: 7397–403.

Ploghaus A, Narain C, Beckmann CF, Clare S, Bantick S, Wise R, *et al* (2001). Exacerbation of pain by anxiety is associated with activity in a hippocampal network. *J Neurosci* **21**: 9896–903.

Podvin S, Yaksh T, Hook V (2016). The Emerging Role of Spinal Dynorphin in Chronic Pain: A Therapeutic Perspective. *Annu Rev Pharmacol Toxicol* **56**: 511–33.

Pogozheva ID, Przydzial MJ, Mosberg HI (2005). Homology modeling of opioid receptor-ligand complexes using experimental constraints. *AAPS J* **7**: E434-48.

Porreca F, Ossipov MH, Gebhart GF (2002). Chronic pain and medullary descending facilitation. *Trends Neurosci* **25**: 319–25.

Porreca F, Tang QB, Bian D, Riedl M, Elde R, Lai J (1998). Spinal opioid mu receptor expression in lumbar spinal cord of rats following nerve injury. *Brain Res* **795**: 197–203.

Porsolt RD, Bertin A, Jalfre M (1977). Behavioral despair in mice: a primary screening test for antidepressants. *Arch Int Pharmacodyn Ther* **229**: 327–36.

Porta C La, Bura SA, Llorente-Onaindia J, Pastor A, Navarrete F, García-Gutiérrez MS, *et al* (2015). Role of the endocannabinoid system in the emotional manifestations of osteoarthritis pain. *Pain* **156**: 2001–12.

Porta C La, Lara-Mayorga IM, Negrete R, Maldonado R (2016). Effects of pregabalin on the nociceptive, emotional and cognitive manifestations of neuropathic pain in mice. *Eur J Pain (United Kingdom)* **20**: 1454–1466.

Pozza DH, Potes CS, Barroso PA, Azevedo L, Castro-Lopes JM, Neto FL (2010). Nociceptive

behaviour upon modulation of mu-opioid receptors in the ventrobasal complex of the thalamus of rats. *Pain* **148**: 492–502.

Pradhan A a, Befort K, Nozaki C, Gavériaux-Ruff C, Kieffer BL (2011). The delta opioid receptor: an evolving target for the treatment of brain disorders. *Trends Pharmacol Sci* **32**: 581–90.

Preston SD, Waal FBM de (2002). Empathy: Its ultimate and proximate bases. *Behav Brain Sci* **25**: 1-20-71.

Price DD (2000). Psychological and neural mechanisms of the affective dimension of pain. *Science* **288**: 1769–72.

Price DD, Gruen A Von der, Miller J, Rafii A, Price C (1985). A psychophysical analysis of morphine analgesia. *Pain* **22**: 261–9.

Przewlocki R, Przewlocka B (2005). Opioids in neuropathic pain. *Curr Pharm Des* **11**: 3013–25.

Przewłocki R, Przewłocka B (2001). Opioids in chronic pain. *Eur J Pharmacol* **429**: 79–91.

Puighermanal E, Marsicano G, Busquets-Garcia A, Lutz B, Maldonado R, Ozaita A (2009). Cannabinoid modulation of hippocampal long-term memory is mediated by mTOR signaling. *Nat Neurosci* **12**: 1152–8.

Purves D, Augustine GJ, Fitzpatrick D, Hall WC, LaMantia A-S, McNamara JO, *et al* (Sinauer Associates: Sunderland, 2012). *Neuroscience*. .

Qiu S, Chen T, Koga K, Guo Y, Xu H, Song Q, *et al* (2013). An increase in synaptic NMDA receptors in the insular cortex contributes to neuropathic pain. *Sci Signal* **6**: ra34.

Raber P, Devor M (2002). Social variables affect phenotype in the neuroma model of neuropathic pain. *Pain* **97**: 139–50.

Ragnauth A, Schuller A, Morgan M, Chan J, Ogawa S, Pintar J, *et al* (2001). Female preproenkephalin-knockout mice display altered emotional responses. *Proc Natl Acad Sci U S A* **98**: 1958–63.

Rainville P, Duncan GH, Price DD, Carrier B, Bushnell MC (1997). Pain affect encoded in human anterior cingulate but not somatosensory cortex. *Science* **277**: 968–71.

Ramachandran VS, Stewart M, Rogers-Ramachandran DC (1992). Perceptual correlates of massive cortical reorganization. *Neuroreport* **3**: 583–6.

Raman M, Chen W, Cobb MH (2007). Differential regulation and properties of MAPKs. *Oncogene* **26**: 3100–12.

Randall-Thompson JF, Pescatore KA, Unterwald EM (2010). A role for delta opioid receptors in the central nucleus of the amygdala in anxiety-like behaviors.

Psychopharmacology (Berl) **212**: 585–595.

Rashid MH, Inoue M, Toda K, Ueda H (2004). Loss of peripheral morphine analgesia contributes to the reduced effectiveness of systemic morphine in neuropathic pain. *J Pharmacol Exp Ther* **309**: 380–7.

Ren K, Dubner R (2010). Interactions between the immune and nervous systems in pain. *Nat Med* **16**: 1267–76.

Ren W-J, Liu Y, Zhou L-J, Li W, Zhong Y, Pang R-P, *et al* (2011). Peripheral nerve injury leads to working memory deficits and dysfunction of the hippocampus by upregulation of TNF-α in rodents. *Neuropsychopharmacology* **36**: 979–992.

Rezaï X, Faget L, Bednarek E, Schwab Y, Kieffer BL, Massotte D (2012). Mouse δ opioid receptors are located on presynaptic afferents to hippocampal pyramidal cells. *Cell Mol Neurobiol* **32**: 509–16.

Rhudy JL, Grimes JS, Meagher MW (2004). Fear-induced hypoalgesia in humans: effects on low intensity thermal stimulation and finger temperature. *J Pain* **5**: 458–68.

Rhudy JL, Meagher MW (2000a). Fear and anxiety: divergent effects on human pain thresholds. *Pain* **84**: 65–75.

Rhudy JL, Meagher MW (2000b). Fear and anxiety : divergent effects on human pain thresholds. **84**: .

Rhudy JL, Meagher MW (2003). Negative affect: effects on an evaluative measure of human pain. *Pain* **104**: 617–26.

