CHAPTER 2

Spinal plasticity of the nociceptive system: The role of central sensitisation in chronic pain states

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Introduction

Pain is critical to adequately protecting an organism from injuries. An illustration of this principle is the 'classic' bite or burning of the tongue when drinking hot coffee or tea after a visit to the dentist in which local anaesthetics were used. With no proper feedback from our environment we cannot stop damage to ourselves until it is too late and a tissue lesion has already occurred. A more dramatic example is provided by patients who suffer from hereditary sensory and autonomic neuropathies that make them chronically insensitive to pain (Indo et al., 1996; Cox et al., 2006; Cox et al., 2010). In the course of their lives, these patients undergo massive traumas mostly without realising it, endangering their well-being and general health, as fractures go unchecked and open wounds untreated (Bennett and Woods, 2014) – something that can become life-threatening.

At the other end of the spectrum there are congenital conditions that render individuals hypersensitive to pain or put them under constant pain, which severely deteriorates the quality of their life (Bennett and Woods, 2014). Chronic pain hypersensitivity syndromes can have various aetiologies, from peripheral nerve trauma to viral infection; and they affect millions of people worldwide (Breivik et al., 2006; Torrance et al., 2006; Van Hecke et al., 2014). These syndromes occur around an estimated median age of fifty and, while they affect both genders, they are more prevalent in women when all chronic pain syndromes are pooled together (Breivik et al., 2006; Torrance et al., 2006). As life expectancies have significantly been extended in the past few decades, chronic pain syndromes typically affect working-age populations and represent therefore a major public health issue.

Synaptic plasticity within the spinal cord, the first integration centre of the pain information, can significantly alter the nature of the stimuli perceived. More than a simple anatomical relay, the spinal cord is the site of a very dynamic integration of somatosensory inputs. In normal conditions noxious and innocuous stimuli are processed through distinct pathways. This separation is in part due to an extremely complex and highly adaptive network within the spinal cord. The functional state of a spinal nociceptive neuron in particular is the result

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of the balance between numerous excitatory and inhibitory inputs. A change in this balance will in turn cause numerous changes in the functional properties of the spinal nociceptive neurons, thereby altering how these neurons respond not only to noxious stimuli, but also to previously sub-threshold stimuli. For example, a sustained intense noxious stimulation triggers a state of hyperexcitability of the spinal neuronal circuitry known as activity-dependent central sensitisation (Woolf, 1983; Latrémolière and Woolf, 2009; Woolf, 2010). When in a state of central sensitisation, spinal nociceptive neurons display one or all of the following features: an increase in excitability and response to noxious stimuli; an enlargement of their receptor field; and an ability to respond to innocuous stimuli, which means that they shift from a nociceptive-specific state to a functional state where they are activated non-selectively by a wide variety of stimuli. The phenomenon of central sensitisation has provided a mechanistic explanation for many of the temporal, spatial and threshold changes in pain sensibility in various chronic clinical pain settings. Therefore, if we want to develop new and more effective therapeutic strategies for treating chronic pain symptoms, it is critical that we understand the triggers and mechanisms responsible for this switch in the somatosensory system from the physiological state, in which the sensory experiences evoked by low-intensity stimuli (innocuous sensations) and by noxious stimuli (pain) are distinct and separate, to a dysfunctional hypersensitive system in which this discrimination is lost.

In this chapter we will first explain how a noxious stimulus is transmitted from the periphery to the central nervous system (CNS) and how this information can be modified at the spinal level through central sensitisation. We will discuss the cellular basis of this phenomenon and its consequences for pain perception. We will then describe the molecular triggers for central sensitisation in physiological states and the mechanisms responsible for the shift in the excitatory/inhibitory balance that occurs. In a third part we will examine how central sensitisation plays a fundamental role in the development and maintenance of pain hypersensitivity in two chronic pain syndromes: chronic inflammatory pain and neuropathic pain. Finally in the last section we will briefly discuss the possible involvement of this state in other diseases associated with abnormal pain symptoms, and will conclude with a short discussion about how it is conceivable that various perturbations within the CNS promote a central sensitisation state.

Nociceptive pain and nociception

Nociceptive pain is the physiological sensation caused by the activation of neural pathways by stimuli of sufficient intensity to potentially lead to tissue damage (for example a burn, a bite or excessive pressure). The detection per se of these stimuli is named 'nociception' (from the Latin *nocere*, 'to do harm/be harmful' and *cepi* < *capere*, 'to catch, receive') and is a critical protective process that helps prevent injury by generating both a reflex withdrawal from the stimulus and an unpleasant sensation (pain) that promotes avoidance of any further contact with such stimuli. Nociception occurs through highly specialised sensory fibres called nociceptors whose cell bodies are located in the trigeminal ganglia (TG) in the cephalic region and in the dorsal root ganglia (DRG) in extracephalic regions. The sensory neurons detecting innocuous mechanical and thermal stimuli are also located in the TGs and DRGs. All sensory neurons have a characteristic pseudo-unipolar morphology. Upon leaving the soma, the same

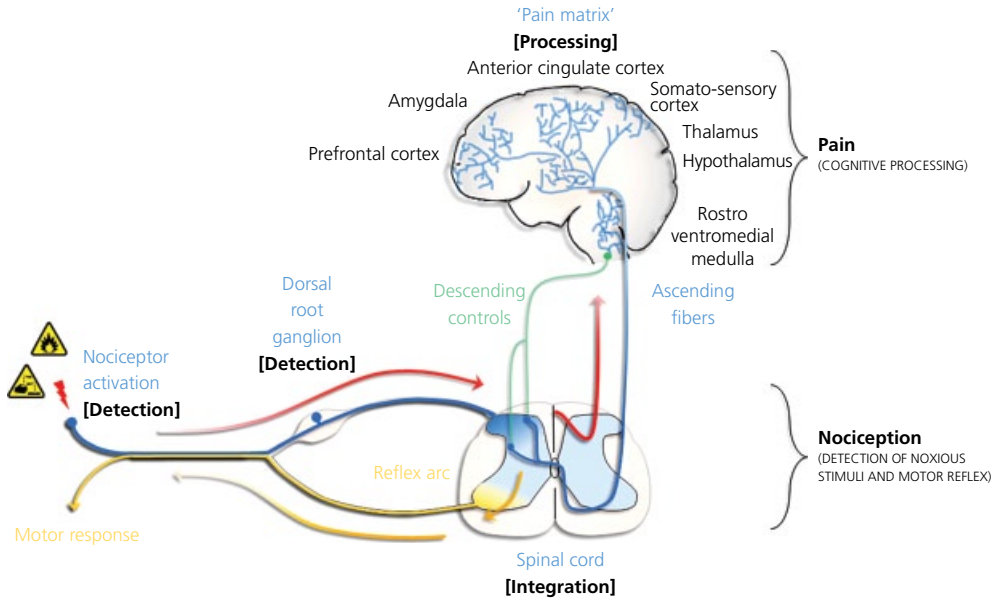


Figure 2.1 Nociception and pain. Nociception corresponds to the detection of a potentially harmful stimulus and the reflex motor response to withdraw a body region from the source of potential danger. Noxious stimuli are detected by specialized nerve fibres called nociceptors, whose cell bodies are located in the dorsal root ganglia. These fibres have a pseudo-unipolar morphology, where one branch of the axon goes to the periphery and the other contacts the second-order neurons in the spinal cord. The withdrawal motor response is triggered through a reflex arc in the spinal cord (marked in yellow). The excitability state of the projection nociceptive spinal neurons can be modulated by local (spinal) and supraspinal controls (the descending controls shown in green, which originate from the midbrain). As a result, the information sent to the brain for processing through the ascending fibres is the result of the integration of many signals that occur within the spinal circuitry. In the brain, several structures, collectively named the 'pain matrix', are involved in the cognitive processing of the nociceptive information that will eventually lead to the pain sensation – a complex experience, subjective in nature. The integration of the sensory signals in the dorsal horn of the spinal cord defines the nature of the nociceptive information sent to the brain, ultimately determining how much pain we will feel or for how long.

axon forms two branches, describing a 'T' shape. One branch innervates the peripheral tissues, where it detects environmental stimuli, while the other branch contacts the neurons within the CNS in order to transmit the neural information (Figure 2.1).

Nociceptors can be divided into two main classes on the basis of their size: Aδ nociceptors and C fibres nociceptors. Aδ nociceptors have a medium-diameter cell body and produce a thinly myelinated axon, allowing for a relatively fast transmission of the neural information (4–30 m/s). They are modality-specific (monomodal), so their activation leads to a well-localised and well-defined 'fast' pain sensation (Julius and Basbaum, 2001). C fibres are unmyelinated, slow-conducting (0.5–2 m/s) sensory fibres that originate in small-diameter neurons (Julius and Basbaum, 2001). They represent 60–90 per cent of all sensory neurons, depending on the species, and can be activated by stimuli of various modalities (thermal, mechanical or chemical); for this reason they are referred to as polymodal (Julius and Basbaum, 2001). Their activation leads to a 'slow' and diffuse pain sensation. Nociceptive C fibres can further be divided into two major classes on the basis of their cellular content: peptidergic and non-peptidergic. Peptidergic

C fibres produce substance P (SP) and calcitonin-related gene peptide (CGRP), two neuropeptides that trigger a massive vasodilatation at the periphery when they are released upon nociceptor stimulation (Holzer, 1988) and cause long-term depolarisation of the second-order neurons when they are released in the spinal cord. Peptidergic C fibres are sensitive to nerve growth factor (NGF), which binds to its tropomyosin receptor kinase A (TrkA) receptor; notably they express the ion channel – the transient receptor potential cation channel member 1 (TRPV1) – which is activated by capsaicin (the active compound found in red hot chili peppers), by acid pH and by heat (above 44°C). These peptidergic fibres are critical to the transduction of noxious heat stimuli (Cavanaugh et al., 2009), which explains why their activation by capsaicin causes a burning pain sensation. Non-peptidergic C fibres also express specific factors such as the ligand-gated ion channel purinergic receptor P2X3 or the G-protein-coupled receptor MRGPRD and are dependent on glia-derived neurotrophic factor (GDNF) (Molliver et al., 1997). Their role is not fully understood, but evidence suggests that they participate in the transduction of mechanical noxious stimuli (Cavanaugh et al., 2009). Recent advances in gene expression profiling from single-cell RNA sequencing have allowed to further define subclasses of sensory neurons on the basis of the pattern of the major genes they express (Usoskin et al., 2014).

The first relay for the transmission nociceptive information is the dorsal horn of the spinal cord, which displays a laminar organisation, mostly based on the fibres that contact the spinal neurons (Figure 2.2). Schematically, lamina I is contacted by both Aδ nociceptors and peptidergic C fibres – both nociceptors and fibres that detect innocuous temperatures (4 to 44°C). Lamina II is a region extremely rich in interneurons that can be further divided into two subregions. The outer region, named lamina IIo, is contacted mostly by peptidergic C fibres, whereas the inner region, called lamina IIi, is almost exclusively contacted by non-peptidergic C fibres. A subpopulation of excitatory interneurons that express the protein kinase C gamma (PKCγ) isoform (Malmberg et al., 1997) are located in the most ventral part of lamina IIi and are the target of non-nociceptive fibres only (Neumann et al., 2008). Laminae III and IV do not receive inputs from nociceptive fibres, but mostly from large-diameter myelinated fibres that convey fine tactile information (Aβ fibres). Finally lamina V, populated by convergent neurons (also named wide dynamic-range neurons), is contacted by Aβ fibres, Aδ nociceptors, and some – but only a few – peptidergic C fibres (Figure 2.2). There are relatively few ascending projection neurons that transfer the nociceptive information to the brain, and these are located mainly in laminae I and V (Gauriau and Bernard, 2002), although a few projection neurons have also been identified in lamina III (Todd, 2010). These projection neurons are under numerous modulatory inputs from local excitatory and inhibitory interneurons scattered throughout the dorsal spinal cord with a high density in lamina II (the region dominated by nociceptor input), as well as from descending controls originating in supraspinal structures (Millan, 2002). The fine anatomical organisation of the connectivity network within the spinal cord is not yet fully elucidated. See A Todd in this book for a comprehensive description and analysis of the anatomy of the spinal somatosensory pathways with particular emphasis on neuronal populations and their synaptic connections. What is clear, however, is that the signals sent by the projection neurons that will ultimately determine in the brain how much pain we feel in response to what stimuli and for how long are the result of the integration of these local and distant excitatory and inhibitory synaptic

