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Neurobiology of Pain

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2.1 Physiology of Pain

Pain is a physiological experience, designed to alert us from potential damages to our body, so it has a clear protective role. Pain is defined by the IASP (International Association for the Study of Pain) as *an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage*. When the pain circuits are correctly working they aware us from external (abnormal heating, pinch stimuli, etc.) or internal stimuli (cardiac ischemia) that would potentially hurt the tissues. Ideally, the sensation we perceive should be unpleasant enough, so it cannot be ignored, and the sensation should continue as long as the stimulus is present. Different types of “normal” pain can be distinguished depending on their origin and characteristics: acute (or pricking), chronic (or burning), and continuous or visceral.

2.1.1 Nociceptors and Nociceptive Fibers

There are two main types of nerve fibers conveying pain information: C fibers and A δ fibers (Table 2.1). In both cases, the stimuli may come from the skin, muscle, and joint tissues or certain visceral structures. They do not present a clear ending receptor structure and are commonly identified as free nerve endings.

Table 2.1 Classification of the primary afferent axons in the peripheral nervous system

Fiber	Myelin	Diameter (μm)	Velocity (m/s)	Function
A α	Yes	13–20	80–120	Proprioceptors of skeletal muscle
A β	Yes	6–12	35–75	Touch, mechanoreceptors, proprioceptors
A δ	Yes	1–5	5–30	Pain, mechanical and thermal receptors
C	No	0.2–1.5	0.5–2	Pain, warm thermoreceptors

A δ fibers are thinly myelinated, so they can conduct a *fast pain* signal, at 5–30 m/s. In this case, A δ nociceptive fibers convey nociceptive information as well as information coming from intense mechanical or thermal stimulation. This fast pain has been reported as the *first pain*, the initial sharp painful sensation just after the contact with the noxious stimuli.

C fibers are related with a *slow pain*, since these unmyelinated fibers conduct impulses at less than 2 m/s, normally evoked by thermal, mechanical, and chemical stimuli. Most of them act as **polymodal nociceptors**, although a proportion seems to be sensitive only to mechanical or thermal stimuli. This slow pain is also called *second pain*, and evokes a more diffuse and longer lasting painful sensation than the pain evoked by the A δ fibers.

The unmyelinated C nociceptive afferents can be divided into two major subpopulations, the peptidergic and the nonpeptidergic. The peptidergic nociceptors express substance P (SP) and calcitonin gene-related peptide (CGRP), and the nonpeptidergic ones possess fluoride-resistant acid phosphatase (FRAP) activity, bind the lectin IB4 and express purinergic P2X3 receptors. These two populations differ in neurotrophic support in the adult. In fact, during development, both populations require nerve growth factor (NGF) for survival, but shortly after birth only the peptidergic continue to respond to NGF, whereas the nonpeptidergic population starts to respond instead to glial cell line-derived neurotrophic factor (GDNF). Accordingly, the peptidergic population expresses the NGF high-affinity receptor trkA, whereas the nonpeptidergic expresses GDNF receptors. Although the distinction between two populations of primary sensory fibers, peptidergic and nonpeptidergic, seems attractive, it is not completely accurate as a small proportion of peptidergic sensory fibers (those that colocalize CGRP and somatostatin (SOM)) do not respond to NGF in the adult and bind the lectin IB4. In the spinal cord, sensory fibers that express SP and CGRP terminate mostly in laminae I, outer II, and V; those that colocalize CGRP and SOM terminate in laminae I and II, and those that contain FRAP, bind the lectin IB4 or express the purinergic P2X3 receptor terminate mostly in the inner lamina II.

The peripheral terminal of the nociceptor, embedded in the tissue, is where the noxious stimuli are detected and transduced into electrical

impulses. This leads to the train of events that allows for the conscious sensation of pain. The sensory specificity of nociceptors is established by the high threshold to particular types of stimuli. Only when the high threshold has been reached by either chemical, thermal, or mechanical events are the nociceptors activated.

The transduction of the nociceptive information starts in the periphery, where a stimulus is able to activate the nociceptor endings, by stretching or bending the nociceptor surface or by activating ion channels present in its membrane. At the site of injury some algogenous substances are released, such as proteases, bradykinin, ATP, and potassium ions. Due to the variety of stimuli that can elicit nociceptive signaling (thermal, mechanical, and chemical stimuli), different specific receptors have been described. Both A δ and C fibers present transient receptor potential (TRP) receptors, which resemble voltage gated ion channels, presenting six transmembrane domains and a central pore which allows an influx of sodium and calcium that initiates the generation of action potentials. One subtype of these channels is the vanilloid receptor (TRPV1), that responds to capsaicin but is also activated by acidification of the extracellular medium, by heat (thermal threshold $>43^{\circ}\text{C}$) and by anandamide, an endogenous cannabinoid. Other channels of the TRP family activated by temperature increase are TRPV3 and TRPV4, with thresholds of 33°C and 25°C , respectively. There are also nociceptors activated by intense cold, below 15°C , that causes painful sensations with a burning or stabbing component. The TRPA1 channel is activated both by temperatures below 18°C and by irritant compounds.

Other channels present in nociceptive terminals are those of the degenerin family (Deg/ENaC), which are epithelial sodium channels activated by mechanical forces. Some of these channels also respond to decrease in the pH of the extracellular medium and are therefore called acid sensing channels (ASICs). Also important as stimulus transducer channels are those belonging to a diverse family of channels for potassium ions ($\text{K}_{2\text{P}}$ channels). These channels are open at rest, and are closed by the stimulus, causing depolarization of the fiber. Their activity can be modified by mechanical, thermal stimuli, intracellular and extracellular pH changes, and hypoxia.