Rhudy JL, Williams AE, McCabe KM, Russell JL, Maynard LJ (2008). Emotional control of nociceptive reactions (ECON): do affective valence and arousal play a role? *Pain* **136**: 250–61.

Richards EM, Mathews DC, Luckenbaugh DA, Ionescu DF, Machado-Vieira R, Niciu MJ, *et al* (2016). A randomized, placebo-controlled pilot trial of the delta opioid receptor agonist AZD2327 in anxious depression. *Psychopharmacology (Berl)* **233**: 1119–30.

Riley JL, Robinson ME, Wise EA, Myers CD, Fillingim RB (1998). Sex differences in the perception of noxious experimental stimuli: A meta-analysis. *Pain* **74**: 181–187.

Rittner HL, Brack A, Stein C (2008). Pain and the immune system. *Br J Anaesth* **101**: 40–44.

Rivat C (2016). Neuropathic Pain: When Nerve Lesion Turns off Peripheral Analgesia. *Anesthesiology* **125**: 627–9.

Rivat C, Ballantyne J (2016). The dark side of opioids in pain management: basic science explains clinical observation. *Pain reports* **1**: e570.

Robbins TW, Everitt BJ (2002). Limbic-striatal memory systems and drug addiction.

Neurobiol Learn Mem **78**: 625–36.

Rodríguez de Fonseca F, Arco I Del, Bermudez-Silva FJ, Bilbao A, Cippitelli A, Navarro M (2005). The endocannabinoid system: physiology and pharmacology. *Alcohol Alcohol* **40**: 2–14.

Roeckel L-A, Coz G-M Le, Gavériaux-Ruff C, Simonin F (2016). Opioid-induced hyperalgesia: Cellular and molecular mechanisms. *Neuroscience* **338**: 160–182.

Roeckel L-A, Utard V, Reiss D, Mouheiche J, Maurin H, Robé A, *et al* (2017). Morphine-induced hyperalgesia involves mu opioid receptors and the metabolite morphine-3-glucuronide. *Sci Rep* **7**: 10406.

Roeska K, Ceci A, Treede R-D, Doods H (2009). Effect of high trait anxiety on mechanical hypersensitivity in male rats. *Neurosci Lett* **464**: 160–4.

Roeska K, Doods H, Arndt K, Treede RD, Ceci A (2008). Anxiety-like behaviour in rats with mononeuropathy is reduced by the analgesic drugs morphine and gabapentin. *Pain* **139**: 349–357.

Rojas-Corrales MO, Berrocoso E, Gibert-Rahola J, Micó JA (2002). Antidepressant-like effects of tramadol and other central analgesics with activity on monoamines reuptake, in helpless rats. *Life Sci* **72**: 143–52.

Rojas-Corrales MO, Gibert-Rahola J, Micó JA (1998). Tramadol induces antidepressant-type effects in mice. *Life Sci* **63**: PL175-80.

Romero-Sandoval A, Nutile-McMenemy N, DeLeo JA (2008). Spinal microglial and perivascular cell cannabinoid receptor type 2 activation reduces behavioral hypersensitivity without tolerance after peripheral nerve injury. *Anesthesiology* **108**: 722–34.

Roques BP, Fournié-Zaluski MC, Wurm M (2012). Inhibiting the breakdown of endogenous opioids and cannabinoids to alleviate pain. *Nat Rev Drug Discov* **11**: 292–310.

Rosenblum A, Marsch LA, Joseph H, Portenoy RK (2008). Opioids and the treatment of chronic pain: controversies, current status, and future directions. *Exp Clin Psychopharmacol* **16**: 405–16.

Rubinstein M, Mogil JS, Japón M, Chan EC, Allen RG, Low MJ (1996). Absence of opioid stress-induced analgesia in mice lacking beta-endorphin by site-directed mutagenesis. *Proc Natl Acad Sci U S A* **93**: 3995–4000.

Rutten K, Tzschentke TM, Koch T, Schiene K, Christoph T (2014). Pharmacogenomic study of the role of the nociceptin/orphanin FQ receptor and opioid receptors in diabetic

hyperalgesia. *Eur J Pharmacol* **741**: 264–71.

Saab CY, Hains BC (2009). Remote neuroimmune signaling: a long-range mechanism of nociceptive network plasticity. *Trends Neurosci* **32**: 110–7.

Saarto T, Wiffen PJ (2007). Antidepressants for neuropathic pain. *Cochrane database Syst Rev* CD005454.

Sadiq SA, Poopatana CA (2007). Intrathecal baclofen and morphine in multiple sclerosis patients with severe pain and spasticity. *J Neurol* **254**: 1464–1465.

Saitoh A, Kimura Y, Suzuki T, Kawai K, Nagase H, Kamei J (2004). Potential anxiolytic and antidepressant-like activities of SNC80, a selective delta-opioid agonist, in behavioral models in rodents. *J Pharmacol Sci* **95**: 374–380.

Saitoh A, Yamada M, Yamada M, Takahashi K, Yamaguchi K, Murasawa H, *et al* (2008). Antidepressant-like effects of the delta-opioid receptor agonist SNC80 ([(+)-4-[(alphaR)-alpha-[(2S,5R)-2,5-dimethyl-4-(2-propenyl)-1-piperazinyl]-(3-methoxyphenyl)methyl]-N,N-diethylbenzamide) in an olfactory bulbectomized rat model. *Brain Res* **1208**: 160–9.

Sala M, Braida D, Leone MP, Calcaterra P, Frattola D, Gori E (1994). Chronic morphine affects working memory during treatment and withdrawal in rats: possible residual long-term impairment. *Behav Pharmacol* **5**: 570–580.

Sandkühler J (2007). Understanding LTP in pain pathways. *Mol Pain* **3**: 9.

Sandkühler J (2009). Models and mechanisms of hyperalgesia and allodynia. *Physiol Rev* **89**: 707–58.

Sato J, Perl ER (1991). Adrenergic excitation of cutaneous pain receptors induced by peripheral nerve injury. *Science* **251**: 1608–10.

Schepers RJ, Mahoney JL, Gehrke BJ, Shippenberg TS (2008). Endogenous kappa-opioid receptor systems inhibit hyperalgesia associated with localized peripheral inflammation. *Pain* **138**: 423–39.