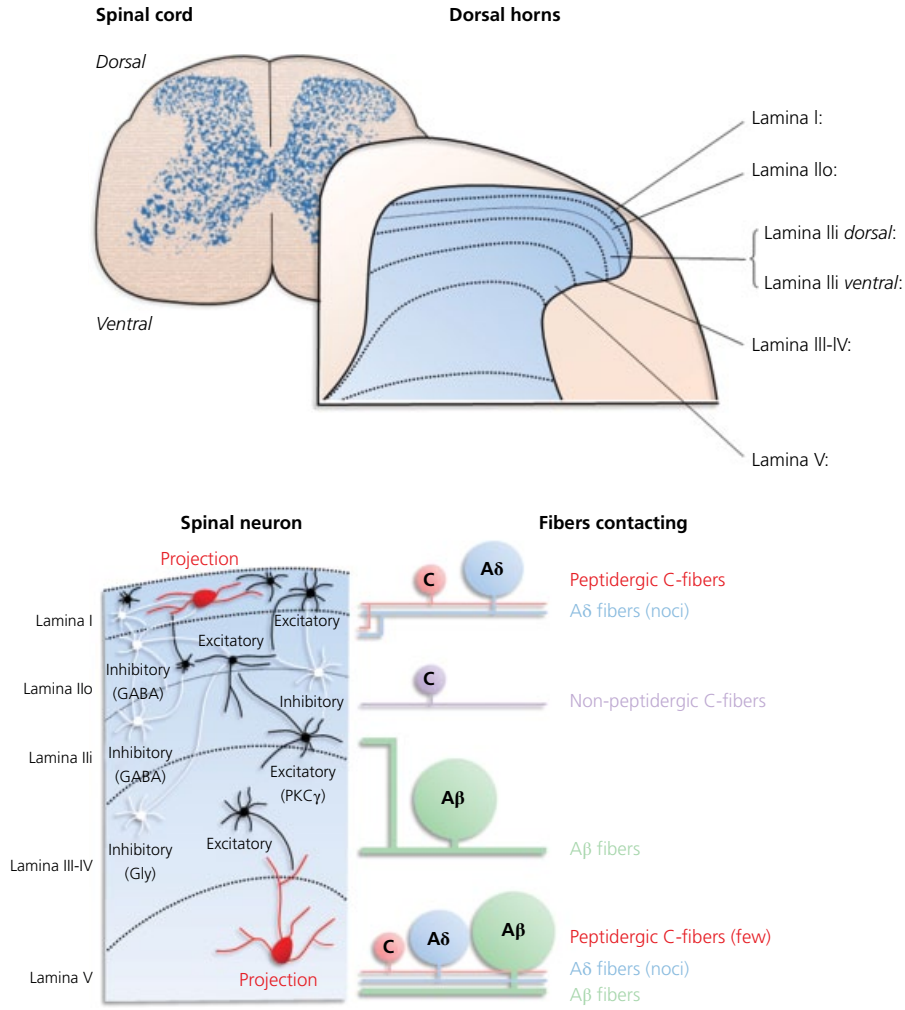


Figure 2.2 Laminar organization of the dorsal horn of the spinal cord, types of neurons and contacting sensory fibres. **Top Panel**: anatomy of the spinal cord at the lumbar level. In blue are the cell somata (bodies) that form the 'grey matter', surrounded by ascending and descending fibres tracks (the 'white matter'). The dorsal horns are organized in lamina, on the basis of their fine anatomy (density and size of neurons) and the fibres that contact them. Laminar boundaries are marked by dotted lines. **Bottom Panel**: there are few projection nociceptive neurons, and they are located mainly in laminae I and V (projection neurons are shown in red). Lamina II, the region dominated by nociceptor input, consists of excitatory glutamatergic (back) and inhibitory GABAergic (white) interneurons. Interneurons that can modulate the projection nociceptive neurons are also found in laminae III–IV, notably the glycinergic inhibitory interneurons. Laminae I and IIo are contacted by peptidergic C fibre nociceptors (in red) and by Aδ nociceptors (in blue), whereas the dorsal part of lamina IIi is only contacted by non-peptidergic C fibres (in purple). The excitatory interneurons of the ventral part of lamina IIi that specifically express the PKCγ isoform are contacted by a small contingent of Aβ fibres (in green), whereas most of these fibres project into laminae III–IV (non-nociceptive lamina). Projection neurons of lamina V (known as 'dynamic wide range' or 'convergent' neurons) are contacted by a small number of peptidergic C fibres, but mostly by Aδ and Aβ fibres.

inputs (Millan, 2002; Torsney and MacDermott, 2006). In other words, the pain we experience is not solely defined by the sensory fibres that are activated but ultimately by the nature of the integrated signal emitted by the spinal nociceptive projection neurons. The state of excitability of the spinal projection neuron is therefore critical to a proper discrimination of noxious stimuli. Altering this state will change how peripheral signals are integrated and will ultimately determine which stimuli are considered painful and which ones are not.

Synaptic plasticity of the nociceptive system

A fundamental characteristic of the nociceptive system is that it can be sensitised, notably after repeated or sustained noxious stimulations or in the presence of pro-inflammatory factors. The sensitisation of nociceptors is called peripheral sensitisation (Gold and Gebhart, 2010; Basbaum et al., 2009) and plays a major role in external injuries where there is a local inflammation at the wound site (Basbaum et al., 2009). Numerous immune cells and the injured epithelial cells release in concert pro-inflammatory factors such as NGF, serotonin (5-HT), prostaglandins, bradykinin, histamine, adenosine tri-phosphate (ATP) and protons (tissue acidosis) collectively referred to as an inflammatory soup (Kessler et al., 1992), which sensitise the nociceptors and lower their activation threshold (Woolf and Ma, 2007). The sensitised nociceptive fibres contribute to symptoms such as heat hyperalgesia, an increased pain response to a noxious heat stimulus; heat allodynia, when an innocuous temperature causes a sensation of pain; and hypersensitivity to pressure applied directly at the site of the wound (Woolf and Ma, 2007). This increased sensitivity is restricted to nociceptors that are in contact with the inflammatory agents. For example, the intradermal injection of NGF at low doses causes heat and pressure hyperalgesia, in line with the effects of nociceptive C fibres sensitisation, whereas fine tactile sensations mediated by Aβ fibres are not affected (Dyck et al., 1997).

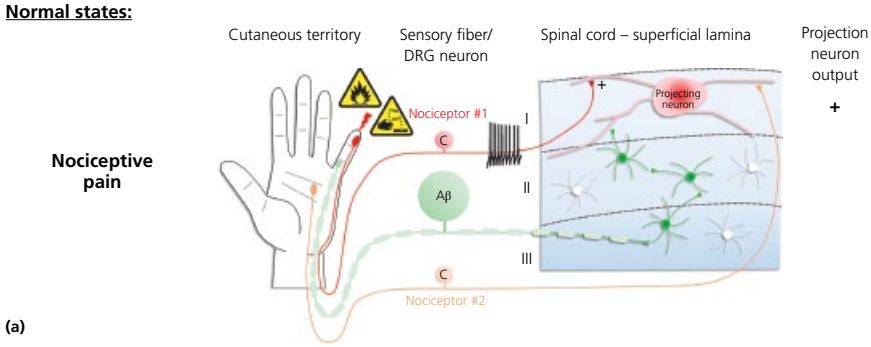
The enhancement in the functional status of nociceptive neurons can occur directly, at the spinal cord level, and is then referred to as central sensitisation (Latrémolière and Woolf, 2009, 2010). This form of plasticity is prominent in projection nociceptive neurons of lamina I in the dorsal horn of the spinal cord (Cook, Woolf and Wall, 1986; Woolf and King, 1990; Dougherty and Willis, 1992; Lin et al., 1997; Mantyh et al., 1997; Lin et al., 1999) but has also been detected in projection neurons located in lamina V (Pezet et al., 2008; Roh et al., 2008) and in the spinal nucleus *pars caudalis* (Sp5c) (Burstein and Jakubowski, 2004). Central sensitisation is characterised by three main functional features: an increase in the excitability of spinal nociceptive neurons and in their response to noxious stimuli; the enlargement of their receptor field; and their ability to respond to innocuous stimuli (Latrémolière and Woolf, 2009). The receptor field of a nociceptive spinal neuron is defined by the sensory inputs that can produce a significant action potential output from this cell. In other words it corresponds to the sensory fibres whose activation will systemically lead to the generation of a pain signal from the nociceptive neuron and will therefore define the area of peripheral tissue 'probed' by the spinal nociceptive neuron. In normal conditions, the nociceptive-specific neurons of lamina I are mostly activated by large mono- and polysynaptic inputs from nociceptors (Dahlhaus, Ruscheweyh and Sandkuhler, 2005; Torsney and MacDermott, 2006), but they also receive inputs from nociceptors outside of their receptive field, as well as polysynaptic

inputs from low-threshold afferents (Aβ fibres; Torsney and MacDermott, 2006). These additional inputs cause a small amplitude response from the cell, but they are insufficient to drive a functional output on their own. They represent structural but functionally silent connections between the nociceptive neurons and the sensory fibres (Figure 2.3). Their recruitment during central sensitisation is the substrate for receptive field plasticity and provides a mechanistic explanation for pain symptoms such as secondary hyperalgesia and mechanical allodynia (Latrémolière and Woolf, 2009). Secondary hyperalgesia is defined by an increased pain sensitivity in territories adjacent to the site of injury. This phenomenon is not caused by the local diffusion of inflammatory mediators, as it persists even after local blood flow is blocked (Lamotte et al., 1991). Blocking the nociceptive fibres located outside the injury site abolishes this symptom, indicating that it is mediated through the recruitment of novel nociceptors (Figure 2.3). Similarly, mechanical allodynia, defined by the ability of innocuous stimuli such as a light touch or a gentle brush of the skin to cause pain, corresponds to the recruitment of polysynaptic inputs from Aβ fibres that are now sufficient to activate potentials through the nociceptive neurons (Torebjork, Lundberg and Lamotte, 1992; Koltzenburg, Torebjork and Wahren, 1994; Woolf and Doubell, 1994; Ziegler et al., 1999) (Figure 2.3). Although mechanical allodynia is one of the major abnormal pain sensations experienced in chronic pain diseases, it also occurs after minor and transient injuries, where it serves a protective purpose. For example, the development of mechanical allodynia in the irradiated area after sunburn is caused by the central sensitisation of spinal nociceptive neurons (Gustorff et al., 2004; Gustorff et al., 2013). This hypersensitivity to such low-threshold stimuli forces the organism to adapt its global behaviour so as to fully isolate and protect the injured area until recovery. When recovery has occurred, the nociceptive system returns to its original baseline levels, where high threshold stimuli are again required for causing nociceptive pain.

Homosynaptic versus heterosynaptic potentiation

Because the activation of Aβ fibres does not normally trigger central sensitisation (Woolf, 1983) but can activate nociceptive neurons once they have been sensitised (Thompson, Woolf and Sivilotti, 1993), this represents a form of heterosynaptic potentiation (Ji et al., 2003; Latrémolière and Woolf, 2009), where activity in one synapse enhances activity in previously non-activated synapses, typically by 'sensitising' the entire neuron (Figure 2.4). This contrasts with the phenomenon of homosynaptic potentiation, which corresponds to an use-dependent facilitation of one synapse evoked by the activation of that same synapse, and is referred to as long-term potentiation (LTP) (Bliss and Collingridge, 1993) (Figure 2.4). At the spinal cord level, homosynaptic facilitation is observed in lamina I projection neurons after C fibres stimulation (Ikeda et al., 2003; Ikeda et al., 2006; Drdla and Sandkuhler, 2008). While LTP between C fibres nociceptors and projection nociceptive neurons provides a mechanistic explanation for behavioural manifestations such as hyperalgesia, it cannot account for the expansion of nociceptive receptor fields or for the recruitment of innocuous fibres, two major features of central sensitisation (Figure 2.4). The mechanisms underlying the heterosynaptic potentiation observed during central sensitisation are not yet understood. They could involve intrinsic properties of spinal nociceptive projection neurons or be the consequence of specific anatomical characteristics of

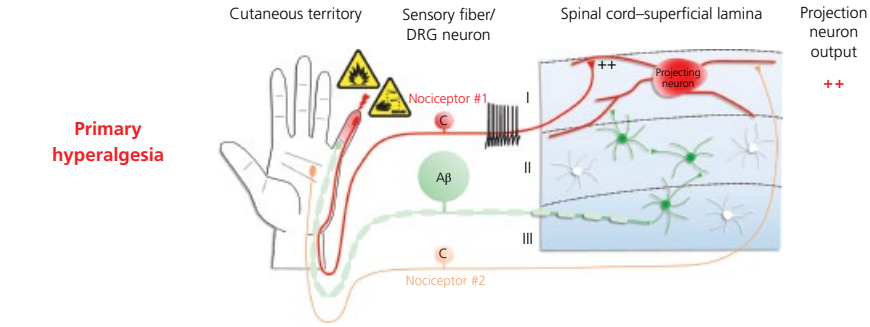
Normal states:



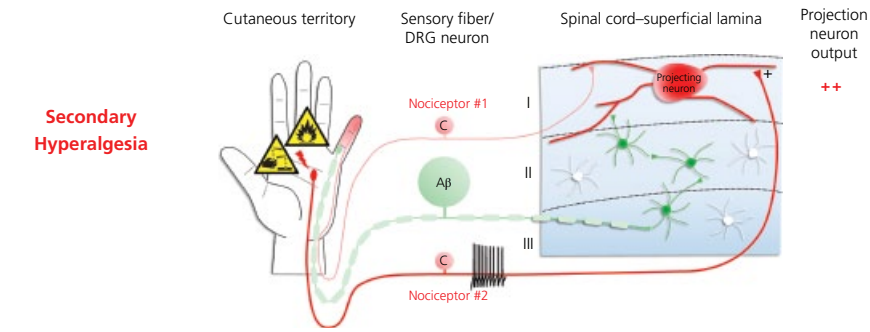
(a)

Central sensitisation:

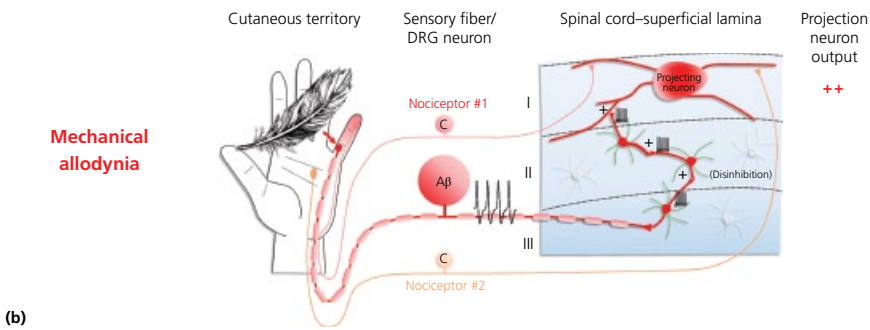
B1 - Increased sensitivity:



B2 - Increase in nociceptive receptive fields:



B3 - Recruitment of non-nociceptive fields:



(b)

the spinal nociceptive circuitry (Latrémolière and Woolf, 2009, 2010; Sandkuhler, 2010; Naka, Gruber-Schoffnegger and Sandkuhler, 2013). At a more integrated level, the fact that spinal nociceptive neurons are prone to developing heterosynaptic facilitation when stimulated represents a fundamental difference from the homosynaptic facilitation exhibited by most of the other neurons of the CNS – like the LTP produced by hippocampal neurons, which is thought to underlie memory formation (McGaugh, 2000; Goosens and Maren, 2002; Collingridge, Isaac and Wang, 2004; Malenka and Bear, 2004). Memory is a convergent neural operation where the storage of defined information is the consequence of coincident temporal and spatial association between specific stimuli. This convergent phenomenon is critical for the establishment of memory engrams: each specific stimulus needs to be encoded in its context, together with other stimuli that occurred at the same time. For one combination of events – all encoded by specific synapses – one unique engram is formed. In contrast, central sensitisation represents a divergent neural operation, where a conditioning nociceptive input triggers diffuse changes such that any other unstimulated synaptic inputs can now generate pain.