2.1.2 Nociceptive Spinal Cord Circuits

The different subpopulations of primary afferent fibers convey nociceptive information through parallel spinal pathways. At every stage of the pain pathway – from sensory nerve to spinal cord, from spinal cord to brainstem and from brainstem to the forebrain – information signaling injury is subdivided

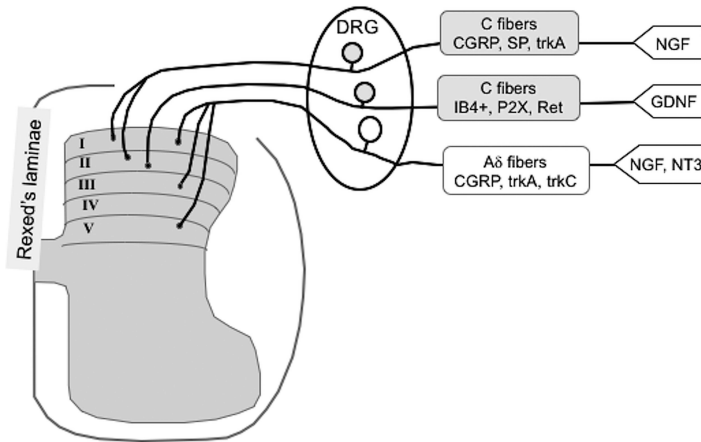


Figure 2.1 Representation of the three main types of nociceptive fibers, their markers and the laminae of the dorsal horn where they mainly project central terminals.

or shared between these parallel systems. Molecular dissection has begun to reveal distinct functions for these separate pathways and their contribution to the final behavioral outcome.

Nociceptive neurons have their body in the dorsal root ganglia, where they can be divided in three main populations (Figure 2.1): (i) mid-size neurons with A δ axons, that express CGRP, and TRPV2 receptors, (ii) small neurons with unmyelinated C axons, expressing neuropeptides (SP, CGRP) and TRPV1 receptors, and (iii) small neurons nonpeptidergic, with C axons, that express receptors TRPV1 and P2X. It has been suggested that the C peptidergic pathway would be particularly important in conveying inflammatory pain, whereas the nonpeptidergic pathway would play a role in neuropathic pain.

Peptidergic primary afferents connect with the pathway that derives largely from lamina I neurons of the dorsal horn expressing the NK1 receptor, and terminates within the thalamus, the parabrachial area, and the periaqueductal grey matter (PAG). These latter areas in turn project on brain areas such as the hypothalamus and amygdala that modulate the affective dimensions of pain and control autonomic activity. The lamina I pathway is mainly involved in signaling the intensity of pain, therefore these second-order neurons are capable of reliably detecting and transmitting precise quantitative information about noxious pressure and noxious heat to higher centers. Of interest, the selective destruction of lamina I results in loss of the increased sensitivity to stimulation that follows inflammation or manipulation of the peripheral nerve.

On the other hand, the IB4 binding subpopulation of nonpeptidergic C fibers contact with neurons in inner lamina II of the spinal cord. These interneurons in turn contact projection neurons of lamina V, and many of these send axons to fourth-order neurons in the amygdala, hypothalamus, bed nucleus of the stria terminalis and globus pallidus. Interneurons in this lamina also show increase in protein kinase Cgamma (PKC γ) following inflammation which results in mechanical hypersensitivity. However, these interneurons predominantly receive inputs from myelinated, rather than unmyelinated, primary afferent terminals. In contrast, calbindin positive interneurons of lamina III are located postsynaptic to the IB4-positive subpopulation of nonpeptidergic C fibers, but not to myelinated afferents.

Type A nociceptive fibers send collateral branches to contact with the wide dynamic range (WDR) neurons or nociceptive neurons type 2, located mainly in lamina V. Taken together, these observations illustrate the very complex connectivity of primary afferents with the interneurons of the dorsal horn of the spinal cord.

2.1.3 Nociceptive Ascending Pathways

The nociceptive information arriving from the periphery travels along the peripheral axonal branch of primary nociceptive neurons, whose soma are located in the dorsal root ganglia, and the central axon entering into the spinal cord by the dorsal roots. After the dorsal root entry, they travel within the zone of Lissauer, in which axons move up or down a pair of segments before entering the gray matter of the dorsal horn, in a region called substantia gelatinosa. Central nociceptive terminals contact to second-order neurons mainly placed in laminae I and II (pure nociceptive), and V (mixed nociceptive and mecanosensory, WDR). Sensory fibers that are peptidergic terminate mostly in laminae I and outer II, and a few in lamina V; those that are nonpeptidergic terminate mostly in the inner lamina II. The main neurotransmitter involved in these first relay is glutamate, but also substance P, acting as cotransmitter in peptidergic nociceptors, is important to experience moderate to intense pain.

