# SECTION I MECHANISMS AND EPIDEMIOLOGY

# 1 Nociception: basic principles

RIE SUZUKI, SHAFAQ SIKANDAR, AND ANTHONY H. DICKENSON
*University College London*

## Introduction

Pain has been a major concern in the clinic for many decades. In recent years, considerable progress has been made with respect to our understanding of both acute and chronic pain mechanisms. This has largely been attributed to advancements in molecular biology and genomic techniques, as well as the use of animal models, which has allowed us to explore mechanisms and networks of neurons involved in pain processes. This has fundamentally altered our understanding of the pathophysiology of pain mechanisms and has led to the hope of development of novel analgesics.

The study of the receptor systems involved in the transmission of pain and its modulation involves investigation of processes occurring at the peripheral endings of sensory neurons, as well as central events. The mechanisms of inflammatory, visceral and neuropathic pain are different from those of acute pain, and cancer pain may both overlap and differ in some respects with these broad categories. Furthermore, there is considerable plasticity in both the transmission and modulating systems in these prolonged pain states so that systems change over time. The search for new treatments for these pain states requires the development of valid animal models. For such models to be valid, a number of criteria must be fulfilled. First, the model must provide reproducible and quantifiable behavioral data. Second, the model must produce behaviors in the animal that resemble some of the pain syndromes observed in humans (e.g., allodynia, hyperalgesia). Third, the behavioral data must correlate with pain responses seen and therapies used in humans. Through the use of these animal models, we can broaden our understanding of pain mechanisms and possibly identify or develop potential agents for treatment.

## Mechanisms of pain and analgesia

### The anatomy and physiology of pain

The somatosensory primary afferent fibers, which convey sensory information to the spinal cord, can be grouped into several classes according to the transduction properties of the individual nerve fiber. The properties of each afferent fiber are summarized in Table 1.1.

The afferent fibers differ in their conduction velocities and degrees of myelination, and can be distinguished by their diameter. The large-diameter Aβ-fibers are myelinated by Schwann cells and hence have a fast conduction velocity. This group of nerve fibers innervates receptors in the dermis and is involved in the transmission of low-threshold, non-noxious information, such as touch. The Aδ-fiber is less densely myelinated and conveys both non-noxious and noxious sensory information. The unmyelinated C-fiber conveys high-threshold noxious inputs and has the slowest conduction velocity of all three fiber types.

On entry into the spinal cord, each primary afferent fiber (Aβ-, Aδ-, or C-fiber) exhibits a specific termination pattern in the dorsal horn (Fig. 1.1). This has been studied extensively through the use of specific markers. Dorsal root

Table 1.1. *Classification of somatosensory primary afferent fibers innervating the skin*

| Primary afferent fiber type | Mean diameter (μm) | Myelination | Mean conduction velocity (m/s) |
|---|---|---|---|
| Aβ | 6–12 | Myelinated | 25–70 |
| Aδ | 1–5 | Thin myelination | 10–30 |
| C | 0.2–1.5 | None | <2.5 |

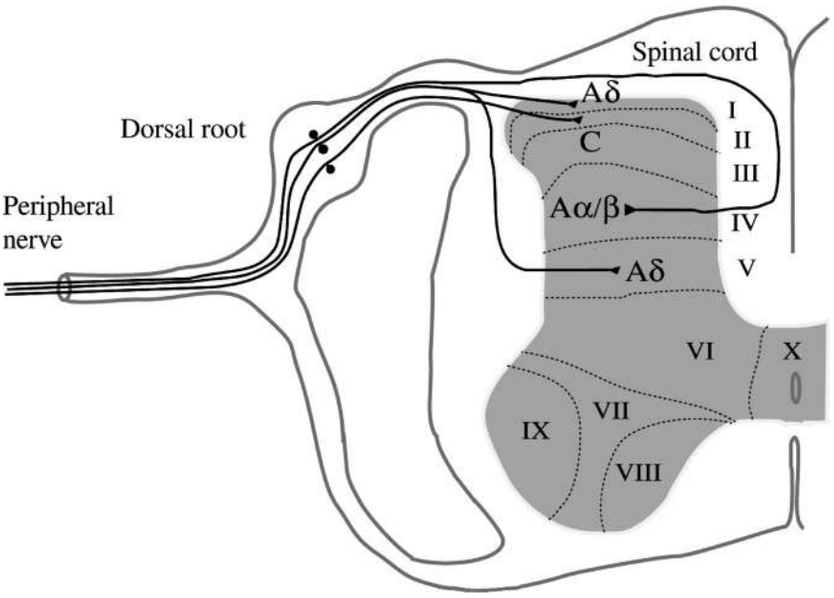


Fig. 1.1. Cross-section of the lumbar spinal cord illustrating the termination sites of afferent fibers in the dorsal horn and the organization of the gray matter into laminae I to X.

afferents send most of their collaterals into the segment of entry. However, there is also a degree of rostrocaudal distribution, and some collaterals may spread to several segments above or below the target segment. Thus, there is an anatomical substrate for the spreading of pain beyond the segment in which it originates.