To be triggered, central sensitisation requires input from many C fibres nociceptors over tens of seconds; a single stimulus, such as a pinch, is insufficient (Woolf, 1983). Whereas peripheral tissue injury is not necessary to trigger central sensitisation, in effect the degree of noxious stimulation required almost always produces frank tissue injury. When such injury occurs, the role of the nociceptive system, now sensitised, is perhaps equally important as its preventive detection function. Central sensitisation enhances the overall defense role of the nociceptive system by placing the organism into a hyperalert state where any stimulus applied on the site of injury or in its close vicinity is perceived as a potential threat and triggers an avoidance reflex. Central sensitisation therefore represents an additional level of protection for an injured organism, one that is activated when the first line of defense – the detection of potentially harmful stimuli – has failed and damage has occurred.

Figure 2.3 Characteristics of a nociceptive neuron in a state of central sensitisation. **(a)** Schematic example of a projection neuron of lamina I (in red) contacted by a C fibre nociceptor #1 (in red) that can activate the nociceptive neuron when stimulated on its innervation territory (last digit). This nociceptor contributes to the receptive field of the projection neuron. C-fibre nociceptor #2 (in orange) innervates another cutaneous territory (middle of the hand) and contacts the projection neuron. Stimulation of nociceptor #2 is insufficient to activate the projection neuron and does not contribute to its receptive field. The Aβ myelinated fibre (in green) projects to lamina III; its stimulation does not activate the projection neuron. The activation of the projection nociceptive neuron is specific to noxious stimuli that activate the nociceptor 1. **(b)** Three main characteristics define a neuron in a state of central sensitisation: an increased sensitivity to nociceptive stimuli, an enlargement of the receptive fields and the recruitment of non-nociceptive fibres. **B1**: The stimulation of the nociceptor #1 now strongly activates the projection neuron in a state of central sensitisation (in bright red), causing primary hyperalgesia (a noxious stimulus causes increased pain response). **B2**: The activation of nociceptor #2 is now sufficient to activate the sensitized projection neuron, which has therefore 'extended' its receptive field. Because nociceptor #2 is outside of the normal receptive field of the projection neuron, the pain caused by its activation is referred to as secondary hyperalgesia. **B3**: The activation of the Aβ fibre by an innocuous stimulus can activate the projection neuron through a polysynaptic pathway from lamina III to lamina I (shown in red). This polysynaptic pathway is revealed through the phenomenon of disinhibition and causes allodynia (when an innocuous stimulus, such as a light touch represented by the feather, causes pain). In this state the projection nociceptive neuron is therefore not 'pain-specific': both noxious and innocuous stimuli activate it, ultimately causing pain.

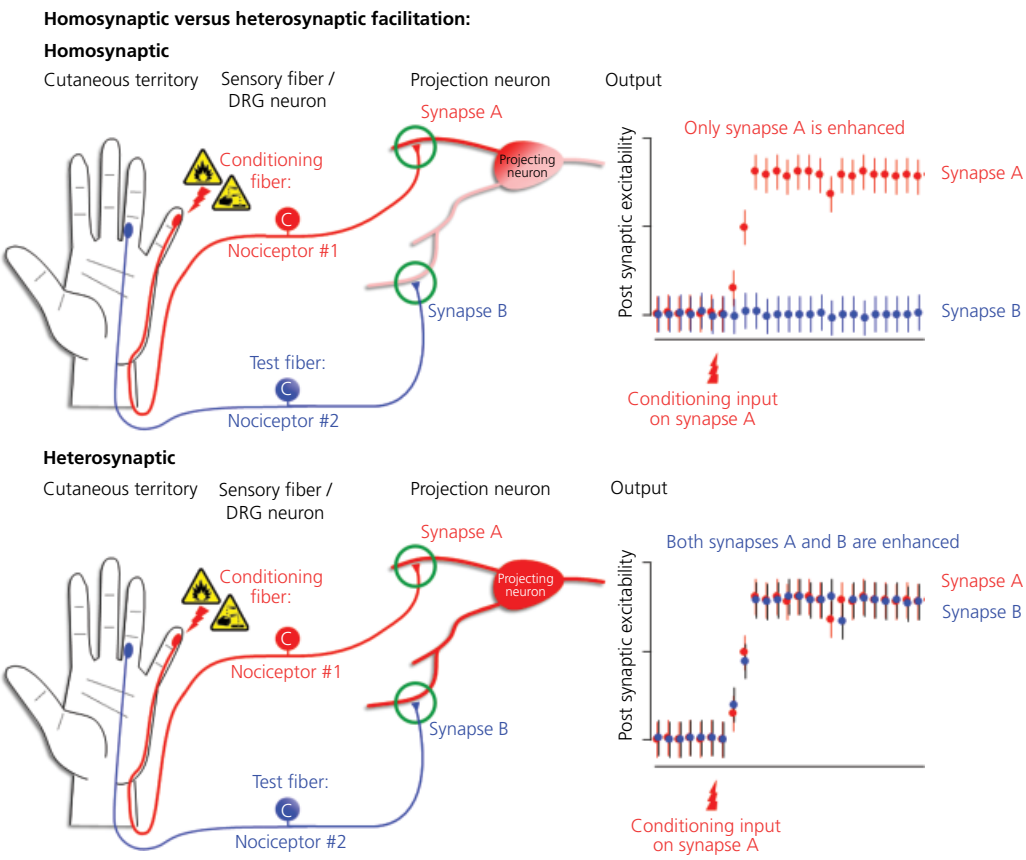


Figure 2.4 Schematic representation of a homosynaptic versus a heterosynaptic potentiation. **Top panel**: homosynaptic potentiation is a form of use-dependent facilitation where the conditioning fibre (nociceptor #1, in red) potentiates the strength of the same synapse (synapse A). The non-conditioned synapse (synapse B) is not potentiated, so the stimulation of the test fibre (nociceptor #2, in blue) does not change the output of the projection neuron. This type of potentiation is commonly called 'long-term potentiation' (LTP). LTP-like homosynaptic potentiation can contribute to primary hyperalgesia: nociceptor #1 causes more activation upon stimulation. **Bottom panel**: heterosynaptic potentiation represents a form of activity-dependent facilitation in which activity in the conditioning fibre (nociceptor #1, in red) potentiates all synapses of the neuron (synapses A and B). Stimulation of the test fibre (nociceptor #2, in blue), which has not been stimulated before, can drive an increased output from the projection neuron. Heterosynaptic potentiation is responsible for the major sensory manifestations of central sensitisation: pain in response to low-threshold afferents (allodynia) and spread of pain sensitivity to non-injured areas (secondary hyperalgesia).

Mechanisms of central sensitisation

Central sensitisation can be triggered by a strong gain in nociceptive inputs or by a reduction of the endogenous inhibitory brake on the spinal nociceptive neurons. Typically any intense and sustained input from C-nociceptors is sufficient for triggering central sensitisation. In experimental settings this can be provoked by a sustained hot stimulation (49°C for 10s) or

by selectively activating nociceptive fibres without causing actual peripheral damage – for example by recruiting C fibres through electrical stimulation (1 Hz for 10–20s) (Woolf and Thompson, 1991), or through chemical activation by irritant compounds such as allyl isothiocyanate (mustard oil) and formalin, which both act through the TRPA1 channel (Jordt et al., 2004; McNamara et al., 2007), as well as by capsaicin (Simone, Baumann and Lamotte, 1989; LaMotte et al., 1991), which activates TRPV1 channels (Caterina et al., 1997). Triggering central sensitisation by a nociceptive barrage is referred to as activity-dependent central sensitisation.

Key excitatory neurotransmitters and receptors

Glutamate is the main transmitter of primary afferent neurons and binds to several receptors on postsynaptic neurons in the dorsal horn of spinal cord, including ionotropic amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA), N-methyl-D-aspartate (NMDA) and several G-protein-coupled glutamate receptor subtypes (mGluR). In the superficial lamina of the dorsal horn the three types of receptors are present in almost every synapse. Typically AMPA receptors (AMPARs) and NMDA receptors (NMDARs) are present at the core of the synapse, and metabotropic receptors sit at the periphery (Alvarez et al., 2000). In basal conditions, excitatory neurons of lamina I/II appear to express mostly the low Ca^{2+}-permeable GluR2 AMPAR subunits, whereas the inhibitory interneurons appear to preferentially express the highly Ca^{2+}-permeable GluR1 AMPAR subunits (Kerr, Maxwell and Todd, 1998; Tong and MacDermott, 2006). NMDARs are expressed in virtually all glutamatergic synapses in the dorsal horns of the spinal cord (Antal et al., 2008) and constitute a complex formed by two mandatory NR1 subunits associated with two NR2A, NR2B or NR2C subunits (Stephenson, Cousins and Kenny, 2008). The distribution of NMDAR heteromers is relatively lamina-specific, which suggests distinct physiological roles for the different subtypes: NR2A subunits are heavily found in laminae III–IV (Nagy, Watanabe, et al., 2004); NR2B are highly expressed in laminae I–II (Momiyama, 2000; Nagy, Watanabe, et al., 2004); and NR2D are mostly found in lamina I neurons (Hildebrand et al., 2014). Next to these ionotropic receptors sit the slower-acting metabotropic glutamate receptors. Most of the eight existing isoforms are expressed in the spinal cord (Valerio et al., 1997; Berthele et al., 1999; Alvarez et al., 2000), but group I mGluRs (mGluR1 and 5), coupled with Gαq-proteins, have a prominent role in pain processing and central sensitisation (Neugebauer, Chen and Willis, 1999; Karim, Wang and Gereau, 2001; Price et al., 2007).

At baseline states the NMDAR channel is blocked by a magnesium (Mg^{2+}) ion sitting in the receptor pore (Mayer, Westbrook and Guthrie, 1984). Acute pain responses caused by the detection of intense thermal, mechanical or noxious chemicals (nociceptive pain) do not require the activation of the NMDARs (South et al., 2003), which suggests that they are mostly mediated by the activation of the AMPARs. Activation of the NMDARs is, however, essential to any form of synaptic plasticity in spinal nociceptive neurons. Blocking the NMDARs through pharmacological (Woolf and Thompson, 1991; Ma and Woolf, 1995) or genetic tools (South et al., 2003) fully prevents any form of synaptic plasticity of the nociceptive system. NMDAR blockade by Mg^{2+} is voltage-dependent and can only be removed

when the membrane potential is sufficiently depolarised (Mayer et al., 1984). Such depolarisation typically occurs after a very intense or prolonged noxious stimulus and involves the activation of the AMPARs through glutamate effects as well as through other key neurotransmitters.

Most of the nociceptive fibres that contact projection spinal neurons express the neuropeptide SP (Todd, 2010). Noxious stimuli trigger the release of SP (Afrah et al., 2002), which binds to the neurokinin-1 (NK1) G-protein–coupled receptor expressed by the spinothalamic, spinoparabrachial, and spino-PAG neurons – the major populations of spinal projection neurons that are essential to the development of central sensitisation (Mantyh et al., 1997; Khasabov et al., 2002; Willis, 2002). The binding of SP to its receptor causes a long-lasting membrane depolarisation (Henry, 1976), thereby allowing the temporal summation of C fibre-evoked synaptic potentials (Dougherty and Willis, 1991; Xu, Dalsgaard and Wiesenfeld-Hallin, 1992a, 1992b). In addition to SP, both CGRP and brain-derived neurotrophic factor (BDNF), also synthesised by small-diameter sensory neurons, can further promote a temporal summation of postsynaptic excitatory potentials upon nociceptive stimulations (Zhou and Rush, 1996; Kerr et al., 1999; Thompson et al., 1999; Balkowiec and Katz, 2000; Heppenstall and Lewin, 2001) (Figure 2.5). This sustained release of glutamate and neuropeptides by nociceptors at a low frequency (0.3–5 Hz) increases the postsynaptic excitability of the nociceptive neuron, a phenomenon known as 'wind-up' (Mendell and Wall, 1965). Such stimulation paradigm is within the physiological range of the activity of C fibre nociceptors upon noxious stimulation (Iggo, 1960; Kress et al., 1992), although they can transiently exhibit a peak of their frequency discharge (greater than 10 Hz; Kress et al., 1992). The gradual increase in membrane potential observed during wind-up allows a temporal and a spatial summation of the excitatory inputs onto the nociceptive neuron. This eventually leads to sufficient membrane depolarisation to remove the Mg^{2+} from NMDAR, which activates this channel (Davies and Lodge, 1987; Dickenson and Sullivan, 1987).

Entry of calcium and signaling cascades

In this state, glutamate binding to NMDARs causes the channel to open, allowing a massive sodium and Ca^{2+} influx into the cell; this leads to a long-lasting membrane depolarisation (Dingledine, 1983). Whereas wind-up is critical to the establishment of activity-dependent central sensitisation, it does not by itself correspond to a state of generalised excitability, but rather represents the excitability state of a neuron during the conditioning paradigm by nociceptors activity. This activation of NMDARs represents a major boost in synaptic strength and is the key trigger for activity-dependent plasticity of the spinal nociceptive neurons (Woolf and Thompson, 1991).