From the second-order neuron the thermal and nociceptive information crosses the midline and ascends to the brain in the spinothalamic tract, part of the anterolateral system. This decussation occurs at the spinal level and in two or three segments all the fibers are in the contralateral side. The ascending axons travel through the medulla, the pons and the midbrain without synapsing, until reaching the thalamus. From here, the information is conveyed to the primary somatosensory cortex (Figure 2.2). This route

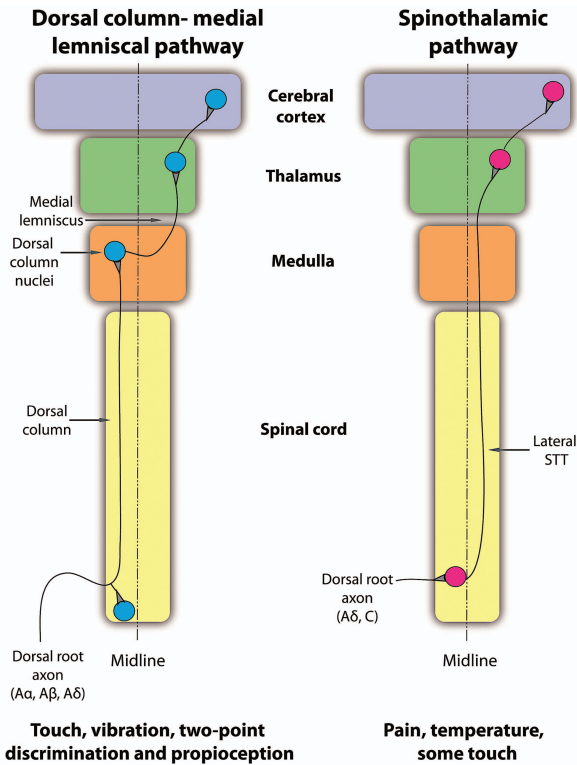


Figure 2.2 Schematic of the ascending pathways of somatic sensations. Low threshold mechanoreceptive afferents follow the dorsal column system, that decussates at the medulla, whereas thermal and pain afferents constitute the anterolateral system, crossed at segmental cord levels.

is followed in order to transmit the gross information of pain, the essential information for the brain to note stimuli that threaten the integrity of the body.

Axons from the second-order spinal neurons make relays on different structures in order to mediate different aspects of the sensory and behavioral response to pain. One of these aspects is the sensory discrimination of pain, in terms of location, intensity, duration, and quality. The main responsible of this discrimination is the thalamus, in particular the ventral posterior lateral (VPL) nucleus, and its projections to the primary somatosensory cortex (SI). Another important aspect is the affective or motivational, more related with the emotion that pain provokes in the individual who is suffering it (unpleasant feeling, fear, anxiety, and secondary autonomic reactions). In this

case, the information travels by the spinoreticular and spinomesencephalic tracts, reaching several structures, such as the reticular formation, the superior colliculus, PAG, hypothalamus, and amygdala. In addition, another group of neurons constituting the anterior spinothalamic tract, reach the midline thalamic nuclei, that will connect with the anterior cingulate cortex and the insular cortex.

Apart from the anterolateral system, another fraction of information entering from the periphery travels along the dorsal columns, which will provide a more qualitative information of the stimulus that is reaching the nociceptive signaling system. This route is used essentially by mechanoreceptive afferents, which provide information about touch, vibration, and proprioception, and is known as the dorsal column-medial lemniscus system. This information travels directly by the central axons of primary sensory neurons in the dorsal columns ipsilateral to the site of entrance until the dorsal column nuclei in the medulla, where it decussates to reach the thalamus in the contralateral side and later on the cortex. This system also participates in the discriminative aspects of pain.

2.1.4 Descending Control of Pain

Once the nociceptive information arrives to the higher level centers, it is integrated in order to elicit a complex physiological response in front of the noxious stimuli, and also modulated in order to reduce the intensity of the painful sensation. The main mechanisms for pain modulation conform the descending pathway (Figure 2.3). One of the most important regions is the PAG in the midbrain, but there are other regions in the brainstem also involved in this process: parabrachial nucleus, medullary reticular formation, locus coeruleus, and raphe nuclei. These centers use noradrenaline, serotonin, dopamine, histamine, and acetylcholine to exert both excitatory and inhibitory effects on different sets of neurons in the dorsal horn. Then, they can act on synaptic terminals of nociceptive afferents, interneurons (excitatory and inhibitory), synaptic terminals of other descending pathways, and projection neurons. These contacts do not only act inhibiting the transmission of nociceptive information but also modulating it, as well as controlling the balance between excitation and inhibition in the spinal cord.

The main action of the PAG is to modulate nociceptive signaling in the dorsal horn by releasing endogenous opioids (encephalin, endorphins, and dynorphins) on the dendrites of nociceptive neurons and WDR neurons, causing hyperpolarization, thus inhibiting, of the second-order neurons. They

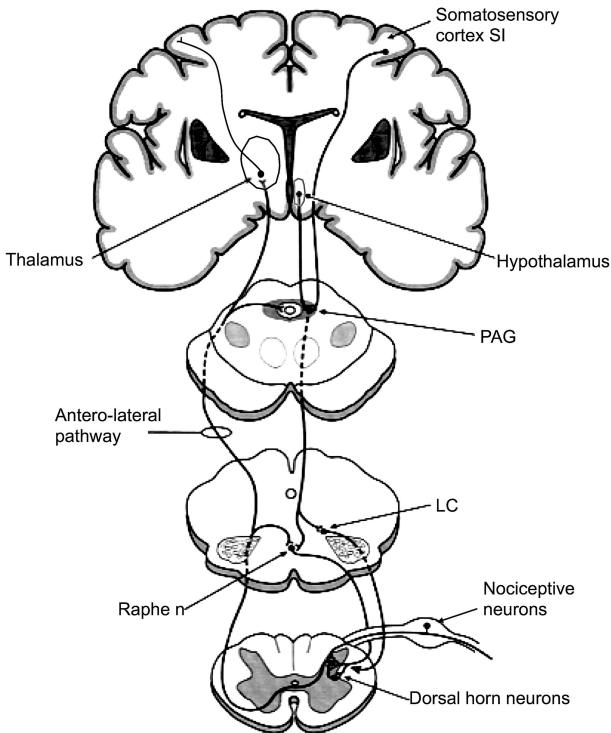


Figure 2.3 Ascending (left side) and descending (right side) pain pathways.

also release glycine on the terminals of primary afferents (A and C fibers), inducing presynaptic inhibition that reduces the release of neurotransmitters on the second-order neurons. Finally, the secretion of glutamate from the PAG excites the GABAergic interneurons in lamina II of the dorsal horn. This promotes the release of GABA on the second-order neurons, hyperpolarizing and therefore inhibiting them.