The large-diameter Aβ-fiber enters the spinal dorsal horn through the medial division of the dorsal root and terminates in laminae III and IV, where it forms a characteristic termination pattern.[1] The densest arborization appears to occur in lamina III. Some also extend to laminae VIII and IX of the ventral horn, where they synapse directly onto motor neurons and form the basis of monosynaptic reflexes.[2] The terminals of Aδ-fibers, on the other hand, form a plexus at the surface of the spinal cord in laminae I and IIo. Unmyelinated C-fibers enter the spinal cord through the lateral part of the dorsal white matter and terminate in the superficial dorsal horn. Current evidence suggests that lamina II is the main termination area for cutaneous primary afferent C-fibers, whereas that for Aδ-fibers is in lamina I.[1]

These peripheral fibers activate spinal cord neurons, which in turn produce local spinal autonomic and motor reflexes. Spinal neurons can be classified into three broad categories: low-threshold only; nociceptive-specific (NS), in that these cells respond only to noxious mechanical, thermal, and chemical stimuli; and wide dynamic range (WDR) neurons that additionally code innocuous stimuli but increase their activity into the noxious range. The majority of lamina I nociceptive specific (NS) neurons in the superficial spinal cord are projection neurons that ascend to the parabrachial area, with limited terminations in other brainstem/midbrain regions.[3] The parabrachial area is a key supraspinal target implicated in the emotional and autonomic aspects of nociceptive processing. Because these limbic parts of the brain are important in mood, anxiety, fear, the sleep–wake cycle, and central autonomic control, the ability of spinal neurons to contact these zones is a likely basis for the comorbidities that accompany pain. In turn, the parabrachial and cuneiform areas contact the amygdala and hypothalamus and indirectly drive descending modulatory descending pathways. WDR neurons tend to project to areas of the brain that generate the sensory-discriminative components of pain, such as the thalamus and cortex. The activation of spinal neurons therefore produces local motor activity, and the parallel ascending pathways elicit the sensory and affective aspects of pain.[4]

## Pharmacology of pain transmission

### Peripheral events

The transmission of acute pain involves activation of sensory receptors on peripheral C-fibers, the nociceptors, which include a number of sensors for heat, mechanical, and chemical stimuli. However, once tissue damage and inflammation occur, the actions of prostanoids, bradykinin, and

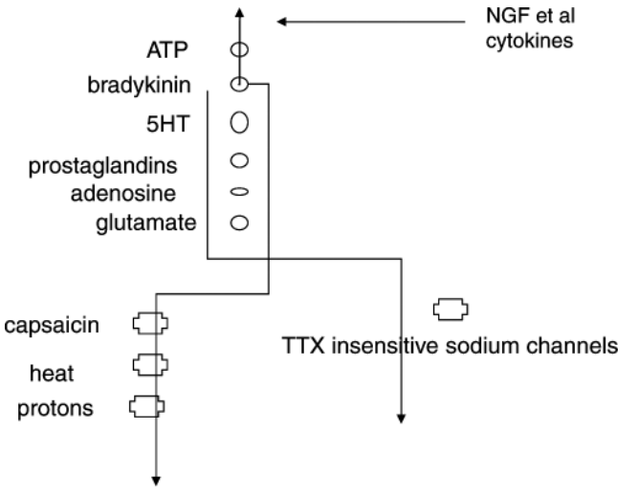


Fig. 1.2. A schematic diagram of a C-fiber showing some of the peripheral mediators of pain and inflammation in the periphery.