Heightened intracellular levels of Ca^{2+} are critical to initiating activity-dependent central sensitisation (Ikeda et al., 2003). Most of this increase is due to the massive entry of calcium from the extracellular milieu through NMDARs (MacDermott et al., 1986), AMPARs (Hartmann et al., 2004) and voltage-gated calcium channels (Coderre and Melzack, 1992), but also, to some extent, from the endoplasmic reticulum (Fagni et al., 2000) upon activation

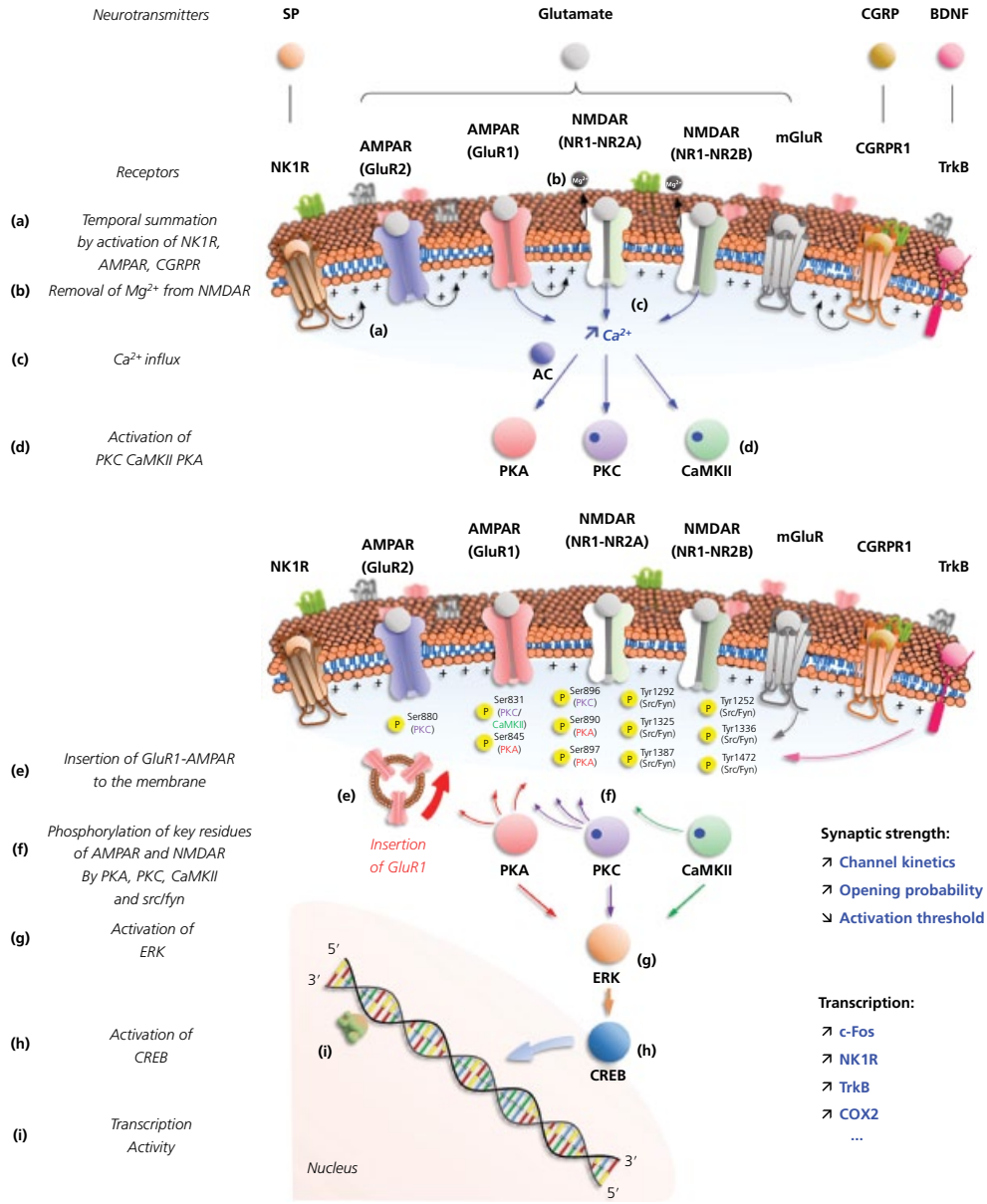


Figure 2.5 Schematic representation of the neurotransmitters, receptors and cellular cascades involved in central sensitisation. **Top panel**: neurotransmitters, receptors and cellular cascades involved in central sensitisation. **(a)** a barrage of activity in the nociceptors releases glutamate, SP and CGRP that bind to their receptors and cause a gradual increase in membrane potential, allowing a temporal and a spatial summation of the excitatory inputs onto the nociceptive neuron. **(b)** This leads to sufficient membrane depolarization to remove the Mg^{2+} from NMDAR, which activates this channel and results in **(c)** a rapid increase of $[Ca^{2+}]i$. **(d)** Rise in intracellular calcium activates PKC, CaMKII and PKA (through adenylyl cyclase (AC) activation). **Bottom panel**: **(e)** PKA activation promotes the insertion of Ca^{2+}-permeable GluR1-containing AMPARs into the membrane from vesicles stored under the synapse, further promoting Ca^{2+} entry into the cell. **(f)** PKA, PKC and CaMKII phosphorylate key residues of AMPAR and NMDAR NR1 subunits. NR2A and NR2B NMDAR subunits are phosphorylated by Src/Fyn kinases. These phosphorylations change the threshold and activation kinetics of NMDA and AMPA receptors, boosting synaptic efficacy. **(g)** PKA, PKC and CaMKII converge to activate the ERK pathway, which leads to the activation of CREB **(h)** and other transcription factors that drive the expression of several genes, including c-Fos, NK1, TrkB, and Cox-2, so as to produce a long-lasting strengthening of the synapse **(i)**.

of the phosphatidylinositol-3-kinase (PI3K) pathway (Pezet et al., 2002) triggered by group 1 metabotropic glutamate receptors (Guo et al., 2004).

The rise of intracellular levels of calcium leads to the activation of the calcium-dependent protein kinase C (PKC) and of the calcium–calmodulin-dependent protein kinase II (CAMKII) that phosphorylate NMDA and AMPA glutamate receptors on key residues (Carvalho, Duarte and Carvalho, 2000; Chen and Roche, 2007) (Figure 2.5). PKC phosphorylates NMDAR subunit NR1 on residue Ser896 (Leonard and Hell, 1997; Tingley et al., 1997; Carvalho et al., 2000), AMPAR subunit GluR2 on residue Ser880, and AMPAR subunit GluR1 on residue Ser831 – this residue being also the target of CAMKII (Carvalho et al., 2000). The phosphorylation of those serine residues leads to dramatic changes of the activation threshold, channel kinetics, and voltage dependence properties of the AMPARs and NMDARs, causing an increase of their activity and an overall postsynaptic hyperexcitability of the nociceptive neuron (Yu and Salter, 1999; Fang et al., 2002; Zou, Lin and Willis, 2002; Fang, Wu, Ling, et al., 2003; Fang, Wu, Zhang, et al., 2003; Sun, Lawand and Willis, 2003; Brenner et al., 2004; Sun et al., 2004; Zou, Lin and Willis, 2004; Jones and Sorkin, 2005; Ultenius et al., 2006; Liu et al., 2008). Once activated, PCK also reduces the Mg^{2+} block of NMDARs and increases their probability of channel opening, so that there are higher chances of NMDAR activation upon future glutamate release (Chen and Huang, 1992) – a mechanism that could explain why established central sensitisation can be maintained through a low activity of C fibres that normally cannot trigger this state (Koltzenburg, Lundberg and Torebjork, 1992).

The nociceptor-induced elevation in intracellular Ca^{2+} also activates adenylyl cyclases 1 and 8, whose cAMP production in turn activates protein kinase A (PKA) (Zou et al., 2002; Sun et al., 2003; Sun et al., 2004; Wei et al., 2006). PKA phosphorylates the NMDAR subunit NR1 on residues Ser890 and Ser897 (Leonard and Hell, 1997; Tingley et al., 1997), which increases the response of these receptors to glutamate (Chen and Huang, 1992; Raman, Tong and Jahr, 1996). NMDAR excitability can also be modulated through the phosphorylation of the NR2A and NR2B subunits, which are heavily expressed in the spinal cord by nonreceptor tyrosine kinases such as Src or Fyn. This leads to an increase in the probability of receptor opening as well as to changes in its conductance properties (Guo et al., 2002; Guo et al., 2004; Salter and Kalia, 2004; Chen and Roche, 2007) and prevents their endocytosis (Chen and Roche, 2007), further strengthening synaptic excitability (Figure 2.5).

In addition to phosphorylation of the receptors, which affects their intrinsic properties, central sensitisation also causes structural changes of the synapse. At basal states neurons in the superficial lamina of the dorsal horn mostly express GluR2 AMPAR subunits, which are relatively low Ca^{2+}-permeable. After nociceptive stimulation there is a rapid increase in the trafficking of GluR1 AMPAR subunits into the synapse (Figure 2.5). The phosphorylation of the AMPAR GluR1 residue Ser845 by PKA, together with the Ser831 by CAMKII, promotes the insertion of GluR1 into the synapse (Esteban et al., 2003; Galan, Laird and Cervero, 2004), which causes an increase in synaptic strength (Banke et al., 2000; Fang, Wu, Zhang, et al., 2003), as well as larger Ca^{2+} influx upon stimulation.

Stimuli that cause central sensitisation also cause a massive activation of extracellular signal-regulated kinase (ERK) in nociceptive neurons (Fang, Wu, Zhang, et al., 2003;

Kawasaki et al., 2004; Slack et al., 2005; Wei et al., 2006; Walker et al., 2007). This activation of the ERK pathway is the convergence point of cellular pathways of several activated receptors such as the NMDAR, group I mGluR, TrkB, NK1, or CGRP1R (Ji et al., 1999; Karim et al., 2001; Hu and Gereau, 2003; Lever et al., 2003). Once phosphorylated, ERK activates the cAMP response element-binding protein (CREB) (Shaywitz and Greenberg, 1999) that will drive the expression of several genes, including c-Fos, NK1, TrkB, and Cox-2 (Ji et al., 2002), thereby promoting the maintenance of the hyperexcitability state (Figure 2.5). These transcriptional changes might also contribute to the fact that the hyperexcitability caused by central sensitisation strongly outlasts the duration of the nociceptive stimulation, to an extent that is not likely to be explained solely by the phosphorylation of receptors (Simone et al., 1989; Woolf and King, 1990; Ji et al., 2003). In addition, they indicate that a nociceptive neuron in a state of central sensitisation has the cellular machinery ready for long-term structural changes.

Taken together, these changes caused by a barrage of nociceptive inputs produce dramatic functional alterations in the nociceptive neurons. In normal conditions, however, they are reversible. Phosphatases dephosphorylate receptors and ion channels so as to reset their activity to baseline levels; the increased trafficking of receptors to the membrane reverses through endocytosis; and, in time, the nociceptive synapses return to their basal state. Activity-dependent central sensitisation is therefore a transient phenomenon.

Loss of inhibitory signals (disinhibition)

The other major way to trigger a state of central sensitisation in spinal nociceptive neurons is by removing the inhibitory brake that is normally exerted on them. The nociceptive neurons of the spinal cord are indeed under strong inhibitory controls that originate locally (segmental inhibition) or distally (descending inhibition) (Roberts, Beyer and Komisaruk, 1986; Dickenson, Chapman and Green, 1997). Segmental inhibition is constituted by inhibitory GABAergic and glycinergic interneurons located in the dorsal horns (Sivilotti and Nistri, 1991) that drive a very high tonic inhibition of local nociceptive neurons; but their activity is also modulated by sensory inputs (Sivilotti and Woolf, 1994). GABAergic interneurons are predominant in superficial laminae (I–II; Tamamaki et al., 2003), where they release GABA locally, whereas glycinergic neurons have their soma mostly located in deeper laminae (III and lower; Todd, 1990) but are nonetheless critical for modulating lamina I projection neurons (Chery and De Koninck, 1999; Zeilhofer, Wildner and Yevenes, 2012). Due to their anatomical localisation, GABAergic interneurons are mostly contacted by nociceptive fibres (Alvarez, Kavookjian and Light, 1992) and glycinergic interneurons by large myelinated fibres (Todd, 1990; Watson, 2004). Upon release, GABA can act through three receptors: the ionotropic GABAA receptor and GABAC receptor and the metabotropic GABAB receptor (Bowery, Hudson and Price, 1987; Bormann, 2000). The chloride-permeable GABAAR is the prominent class of GABA receptors found in the dorsal horns of the spinal cord (Bohlhalter et al., 1996), where their activation causes strong analgesia (Knabl et al., 2008; Knabl et al., 2009). Similarly, the binding of glycine to its ionotropic receptor (glyR) causes the opening of this channel, which in turn allows a massive entry of chloride into the cell, leading to its inhibition (Chery and De Koninck, 1999).

A loss of segmental spinal inhibitory inputs leads to the phenomenon known as disinhibition, where dorsal horn neurons become more susceptible to activation through both nociceptive and non-nociceptive inputs (Figure 2.3). Disinhibition is a critical mechanism for triggering and maintaining central sensitisation (Yaksh, 1989; Sivilotti and Woolf, 1994; Malan, Mata and Porreca, 2002; Baba et al., 2003; Torsney and MacDermott, 2006). Indeed, an intrathecal injection of biccuculine (antagonists of GABAAR), strychnine (antagonists of GlyR) or phaclofen (antagonists of GABABR), or the genetic ablation or silencing of GlyR2-expressing spinal cord neurons in naïve animals induces spontaneous pain-like behaviours and causes mechanical allodynia to light touch stimuli (Beyer, Roberts and Komisaruk, 1985; Roberts et al., 1986; Sivilotti and Woolf, 1994; Loomis et al., 2001; Malan et al., 2002; Foster et al., 2015). When the inhibitory GABA/glycine inhibition tone is removed, this allows a polysynaptic activation of lamina I projection neurons by large-diameter morphine-insensitive fibres (Sherman and Loomis, 1994) – a process that involves the PKCγ excitatory interneurons (Miraucourt, Dallel and Voisin, 2007) located in lamina IIi and contacted by Aβ fibres (Neumann et al., 2008). Punctuate mechanical allodynia can therefore be triggered without any structural changes in the spinal cord, only by reducing the normal inhibition of the spinal nociceptive projection neurons.

Partial disinhibition also occurs during the establishment of activity-dependent central sensitisation, in which the activation of PKC causes a reduction in the sensitivity of GABA and glycine receptors to their ligands and a consequent reduction of their inhibitory tone on the nociceptive neurons (Lin, Peng and Willis, 1996).