The PAG also causes depolarization of serotonergic neurons in the raphe magnocellular nucleus (RMN), which project to second-order neurons in the dorsal horn via the raphespinal tract. The binding of serotonin to receptors 5-HT₁ and 5-HT₂ induces an increase in the conductance of potassium and therefore the hyperpolarization of the second-order nociceptive neurons. It also interacts with 5-HT₃ receptors in the dendrites of GABAergic interneurons in lamina II, inducing the release of GABA and the inhibition of second-order neurons. Something similar happens when PAG neurons secrete

glutamate on the locus coeruleus neurons. Once depolarized, these neurons release noradrenaline, which causes hyperpolarization of the nociceptive second-order neurons by binding to α -adrenergic receptors.

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

Similarly, it has also been described a mechanism by which pain can inhibit pain. This phenomenon is called “diffuse noxious inhibitory control” (DNIC), or heterotopic noxious conditioning stimulation (HCNS) (Sprenger et al., 2011), and implies a spinal-medullary-spinal pathway. DNIC systems permit that a spinal neuron can be inhibited by a nociceptive stimulation applied in another part of the body (outside its receptive field), thus inhibiting the pain sensation after the application of a remote pain stimulation. WDR neurons and trigeminal nociceptive neurons play a key role in this phenomenon, which is regulated by serotonergic pathways, and probably by opioids. DNIC effects are usually contralateral and extrasegmental, and highly dependent on the intensity of the stimulus. DNIC mechanisms may reflect alterations in the function of central descending inhibitory systems that could be potentially involved in chronic pain. In fact, research based on the use of DNIC has shown dysfunctions in this system in chronic pain conditions, such as fibromyalgia or irritable bowel syndrome (van Wijk and Veldhuijzen, 2010).

Other elements are also involved in pain regulation, such as the endogenous opioids. Several brainstem regions, most of them conforming the descending system of pain control, are susceptible to the action of these molecules, provoking an important analgesic effect. Endogenous opioids are classified in three groups, called enkephalins, endorphins, and dynorphins, which present different distribution along the nociceptive system. Enkephalins, for example, can be released by local neurons on the dorsal horn, then impeding the release of neurotransmitters from the terminals onto the projection neurons, and therefore diminishing their level of activity. This local circuit can also be the target of other descending inhibitory projections, therefore constituting a powerful control mechanism of the amount

of nociceptive information able to reach superior centers. Endorphins are released in pain states within some brain regions, but they can also provide tonic analgesic effect in the dorsal horn. Dynorphins have been described to increase after neural injuries, and are related to the development of thermal hyperalgesia by acting on the NMDA receptors and driving to spinal sensitization (Ossipov et al., 2000).

2.2 Neurobiology of Neuropathic Pain

Neuropathic pain is defined as that pain provoked by a lesion or a dysfunction in the nervous system. Although sharing features with other kind of pain (inflammatory or cancer pain), neuropathic pain presents some particular characteristics. Nociceptive and inflammatory pain can be both symptoms of peripheral tissue injury, and present a clear defensive, beneficial component, whereas neuropathic pain is a symptom of neurological disease or injury, either affecting the peripheral or the central nervous system (Cervero, 2009), and instead of a defensive component it is considered as a maladaptive response.

Neuropathic pain states are characterized by an almost complete lack of correlation between the intensity of peripheral noxious stimuli and of pain sensation, and are produced by neurological lesions that cause abnormal impulse activity generated in nerve sprouts, neuromas, or dorsal root ganglion cells, ephaptic coupling between adjacent nerve fibers and abnormal responses of peripheral nociceptors and CNS neurons. Neuropathic pain syndromes produce pain sensations well outside the range of the sensations produced by the normal nociceptive system, even after serious peripheral injury or inflammation (Cervero, 2009). Neuropathic pain may include spontaneous, or stimulus-independent pain that has been described as shooting, burning, lancinating, prickling, and electrical. Evoked, or stimulus-dependent, neuropathic pain includes allodynia, defined by the IASP as “pain due to a stimulus which does not normally provoke pain.” These stimuli may be nonnoxious heat, light touch, or even a puff of cool air. Moreover, mechanical allodynia may be static, as evoked by light touch, or dynamic, as evoked by a light brush of the skin. Hyperalgesia is identified when a stimulus that normally produces nociceptive responses produces exaggerated responses.