5-hydroxytryptamine (5-HT) on their excitatory receptors play a major role in sensitization and activation of C-fibers (Fig. 1.2). Other factors, such as nerve growth factor (NGF) and cytokines, also are important at the peripheral level, and resultant changes in the phenotype of the sensory neurons may be another important process. In common with all nerve fibers, C-fibers need to generate action potentials in response to stimuli, yet these nociceptive fibers have unique sodium channels, whereas some C-fibers are "sleeping"; that is, they do not respond to natural stimuli until after inflammation. Peripheral input that drives pain sensation depends on the presence of voltage-gated sodium channels, and altered sodium channel activity plays an important role in inflammatory and neuropathic pain.[5] Voltage-gated $Na^+$ channels propagate action potentials along neurons and spur hyperexcitability after nerve injury. Moreover, based on the efficacy of drugs such as lignocaine and carbamazepine, sodium channels are important targets for drugs, but the ubiquitous nature of the channels leads to low therapeutic windows. Different voltage-gated $Na^+$ channel isoforms, with different kinetic and pharmacological properties, have been delineated in sensory neurons, leading to the potential for more selective agents. The $Na_v1.8$ and 1.9 $\alpha$ subunits are expressed exclusively in small unmyelinated fibers and are resistant to block by tetrodotoxin (TTX), whereas the $Na_v1.7$ $\alpha$ subunit, which is susceptible to block by TTX, is expressed in sensory *and* sympathetic neurons. Tissue and nerve damage may lead to a change in the expression and function of $\alpha$ subunits and a resultant change in neuronal excitability to the detriment of the sensory system. Tellingly, inherited "gain-of-function" mutations in the $Na_v1.7$ $\alpha$ subunit in humans result in erythermalgia, a painful condition characterized by intolerable burning sensations in the extremities, whereas other mutations in this channel result in paroxysmal extreme pain disorder and loss-of-function mutations produce analgesia.[6]

### Peripheral mechanisms of inflammatory pain

Polymodal C-fiber receptors can be activated selectively by noxious thermal and mechanical stimuli. In the case of activation by noxious heat, we now suspect that the family of transient receptor potential vanilloid channels that also responds to the extract of hot peppers – capsaicin – may be responsible for the generation of action potentials after application of heat. Others within this family are responsive to warming, cooling, and noxious cold. Thus, the peripheral terminals of small-diameter neurons may be excited by a number of applied stimuli and also by endogenous chemical mediators, especially in conditions of tissue damage. These can be released from local non-neuronal cells, the afferent fibers themselves, and products from immune cells triggered by activation of the body's defense mechanisms. These chemical mediators then interact to cause a sensitization of nociceptors so that afferent activity induced by a given stimulus is increased. This produces primary hyperalgesia, a zone of increased sensitivity to painful stimuli in the center of the damaged tissue.[7]

One of the most important components in inflammation is the production of arachidonic acid metabolites. Arachidonic acid, a component of cell membranes, is liberated by phospholipase $A_2$ and is subsequently metabolized by two main pathways controlled by two enzymes, cyclooxygenase (COX) and lipoxygenase. This metabolism gives rise to a large number of eicosanoids (leukotrienes, thromboxanes, prostacyclins, and prostaglandins). These chemicals are still poorly understood, but it is clear that they do not normally activate nociceptors directly but, by contrast, reduce the C-fiber threshold and so sensitize them to other mediators and stimuli. The use of both steroids and nonsteroidal anti-inflammatory drugs (NSAIDs) is based on their ability to block the conversion of arachidonic acid to these mediators.[8] However, these drugs can only prevent further conversion and will not change the effects of eicosanoids that have already been produced. The action of most NSAIDs is to inhibit COX-1, but as this form is the constitutive enzyme, COX-1 inhibition results in the gastric side effects of NSAIDs, whereas their ability to block the inducible COX-2 enzyme appears to be a major contributor to analgesia. The new generation of selective COX-2 inhibitors was thought to have improved therapeutic profiles, as this form of the enzyme is induced at the site of tissue damage

and so spares gastric function; however, doubts as to their cardiovascular safety have arisen. Interestingly, COX-2 is normally present in the brain and spinal cord and so may be responsible for some of the central analgesic effects of NSAIDs.

Bradykinin is another chemical with important peripheral actions, but as yet it cannot be manipulated in any direct way by drugs. It is a product of plasma kininogens that find their way to C-fiber endings following plasma extravasation in response to tissue injury. Bradykinin receptors have been characterized and here again, there are two forms. The $B_1$-receptor is constitutively expressed less than the $B_2$-receptor, but in chronic inflammation, it is upregulated. Pain may arise via the activation of the $B_2$-receptor, which is abundant in most tissues; this can activate C-polymodal receptors. The response to bradykinin may be enhanced by prostaglandins, heat, and serotonin, indicating the extent of interactions among these peripheral pain mediators.[9]

After tissue damage, there is an accumulation of hydrogen ions; the pH is lowered in inflammation and ischemia. These protons may activate nociceptors directly via their own family of ion channels and sensitize them to mechanical stimulation. Acid-sensing ion channels are a family of sodium channels that are activated by protons. Of special interest is one type found only in small dorsal root ganglion neurons that are responsible for activation of nociceptors.