The dorsal horns of the spinal cord are also contacted by several supraspinal projections that can modulate the excitability of the nociceptive system (Millan, 2002; Gebhart, 2004). In baseline conditions, these descending controls are mostly driving a tonic inhibition of the nociceptive neurons; but their activation upon nociceptive stimuli can either inhibit or amplify the excitability of the spinal circuitry (Gebhart, 2004). The neurons of the locus coeruleus that project into the spinal cord are noradrenergic (Kwiat and Basbaum, 1992; Millan, 1999). Their activation by noxious stimuli causes the release of norepinephrine, which in turn causes analgesia (Yaksh, 1985; Sullivan, Dashwood and Dickenson, 1987; Men and Matsui, 1994a) through its binding to α2-adrenergic receptors (Yaksh, 1985). Descending neurons of the raphe magnus produce serotonin (Willis et al., 1984; Rivot, Calvino and Besson, 1987) but can also express GABA, dynorphin and enkephalin (Millan, 2002). Descending controls from the raphe magnus nucleus are strongly activated by nociceptive stimuli (Puig, Rivot and Besson, 1992; Taguchi and Suzuki, 1992; Men and Matsui, 1994b). Once activated, they cause an inhibition of the spinal nociceptive neurons (Fields et al., 1977) through the release of serotonin – as well as through the co-release of other neurotransmitters. The inhibitory effects observed after the intrathecal injection of serotonin are mostly caused by the activation of Gi/o-coupled 5-HT_{1A}R expressed at the soma of nociceptive neurons (Bardin, Lavarenne, et al., 2000; Bardin, Schmidt, et al., 2000), whereas the activation of the Gi/o-coupled 5-$HT_{1B/1D}$R, expressed at the presynaptic level (Potrebic et al., 2003) presumably reduces the release of neurotransmitters from sensory fibre terminals (Carmichael, Charlton and Dostrovsky, 2008). 5-HT_3 receptors, the only serotonin receptors that are ligand-gated ion channels, cause a membrane depolarisation upon activation (Maricq et al., 1991). At the spinal cord level, 5-HT_3R is expressed in sensory fibres

terminals (Hamon et al., 1989) and in various nociceptive neurons, both excitatory and inhibitory (Laporte et al., 1996; Morales, Battenberg and Bloom, 1998; Tsuchiya, Yamazaki and Hori, 1999). Serotonin released in the spinal cord can therefore either inhibit or facilitate nociceptive transmission. The net effect of serotonin will eventually depend on which cellular targets are contacted, the types of receptors expressed, and the overall state of excitability of the raphe magnus neurons, which can itself be modulated by numerous brain areas (Heinricher et al., 2009; Rodriguez-Gaztelumendi et al., 2014).

It clearly appears on the whole that the nociceptive neurons are finely regulated by both inhibitory and excitatory controls. A loss of these controls, or a shift in a balance in favour of an increased excitability, would facilitate the transition of nociceptive neurons into a state of central sensitisation.

Central sensitisation in pathological states

Central sensitisation represents an additional level of protection for an injured organism. This phenomenon is triggered when the first line of defense, designed to detect potential harmful stimuli, has failed and damage has occurred. Once the nociceptive neurons of the spinal cord are in a state of central sensitisation, they no longer transmit information that reflects with precision the nature and intensity of environmental stimuli. Instead they transform any stimuli they receive into alert messages and convey a message that is uncoupled with its environment. When there is a transient injury, this represents an extremely efficient way to maintain a whole body area protected from any stimuli until its full recovery.

If this state of hypersensitivity is maintained over extended periods of time or is abnormally triggered, the resulting pain is no longer protective. This can happen when the source of the nociceptive input does not resolve, when the nociceptive system itself is damaged, or in any condition where there is an unbalance in the excitatory/inhibitory modulatory systems of the nociceptive system that affects spinal circuitry. Such an exaggerated hypersensitive state represents a veritable burden when it is maintained chronically. Then pain is not a protective alarm system any more, but becomes an ongoing nuisance for the organism and isolates it from its environment. In various disease states these changes have become permanent, literally transforming the first nociceptive relay into a new, hyperactive synapse that cannot discriminate and determine the nature of the information. Several key features of central sensitisation are present in pathologies with chronic pain symptoms such as neuropathic pain, chronic inflammatory diseases (rheumatoid arthritis, osteoarthritis), complex regional pain syndrome (CRPS), pancreatitis, whiplash injury, fibromyalgia, low-back pain or migraine (Woolf, 2010).

Because several redundant factors can trigger and maintain central sensitisation, it is perhaps not surprising that many changes that affect the spinal cord circuitry can cause chronic pain hypersensitivity. NMDAR (Seltzer et al., 1991; Mao et al., 1993; Cheng et al., 2008; Qu et al., 2009), AMPAR (Lu et al., 2008; Park et al., 2008), group I mGluR (Neugebauer, Lucke and Schaible, 1994; Young et al., 1997; Dogrul et al., 2000; Fundytus et al., 2002; Adwanikar, Karim and Gereau, 2004; de Novellis et al., 2004; Zhu et al., 2004; Giles, Trezise and King, 2007), group II–III mGluR (Simmons et al., 2002; Chen and Pan, 2005; Goudet et al., 2008; Zhang,

Chen and Pan, 2009), SP and CGRP (Abbadie et al., 1996; Abbadie et al., 1997; Lee and Kim, 2007) have all been shown to participate in the development and maintenance of central sensitisation in various preclinical models of chronic pain, through the same molecular and cellular mechanisms involved in activity-dependent central sensitisation. The following sections will describe mechanisms that are unique to chronic pain states due to either chronic inflammatory or peripheral nerve injury with regard to the development or maintenance of central sensitisation.

Chronic inflammatory pain

Chronic inflammatory pain is due to the ongoing activation of nociceptors when the source of the inflammation does not resolve. At the periphery, this persistent inflammation can result from the organism's incapacity to heal from an actual injury or from the development of an auto-immune reaction that will constantly produce and activate additional immune factors targeted against the organism's own cells. Inflammation causes peripheral sensitisation, which lowers the activation threshold of nociceptors and is often associated with hyperalgesia. However, it is the ongoing stimulation of nociceptive fibres together with additional pathways within the CNS that is responsible for the development of a chronic central sensitisation state and associated symptoms such as secondary hyperalgesia and mechanical allodynia. In what follows we will present the changes that cause either a gain in excitability or a loss of inhibition in the nociceptive system, which together lead to the maintenance of an abnormal hyperexcitable state.

Gain in excitability

Persisting peripheral inflammation causes several types of sensory neurons to dramatically change their transcription and translation pattern (Rodriguez Parkitna et al., 2006). In addition to the increase in transduction sensitivity and membrane excitability in nociceptive fibres, some sensory neurons undergo a phenotypic switch. Large-diameter neurons begin to express SP and BDNF (Neumann et al., 1996; Mannion et al., 1999), two peptides critical to the wind-up phenomenon. As a result, an activation of these fibres by innocuous stimuli leads to the release of these neurotransmitters, which could trigger and maintain central sensitisation (Indo et al., 1996; Neumann et al., 1996; Baba, Doubell and Woolf, 1999) (Figure 2.6).

A central pathway important for the development of inflammatory pain hypersensitivity involves the induction of cyclo-oxygenase-2 (COX2) in dorsal horn neurons. COX2 drives the production of prostaglandin E2 (PGE2) (Vasko, Campbell and Waite, 1994; Samad et al., 2001), which binds to its EP2 receptor located on dorsal horn neurons to potentiate both AMPAR and NMDAR currents and to activate non-selective cation channels that depolarise the potential membrane of nociceptive neurons (Baba et al., 2001). Genetic deletion of COX-2 only in neurons strongly attenuates the development of mechanical allodynia without altering heat hyperalgesia (Vardeh et al., 2009), which suggests an important role of this pathway in the development of central sensitisation in chronic inflammatory pain (Figure 2.6).

Under normal conditions microglia are the only immunocompetent cells of the nervous system, and they remain in a quiescent state while surveying the local milieu (DeLeo and Yezierski, 2001;

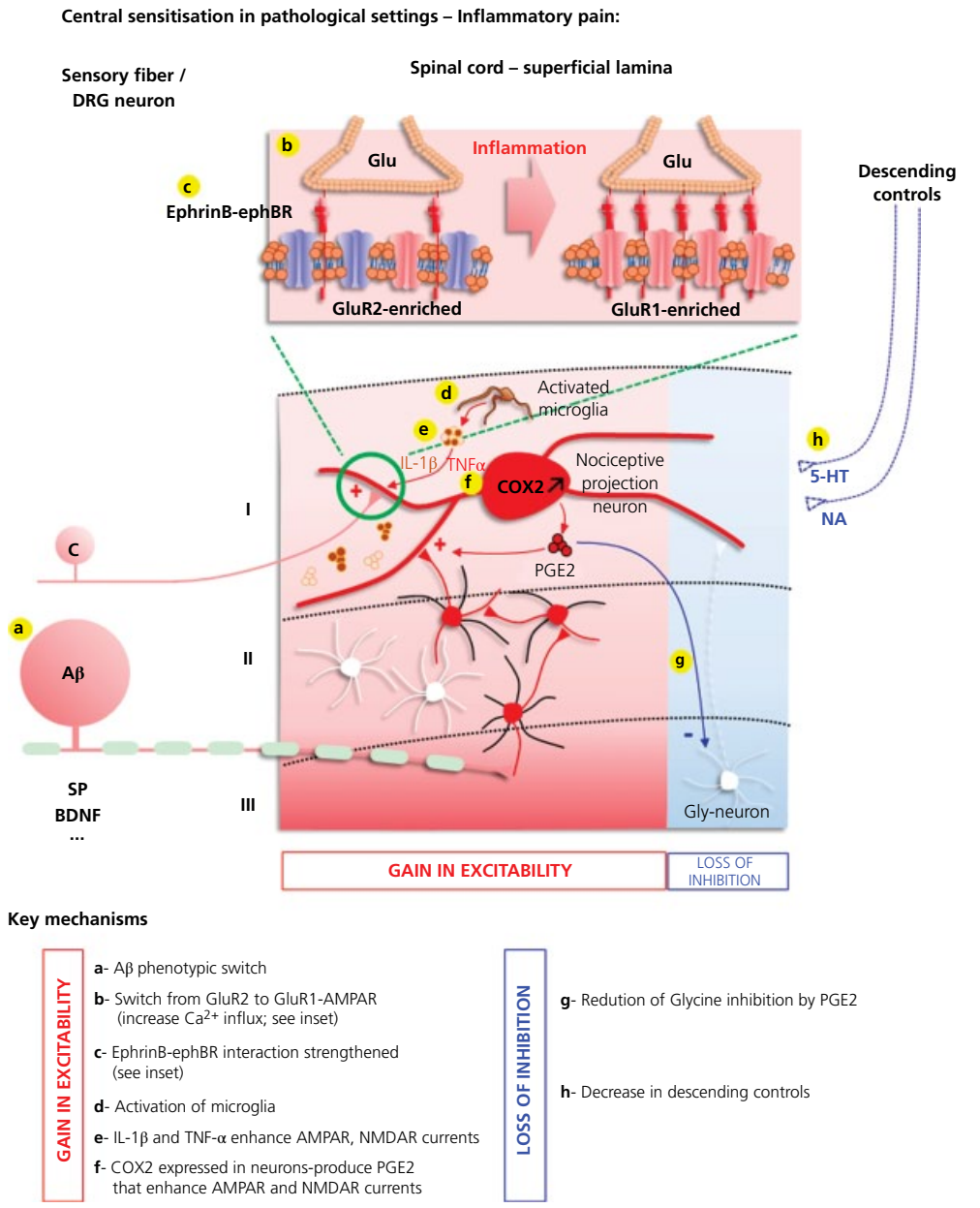


Figure 2.6 Schematic representation of the changes contributing to central sensitisation in the superficial lamina in chronic inflammatory states. **Gain in excitability**: Large DRG neurons undergo a phenotypic switch and **(a)** start to express SP and BDNF, so stimulation of these afferents acquires the capacity to generate long-term postsynaptic depolarization. **(b)** Neurons start to express GluR1-containing AMPAR at their synapse (see inset), which results in an increase of the Ca^{2+} influx on their activation. **(c)** The presynaptic element of the nociceptive synapse is also structurally strengthened though the EphrinB–ephBR interaction (see inset). The kinase activity of EphBR increases NMDAR activity, which potentiates synaptic activity. **(d)** Microglial cells are activated and release factors such as pro-inflammatory cytokines IL-1β and TNF-α, which **(e)** contribute to the development of central sensitisation by enhancing excitatory and reducing inhibitory currents. **(f)** Induction of cyclooxygenase-2 (Cox-2) in dorsal horn neurons drives the production of prostaglandin E2 (PGE2), which potentiate AMPAR and NMDAR currents, boosting post-synaptic excitability. **Loss of inhibition**: **(g)** PGE2 produced by dorsal horn neurons reduces glycine-mediated currents. **(h)** Inhibitory 5-HTergic and NAergic descending controls are diminished. Projection lamina I neuron is shown in red. Excitatory interneurons are in black (the polysynaptic activation pathway from Aβ is highlighted in red) and the inhibitory interneurons are in white. Laminae are marked I–III and separated by dotted lines.

Watkins, Milligan and Maier, 2001; Nimmerjahn, Kirchhoff and Helmchen, 2005). After peripheral inflammation these cells rapidly change their shape, their function and the factors they release (Watkins et al., 2001). In particular, the activation of the p38 MAPK pathway (Svensson, Hua, et al., 2003; Svensson, Marsala, et al., 2003) leads to the synthesis and release of pro-inflammatory factors, among them IL-1β and TNF-α, which contribute to the development of central sensitisation by enhancing excitatory currents (Honore et al., 2000; Raghavendra, Tanga and DeLeo, 2004; Kawasaki et al., 2008; Gruber-Schoffnegger et al., 2013) and by activating or maintaining Cox-2 activity (Figure 2.6). Blockade of microglial activation through the intrathecal administration of minocycline or through the administration of IL-1β receptor antagonist (IL-1ra) reduces the development of pain behaviours, notably thermal hyperalgesia and mechanical allodynia (Hua et al., 2005; Honore et al., 2006; Schreiber, Beitz and Wilcox, 2008; Zhang et al., 2008), whereas the intrathecal injection of IL-1β, but not of TNF-α is sufficient to cause mechanical allodynia (Reeve et al., 2000).