Epidemiological studies on the prevalence of neuropathic pain indicate a high incidence of about 5% of the general population (Bouhassira et al., 2008; Dieleman et al., 2008). Associated risk factors include gender, age, and

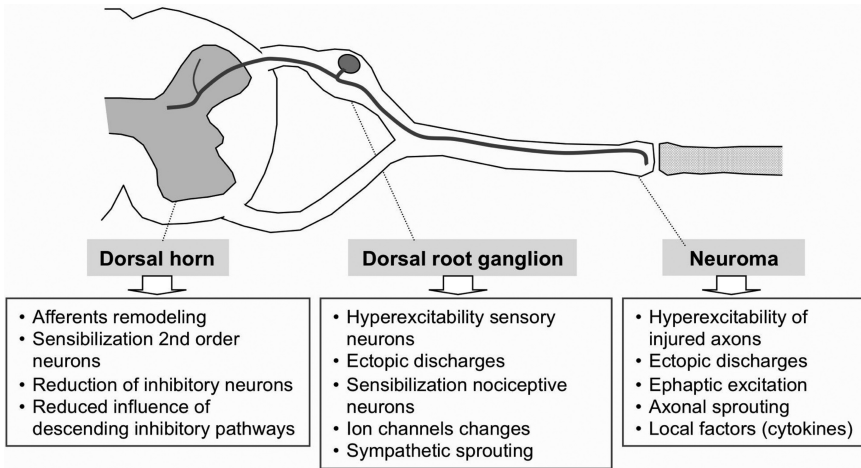


Figure 2.4 Schematic representation of the main peripheral mechanisms involved in the generation of neuropathic pain after a peripheral nerve injury.

anatomical site of the injury. Emotional and cognitive factors influence how patients react to chronic pain (Haythornthwaite et al., 2003), but it is much less certain if these factors contribute to the risk of developing pain.

The different forms through which neuropathic pain manifests suggest that different mechanisms are likely to mediate the different features of the condition. Features that appear most often related to neuropathic pain include tingling or numbness, pain evoked by heat or cold, and sensation of heat or a burning-like quality.

2.2.1 Mechanisms of Neuropathic Pain

Five key processes are considered in the neurobiological approach to the mechanisms of pain and hyperalgesia following a peripheral nerve injury (Figure 2.4): (1) the process of nociceptor activation and sensitization, responsible for the initial signaling of injury and the peripheral changes in the nociceptive system induced by a noxious stimulus; (2) the process of central amplification of nociceptive signals, known as central sensitization, generated by synaptic strengthening of connections between CNS neurons and responsible for the enhanced excitability that accompanies persistent pain states; (3) the process whereby activity in low-threshold sensory receptors from undamaged peripheral areas can access the nociceptive system and evoke pain sensations and hyperalgesic states (e.g., touch-evoked pain, tactile

allodynia); (4) the loss of endogenous inhibition produced at spinal level paired with changes in descending inhibitory pathways; and (5) neuroplastic changes at subcortical and cortical levels (Navarro et al., 2007).

2.2.2 Nerve Injury-induced Changes in Transduction

Peripheral nerve injury leads to a redistribution of transducers in the wrong place within a neuron, and this event carries two consequences: the emergence of mechanical sensitivity at sites that are normally mechanically insensitive and mechanical allodynia. Even mechanical stimuli associated with physiological functions, such as movement of tissue associated with blood flow, may also stimulate these transducers.

Normal pain sensations are normally elicited by activity in unmyelinated (C) and thinly myelinated (A δ) primary afferent neurons. These nociceptors are usually silent in the absence of stimulation, and respond best to stimuli that are potentially noxious. In neuropathic pain disease, after a peripheral nerve lesion, axotomized neurons become abnormally sensitive and develop pathological spontaneous activity generated at any anatomical level proximal to the lesion. These pathological changes are underpinned by dramatic molecular and cellular changes at the primary afferent nociceptor that are triggered by the nerve lesion.

A long time ago, spontaneous ectopic activity was demonstrated in awakened human amputees with phantom limb pain, by microneurographic single-fiber recordings from afferent fibers projecting into the neuroma, as well as barrages of action potential firing. After a nerve injury there is an increase in membrane excitability. Spontaneous discharges in DRG neurons have been recorded from cells of both intact and injured nerves. There are phenotypic changes in injured neurons but also in uninjured ones, driven by cytokines and growth factors released by denervated Schwann cells.

An embryonic channel, Nav1.3, is upregulated in damaged peripheral nerves, and this is thought to promote ectopic spontaneous activity in primary afferent neurons. Also, genes for the voltage-gated sodium channels Nav1.8 and Nav1.9 are expressed selectively in nociceptive primary afferent neurons. These fibers acquire a unique sodium-channel expression profile after nerve injury, with upregulation of Nav1.3 and downregulation of Nav1.7, Nav1.8, and Nav1.9. Changes of these channels are responsible for the lowering of the action-potential threshold and consequent hyperexcitability, playing an important role in the genesis of neuropathic pain (Omana-Zapata et al., 1997).

Entrance of calcium ions into the nerve endings regulates growth-related proteins and can participate also in the increased excitability of the injured neurons. In vitro, N and L-type calcium channels have been found to contribute to CGRP release from injured nerves. Blockade of N-, T-, and P-type calcium channels reduces neuropathic pain.

Related to thermal stimuli, normal body temperature can elicit spontaneous activity of primary afferents as a result of a change in the activation threshold of the noxious heat-sensitive TRPV1 channel. Damage to peripheral nerves provokes upregulation of TRPV1 that are located predominantly on uninjured C fibers and A fibers. These changes might contribute to the development of C-nociceptor sensitization and the associated symptom of heat hyperalgesia.

In transduction for mechanical stimuli, ASICs seem to be involved in static mechanical hyperalgesia. Nerve lesion fibers developing adrenergic sensitivity. It is known that in amputees, the perineuronal administration of norepinephrine induced intense pain. Neuroma after injury has both afferent C fibers and efferent post-ganglionic sympathetic C fibers which release noradrenaline and adrenaline. With high sympathetic activity there is a raised sensitivity of the regenerating sprouts toward the detection of nociceptive substances, such as bradykinin, serotonin, histamine, and capsaicin evoking depolarization and ectopic firing. Afferent excitability can also be increased by the combination of a downregulation of inhibitor transducers, such as opioid receptors, and the upregulation of excitatory transducers, such as the ATP receptor P2X3.