Mast cells can release histamine, which causes vasodilation, edema, and itch. Adenosine also is involved in inflammatory conditions. Substance P (SP) and calcitonin gene-related peptide (CGRP) released from the peripheral terminals of primary afferents (via axon reflex) cause neurogenic inflammation. These peptides produce vasodilation, plasma extravasation, and mast cell degranulation. Adenosine 5′-triphosphate (ATP) can cause direct nociceptor activation. The vascular changes produced by SP, CGRP, prostaglandins, and bradykinins lead to vasodilation and plasma extravasation that underlie the swelling that accompanies tissue damage.[10]

Serotonin is released from a number of non-neuronal cells, such as platelets and mast cells, and can produce an excitation of nociceptive afferents via the activation of a large number of receptors (5-$HT_{1A}$, 5-$HT_2$, and 5-$HT_3$), as well as sensitizing nociceptors, especially to bradykinin. 5-HT's key role, but not its mechanism of action, in the pain associated with migraine and other headaches is well established, but little is known about the actions of this mediator in other noncranial pains. The aura of neurological symptoms and/or signs is thought to be caused by a vascular or a neuronal mechanism, or a combination of the two. One theory suggests that changes in the vasculature are responsible for causing migraine. A related idea is that peripheral nerves are the source of the problem and then cause the associated vascular changes via release of 5-HT and other inflammatory mediators. A third theory suggests that the primary abnormality is neuronal but originates within the brain itself.[11]

Sumatriptan, which is commercially available for the treatment of migraine, is an agonist at 5-$HT_{1B}$ and 5-$HT_{1D}$ receptors. It has three distinct pharmacological actions. Stimulation of the presynaptic 5-$HT_{1D}$ receptors on trigeminal Aδ-fibers inhibits the release of CGRP, which inhibits dural vasodilation. 5-$HT_{1D}$ receptors on trigeminal C-fibers also are stimulated, inhibiting the release of SP and, therefore, blocking neurogenic inflammation and dural plasma extravasation. A further possible action is a direct attenuation of excitability of trigeminal nuclei, as 5-$HT_{1B/1D}$ receptors in the brainstem are stimulated. Stimulation of these receptors is caused by second-generation triptans that cross the blood–brain barrier, such as zolmitriptan. They all bind to neurons in the trigeminal nucleus caudalis and in the upper cervical cord.[12]

Direct vasoconstriction is mediated by the stimulation of vascular 5-$HT_{1B}$ receptors. These receptors also are found systemically, and coronary arteries also undergo vasoconstriction. Sumatriptan constricts cerebral arteries, but if the vasculature is normal, this does not affect cerebral blood flow.

Other factors, such as NGF and cytokines, also are important at the peripheral level. Changes in the phenotype of sensory neurons may be produced by these mediators. This contributes to the complex changes in the transduction of painful stimuli.

### Peripheral mechanisms of neuropathic pain

After nerve injury, there is considerable plasticity in the peripheral and central nervous system (CNS), which may be related to the pathogenesis of neuropathic pain states. The mechanisms underlying these chronic pain states are heterogeneous. The complex nature of these syndromes is largely responsible for the limited number of therapeutic strategies.

The sequence of events that follow peripheral nerve injury, and consequently contribute to the development of neuropathic pain, can be seen at various levels of the nervous system. Nerve injury is associated with anatomical, neurochemical, pharmacological, and electrophysiological changes.[13] After a nerve lesion develops, there are alterations in the anatomy of the peripheral nerves; demyelination occurs through phagocytosis by macrophages. A

number of neurochemical changes also take place. Studies have reported a complex change in the expression of neuropeptides in the dorsal root ganglia (DRG), including a reduction in the levels of SP and CGRP. In contrast, there is evidence for an upregulation of galanin, as well as for a novel induction of vasoactive intestinal polypeptide and neuropeptide Y. These changes may be related to the degenerative and regenerative processes that take place in the central and peripheral branches of the sensory neuron.