Peripheral inflammation causes a critical rearrangement of the excitatory synapses of the neurons of the superficial lamina that increases the strength of nociceptive transmission (Larsson and Broman, 2008; Vikman, Rycroft and Christie, 2008; Park et al., 2009). The majority of the AMPARs expressed by these neurons usually contain the relatively Ca^{2+}-impermeable GluR2 subunit (Petralia et al., 1997; Nagy, Al-Ayyan, et al., 2004). In peripheral inflammation states there is a shift from GluR2 to GluR1-containing AMPARs expressed at the membrane (Larsson and Broman, 2008; Vikman et al., 2008; Park et al., 2009). This shift is orchestrated by the action of PKA and PKC (Park et al., 2009; Kopach et al., 2013). The activation of the PKA is dependent on the TNF-a (Choi et al., 2010), which is probably produced and released by activated microglial cells (Sawada, Suzumura and Marunouchi, 1992; Raghavendra et al., 2004), and the activation of the PKC is triggered by the Ca^{2+} influx through NMDARs (Park et al., 2009). Once activated, the PKC phosphorylates the GluR2 subunit, thereby reducing its affinity for the scaffolding protein GRIP-1, which is needed to cluster the AMPARs at the synapse (Dong et al., 1997; Hirai, 2001). At the same time, the increase in intracellular Ca^{2+} activates the protein PICK-1 (Chung et al., 2000; Hanley and Henley, 2005; Park et al., 2009), which promotes the endocytosis of GluR2-containing AMPARs that removes them from the membrane (Matsuda, Mikawa and Hirai, 1999; Chung et al., 2000; Lin and Huganir, 2007; Terashima et al., 2008; Park et al., 2009). The reinsertion of the endocytosis vesicle requires the action of the N-ethylmaleimide-sensitive fusion protein (NSF) (Nishimune et al., 1998; Song et al., 1998; Noel et al., 1999; Huang et al., 2005), but the expression of this protein is strongly reduced in the spinal cord during inflammation (Katano et al., 2008). This further favours the insertion of GluR1-containing AMPARs at the synapse – but also at extrasynaptic sites of tonically active lamina II neurons (Kopach et al., 2011) – by PKA (Yang et al., 2011). Once established, GluR1-containing AMPARs are maintained at the membrane as long as there is synaptic activity (Ehlers et al., 2007; Man, Sekine-Aizawa and Huganir, 2007). With this expression pattern of AMPAR subunits, glutamate binding elicits a massive entry of Ca^{2+} into the cell, to levels comparable with NMDAR activation (Luo et al., 2008), which contributes to triggering and maintaining central sensitisation (Figure 2.6). This structural strengthening of the spinal excitatory synapse is also mediated through the rearrangement of the subcellular organisation

of metabotropic glutamate receptors, where group I mGluR5 are inserted into the membrane and mGluR1 are clustered closer to the synapse (Pitcher, Ribeiro-Da-Silva and Coderre, 2007). The resulting increased activation of mGluR promotes the excitability of NMDAR through the phosphorylation of its NR2B subunit, which is mediated in part by the activation of the Src cascade (Guo et al., 2002; Guo et al., 2004). Specific blockading of the Src cascade significantly attenuates signs of central sensitisation like mechanical allodynia without affecting nociceptive pain (Guo et al., 2002; Liu et al., 2008).

The presynaptic element of the nociceptive synapse is also structurally strengthened, notably though the EphrinB–EphBR interaction mostly present between C fibres and laminae I–II neurons (Figure 2.6). EphBRs are receptor tyrosine kinases only expressed by postsynaptic neurons, whereas EphrinB is anchored to the presynaptic membrane (Kullander and Klein, 2002). The kinase activity of EphBRs is critical to maintaining the clustering of NMDARs at the synapse (Dalva et al., 2000). The stimulation of EphBRs increases NMDAR activity, which potentiates the Ca^{2+} influx into the neuron and causes the phosphorylation of CREB through the activation Src (Takasu et al., 2002). The activation of EphBRs in naïve animals leads to a facilitation of C fibre input onto nociceptive neurons and to an increased sensitivity to heat, but does not cause mechanical allodynia (Battaglia et al., 2003). Inhibition of this pathway can prevent and reverse pain hypersensitivity in preclinical pain models of inflammatory pain without altering nociceptive pain (Battaglia et al., 2003; Slack et al., 2008). (For an extensive review of the experiments on the EphrinB/EphBR system and the spinal processing of noxious stimuli, see Chapter 7 in this volume.)

The overall hyperexcitability of the nociceptive neurons is further enhanced by an increase in descending facilitation, which is mediated notably through BDNF (Urban and Gebhart, 1999, Guo et al., 2006).

Disinhibition

The production of prostaglandin E2 by COX2 in neurons of the spinal cord selectively blocks glycinergic receptors containing α3 subunits (Ahmadi et al., 2002; Muller, Heinke and Sandkuhler, 2003; Harvey et al., 2004), thereby reducing the glycinergic-mediated inhibitory tonus on nociceptive neurons and promoting the phenomenon of disinhibition (Zeilhofer and Zeilhofer, 2008). In addition to this reduction in segmental inhibition, peripheral inflammation causes, over time, a severe loss of the descending inhibition normally mediated by NE and 5-HTergic fibres (Wei, Dubner and Ren, 1999) (Figure 2.6).

Neuropathic pain

Peripheral neuropathic pain is an extremely complex disease that can occur after lesions to the somatosensory system (Treede et al., 2008). The origin of the nerve lesion can be diverse: mechanical trauma (e.g., section, compression, crush), metabolic diseases (e.g., diabetes), neurotoxic chemicals, infection (e.g., HIV) or tumours. The nociceptive system itself is damaged and no longer processes sensory information adequately (Costigan, Scholz and Woolf, 2009; von Hehn, Baron and Woolf, 2012). As a result, there is a loss of tactile fine sensory perception (part of the so-called ‘negative symptom’), but also the apparition of pain hypersensitivity and

spontaneous pain attacks – positive symptoms that can persist indefinitely (Costigan, Scholz et al., 2009; for a detailed overview of neuropathic symptoms and pathology, see Chapter 3 in this volume). Central sensitisation plays a critical role in many aspects of neuropathic pain pathophysiology (Woolf, 2010). Key symptoms like mechanical allodynia and cold allodynia are mostly generated centrally, by nociceptive neurons in a chronic state of central sensitisation (Latrémolière and Woolf, 2009; Woolf, 2010). Upon peripheral nerve injury there are considerable changes that contribute to the development of abnormal pain sensitivity. In the following sections we will highlight those that can directly trigger or maintain a state of central sensitisation.

Gain in excitability

Nerve injury caused by a physical trauma – for example, nerve section or compression – causes a massive release of glutamate by the injured fibres into the spinal cord during the first hour after axotomy (Inquimbert et al., 2012). This amount of glutamate, once released, binds and activates AMPARs and NMDARs, which can trigger the cascades leading to central sensitisation. Pre-treatment with a NMDAR blocker such as ketamine or MK801 can prevent the establishment of pain hypersensitivity in various animal models of neuropathic pain (Smith et al., 1994; Kim et al., 1997; Munglani et al., 1999; Shields, Eckert, 3rd and Basbaum, 2003), and blocking the effects of an early axotomy-mediated glutamate release is sufficient to attenuate the development of hypersensitivity for several weeks (Munglani et al., 1999). Neither a preemptive nerve blocking through bupivacaine-loaded microspheres nor analgesic treatment with pregabalin (which blocks α2-δ1 voltage-dependent calcium channels: Field et al., 2006) prevents the development of neuropathic pain (Suter et al., 2003; Yang et al., 2014). This indicates that, whereas NMDAR activation is critical to initiating central sensitisation after peripheral nerve trauma, neuronal activity of the sensory fibres is not necessarily required.

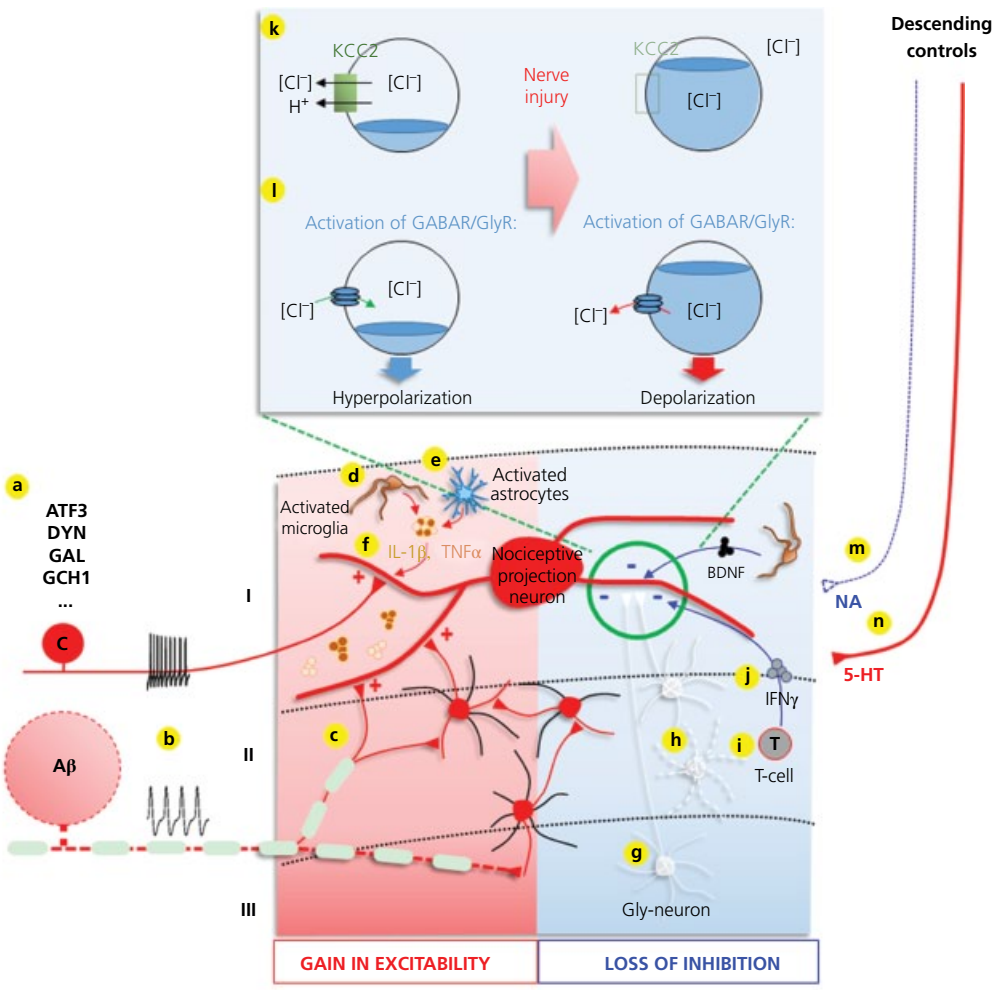
After peripheral nerve injury, both injured and non-injured sensory neurons in the dorsal root ganglion of the damaged nerve exhibit a massive change in transcription, which alters their membrane properties, their growth and their transmitter function (Fukuoka et al., 2001; Costigan et al., 2002; Xiao et al., 2002; Obata et al., 2003; Obata et al., 2004). In particular, there is a considerable production of BDNF, which enhances nociceptive transmission both presynaptically, by improving vesicular release through the activation of the Src and NMDARs, and postsynaptically, by enhancing NMDAR response through phosphorylation of its NR2B subunit (Kerr et al., 1999; Geng et al., 2010). At the spinal cord level, the BDNF activates the ERK cascade in nociceptive neurons of the superficial laminae, which drive the transcriptional activity required for long-term synaptic consolidation (Zhou et al., 2008). Enzymes responsible for determining the intracellular levels of the pteridine (6R)-L-erythro-5,6,7,8-tetrahydrobiopterin (BH4), an essential co-factor for aromatic amino acid hydroxylases (tyrosine and tryptophan hydroxylases) and nitric oxide synthase, are up-regulated in injured neurons (Tegeder et al., 2006), but also in macrophages that infiltrate the injured nerve and the DRGs (Latrémolière et al., 2015). An elevation in BH4 levels triggers an increased nitric oxide (NO) production (Tayeh and Marletta, 1989), which in turn sensitises ion channels such as TRPV1 and TRPA1 by nitrosylation (Miyamoto et al., 2009); these channels could mediate in part the NO-mediated thermal pain hypersensitivity caused by elevated BH4 levels only in sensory

neurons (Latrémolière and Costigan, 2011; Latrémolière et al., 2015). In addition, a lowered activation threshold for TRPV1 can lead to an increased probability that the channels would open at temperatures within the physiological range (Waxman et al., 1999), which can cause abnormal spontaneous neuronal activity (Hitomi et al., 2012).

Activity of the sensory fibres that is not triggered by external stimuli is known as ectopic activity and probably contributes to maintaining established central sensitisation (Koltzenburg et al., 1992; Devor, 2009). Neuropathic pain is often associated with two types of spontaneous pain symptoms: an ongoing 'burning' type of pain that could be mediated by the ectopic (and ongoing) activity of nociceptive C fibres (Baron, 2006); and a paroxysmal, 'electric' type of pain attacks that are thought to be caused by a burst of activity in larger, faster conducting sensory fibres (Baron, Binder and Wasner, 2010). In normal states, both myelinated and non-myelinated fibres exhibit very low tonic activity (Tanelian and MacIver, 1991). After peripheral nerve injury, however, they start displaying a high spontaneous discharge rate of action potentials. This ectopic activity can occur in large injured sensory fibres (Devor, 2009; Djouhri et al., 2012), in small-diameter injured C fibres (Seltzer and Devor, 1979; Omana-Zapata et al., 1997; Pan, Eisenach and Chen, 1999) or in small-diameter non-injured C fibres in close vicinity to damaged fibres (Wu et al., 2002; Djouhri et al., 2006; Djouhri et al., 2012) (Figure 2.7). Peripheral nerve blockade totally abolishes spontaneous pain symptoms in patients with neuropathic pain, which confirms its peripheral origin (Haroutounian et al., 2014). The precise mechanisms responsible for the genesis of ectopic activity are not yet fully understood, but evidence suggests a role for depolarising sodium channels ('Na(v)s') and for hyperpolarising potassium channels ('K(v)s'), which are both critical to neuronal excitability. The two TTX-resistant Na(v)1.8 and Na(v)1.9 exhibit changes in their axonal transport after nerve injury, so that there is an accumulation of these channels at the site of injury. This triggers local action potentials that can propagate to nearby fibres (Novakovic et al., 1998; Gold et al., 2003). Potassium channels, which are critical to maintaining normal resting membrane potential (Kang and Kim, 2006; Dobler et al., 2007), are heavily down-regulated after nerve injury (Everill and Kocsis, 1999), which significantly depolarises the cell membrane (Tulleuda et al., 2011) and allows a spontaneous firing of sensory neurons (Acosta et al., 2014). Ectopic activity in the injured nerve has been described in early and chronic phases of neuropathic pain. The administration of anticonvulsants (gabapentin, lamotrigine) reduces mechanical allodynia (Field et al., 1999; Christensen et al., 2001), possibly in part by disrupting the maintenance of central sensitisation (Koltzenburg et al., 1992; Rogawski and Loscher, 2004).