After Wallerian degeneration, products such as nerve growth factor (NGF) are released in the vicinity of spared fibers triggering the release of tumor-necrosis factor- α (TNF- α), channels and receptors expression (sodium channels, TRPV1 receptors, adrenoceptors. . .) thereby altering also the properties of uninjured afferents (Wu et al., 2002). Research is more focused now on the variety of changes that might occur in uninjured axons, as these neurons are still connected with their peripheral organs and could have a pivotal role in the generation of neuropathic pain.

2.2.3 Central Sensitization

Neuropathic pain may arise either as a result of peripheral sensitization of intact afferents or due to central sensitization. Central sensitization has been defined as “a prolonged but reversible increase in the excitability and synaptic

efficacy of neurons in central nociceptive pathways.” The enhanced synaptic transmission is manifested by long-term potentiation (LTP) following a short train of stimulation of C fibers. The transition of LTP between spinal interneurons involves glutamate and neurokinin 1 receptors.

The function of nociceptive pathways is increased due to high membrane excitability, synaptic efficacy, and reduced central inhibition. The net effect of central sensitization is to recruit previously subthreshold synaptic inputs to nociceptive neurons, generating an increased or augmented action potential output: a state of facilitation, potentiation, augmentation, or amplification (Latremoliere and Woolf, 2009). It is manifested as allodynia (touch-evoked pain), enhanced temporal summation (escalating pain in response to a constant stimulus), hyperalgesia (exaggerated pain experience to a standardized noxious stimulus), and secondary hyperalgesia (pain and hypersensitivity beyond the dermatome of the nerve injury).

Primary nociceptive neurons release glutamate, SP, CGRP, and ATP as neurotransmitters and neuromodulators. After injury, hyperexcitability is established with greater release of neurotransmitters in the spinal cord. They interact with NMDA, AMPA, mGluR, NK1R, and P2X receptors causing depolarization of nociceptive second-order neurons and scattering throughout the spinothalamic pathway.

Peripheral nerve injury leads to an increase in the general excitability of wide dynamic range neurons in the dorsal horn with multiple synaptic inputs from nociceptive and non-nociceptive system. This central sensitization is initiated and maintained by activity in pathologically sensitized C fibers. After peripheral nerve injury there are presynaptic functional changes that increase synaptic strength: the synthesis of transmitters and neuromodulators and more calcium. $A\beta$ touch fibers express increased levels of neuropeptides, such as CGRP and SP, and enhance activity of excitatory amino acid transmission via NMDA receptors.

Healthy nerve terminals uptake NGF and other growth factors from their target cells and transmit them by axonal transport to the DRG neurons. After nerve transection, this growth factors supply is interrupted, so that gene transcription and protein synthesis are altered. At the level of transcription control in the DRG neurons, the c-jun gene can be induced 1 day after axotomy. It is well known that c-Jun expression in the DRG neurons after nerve transection is associated with changes in neuropeptide levels: SP and CGRP decrease; galanin and NO synthase (NOS) increase dramatically during weeks following axotomy. The induction of c-jun of axotomized neurons has been closely related with inhibitory transynaptic neuron death or apoptosis by NGF starvation.

The increased release and production of NOS at the intraspinal presynaptic terminal may facilitate afferent synaptic transmission to the dorsal horn neurons contributing to spinal neuronal sensitization and hyperalgesia. Repetitive noxious stimulation leads to the increased activity of NMDA and AMPA receptors, which produce an influx of extracellular Ca^{2+} and activation of PKC in dorsal horn neurons. The increased intracellular Ca^{2+} induces the expression of *c-fos*. Fos protein is involved in the transcriptional control of genes that encode a variety of neuropeptides, including enkephalin and dynorphin. Enkephalin typically produces antinociceptive effects. Dynorphin has direct excitatory effects on spinal projection neurons and may also produce inhibition via a negative feedback mechanism on dynorphin-containing neurons. The net effect of these changes may have complex modulations in the development of central plasticity.

Postsynaptically, second-order dorsal horn neurons also abnormally express Nav1.3 after peripheral nerve injury. Physiologically, dorsal horn neurons receive a strong inhibitory control by GABA releasing interneurons that are lost by apoptosis after partial peripheral nerve injury, thus favoring central sensitization. Other postsynaptic changes involve phosphorylation of NMDA subunits and increased receptor density due to trafficking and enhanced synthesis of ion channels and scaffold proteins. These changes underlying central sensitization occur in the dorsal horn, amygdala, anterior cingulate gyrus, and prefrontal cortex.

Continuous and sustained afferent inputs, due to hyperactivity of the damaged nociceptive fibers, into the spinal cord cause a state of spinal sensitization. This is related to the demonstrated phenomenon of wind-up, in which noxious stimuli applied to the skin also enhance the excitability of dorsal horn units, producing exaggerated responses to subsequent stimuli (Mendell and Walsh, 1965). Another mechanism of intraspinal disinhibition following peripheral nerve injury involves a trans-synaptic reduction in the expression of the potassium-chloride cotransporter KCC2 in lamina I neurons, which disrupts anion homeostasis in these neurons (Mòdol et al., 2014). The effect is that GABA release from normally inhibitory interneurons and now exerts an excitatory action increasing central sensitization. Dorsal horn neurons receive a powerful facilitatory but mostly inhibitory descending modulating control from supraspinal brainstem centers. A loss of function in descending inhibitory serotonergic and noradrenergic pathways contributes to central sensitization and pain chronification. Peripheral nerve injury activates spinal cord glia and causes these cells to enhance pain by releasing proinflammatory cytokines and glutamate producing also central sensitization.