Peripheral nerve damage leads to a downregulation of 1.8 and 1.9 sodium channel transcripts in the DRG (despite the translocation, insertion, and clustering of $Na^+$ channels containing these subunits at injury and neuroma sites), with a concomitant upregulation of the embryonic TTX-S α 1.3 subunit.[14,15] Various studies suggest that ectopic activities in sensory neurons are mediated by $Na_v1.3$ α subunits, and not 1.7, 1.8, or 1.9 α subunits. (Interestingly, neuropathic pain develops normally in mice lacking $Na_v1.7$ and/or 1.8 subunits despite their known involvement in setting mecha nical sensory thresholds.)[16] $Na^+$ channels with the 1.3 subunit have the correct biophysical properties to support rapid firing; they are upregulated in all models of neuropathic pain, and their spontaneous firing can be blocked by TTX.

One prominent feature of nerve injury is a marked increase in neuronal excitability, which is manifested as abnormal ectopic activities. Ectopic impulses appear to originate from the DRG as well as from the neuroma of the injured peripheral nerve.[17] The incidence and level of spontaneous activity depend on several factors, including animal species, time elapsed from injury onset, type of nerve injury, and the nerve studied. One factor contributing to the electrogenesis of ectopic discharges is thought to be the alteration in the expression of voltage-gated sodium channels on peripheral afferents after nerve injury. Immunohistochemical studies have demonstrated that nerve injury induces remodeling of axolemmal sodium channels, and channels have been shown to accumulate in neuromas, especially in regions of demyelination.[18]

Activation of these previously quiescent channels may therefore induce abnormal repetitive firing in injured neurons and potentially act as ectopic impulse generators. The change in expression of both TTX-S and TTX-R channels implies that the kinetics and voltage-dependent characteristics of sodium currents may be considerably altered in neuropathic pain states. This, together with the pathological accumulation of sodium channels at the neuroma, may promote inappropriate action potential initiation and may form the basis of the therapeutic use of local anesthetics and excitability blockers, such as carbamazepine, for neuropathic pain states.[19]

Another feature of nerve injury is axonal sprouting, whereby injured axons undergo regeneration and reinnervation of target peripheral tissues including the deafferentated territory. In addition, nerve injury can induce adjacent undamaged sensory axons to sprout collateral fibers into an area that has been denervated owing to the nerve lesion over a limited distance (collateral sprouting).

However, although studies have reported a structural reorganization of the spinal cord after nerve injury such that the central terminals of axotomized Abeta-fibers sprout into lamina II, an area of the cord that normally processes only C-fiber input,[20] technical issues with the tracer refute the suggestion that this is a basis for allodynia.[21]

### Central mechanisms of pain

As peripheral nerve fibers become activated by tissue or nerve damage, action potentials are generated in the nerve and arrive in their central endings of the fibers in the spinal cord. Here, the electrical events switch to chemical transmitter release through calcium channels that open in the membrane. As calcium channels are activated, they cause transmitter release, and the transmitters then activate their receptors in the spinal cord, causing important increases in neuronal excitability. The respective neurons then generate action potentials that are passed onto the next nerve cell, transmitter is again released, and the messages pass on upward to many areas of the brain involving thousands and thousands of nerve cells scattered throughout the brain.

Whatever the pain state and alterations in function that follow, abnormal peripheral nerve activity will then affect calcium channel function and alter the release of transmitter into the spinal cord. Indeed, numerous studies have shown upregulation of calcium channels after nerve injury, a possible compensation for the loss of normal input produced by the nerve injury. Recent studies with agents that block neuronal voltage-sensitive calcium channels would also suggest that this leads to an increase in central neuronal excitability.[22] N-type channels, blocked by omega-conotoxin, appear to be important in behavioral allodynia and play a major role in the neuronal responses to low- and high-threshold natural stimuli and in the C-fiber–evoked central hyperexcitability. Blockers of this channel are considerably more effective after nerve injury.[23] Because the channel is voltage operated, these results again suggest increased excitability of the spinal cord neurons after injury. Ziconotide is an example of a drug that works to block calcium channels. Its use is restricted to the spinal route.[24] Gabapentin and pregabalin are drugs licensed for neuropathic pain that have analgesic activity in neuropathic pain

states from varying origins. Recent randomized controlled trials of both drugs, one in patients with postherpetic neuralgia and another in patients with diabetic neuropathy, concluded that the drugs are effective in the treatment of these pain states. The mechanism of action of gabapentin and pregabalin is now clearly established; these drugs interact with calcium channels to reduce the release into the spinal cord of transmitter produced by peripheral pain stimuli, reducing the excitability of neurons that are sending messages to the brain. This interaction occurs through the ability of these compounds to bind to the $\alpha_2\delta$ subunit of calcium channels.[22] This, in theory, would lead to a change in the function of all calcium channels if not for the state dependency of these compounds that are able to selectively alter abnormal activity. This appears to result from an upregulation of the $\alpha_2\delta$ subunit in damaged nerve fibers and activity in descending excitatory 5-HT pathways from the brainstem, which then permit actions of the drug on pathological activity while sparing normal function.[25]