The activation of genetic programs designed to promote intrinsic axonal growth in injured neurons in order to reinnervate peripheral targets also gives the opportunity for rearrangements within the spinal cord. It has been suggested that myelinated Aβ fibres, normally confined within laminae III–IV, make direct contact with nociceptive neurons located in laminae I–II (this is the so-called 'sprouting' phenomenon: Woolf, Shortland and Coggeshall, 1992; Woolf et al., 1995; Lekan, Carlton and Coggeshall, 1996; Shortland, Kinman and Molander, 1997) (Figure 2.7). The original experiments describing sprouting used the cholera toxin B subunit as a selective tracer for A-fibres. The selectivity of this toxin is, however, altered after peripheral nerve injury (Tong et al., 1999; Shehab, Spike and Todd, 2003), which has probably led to an overestimation of the sprouting phenomenon (Hughes et al., 2003). Nevertheless, one of the

Central sensitisation in pathological settings – Neuropathic pain:



Key mechanisms:

GAIN IN EXCITABILITY

a- Changes in transcription profile

b- Ectopic activity of sensory fibers

c- Aβ fibers sprout into lamina II

d- Microglial activation

e- Astrocyte activation

f- IL-1β and TNF-α enhance AMPAR, NMDAR currents

LOSS OF INHIBITION

g- Reduction of Glycine inhibition

h- Apoptosis of GABAergic interneurons

i- Infiltration of T-cells that produce IFNγ

j- IFNγ reduces GABAergic currents

k- Loss of KCC2 causes a increase in $[Cl^-]_i$ (see inset top line)

l- Loss of GABAAR/GlyR effects (see inset bottom line)

m- Decrease in descending NA controls

n- Shift of descending 5-HT controls into excitatory

key clinical and preclinical manifestations of neuropathic pain is mechanical allodynia; and both immunostaining for c-Fos in the nociceptive neurons (a marker of neuronal sustained activation: see Hunt, Pini and Evan, 1987) and electrophysiological recordings have clearly established that peripheral nerve injury causes large myelinated fibres to activate nociceptive neurons in the superficial laminae (Bester, Beggs and Woolf, 2000; Okamoto et al., 2001; Kohno et al., 2003). The lack of evidence for a massive direct rewiring of myelinated fibres onto nociceptive neurons suggests that a functional plasticity is more plausibly responsible for the development of allodynia – which points to a critical role of central sensitisation in this behavioural state.

Peripheral nerve injury triggers an extensive gliosis in the spinal cord segments contacted by the damaged nerve (Garrison, Dougherty and Carlton, 1994; Colburn et al., 1997), especially near the entry point of the dorsal roots (Tsuda et al., 2003; Beggs and Salter, 2007, 2010). The activation of astrocytes and microglial cells has been correlated with the apparition of pain hypersensitivity in various preclinical neuropathic pain models (Sweitzer, Schubert and DeLeo, 2001; Sweitzer and DeLeo, 2002; Raghavendra, Tanga and DeLeo, 2003; Milligan et al., 2004; Ledeboer et al., 2005; Latrémolière et al., 2008). Preventing the activation of glial cells, especially microglia, at the time of nerve injury attenuates the development of abnormal pain behaviours (Raghavendra et al., 2003; Ledeboer et al., 2005; Latrémolière et al., 2008; Beggs and Salter, 2010). The initial recruitment and activation of spinal glial cells is triggered by primary afferent fibres, and a total nerve blockade of the sciatic nerve significantly reduces microglial activation and proliferation after axotomy (Wen et al., 2007; Suter et al., 2009). The targeted blockade of TRPV1-positive C fibres is not, however, sufficient to prevent the microglial activation induced by nerve injury (Suter et al., 2009). This suggests that large A fibres release specific factors required for microglial activation (Suter et al., 2009), or that

Figure 2.7 Schematic representation of the changes contributing to central sensitisation in the superficial lamina in neuropathic pain. **Gain in excitability**: After peripheral nerve injury, both injured and non-injured sensory neurons in the dorsal root ganglion of the damaged nerve exhibit a massive change in transcription that alters their membrane properties, growth and transmitter function **(a)**, and also leads to the development of ectopic activity **(b)**. Some myelinated Aβ fibres sprout from deep to superficial laminae, to make contact with nociceptive neurons **(c)**. Recruitment and activation of microglial cells **(d)** and astrocytes **(e)** is an essential step in the development of pain after nerve injury and triggers central sensitisation by releasing pro-inflammatory cytokines (IL-1β and TNF-α **(f)**), which increase neuronal excitability. **Loss of inhibition**: Several mechanisms contribute to the phenomenon of disinhibition after nerve injury: there is a severe reduction in the inhibitory glycinergic currents onto the projection lamina I neuron **(g)**, as well as a loss of inhibitory GABAergic interneurons caused by excitotoxic apoptosis **(h)**. The infiltration of activated T-cells **(i)** produces and releases IFNγ, which also reduces GABAergic currents **(j)**. **(k)** KCC2 is critical to maintaining a low concentration of intracellular Cl^- (see inset). After peripheral nerve injury, KCC2 is down-regulated, which results in an increase in intracellular Cl^-. **(l)** In normal conditions (left), the opening of Cl^--permeable channels (such as GABAA and glycine receptors) leads to an entry of Cl^- that hyperpolarizes the cell membrane. After nerve injury (right), the opening of Cl^- channels by activation of GABAR or GlyR now leads to an efflux of Cl^-, which causes membrane depolarization. As a consequence, GABA and glycine excite the neurons upon binding to their receptors. **(m)** Descending inhibitory NAergic controls are reduced, and **(n)** descending 5-HTergic controls shift from inhibitory to excitatory. Projection lamina I neuron is shown in red. Excitatory interneurons are in black (the polysynaptic activation pathway from Aβ is highlighted in red) and the inhibitory interneurons are in white. Dashed neurites represent a loss of fibres. Lamina are marked I–III and separated by dotted lines.

the release of neurotransmitters, even from a limited number of sensory fibres, is sufficient to cause a spill-over of glutamate and ATP in the superficial dorsal horns (Nie and Weng, 2010) that could trigger microglial activation (Tsuda et al., 2003; Davalos et al., 2005; Ferrini et al., 2013). Another factor that contributes to the recruitment and activation of microglial cells is the chemokine fractalkine. Fractalkine is produced by sensory neurons. It is released upon nerve injury into the spinal cord, where it binds to its receptor, CX3CR1, which is expressed only by glial cells (Dorf et al., 2000; Verge et al., 2004); and it causes the activation of the p38 MAPK pathway in those cells (Zhuang et al., 2007). The peptide neuregulin-1 is also up-regulated and released by sensory neurons after nerve trauma. It acts as a chemo-attractant for nearby microglial cells by activating the PI3K pathway and causes the proliferation of these cells through the ERK pathway (Calvo et al., 2011). In addition, the binding of neuregulin-1 to the ErbB2 receptors triggers the activation of the microglial cells (Calvo et al., 2010). Together, these factors converge to cause a massive and sustained activation of glial cells in the dorsal horns of the spinal cord. Activated glial cells in turn produce and release a broad range of molecules, from tropic factors to neurotransmitters, cytokines and reactive oxygen species that contribute to the development and the maintenance of spinal neurons excitability (Watkins and Maier, 2002; Scholz and Woolf, 2007; Milligan and Watkins, 2009; Austin and Moalem-Taylor, 2010, Gao and Ji, 2010). Direct activation of spinal microglial cells through intrathecal injection of the human immunodeficiency virus-1 (HIV-1) envelope glycoprotein gp120 is sufficient to trigger a severe mechanical allodynia (Milligan et al., 2001), a behavioural manifestation strongly associated with central sensitisation. This is mostly mediated by the action of IL-1β and of TNF-α and by the activation of the neuronal isoform of NOS (Holguin et al., 2004; Milligan et al., 2005). IL-1β and TNF-α directly increase neuronal excitability by enhancing AMPAR and NMDAR currents (Kawasaki et al., 2008; Gruber-Schoffnegger et al., 2013), whereas NO increases pain sensitivity through an action on NMDARs (Kitto, Haley and Wilcox, 1992; Meller, Dykstra and Gebhart, 1996; Schmidtko, Tegeder, and Geisslinger, 2009) (Figure 2.7).

After peripheral nerve injury, astrocytes also become activated, and for a more prolonged time course than microglia. They appear to play a critical role in the maintenance of neuropathic pain hypersensitivity (Zhuang et al., 2005; Zhang and De Koninck, 2006; Gao et al., 2009) (Figure 2.7). Activated astrocytes can release several pro-inflammatory cytokines (Aloisi et al., 1992; Sawada et al., 1992; Schwaninger et al., 1999) that enhance excitatory synapses at the postsynaptic level (Milligan et al., 2001), or the chemokine CXCL1 (Gao et al., 2009; Zhang et al., 2013) that promotes presynaptic release of neurotransmitter (Chen, Park, et al., 2014). Interestingly, CXCL1 release from astrocyte is mediated by hemichannels (GAP junctions) containing the Cx43 connexin; and the administration of a Cx43 mimetic peptide that blocks connexin channel-facilitated intercellular communication reduces mechanical allodynia (Chen, Park, et al., 2014). Cx43 is the major connexin isoform; it forms gap junctions in astrocytes (Dermietzel et al., 1989) and plays a major role in the interastrocyte communication through calcium waves (Naus et al., 1997). Calcium waves propagate the information in a more widespread fashion than synaptic connections (Charles et al., 1991), which could promote a state of generalised hyperexcitability of the nociceptive neurons (central sensitisation). The administration of compounds that can decouple gap junctions reduces

the symptoms of 'mirror-image pain' when the non-injured side develops hypersensitivity after injury (Spataro et al., 2004; for a detailed overview of neuroimmune interaction in neuropathic pain, see Chapter 4 in this volume).

Disinhibition

In neuropathic pain states, there is substantial loss of the segmental inhibitory controls normally carried by GABAergic and glycinergic currents (Moore et al., 2002). Several mechanisms converge to produce this nerve injury-induced disinhibition of the spinal nociceptive neurons.

In the weeks following the axotomy, there is a gradual loss of inhibitory interneurons in the dorsal horn of the spinal cord (Sugimoto, Bennett and Kajander, 1990; Scholz et al., 2005) (Figure 2.7). This neuronal death is possibly the result of an NMDAR-induced excitotoxicity that develops over time, rather than the result of the large amount of glutamate released at the time of nerve injury (Scholz et al., 2005). This loss of inhibitory interneurons appears to require a permanent denervation. Neuropathic pain models where regeneration of the injured fibres can occur do not exhibit signs of neuronal apoptosis (Polgár et al., 2003; Polgár et al., 2004). Interestingly, the reduction in glycinergic neurotransmission after nerve injury is not mediated by the activation of EP2 receptors, which further indicates that inflammatory and neuropathic pain mechanisms differ (Hosl et al., 2006). The molecular mechanisms responsible for the loss of glycinergic tone in neuropathic pain states have not yet been identified, but they contribute, together with the loss of GABAergic tone, to the opoids' lack of efficacy in relieving neuropathic pain symptoms (Chen, Chen and Pan, 2005). The restoration of an adequate inhibitory tone through transplantation of immature telencephalic GABAergic interneurons progressively abolishes nerve injury-induced mechanical allodynia (Braz et al., 2012), but it does not reduce inflammatory-mediated pain behaviours caused by intra-plantar formalin injection (Braz et al., 2012).

Another mechanism that contributes greatly to the reduction in segmental inhibition in lamina I neurons after nerve injury is caused by a BDNF-dependent change in the homeostatic chloride concentration gradient in the spinal cord neurons (Figure 2.7). Under normal conditions the intracellular concentrations of Cl- are maintained by the opposed effects of Cl^- co-transporter K^+-Cl^- exporter 2 channels (KCC2) and Na^+-K^+-Cl^- exporter 1 channels (NKCC1) (Price, Cervero and De Koninck, 2005). KCC2 cotransports Cl- and K^+ ions out of the cells, whereas NKCC1 is responsible for an influx of K^+, Na^+ and Cl^- into the cells. The net effect of these two co-transporters is a steady state with low intracellular Cl^- concentration and high extracellular Cl^- concentration. Because both GABAAR and glyR are chloride-permeable channels that open upon binding of their ligand (Zeilhofer et al., 2012), their activation triggers a massive entry of Cl- into the cell that causes a membrane hyperpolarisation (Figure 2.7). After peripheral nerve injury, however, activated microglial cells produce and release large amounts of BDNF (Coull et al., 2003). The binding of BDNF to the TrkB receptors increases NMDAR transmission (Kerr et al., 1999), which leads to a massive calcium entry and to the Ca^{2+}-dependent activation of calpain, which in turn cleaves KCC2 (Zhou et al., 2012). Reduced levels of KCC2 in the spinal cord significantly diminish the efflux of Cl^- from the cell, so that intracellular concentrations are abnormally high, which leads to a loss of the

Cl^- gradient between intra and extracellular milieus (Coull et al., 2003; Miletic and Miletic, 2008) (Figure 2.7). As a result, the binding of GABA or glycine on their receptors causes a minimal entry of Cl^- into the cell; this entry is insufficient to hyperpolarise the nociceptive neurons or can even cause a depolarisation (Coull et al., 2003; Coull et al., 2005). Altogether, this loss of both GABA and glycine inhibitory effects leaves the nociceptive neurons in a state of disinhibition, allowing the development and maintenance of central sensitisation. Inhibition of KCC2 in uninjured animals is sufficient to cause disinhibition and increases pain sensitivity (Austin and Delpire, 2011), whereas rescuing K-Cl co-transport in neuropathic pain states restores spinal nociceptive neurons to a normal sensitivity (Lavertu, Cote and De Koninck, 2014). More importantly, this allows the neurons of the superficial lamina to return to a nociceptive-specific state, which suggests that the restoration of proper inhibitory controls could help resetting the neurons from their central sensitisation state (Lavertu et al., 2014).

After peripheral nerve injury there is, over time, a progressive infiltration of immune-competent cells into the dorsal horns of the spinal cord, notably T-cells (Watkins et al., 2007; Cao and DeLeo, 2008; Costigan, Moss, et al., 2009). These T-cells produce specific cytokines such as IFN-γ, which cause a disinhibition of the nociceptive neurons (Vikman et al., 2003) by reducing GABAergic currents in the dorsal horn (Vikman, Dugan and Siddall, 2007) through the activation of IFN-γ receptors expressed by nociceptive neurons (Vikman et al., 1998) (Figure 2.7).