Scherrer G, Imamachi N, Cao Y-Q, Contet C, Mennicken F, O'Donnell D, *et al* (2009). Dissociation of the opioid receptor mechanisms that control mechanical and heat pain. *Cell* **137**: 1148–1159.

Scherrer G, Tryoen-Tóth P, Filliol D, Matifas A, Laustriat D, Cao YQ, *et al* (2006). Knockin mice expressing fluorescent delta-opioid receptors uncover G protein-coupled receptor dynamics in vivo. *Proc Natl Acad Sci U S A* **103**: 9691–6.

Scholz J, Woolf CJ (2007). The neuropathic pain triad: neurons, immune cells and glia. *Nat Neurosci* **10**: 1361–1368.

Schütz SG, Robinson-Papp J (2013). HIV-related neuropathy: current perspectives. *HIV AIDS (Auckl)* **5**: 243–51.

Schwarzer C (2009). 30 years of dynorphins--new insights on their functions in neuropsychiatric diseases. *Pharmacol Ther* **123**: 353–70.

Schwier C, Kliem A, Boettger MK, Bär K-J (2010). Increased cold-pain thresholds in major depression. *J Pain* **11**: 287–90.

Seifert F, Maihöfner C (2009). Central mechanisms of experimental and chronic neuropathic pain: Findings from functional imaging studies. *Cell Mol Life Sci* **66**: 375–390.

Seminowicz DA, Laferriere AL, Millecamps M, Yu JSC, Coderre TJ, Bushnell MC (2009). MRI structural brain changes associated with sensory and emotional function in a rat model of long-term neuropathic pain. *Neuroimage* **47**: 1007–14.

Seminowicz DA, Mikulis DJ, Davis KD (2004). Cognitive modulation of pain-related brain responses depends on behavioral strategy. *Pain* **112**: 48–58.

Serpell M, Ratcliffe S, Hovorka J, Schofield M, Taylor L, Lauder H, *et al* (2014). A double-blind, randomized, placebo-controlled, parallel group study of THC/CBD spray in peripheral neuropathic pain treatment. *Eur J Pain* **18**: 999–1012.

Serra Catafau J (Editorial Médica Panamericana: 2007). *Tratado de dolor neuropático*. .

Severino A, Chen W, Hakimian JK, Kieffer BL, Gaveriaux-Ruff C, Walwyn W, *et al* (2018). Mu-opioid receptors in nociceptive afferents produce a sustained suppression of hyperalgesia in chronic pain. *Pain* **159**: 1607–1620.

Shapira NA, Keck PE, Goldsmith TD, McConville BJ, Eis M, McElroy SL (1997). Open-label pilot study of tramadol hydrochloride in treatment-refractory obsessive-compulsive disorder. *Depress Anxiety* **6**: 170–3.

Shapira NA, Verduin ML, DeGraw JD (2001). Treatment of refractory major depression with tramadol monotherapy. *J Clin Psychiatry* **62**: 205–6.

Shen H, Aeschlimann A, Reisch N, Gay RE, Simmen BR, Michel BA, *et al* (2005). Kappa and delta opioid receptors are expressed but down-regulated in fibroblast-like synoviocytes of patients with rheumatoid arthritis and osteoarthritis. *Arthritis Rheum* **52**: 1402–1410.

Sherry DD, Wallace CA, Kelley C, Kidder M, Sapp L (1999). Short- and long-term outcomes of children with complex regional pain syndrome type I treated with exercise therapy. *Clin J Pain* **15**: 218–23.

Shi M, Qi W-J, Gao G, Wang J-Y, Luo F (2010a). Increased thermal and mechanical

nociceptive thresholds in rats with depressive-like behaviors. *Brain Res* **1353**: 225–33.

Shi M, Wang J-Y, Luo F (2010b). Depression shows divergent effects on evoked and spontaneous pain behaviors in rats. *J Pain* **11**: 219–29.

Shinder V, Govrin-Lippmann R, Cohen S, Belenky M, Ilin P, Fried K, *et al* (1999). Structural basis of sympathetic-sensory coupling in rat and human dorsal root ganglia following peripheral nerve injury. *J Neurocytol* **28**: 743–61.

Shirayama Y, Ishida H, Iwata M, Hazama G-I, Kawahara R, Duman RS (2004). Stress increases dynorphin immunoreactivity in limbic brain regions and dynorphin antagonism produces antidepressant-like effects. *J Neurochem* **90**: 1258–68.

Simmons D, Self DW (2009). Role of mu- and delta-opioid receptors in the nucleus accumbens in cocaine-seeking behavior. *Neuropsychopharmacology* **34**: 1946–57.

Simon EJ, Hiller JM, Edelman I (1973). Stereospecific Binding of the Potent Narcotic Analgesic [31H]Etorphine to Rat-Brain Homogenate. **70**: 1947–1949.

Simonin F, Valverde O, Smadja C, Slowe S, Kitchen I, Dierich A, *et al* (1998). Disruption of the kappa-opioid receptor gene in mice enhances sensitivity to chemical visceral pain, impairs pharmacological actions of the selective kappa-agonist U-50,488H and attenuates morphine withdrawal. *EMBO J* **17**: 886–97.

Singer T, Seymour B, O'Doherty J, Kaube H, Dolan RJ, Frith CD (2004). Empathy for Pain Involves the Affective but not Sensory Components of Pain. *Science (80-)* **303**: 1157–1162.

Sladky R, Höflich A, Atanelov J, Kraus C, Baldinger P, Moser E, *et al* (2012). Increased Neural Habituation in the Amygdala and Orbitofrontal Cortex in Social Anxiety Disorder Revealed by fMRI. *PLoS One* **7**: e50050.

Smith PA (2014). BDNF: No gain without pain? *Neuroscience* **283**: 107–123.

Soler MD, Saurí-Ruiz J, Curcoll-Gallemí ML, Benito-Penalva J, Opisso-Salleras E, Chamarro-Lusar A, *et al* (2007). [Characteristics of chronic neuropathic pain and their relationship with psychological well-being in spinal cord injury patients]. *Rev Neurol* **44**: 3–9.

Sorge RE, Mapplebeck JCS, Rosen S, Beggs S, Taves S, Alexander JK, *et al* (2015). Different immune cells mediate mechanical pain hypersensitivity in male and female mice. *Nat Neurosci* doi:10.1038/nn.4053.