Nociceptive sensory information arriving from primary afferent fibers enters the spinal cord via the dorsal horn. On entering the spinal cord, nociceptive signals undergo considerable convergence and modulation. The pharmacology of the spinal cord is extremely rich and contains a diverse range of neurotransmitters and receptors, which may be excitatory or inhibitory depending on the consequence of their activation and their location on the neuronal circuitry. The transmission of pain therefore can be seen as a complex process involving the interplay between excitatory and inhibitory systems acting at different levels of the CNS. All these systems are subject to plasticity, and alterations in pharmacological systems may occur during pathological conditions.[26]

In the spinal cord, nociceptive signaling systems undergo convergence and modulation through interactions that involve peripheral inputs, interneurons, and descending controls. One consequence of this modulation is that the relationship between stimulus and response to pain is not straightforward. The response of output cells could be greatly altered via the interaction of various pharmacological systems in the spinal cord.

### Excitatory transmission

The excitatory amino acids glutamate and aspartate have been implicated in the transmission of nociceptive information in acute and chronic pain states.[27] Several receptors for glutamate have been identified in the brain and spinal cord, including the ionotropic glutamate receptors (*N*-methyl-D-aspartate [NMDA], alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid [AMPA], kainate) and the metabotropic glutamate receptors. The three ionotropic receptor types have a prominent localization in the superficial dorsal horn (laminae I–III) and in deeper layers (laminae IV–VI). The parallel neuroanatomical distribution of these receptors in laminae I–III of the spinal cord provides support for functional interactions between NMDA and non-NMDA receptors in modulating nociceptive transmission.

The excitatory amino acids are found in most sensory fibers, including both large- and small-diameter fibers. In the latter case, they are co-localized with peptides, such as SP. The coexistence of these two transmitters suggests that they are released together in response to a noxious stimulus and thereby contribute to the transmission of pain. Whereas AMPA receptors are activated in response to brief acute stimuli and are involved in the fast events of pain transmission, NMDA receptors are activated only after repetitive noxious inputs, under conditions in which the stimulus is maintained.[27] NMDA receptors have been implicated in the spinal events underlying "wind-up," whereby the responses of dorsal horn neurons are significantly increased after repetitive C-fiber stimulation despite the constant input (Fig. 1.3).[28] This increased responsivity of dorsal horn neurons is probably the basis for central hyperexcitability and is responsible for the amplification and prolongation of neuronal responses in the spinal cord.[26,29]

The NMDA receptor has a heteromeric structure composed of two subunit types: the NR1 subunit and one of four subunits (NR2A–NR2D). It is an ionotropic receptor coupled to a cation channel, which is blocked by physiological levels of $Mg^{2+}$ at the resting membrane potential. The channel is blocked in a voltage-dependent manner. The receptor can operate only after sufficient repeated depolarization. The removal of the $Mg^{2+}$ block is mediated by peptides, likely SP and CGRP, which are co-released with glutamate. After a brief acute stimulus, pain transmission from C-fibers is largely mediated by the action of glutamate on AMPA receptors. When the stimulus is sustained or its intensity is increased, however, the action of SP on NK-1 receptors produces sufficient membrane depolarization so that the $Mg^{2+}$ block can be removed and the NMDA receptor activated.[26] SP (and also other peptides in the spinal cord) therefore plays an important role in recruiting NMDA receptors and contributes to the cascade of events leading to the enhancement and prolongation of the neuronal response. Indeed, the administration of SP receptor antagonists has been shown to produce antinociception and decrease spinal excitability.[30]

Functional modulation of the NMDA receptor can be achieved through actions at various recognition sites.