In addition to the reduction of segmental inhibitory controls, there is an increase in descending excitatory controls from the rostral ventromedial medulla (RVM) along with a reduction in descending inhibitory controls (Gardell et al., 2003; Vogel et al., 2003; Vera-Portocarrero et al., 2006; Sikandar, Bannister and Dickenson, 2012; Kim et al., 2014), further enhancing the hyperexcitability of spinal nociceptive neurons (Figure 2.7). The effects of serotonin released from the raphe magnus neurons in particular appear to display a strong shift towards a gain in excitability. The specific disruption of the serotonin production through injection of shRNA (RNAi) of TPH2 into the RVM reveals a pronociceptive effect of 5-HT in the second phase of the formalin test without alteration of baseline nociception; this suggests a specific role in spinal plasticity (Wei et al., 2010). In support of this view, TPH2 blockade in the RVM strongly reduces thermal hyperalgesia, but also mechanical allodynia after peripheral nerve injury (Wei et al., 2010; Kim et al., 2014). Lesion of the dorsolateral funiculus, which contains in part descending fibres from serotoninergic neurons (Basbaum and Fields, 1979), strongly attenuates spontaneous signs of pain and mechanical allodynia after peripheral nerve injury (Wang et al., 2013). One potential mechanism through which serotonin increases nociceptive neurons excitability is the sensitisation of TRPV1 channels expressed by primary afferent fibres through 5-HT3R, which enhance synaptic transmission (Kim et al., 2014). Because nerve injury causes large-diameter neurons to express nociceptive-specific factors, including TRPV1, this could contribute to the maintenance of central sensitisation by innocuous stimuli (Kim et al., 2014). In addition, TRPV1 channels appear to be expressed by some GABAergic inhibitory interneurons (Kim et al., 2012). TRPV1 activation triggers the disinhibition phenomenon by causing a long-term depression (LTD) between the interneurons and the projection nociceptive neurons of lamina I; preventing this reduces the development of mechanical

allodynia caused by nerve injury (Kim et al., 2012). Because 5-HT3R are also expressed by inhibitory interneurons, it is possible then that this mechanism participates to the pro-nociceptive action of 5-HT in neuropathic states.

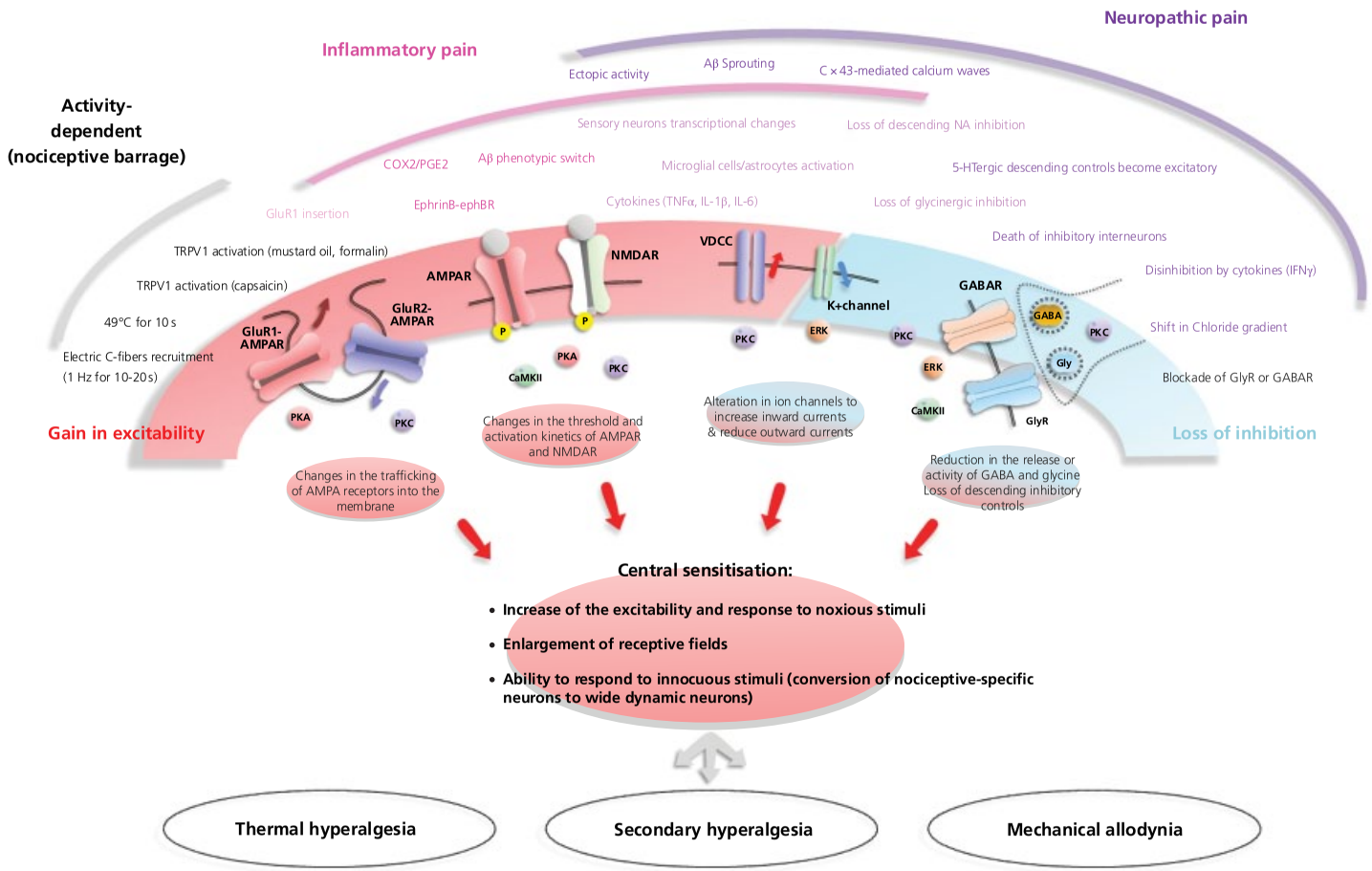
Other diseases with chronic pain syndromes

There is an increasing body of evidence to suggest a critical role of central nociceptive plasticity in the development and maintenance of pain hypersensitivity syndromes observed in diseases such as CRPS, pancreatitis, whiplash injury, fibromyalgia, low back pain or migraine (Woolf, 2010). For most of these pathologies there are no adequate preclinical animal models that could identify the structures or mechanisms causing the pain symptoms. Several clinical signs and behavioural manifestations point, however, to the existence of central sensitisation and suggest that approaches otherwise efficient at altering this state in other conditions might be effective in the treatment of pain symptoms as well. The next section will briefly describe some recent key results and observations that support the hypothesis of the role of central sensitisation in these painful conditions.

Complex regional pain syndrome may develop after limb trauma – even after apparently minor injuries – and is characterised by chronic pain and sensory–motor and autonomic disturbances (Birklein, 2005; Marinus et al., 2011). Patients with CRPS can develop mechanical allodynia (Maihofner, Handwerker and Birklein, 2006) and contralateral pain in the chronic stages of the disease (Huge et al., 2008); this strongly suggests a spinal involvement. In support of this view, the intradermal injection of capsaicin in patients with CRPS causes a higher hypersensitivity than in healthy subjects, but also bilateral pain (Terkelsen et al., 2014), whereas the capsaicin-induced neurogenic inflammation is the same (Terkelsen et al., 2014). This defect in central pain controls was also found in another cohort of patients, where quantitative sensory testing of the affected region – but also of non-affected areas – showed a strong widespread muscle hyperalgesia (Van Rooijen, Marinus and Van Hilten, 2013). Two recent clinical trials tested the effect of intravenous ketamine infusions and found that these treatments produced significant pain relief (Schwartzman et al., 2009; Goldberg et al., 2010).

Pancreatitis is associated with chronic abdominal pain in the majority of patients (Lankisch, 2001), and the pain symptoms appear to be quite refractory to several current lines of medication (Gress et al., 1999). There is a growing body of evidence indicating that most, if not all the pain symptoms in chronic pancreatitis are central in nature (Drewes et al., 2008). For example, quantitative sensory testing through electric pain detection and pain tolerance paradigms revealed that patients with chronic pancreatitis had lower pain thresholds, including in cutaneous territories non-referred to the pancreas, and a significant loss of descending inhibitory controls (Bouwense, Ahmed Ali, et al., 2013; Bouwense, Olesen, et al., 2013). Temporal summation is strongly facilitated in patients with pancreatitis, as well as the expansion of their receptive fields (Dimcevski et al., 2007). A recent clinical trial revealed that a chronic pregabalin treatment could attenuate pain hypersensitivity, especially in dermatomes outside of the pancreas territory. This suggests an effect on descending rather than segmental controls (Bouwense et al., 2012).

Protective	Pathological



Patients suffering from either whiplash injury, which typically happens after car accidents, or fibromyalgia present a defect in centrally mediated inhibitory controls (Banic et al., 2004). Chronic whiplash-associated disorders appear to be accompanied by an almost complete loss of several types of descending controls (Daenen, Nijs, Cras, et al., 2013; Daenen, Nijs, Roussel, et al., 2013), which could explain the occurrence of pain symptoms in apparently healthy body areas. In patients with intractable neck pain, the anaesthetic infiltration of trigger points reduces the hypersensitivity observed in non-injured areas (Freeman, Nystrom and Centeno, 2009) – a phenomenon that confirms the central origin of this pain (Curatolo et al., 2001). Similarly, several symptoms in fibromyalgia point to a loss of descending inhibitory controls and to a generalised defect in spinal inhibition. Fibromyalgia patients have lower pressure thresholds and increased temporal summation to muscle stimulation (Graven-Nielsen et al., 2000; Staud et al., 2003), which can be reduced through systemic administration of ketamine (Graven-Nielsen et al., 2000). Experimental pain triggered by temporal summation protocol causes more pain in patients suffering from fibromyalgia and produces a greater extension of the nociceptive receptive fields (Staud et al., 2004). In addition, in this experimental setting, the hypersensitivity can be maintained using lower frequency than in healthy subjects, which again suggests that inhibitory controls are defective (Staud et al., 2004). Blocking sensory fibres through local intramuscular injections of an anaesthetic attenuates secondary hyperalgesia, a phenomenon that indicates that sensory input from muscles participates in the maintenance of the central hyperexcitability (Staud et al., 2009).

Concluding remarks

A summary of the factors causing central sensitisation through either a gain in excitability or a loss of inhibition in normal states and in chronic pain states is described in Figure 2.8.

Functional plasticity of the spinal nociceptive system is a critical feature of physiological pain. There are relatively few nociceptive neurons that project from the spinal cord to the brain and determine how much pain we feel and for how long. The nature of the signal they send is

Figure 2.8 Schematic representation of the factors that can trigger central sensitisation under normal or pathological conditions; the cellular processes involved; the three main features of central sensitisation; and the associated behavioural manifestations. Factors causing a gain in the excitability of the projection spinal nociceptive neuron are listed along the red arc, whereas those causing a loss of inhibition are listed along the blue arc. Activity-dependent inducers of central sensitisation are represented in black, inflammatory pain-specific mechanisms are represented in pink, neuropathic pain-specific mechanisms are represented in purple and mechanisms shared by inflammatory and neuropathic pains are represented in magenta. Central sensitisation can be triggered by a strong and sustained nociceptive activation, something referred to as activity-dependent central sensitisation. This represents a protective mechanism and is mostly mediated by a gain in the excitability of the spinal nociceptive neuron. Both chronic inflammatory pain and neuropathic pain exhibit many changes that can trigger or maintain central sensitisation. Some mechanisms overlap between the two types of diseases, whereas others are specific. Chronic inflammatory pain has a majority of changes that favour a gain in excitability, and neuropathic pain is associated with a severe loss of inhibition. **At the bottom**: Thermal hyperalgesia, secondary hyperalgesia and mechanical allodynia are symptoms that can be explained by the development of central sensitisation. Secondary hyperalgesia and mechanical allodynia are symptoms that strongly suggest functional changes at the spinal level.

therefore crucial to our reacting adequately to potential threats. The spinal cord is the first centre for the integration of the nociceptive input; the information is not just passively relayed to the brain areas but modified to convey the most relevant message possible. In normal states the nociceptive projection neurons are under strong local and distant (supraspinal) inhibitory and excitatory controls, whose balance affects the overall excitability of the cell. Sustained nociceptive activity triggers a state of hyperexcitability called 'activity-dependent central sensitisation', where nociceptive neurons display three key features: an increase in excitability and response to noxious stimuli; an enlargement of their receptor field; and an ability to respond to innocuous stimuli. In this state of central sensitisation the nociceptive system is no longer coupled to its environment. This can represent an adaptive advantage designed to promote behaviours and strategies that allow for a better protection of an injured body part, as the degree of nociceptive input required to trigger central sensitisation is almost always sufficient to cause tissue damage.

If central sensitisation occurs, or is maintained in the absence of tissue damage, this becomes a pathological and maladaptive plasticity of the nociceptive system. Nociception is not a sense any more, as it does not transmit relevant – or even real – information from the environment. Because nociception gives rise to pain and pain is an extremely complex and integrated cognitive state (Loeser and Melzack, 1999), an 'out-of-tune' nociceptive system will have dramatic chronic consequences that will eventually affect brain structures (Apkarian et al., 2004; Pelled et al., 2007; Wei and Zhuo, 2008; Valet et al., 2009).

Central sensitisation can be triggered by many different mechanisms, which can be schematically classed into two broad categories: a gain in excitability and a loss of inhibitory controls (Latrémolière and Woolf, 2009). Central sensitisation is not caused by a single defining molecular mechanism, but rather by one of many possible combinations that can affect the excitatory–inhibitory balance of the nociceptive spinal neuron. It seems logical therefore to infer that any pathological state associated with spinal alterations could affect the modulatory controls of spinal nociceptive neurons and could cause or facilitate central sensitisation and its associated behavioural manifestations. These alterations could originate directly at the spinal level or could affect supraspinal structures, which will in turn affect the excitatory–inhibitory balance of the nociceptive system.

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