Sotocinal SG, Sorge RE, Zaloum A, Tuttle AH, Martin LJ, Wieskopf JS, *et al* (2011). The Rat Grimace Scale: a partially automated method for quantifying pain in the laboratory

rat via facial expressions. *Mol Pain* **7**: 55.

Spain JW, Newsom GC (1991). Chronic opioids impair acquisition of both radial maze and Y-maze choice escape. *Psychopharmacology (Berl)* **105**: 101–6.

Spiegel I, Mardinly AR, Gabel HW, Bazinet JE, Couch CH, Tzeng CP, *et al* (2014). Npas4 regulates excitatory-inhibitory balance within neural circuits through cell-type-specific gene programs. *Cell* **157**: 1216–29.

Spike RC, Puskár Z, Sakamoto H, Stewart W, Watt C, Todd AJ (2002). MOR-1-immunoreactive neurons in the dorsal horn of the rat spinal cord: evidence for nonsynaptic innervation by substance P-containing primary afferents and for selective activation by noxious thermal stimuli. *Eur J Neurosci* **15**: 1306–16.

Spina L, Longoni R, Mulas A, Chang KJ, Chiara G Di (1998). Dopamine-dependent behavioural stimulation by non-peptide delta opioids BW373U86 and SNC 80: 1. Locomotion, rearing and stereotypies in intact rats. *Behav Pharmacol* **9**: 1–8.

Stein C (1993). Peripheral mechanisms of opioid analgesia. *Anesth Analg* **76**: 182–91.

Stein C, Machelska H (2011). Modulation of Peripheral Sensory Neurons by the Immune System: Implications for Pain Therapy. *Pharmacol Rev* **63**: 860–881.

Stemkowski P, García-Caballero A, Gadotti VDM, M'Dahoma S, Huang S, Black SAG, *et al* (2016). TRPV1 Nociceptor Activity Initiates USP5/T-type Channel-Mediated Plasticity. *Cell Rep* **17**: 2901–2912.

Steru L, Chermat R, Thierry B, Simon P (1985). The tail suspension test: a new method for screening antidepressants in mice. *Psychopharmacology (Berl)* **85**: 367–70.

Stoll AL, Rueter S (1999). Treatment augmentation with opiates in severe and refractory major depression. *Am J Psychiatry* **156**: 2017.

Stone WS, Walser B, Gold SD, Gold PE (1991). Scopolamine- and morphine-induced impairments of spontaneous alternation performance in mice: reversal with glucose and with cholinergic and adrenergic agonists. *Behav Neurosci* **105**: 264–71.

Sturgeon JA, Zautra AJ (2016). Pain Management Social pain and physical pain: shared paths to resilience Overlap of physical & social pain. *Pain Manag* **6**: 63–74.

Sud R, Spengler RN, Nader ND, Ignatowski TA (2008). Antinociception occurs with a reversal in α2-adrenoceptor regulation of TNF production by peripheral monocytes/macrophages from pro- to anti-inflammatory. *Eur J Pharmacol* **588**: 217–231.

Suzuki R, Hunt SP, Dickenson AH (2003). The coding of noxious mechanical and thermal stimuli of deep dorsal horn neurones is attenuated in NK1 knockout mice.

Neuropharmacology **45**: 1093–100.

Suzuki T, Amata M, Sakaue G, Nishimura S, Inoue T, Shibata M, *et al* (2007). Experimental neuropathy in mice is associated with delayed behavioral changes related to anxiety and depression. *Anesth Analg* **104**: 1570–7, table of contents.

Svingos AL, Moriwaki A, Wang JB, Uhl GR, Pickel VM (1996). Ultrastructural immunocytochemical localization of mu-opioid receptors in rat nucleus accumbens: extrasynaptic plasmalemmal distribution and association with Leu5-enkephalin. *J Neurosci* **16**: 4162–73.

Takamura M, Okamoto Y, Okada G, Toki S, Yamamoto T, Ichikawa N, *et al* (2017). Patients with major depressive disorder exhibit reduced reward size coding in the striatum. *Prog Neuro-Psychopharmacology Biol Psychiatry* **79**: 317–323.

Takeda K, Muramatsu M, Chikuma T, Kato T (2009). Effect of memantine on the levels of neuropeptides and microglial cells in the brain regions of rats with neuropathic pain. *J Mol Neurosci* **39**: 380–90.

Tamaddonfard E, Erfanparast A (2017). Role of μ-opioid receptor in parafascicular nucleus of thalamus on morphine-induced antinociception in a rat model of acute trigeminal pain. *Vet Res forum an Int Q J* **8**: 29–34.

Tan AM, Chang Y-W, Zhao P, Hains BC, Waxman SG (2011). Rac1-regulated dendritic spine remodeling contributes to neuropathic pain after peripheral nerve injury. *Exp Neurol* **232**: 222–33.

Tan AM, Choi J-S, Waxman SG, Hains BC (2009). Dendritic spine remodeling after spinal cord injury alters neuronal signal processing. *J Neurophysiol* **102**: 2396–409.

Tan AM, Samad OA, Fischer TZ, Zhao P, Persson A-K, Waxman SG (2012). Maladaptive Dendritic Spine Remodeling Contributes to Diabetic Neuropathic Pain. *J Neurosci* **32**: 6795–6807.

Tanabe M, Takasu K, Yamaguchi S, Kodama D, Ono H (2008). Glycine transporter inhibitors as a potential therapeutic strategy for chronic pain with memory impairment. *Anesthesiology* **108**: 929–37.

Tao R, Auerbach SB (1995). Involvement of the dorsal raphe but not median raphe nucleus in morphine-induced increases in serotonin release in the rat forebrain. *Neuroscience* **68**: 553–61.

Tao R, Ma Z, Auerbach SB (1996). Differential regulation of 5-hydroxytryptamine release by GABAA and GABAB receptors in midbrain raphe nuclei and forebrain of rats. *Br J Pharmacol* **119**: 1375–84.

Tardito D, Perez J, Tiraboschi E, Musazzi L, Racagni G, Popoli M (2006). Signaling pathways regulating gene expression, neuroplasticity, and neurotrophic mechanisms in the action of antidepressants: a critical overview. *Pharmacol Rev* **58**: 115–34.