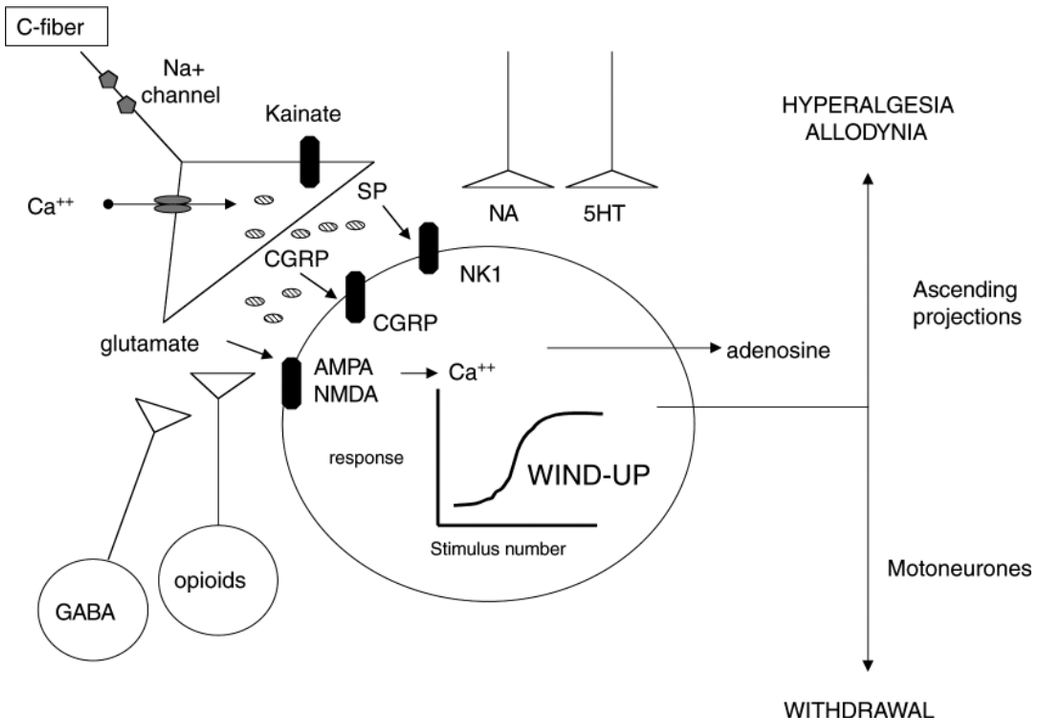


Fig. 1.3. Pharmacology of the spinal cord. The diagram depicts many of the transmitters and receptors involved. Action potentials in a peripheral C-fiber, propagated by sodium channels, arrive in the central terminals and open calcium channels. The influx of calcium causes the release of glutamate, SP, and CGRP, which activate their respective receptors on spinal neurons. NMDA receptor activation produces the wind-up depicted in the figure. Plasticity in these systems may result in hyperalgesia and allodynia. The excitatory events can be controlled by activity or drugs acting on opioid, GABA, and adenosine systems.

Potentially, there are several ways in which the effect of released glutamate can be antagonized through NMDA receptor blockade. Numerous studies have investigated the potential use of antagonists acting through the different recognition sites; however, because of the ubiquitous nature of the receptor, it often has been difficult to achieve therapeutic effects at the target site in the absence of adverse side effects. Evidence suggests that drugs acting at the glycine site in particular appear to lack some of the typical NMDA receptor antagonist side effects. In addition, the fact that there are four subtypes of the receptor (NR1/NR2A–NR2D) might allow the production of drugs with selective actions. If these receptors had different distributions, it might be possible to target pain while avoiding forebrain receptors that may mediate problematic side effects. At present, ketamine, which blocks the channel, although not without problems of tolerability, is the most efficacious blocker of NMDA-induced activity.

Substantial evidence exists for the involvement of NMDA receptors in various pathological pain states. Studies have demonstrated the effectiveness of NMDA receptor antagonists in animal models of inflammation,[31–33] neuropathic pain,[34] allodynia,[35] and ischemia.[36] Both presurgical and postsurgical administration of antagonists was shown to be effective, suggesting that the induction and maintenance of these ongoing pain states depend on NMDA receptor–mediated events.

It is now clear that neuropathic pain states are, at least in part, mediated by NMDA receptor–mediated events based on earlier findings from animal studies.[37] After nerve injury, there appears to be a greater contribution of the NMDA receptor system to neuronal activity, and this may play a role in the spinal hyperexcitability that underlies this condition. Neuropathy may produce a prolonged activation of NMDA receptors as a result of a sustained afferent input to the spinal cord, and this may result in a continuous increase in the release of glutamate through upregulated calcium channels. Hence, a greater proportion of channels are likely to be in their open state during neuropathy, and this could enable NMDA channel blockers to exert greater effects as a result of their use-dependency. In the case of inflammation, the sensitization of peripheral nociceptive fibers means that

a stimulus applied to the periphery will evoke more transmitter release and consequently greater NMDA receptor activation.