2.2.4 Low-threshold A β Fiber-mediated Pain

Neuropathic pain involves a profound switch in sensitivity such that low-threshold A β fibers, which normally signal innocuous sensations, may begin after neural lesions to produce pain sensation (Witting et al., 2006). A number of changes either independently or combined can promote A β fiber-mediated pain: central sensitization, disinhibition, and central afferent terminal sprouting.

Differential block induced by compression of the radial nerve in patients with nerve injury or exposed to experimental pain clearly demonstrated that pain induced by light brushing was mediated through A β fibers, whereas thermal pain was mediated through unmyelinated C fibers. Intrathecal morphine reversed nerve injury-induced thermal, but not tactile, hypersensitivity.

After peripheral nerve injury the most spontaneously active fibers are A β and A δ fibers (>80%), whereas C fibers represent a small (0–30%) portion of the active population. This hypersensitivity includes areas outside the injured nerve territories and occurs largely in the absence of peripheral sensitization. It is typically associated with a loss of C fiber peripheral terminals and disappears when conduction in large myelinated fibers is blocked. After nerve injury polysynaptic and monosynaptic A β fiber inputs in the most superficial laminae I and II of the dorsal horn are increased. These laminae normally receive only input from A δ and C fibers. After an injury, this clear organization can be lost, as some A β fibers arriving to lamina III–IV can produce aberrant sprouting and reach outer laminae. This may imply that some innocuous, tactile information will be processed abnormally in a nociceptive territory, constituting a potential mechanism for allodynia (Costigan and Woolf, 2000).

2.2.5 Changes in Endogenous Inhibitory Pathways, Disinhibition, and Plasticity

After nerve injury there is loss of pre- and postsynaptic inhibition in the spinal cord. This can occur because of death of inhibitory interneurons caused by the excitotoxicity of the lesion, reduction in the release of inhibitory neurotransmitters from surviving interneurons, and reduction in the expression of inhibitory transmitter receptors. In nociceptive lamina I neurons, the transmembrane gradient for chloride ions changes. GABA receptors no longer lead to hyperpolarization, instead depolarization is induced, provoking excitation and spontaneous activity (Keller et al., 2007). Independently, there is loss of GABAergic interneurons by apoptosis compromising the dorsal inhibition.

After GABAergic or glycinergic blockade or removal, tactile allodynia is induced and synaptic currents from A β fibers to nociceptive lamina I neurons increase.

The tonic noradrenergic inhibition that acts on α 2-adrenoceptors appears to be suspended after lesion thus, the net effect of descending adrenergic input goes paradoxically, from inhibition to facilitation. Other descending pathway in the modulation of the nociceptive input are the μ opioid receptors, which reduce their expression together with less sensitivity of dorsal horn neurons to its agonists after nerve injury.

Activated microglia synthesize and release prostaglandins, chemokines, NOS, and cytokines (TNF α , IL1, IL6...) acting as chemical mediators to amplify the microglial reactivity favoring the elevation of these molecules in the dorsal horn. Reactive microglial cells are also responsible for the release of cathepsin-S protease that causes the proteolysis of a transmembrane glycoprotein called fractalkine (CX3CL1) which interacts with its receptor (CX3XR1) located in the reactive microglial cell membrane, maintaining its reactivity and therefore contributing to the preservation of neuropathic pain. Microglia can also provoke neuronal death by producing ROS, proapoptotic cytokines and by a diminished glutamate uptake. Astrocytes also become activated after peripheral nerve injury with a slower onset and more prolonged time course than microglia, but also playing a role in the maintenance of neuropathic pain hypersensitivity.

The energy depletion in the injured neurons decreases the intracellular ATP concentration and consequent K_{ATP} channels activation. The activation causes potassium ions outflow leading to hyperpolarization, less excitability, and reduction in the release of neurotransmitters. However, K_{ATP} expression is decreased after peripheral nerve lesion of myelinated A δ nociceptive fibers enhancing hyperexcitability.

In the thalamus, there is an upregulation of nicotinic and cannabinoid receptors after peripheral nerve injury, suggesting that supraspinal receptors in the thalamus may contribute to the modulation of neuropathic pain responses. On the other hand, μ -opioid receptor mediated G-protein activity is reduced producing desensitization in receptors from this region. Also, NKCC1 and KCC2 dysregulation in the spinothalamic pathway is produced after sciatic nerve section, suggesting that neuropathic pain is maintained by reducing inhibitory inputs not only in the spinal cord but also at thalamus and cortex (Mòdol et al., 2014).

In summary, multiple and different mechanisms operate after nerve injury to increase excitability and reduce inhibition in the central pain pathway.

Apart from the loss of descending inhibition, another feature contributing to the hyperexcitability in the pain pathway after injury is disinhibition. Silent circuits and synapses in normal conditions can become activated after peripheral nerve injuries. The disinhibition can be produced by a shift in the properties of inhibitory receptors, for example, the loss of inhibitory function in GABA receptors, in relation with a shift in the function of chloride transporters NKCC1 and KCC2 (Mòdol et al., 2014).

Neural plasticity occurring after neuronal lesions is also detectable in reflex circuits, which are usually used as an indirect measure of central hyperexcitability. Electrophysiological changes caused by the lesion and following plastic reorganization can produce the appearance of hyperreflexia and an increase in wind-up responses that can eventually lead to spasticity and neuropathic pain (Valero-Cabré and Navarro, 2010; Redondo-Castro et al., 2010).