Taylor AMW, Castonguay A, Taylor AJ, Murphy NP, Ghogha A, Cook C, *et al* (2015). Microglia disrupt mesolimbic reward circuitry in chronic pain. *J Neurosci* **35**: 8442–50.

Taylor BK (2009). Spinal inhibitory neurotransmission in neuropathic pain. *Curr Pain Headache Rep* **13**: 208–14.

Taylor BK, Corder G (2014). Endogenous analgesia, dependence, and latent pain sensitization. *Curr Top Behav Neurosci* **20**: 283–325.

Tejedor-Real P, Mico JA, Maldonado R, Roques BP, Gibert-Rahola J (1995). Implication of endogenous opioid system in the learned helplessness model of depression. *Pharmacol Biochem Behav* **52**: 145–52.

Tejedor-Real P, Micó JA, Maldonado R, Roques BP, Gibert-Rahola J (1993). Effect of mixed (RB 38A) and selective (RB 38B) inhibitors of enkephalin degrading enzymes on a model of depression in the rat. *Biol Psychiatry* **34**: 100–7.

Terenius L (1973). Characteristics of the "receptor" for narcotic analgesics in synaptic plasma membrane fraction from rat brain. *Acta Pharmacol Toxicol (Copenh)* **33**: 377–84.

Terman GW, Shavit Y, Lewis JW, Cannon JT, Liebeskind JC (1984). Intrinsic mechanisms of pain inhibition: activation by stress. *Science* **226**: 1270–7.

Thompson AA, Liu W, Chun E, Katritch V, Wu H, Vardy E, *et al* (2012). Structure of the nociceptin/orphanin FQ receptor in complex with a peptide mimetic. *Nature* **485**: 395–9.

Thomsen KR (2015). Measuring anhedonia: impaired ability to pursue, experience, and learn about reward. *Front Psychol* **6**: 1409.

Tiwari V, Anderson M, Yang F, Tiwari V, Zheng Q, He S-Q, *et al* (2018). Peripherally Acting μ-Opioid Receptor Agonists Attenuate Ongoing Pain-associated Behavior and Spontaneous Neuronal Activity after Nerve Injury in Rats. *Anesthesiology* **128**: 1220–1236.

Toll L, Bruchas MR, Calo' G, Cox BM, Zaveri NT (2016). Nociceptin/Orphanin FQ Receptor Structure, Signaling, Ligands, Functions, and Interactions with Opioid Systems. *Pharmacol Rev* **68**: 419–57.

Tomić M, Pecikoza U, Micov A, Vučković S, Stepanović-Petrović R (2018). Antiepileptic

drugs as analgesics/adjuvants in inflammatory pain: current preclinical evidence. *Pharmacol Ther* doi:10.1016/J.PHARMTHERA.2018.06.002.

Tõnissaar M, Herm L, Eller M, Kõiv K, Rinken A, Harro J (2008). Rats with high or low sociability are differently affected by chronic variable stress. *Neuroscience* **152**: 867–876.

Torebjörk E, Wahren L, Wallin G, Hallin R, Koltzenburg M (1995). Noradrenaline-evoked pain in neuralgia. *Pain* **63**: 11–20.

Torp R, Su JH, Deng G, Cotman CW (1998). GADD45 is induced in Alzheimer's disease, and protects against apoptosis in vitro. *Neurobiol Dis* **5**: 245–52.

Torrance N, Ferguson JA, Afolabi E, Bennett MI, Serpell MG, Dunn KM, *et al* (2013). Neuropathic pain in the community: more under-treated than refractory? *Pain* **154**: 690–9.

Torrance N, Smith BH, Bennett MI, Lee AJ (2006). The epidemiology of chronic pain of predominantly neuropathic origin. Results from a general population survey. *J Pain* **7**: 281–9.

Torrecilla M, Marker CL, Cintora SC, Stoffel M, Williams JT, Wickman K (2002). G-protein-gated potassium channels containing Kir3.2 and Kir3.3 subunits mediate the acute inhibitory effects of opioids on locus ceruleus neurons. *J Neurosci* **22**: 4328–34.

Torregrossa MM, Isgor C, Folk JE, Rice KC, Watson SJ, Woods JH (2004). The delta-opioid receptor agonist (+)BW373U86 regulates BDNF mRNA expression in rats. *Neuropsychopharmacology* **29**: 649–59.

Torregrossa MM, Jutkiewicz EM, Mosberg HI, Balboni G, Watson SJ, Woods JH (2006). Peptidic delta opioid receptor agonists produce antidepressant-like effects in the forced swim test and regulate BDNF mRNA expression in rats. *Brain Res* **1069**: 172–81.

Trang T, Al-Hasani R, Salvemini D, Salter MW, Gutstein H, Cahill CM (2015). Pain and Poppies: The Good, the Bad, and the Ugly of Opioid Analgesics. *J Neurosci* **35**: 13879–88.

Treede R-D, Jensen TS, Campbell JN, Cruccu G, Dostrovsky JO, Griffin JW, *et al* (2008). Neuropathic pain: Redefinition and a grading system for clinical and research purposes. *Neurology* **70**: 1630–1635.

Trigo JM, Zimmer A, Maldonado R (2009). Nicotine anxiogenic and rewarding effects are decreased in mice lacking beta-endorphin. *Neuropharmacology* **56**: 1147–53.

Tsai R, Tai Y, Tzeng J, Cherng C, Yeh C, Wong C (2009). Ultra-low dose naloxone restores

the antinociceptive effect of morphine in pertussis toxin-treated rats by reversing the coupling of mu-opioid receptors from Gs-protein to coupling to Gi-protein. *Neuroscience* **164**: 435–43.

Uchida H, Ma L, Ueda H (2010). Epigenetic gene silencing underlies C-fiber dysfunctions in neuropathic pain. *J Neurosci* **30**: 4806–14.

Uchida H, Matsushita Y, Araki K, Mukae T, Ueda H (2015). Histone deacetylase inhibitors relieve morphine resistance in neuropathic pain after peripheral nerve injury. *J Pharmacol Sci* **128**: 208–11.

Ueda H (2006). Molecular mechanisms of neuropathic pain-phenotypic switch and initiation mechanisms. *Pharmacol Ther* **109**: 57–77.

Ueda H (2008). Peripheral mechanisms of neuropathic pain - involvement of lysophosphatidic acid receptor-mediated demyelination. *Mol Pain* **4**: 11.

Usoskin D, Furlan A, Islam S, Abdo H, Lönnerberg P, Lou D, *et al* (2015). Unbiased classification of sensory neuron types by large-scale single-cell RNA sequencing. *Nat Neurosci* **18**: 145–153.

Vadivelu N, Mitra S, Hines RL (2011). Peripheral opioid receptor agonists for analgesia: a comprehensive review. *J Opioid Manag* **7**: 55–68.

Valverde O, Mantamadiotis T, Torrecilla M, Ugedo L, Pineda J, Bleckmann S, *et al* (2004). Modulation of anxiety-like behavior and morphine dependence in CREB-deficient mice. *Neuropsychopharmacology* **29**: 1122–33.

Vanderah TW (2010). Delta and kappa opioid receptors as suitable drug targets for pain. *Clin J Pain* **26 Suppl 10**: S10-5.

Vanderah TW, Suenaga NM, Ossipov MH, Malan TP, Lai J, Porreca F (2001). Tonic descending facilitation from the rostral ventromedial medulla mediates opioid-induced abnormal pain and antinociceptive tolerance. *J Neurosci* **21**: 279–86.

Vanegas H, Schaible H-G (2004). Descending control of persistent pain: inhibitory or facilitatory? *Brain Res Rev* **46**: 295–309.

Vanelderen P, Rouwette T, Kozicz T, Roubos E, Zundert J Van, Heylen R, *et al* (2010). The role of brain-derived neurotrophic factor in different animal models of neuropathic pain. *Eur J Pain* **14**: 473.e1-9.

Vergura R, Balboni G, Spagnolo B, Gavioli E, Lambert DG, McDonald J, *et al* (2008). Anxiolytic- and antidepressant-like activities of H-Dmt-Tic-NH-CH(CH2-COOH)-Bid (UFP-512), a novel selective delta opioid receptor agonist. *Peptides* **29**: 93–103.

Vetter I, Wyse BD, Monteith GR, Roberts-Thomson SJ, Cabot PJ (2006). The mu opioid

agonist morphine modulates potentiation of capsaicin-evoked TRPV1 responses through a cyclic AMP-dependent protein kinase A pathway. *Mol Pain* **2**: 22.

Vigneri S, Sindaco G, Grua M La, Zanella M, Ravaioli L, Paci V, *et al* (2016). Combined epidural morphine and bupivacaine in the treatment of lumbosacral radicular neuropathic pain: a noncontrolled prospective study. *J Pain Res* **9**: 1081–1087.

Vilardaga J-P, Nikolaev VO, Lorenz K, Ferrandon S, Zhuang Z, Lohse MJ (2008). Conformational cross-talk between α2A-adrenergic and μ-opioid receptors controls cell signaling. *Nat Chem Biol* **4**: 126–131.

Vincent K, Cornea VM, Jong Y-JI, Laferrière A, Kumar N, Mickeviciute A, *et al* (2016). Intracellular mGluR5 plays a critical role in neuropathic pain. *Nat Commun* **7**: 10604.

Volkow ND, McLellan AT (2016). Opioid Abuse in Chronic Pain--Misconceptions and Mitigation Strategies. *N Engl J Med* **374**: 1253–63.

Walker J, Catheline G, Guilbaud G, Kayser V (1999). Lack of cross-tolerance between the antinociceptive effects of systemic morphine and asimadoline, a peripherally-selective kappa-opioid agonist, in CCI-neuropathic rats. *Pain* **83**: 509–16.

Walwyn WM, Chen W, Kim H, Minasyan A, Ennes HS, McRoberts JA, *et al* (2016). Sustained Suppression of Hyperalgesia during Latent Sensitization by μ-, δ-, and κ-opioid receptors and α2A Adrenergic Receptors: Role of Constitutive Activity. *J Neurosci* **36**: 204–21.

Wang D-D, Li Z, Chang Y, Wang R-R, Chen X-F, Zhao Z-Y, *et al* (2010a). Neural circuits and temporal plasticity in hindlimb representation of rat primary somatosensory cortex: revisited by multi-electrode array on brain slices. *Neurosci Bull* **26**: 175–87.

Wang D, Tawfik VL, Corder G, Low SA, François A, Basbaum AI, *et al* (2018). Functional Divergence of Delta and Mu Opioid Receptor Organization in CNS Pain Circuits. *Neuron* **98**: 90–108.e5.

Wang H-B, Zhao B, Zhong Y-Q, Li K-C, Li Z-Y, Wang Q, *et al* (2010b). Coexpression of delta- and mu-opioid receptors in nociceptive sensory neurons. *Proc Natl Acad Sci U S A* **107**: 13117–22.

Wang H-Y, Friedman E, Olmstead MC, Burns LH (2005). Ultra-low-dose naloxone suppresses opioid tolerance, dependence and associated changes in mu opioid receptor-G protein coupling and Gbetagamma signaling. *Neuroscience* **135**: 247–61.

Wang J, Goffer Y, Xu D, Tukey DS, Shamir DB, Eberle SE, *et al* (2011). A Single

Subanesthetic Dose of Ketamine Relieves Depression-like Behaviors Induced by Neuropathic Pain in Rats. *Anesthesiology* **115**: 812–821.

Wang S, Tian Y, Song L, Lim G, Tan Y, You Z, *et al* (2012). Exacerbated mechanical hyperalgesia in rats with genetically predisposed depressive behavior: role of melatonin and NMDA receptors. *Pain* **153**: 2448–57.

Wang W, Qi W-J, Xu Y, Wang J-Y, Luo F (2010c). The differential effects of depression on evoked and spontaneous pain behaviors in olfactory bulbectomized rats. *Neurosci Lett* **472**: 143–7.

Wang Z, Gardell LR, Ossipov MH, Vanderah TW, Brennan MB, Hochgeschwender U, *et al* (2001). Pronociceptive actions of dynorphin maintain chronic neuropathic pain. *J Neurosci* **21**: 1779–86.

Warner NS, Watson JC, Bendel MA, Moeschler SM (2018). Refractory Pain Management in Amyloid-Associated Peripheral Neuropathy. *Reg Anesth Pain Med* **43**: 434–437.