2.2.6 Changes in Subcortical and Cortical Regions

Some groups of brainstem neurons are related with nociceptive modulation, forming the called “brainstem pain modulating system.” It includes the mid-line PAG-RVM system, the more lateral and caudal dorsal reticular nucleus (DRt) and caudal ventrolateral medulla (cVLM). The descending projection of RVM includes the nucleus raphe magnus and the adjacent reticular formation. Serotonin and noradrenaline are the main neurotransmitters in these descending pathways. The predominant source of serotonergic input to the spinal cord arises from the nucleus raphe magnus. Serotonin causes hyperpolarization of afferent nociceptive fiber terminals and dorsal horn projection and it produces excitation in spinal GABAergic interneurons. Noradrenaline causes hyperpolarization of projection neurons and over terminals of primary afferent fibers inducing excitation of dorsal horn inhibitory interneurons. There are two types of neurons in the PAG-RVM system: “ON” cells facilitate and “OFF” cells inhibit pain transmission (Fields et al., 2000).

Neuropathic pain induces hyperexcitation of specific nociceptive and WDR neurons in the spinal cord causing a potentiation of the “ON” neurons response and a decrease of the “OFF” neurons. The preferential activation of “ON” cells located in RVM causes hyperalgesia, whereas hypoalgesia is achieved by the activation of RVM “OFF” cells. A sensitization of “ON” RVM neurons is also induced by overexpression of NMDA/AMPA, Trk-B, and NK1 receptors, whereas μ opioid receptor expression decreases. Under these circumstances, “ON” RVM neurons do not respond to inhibitory signals

from PAG, whereas they are highly stimulated by ascending inputs that release glutamate, SP, and dynorphin over thalamic and brainstem neurons. PAG neurons are also sensitized due to overexpression of several receptors including NMDA/AMPA and SP/NK1 but also overexpression of glutamate and BDNF. These PAG-BDNF-positive neurons project their axons over the “ON” RVM neurons, enhancing their depolarization via Trk-B and NMDA/AMPA receptors.

Furthermore, “OFF” RVM neurons mainly express NMDA/AMPA and TRPV1 receptors. After neuropathic and/or inflammatory painful insults, there are molecular changes in “OFF” RVM neurons such as an overexpression of GABA-A and kappa opioid receptors provoking hyperpolarization and reduction of their antinociceptive effect on spinal cord dorsal horn neurons. The increase of microglial and astroglial reactivity in RVM also contributes to the misbalance of the normally inhibitory descending pathways, by releasing several mediators that facilitate the excitation of “ON” RVM neurons and their excitatory effect on dorsal horn neurons (Wei et al., 2008).

A simple cutaneous nerve anesthetic blockade causes fast and reversible reorganization of dorsal column nuclei and thalamus. In primates, immediately after section of ulnar and median nerves there is a change in hand representation mapping in their brainstem cuneate nucleus, maintained over several days. Reorganization in cortex and thalamus territories is delayed a few weeks to months, depending on the growth of new connections and the expansion of thalamic receptive fields that tend to be larger than in somatosensory cortex. Cortical and subcortical reorganization and plasticity depend on the abnormal projections from peripheral and spinal levels.

Peripheral nerve injuries result in loss of evoked activity by deafferentation in the corresponding cortical map. Then, plastic changes occur resulting in reduction of the cortical area representing the denervated part of the body, in favor of expansion of adjacent representations from intact sources with hardly discernible somatotopy. The brain plasticity is not very accurate per se, as it was demonstrated in several cases of nerve section and repair in humans where sensory mislocalizations persist for many years due to misdirected reinnervation. The reorganization has different time courses depending on the severity of the lesion. After experimental sensory deafferentation of one finger in a human patient, the expansion of cortical representations of intact fingers is very fast and is recovered in minutes when the sensory blockade is stopped. On the other hand, amputees suffer a slower brain plasticity process for a few weeks to become permanent with reduction of affected area and expansion of adjacent cortical regions. The basis of phantom sensations

secondary to limb amputation seems to include reorganization phenomena at the cortical and subcortical levels. Furthermore, a sensory map corresponding to the different portions of the missing limb can be traced in the stump or in the face of some amputee subjects (Ramachandran and Hirstein, 1998).

The first changes in the brain produced by a peripheral nerve injury involve changes in synaptic efficacy, including increased excitatory neurotransmitter release, increased density of postsynaptic receptors, changes in membrane conductance, or removal of inhibitory projection resulting in unmasking of inactive synaptic connections at cortical and subcortical levels. Loss of GABAergic inhibition is the main cause of the short-term plastic changes in the cortex. In the same way that occurs in the spinal cord, there is an upregulation of Nav1.3 in the third-order nociceptive neurons in the thalamus resulting in hyperexcitability and expanded receptive fields. In situations of chronic deafferentation, structural mechanisms like LTP or LTD phenomena, sprouting for the formation of *de novo* connections and synaptogenesis can be reduced by NMDA receptor antagonist administration at the beginning of the plastic process.

At subcortical levels, transneuronal atrophy associated with retraction of axons and compensatory axonal sprouting seems to play a significant influence on the reorganization of somatotopic maps in the brain cortex. Plastic reorganization after nerve injuries has been related with structural changes in dendritic arborization within the cortex. Brain reorganization processes in adult subjects seem to occur primarily through changes in the strength and efficacy of existing synapses, rather than implicate active remodeling of connections.

In summary, neuropathic pain triggers plastic changes in the descending pain modulatory pathway: “ON” cell activation and “OFF” cell inactivation from the PAG-RVM system, to increase pain facilitation in the spinal cord. In addition, reorganization of ascending projections takes place sequentially from the dorsal horn to the brainstem, the thalamus and finally the somatosensory cortex.

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