Webster LR, Israel RJ (2018). Oral methylnaltrexone does not negatively impact analgesia in patients with opioid-induced constipation and chronic noncancer pain. *J Pain Res* **11**: 1503–1510.

Webster LR, Michna E, Khan A, Israel RJ, Harper JR (2017). Long-Term Safety and Efficacy of Subcutaneous Methylnaltrexone in Patients with Opioid-Induced Constipation and Chronic Noncancer Pain: A Phase 3, Open-Label Trial. *Pain Med* **18**: 1496–1504.

Wegert S, Ossipov MH, Nichols ML, Bian D, Vanderah TW, Malan TP, *et al* (1997). Differential activities of intrathecal MK-801 or morphine to alter responses to thermal and mechanical stimuli in normal or nerve-injured rats. *Pain* **71**: 57–64.

Wei F, Li P, Zhuo M (1999). Loss of synaptic depression in mammalian anterior cingulate cortex after amputation. *J Neurosci* **19**: 9346–54.

Weibel R, Reiss D, Karchewski L, Gardon O, Matifas A, Filliol D, *et al* (2013). Mu Opioid Receptors on Primary Afferent Nav1.8 Neurons Contribute to Opiate-Induced Analgesia: Insight from Conditional Knockout Mice. *PLoS One* **8**: 1–18.

Weisenberg M, Aviram O, Wolf Y, Raphaeli N (1984). Relevant and irrelevant anxiety in the reaction to pain. *Pain* **20**: 371–83.

Wellman LL, Forcelli PA, Aguilar BL, Malkova L (2016). Bidirectional Control of Social Behavior by Activity within Basolateral and Central Amygdala of Primates. *J Neurosci* **36**: 8746–56.

Wickman K, Clapham DE (1995). Ion channel regulation by G proteins. *Physiol Rev* **75**:

865–85.

Wieskopf JS, Pan Y-X, Marcovitz J, Tuttle AH, Majumdar S, Pidakala J, *et al* (2014). Broad-spectrum analgesic efficacy of IBNtxA is mediated by exon 11-associated splice variants of the mu-opioid receptor gene. *Pain* **155**: 2063–70.

Wightman R, Perrone J, Portelli I, Nelson L (2012). Likeability and abuse liability of commonly prescribed opioids. *J Med Toxicol* **8**: 335–40.

Willis WD, Coggeshall RE (Springer US: 2004). *Sensory Mechanisms of the Spinal Cord : Volume 1 Primary Afferent Neurons and the Spinal Dorsal Horn*. .

Wilson RI, Nicoll RA (2002). Endocannabinoid Signaling in the Brain. *Science (80-)* **296**: 678–682.

Winters BL, Gregoriou GC, Kissiwaa SA, Wells OA, Medagoda DI, Hermes SM, *et al* (2017). Endogenous opioids regulate moment-to-moment neuronal communication and excitability. *Nat Commun* **8**: 14611.

Wittmann W, Schunk E, Rosskothen I, Gaburro S, Singewald N, Herzog H, *et al* (2009). Prodynorphin-derived peptides are critical modulators of anxiety and regulate neurochemistry and corticosterone. *Neuropsychopharmacology* **34**: 775–85.

Woodbury CJ, Koerber HR (2003). Widespread projections from myelinated nociceptors throughout the substantia gelatinosa provide novel insights into neonatal hypersensitivity. *J Neurosci* **23**: 601–10.

Woodhams SG, Chapman V, Finn DP, Hohmann AG, Neugebauer V (2017). The cannabinoid system and pain. *Neuropharmacology* **124**: 105–120.

Woolf CJ (2011). Central sensitization: Implications for the diagnosis and treatment of pain. *Pain* **152**: S2–S15.

Woolf CJ, Ma Q (2007). Nociceptors--noxious stimulus detectors. *Neuron* **55**: 353–64.

Woolf CJ, Mannion RJ (1999). Neuropathic pain: Aetiology, symptoms, mechanisms, and management. *Lancet* **353**: 1959–1964.

Wu H, Wacker D, Mileni M, Katritch V, Han GW, Vardy E, *et al* (2012). Structure of the human κ-opioid receptor in complex with JDTic. *Nature* **485**: 327–32.

Wu L, Zhang S, Shkhyan R, Lee S, Gullo F, Eliasberg CD, *et al* (2017). Kappa opioid receptor signaling protects cartilage tissue against posttraumatic degeneration. *JCI insight* **2**: e88553.

Wu W-T, Huang Y-H, Chen D-C, Huang Y-H, Chou L-W (2013). Effective management of intractable neuropathic pain using an intrathecal morphine pump in a patient with acute transverse myelitis. *Neuropsychiatr Dis Treat* **9**: 1023–8.

Xie J-D, Chen S-R, Pan H-L (2017). Presynaptic mGluR5 receptor controls glutamatergic input through protein kinase C–NMDA receptors in paclitaxel-induced neuropathic pain. *J Biol Chem* **292**: 20644–20654.

Xie W, Strong JA, Zhang J-M (2009). Early blockade of injured primary sensory afferents reduces glial cell activation in two rat neuropathic pain models. *Neuroscience* **160**: 847–57.

Xu H, Wu L-J, Wang H, Zhang X, Vadakkan KI, Kim SS, *et al* (2008). Presynaptic and postsynaptic amplifications of neuropathic pain in the anterior cingulate cortex. *J Neurosci* **28**: 7445–53.

Xu M, Petraschka M, McLaughlin JP, Westenbroek RE, Caron MG, Lefkowitz RJ, *et al* (2004). Neuropathic pain activates the endogenous kappa opioid system in mouse spinal cord and induces opioid receptor tolerance. *J Neurosci* **24**: 4576–84.

Yalcin I, Aksu F, Bodard S, Chalon S, Belzung C (2007). Antidepressant-like effect of tramadol in the unpredictable chronic mild stress procedure: possible involvement of the noradrenergic system. *Behav Pharmacol* **18**: 623–31.

Yalcin I, Bohren Y, Waltisperger E, Sage-Ciocca D, Yin JC, Freund-Mercier M-J, *et al* (2011). A time-dependent history of mood disorders in a murine model of neuropathic pain. *Biol Psychiatry* **70**: 946–53.

Yamamoto T, Sakashita Y, Nozaki-Taguchi N (2001). Antagonism of ORLI receptor produces an algesic effect in the rat formalin test. *Neuroreport* **12**: 1323–7.

Yang J, Xie M-X, Hu L, Wang X-F, Mai J-Z, Li Y-Y, *et al* (2018). Upregulation of N-type calcium channels in the soma of uninjured dorsal root ganglion neurons contributes to neuropathic pain by increasing neuronal excitability following peripheral nerve injury. *Brain Behav Immun* **71**: 52–65.

Yoo J-H, Lee S-Y, Loh HH, Ho IK, Jang C-G (2004). Altered emotional behaviors and the expression of 5-HT1A and M1 muscarinic receptors in micro-opioid receptor knockout mice. *Synapse* **54**: 72–82.

Younger J, Aron A, Parke S, Chatterjee N, Mackey S (2010). Viewing pictures of a romantic partner reduces experimental pain: involvement of neural reward systems. *PLoS One* **5**: e13309.

Zamponi GW, Snutcht TP (1996). Modulation of voltage-dependent calcium channels by G proteins. *Curr Opin Neurobiol* **8**: 351–356.

Zee A Van (2009). The promotion and marketing of oxycontin: commercial triumph, public health tragedy. *Am J Public Health* **99**: 221–7.

Zeilhofer HU (2008). Loss of glycinergic and GABAergic inhibition in chronic pain—contributions of inflammation and microglia. *Int Immunopharmacol* **8**: 182–187.

Zeng Q, Wang S, Lim G, Yang L, Mao J, Sung B, *et al* (2008). Exacerbated mechanical allodynia in rats with depression-like behavior. *Brain Res* **1200**: 27–38.

Zhang H, Shi Y-G, Woods JH, Watson SJ, Ko M-C (2007a). Central kappa-opioid receptor-mediated antidepressant-like effects of nor-Binaltorphimine: behavioral and BDNF mRNA expression studies. *Eur J Pharmacol* **570**: 89–96.

Zhang H, Torregrossa MM, Jutkiewicz EM, Shi Y-G, Rice KC, Woods JH, *et al* (2006). Endogenous opioids upregulate brain-derived neurotrophic factor mRNA through delta- and micro-opioid receptors independent of antidepressant-like effects. *Eur J Neurosci* **23**: 984–94.

Zhang J, Liang L, Miao X, Wu S, Cao J, Tao B, *et al* (2016). Contribution of the Suppressor of Variegation 3-9 Homolog 1 in Dorsal Root Ganglia and Spinal Cord Dorsal Horn to Nerve Injury-induced Nociceptive Hypersensitivity. *Anesthesiology* **125**: 765–78.

Zhang J, Shi XQ, Echeverry S, Mogil JS, Koninck Y De, Rivest S (2007b). Expression of CCR2 in both resident and bone marrow-derived microglia plays a critical role in neuropathic pain. *J Neurosci* **27**: 12396–12406.

Zhang M, Balmadrid C, Kelley AE (2003). Nucleus accumbens opioid, GABAergic, and dopaminergic modulation of palatable food motivation: contrasting effects revealed by a progressive ratio study in the rat. *Behav Neurosci* **117**: 202–11.

Zhang M, Kelley AE (2000). Enhanced intake of high-fat food following striatal mu-opioid stimulation: microinjection mapping and fos expression. *Neuroscience* **99**: 267–77.

Zhang X, Araujo Lucas G de, Elde R, Wiesenfeld-Hallin Z, Hökfelt T (2000). Effect of morphine on cholecystokinin and mu-opioid receptor-like immunoreactivities in rat spinal dorsal horn neurons after peripheral axotomy and inflammation. *Neuroscience* **95**: 197–207.

Zhang X, Bao L, Shi TJ, Ju G, Elde R, Hökfelt T (1998). Down-regulation of mu-opioid receptors in rat and monkey dorsal root ganglion neurons and spinal cord after peripheral axotomy. *Neuroscience* **82**: 223–40.

Zhao M-G, Ko SW, Wu L-J, Toyoda H, Xu H, Quan J, *et al* (2006). Enhanced Presynaptic Neurotransmitter Release in the Anterior Cingulate Cortex of Mice with Chronic Pain. *J Neurosci* **26**: 8923–8930.

Zhao X-Y, Liu M-G, Yuan D-L, Wang Y, He Y, Wang D-D, *et al* (2009). Nociception-induced spatial and temporal plasticity of synaptic connection and function in the

hippocampal formation of rats: a multi-electrode array recording. *Mol Pain* **5**: 55.

Zhao Z-Q, Gao Y-J, Sun Y-G, Zhao C-S, Gereau RW, Chen Z-F (2007). Central serotonergic neurons are differentially required for opioid analgesia but not for morphine tolerance or morphine reward. *Proc Natl Acad Sci* **104**: 14519–14524.

Zhou Y-Q, Liu Z, Liu Z-H, Chen S-P, Li M, Shahveranov A, *et al* (2016). Interleukin-6: an emerging regulator of pathological pain. *J Neuroinflammation* **13**: 141.

Zhu J, Wei X, Feng X, Song J, Hu Y, Xu J (2008). Repeated administration of mirtazapine inhibits development of hyperalgesia/allodynia and activation of NF-κB in a rat model of neuropathic pain. *Neurosci Lett* **433**: 33–37.

Zhu Z, Wang G, Ma K, Cui S, Wang J-H (2017). GABAergic neurons in nucleus accumbens are correlated to resilience and vulnerability to chronic stress for major depression. *Oncotarget* **8**: 35933–35945.

Zhuang Z-Y, Gerner P, Woolf CJ, Ji R-R (2005). ERK is sequentially activated in neurons, microglia, and astrocytes by spinal nerve ligation and contributes to mechanical allodynia in this neuropathic pain model. *Pain* **114**: 149–59.

Zimmer A, Valjent E, Konig M, Zimmer AM, Robledo P, Hahn H, *et al* (2001). Absence of delta -9-tetrahydrocannabinol dysphoric effects in dynorphin-deficient mice. *J Neurosci* **21**: 9499–505.

Zimmermann M (2001). Pathobiology of neuropathic pain. *Eur J Pharmacol* **429**: 23–37.

ANNEX

REVIEW ARTICLE

Why mu-opioid agonists have less analgesic efficacy in neuropathic pain?

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