A number of studies suggest that there is a subsequent complex change in the pattern of the evoked neuronal responses, as well as in the receptive field size of dorsal horn neurons, after nerve injury. Thus, there are important changes in the spinal processing of neuropathic pain. Alterations in the response profile of spinal neurons appear to be modality dependent and are characterized by both increases and decreases in selected peripheral stimuli. It is not clear what underlies this differential plasticity of spinal neurons, although it is likely that the changes result from de novo acquired neuronal responses as well as from alterations in the existing response profiles of spinal neurons.[38]

Behavioral studies have shown that NMDA receptor activation is required for the induction and maintenance of pain-related behaviors.[39] Thus, it is likely that aberrant peripheral activity is amplified and enhanced by NMDA receptor–mediated spinal mechanisms in neuropathic pain. The degree of hyperexcitability after peripheral nerve damage is hard to gauge, as peripheral fibers, central neurons, and pharmacological systems may all change their properties after injury. As the operation of the NMDA receptor/channel depends critically on the underlying level of excitability, spinal neurons are probably hyperactive and compensate for much of the peripheral nerve damage. Although there is human evidence for the effectiveness of agents acting at the NMDA receptor complex, especially ketamine,[37,40] it appears that although some patients get good pain relief, the majority cannot achieve complete pain control because dose escalation is compromised by the narrow therapeutic window; we await strategies that can increase this therapeutic window.

Although the extent of the loss of myelinated and unmyelinated fibers after nerve injury is unknown, a large proportion of input is expected to be lost as a result of neuropathy. Despite this marked loss of afferent input, however, the overall changes in the responses of spinal neurons appear to be comparatively small. Hence, this may represent an increase in spinal cord excitability, which could compensate for the loss of afferent drive. The high level and incidence of spontaneous activity seen after nerve injury may be one contributing factor to the global spinal hyperexcitability. This activity may well produce an ongoing level of transmitter release in the spinal cord, which may, in turn, favor hyperexcitability of responses to subsequent evoked stimuli. Furthermore, there may be a drop in the mechanical threshold of spinal neurons after nerve injury, which could facilitate neuronal activation with a low-intensity stimulus. This, together with an enlargement of neuronal receptive fields, could form the electrophysiological basis for the mechanism underlying the behavioral manifestation of allodynia, hyperalgesia, and spontaneous pain after neuropathy (Fig. 1.4).[41]

### Central inhibitory systems

The discovery and use of opium date back many centuries. The roles of the μ, δ, and κ receptors have been established. Most clinically used drugs act on the μ receptor, and the δ receptor has remained a target for opioids with fewer potential side effects compared with morphine, although no useful clinical drugs have ensued. The endogenous opioid peptides, the enkephalins, have clear controlling influences on the spinal transmission of pain, whereas the dynorphins have complex actions. Inhibitors of the degradation of the enkephalins have been produced.

To date, four opioid receptor subtypes have been cloned and isolated, which include the μ, δ, and κ receptors[42] and the recently identified ORL-1 (opioid receptor–like) receptor (Table 1.2).[43] The endogenous opioid peptides for these receptors are endorphin, enkephalin, dynorphin, and nociceptin, respectively. The recently discovered ORL-1 receptor, which exhibits considerable sequence homology with the other three classical opioid receptors, shows unique pharmacological properties because it exhibits only a low affinity for naloxone, a universal opioid receptor antagonist. The endogenous peptide for the receptor has been termed nociceptin, or orphanin FQ, and although its functional role is still somewhat unclear, extensive studies are currently being conducted to elucidate its role in pain modulation.[44,45] Overall, this peptide produces spinal analgesia but may well function as an "anti-opioid" at supraspinal sites.

The cloning and isolation of opioid receptors were a major breakthrough in understanding the molecular basis of opioid actions, as well as their localization on nerve fibers. The best-described central sites of action of morphine are at spinal and brainstem (midbrain) loci. Autoradiographic and immunohistochemical studies have shown opioid receptors to be localized primarily in the superficial dorsal horn (laminae I–II); a smaller population has been demonstrated in deeper layers.[46,47] The relative proportion of opioid receptor subtypes in the rat spinal cord has been reported to be approximately 70%, 20%–30%, and 5%–10% for μ, δ, and κ receptors, respectively. The majority of these receptors appear to be located on presynaptic terminals of fine afferent

Table 1.2. *Opioid receptors and their ligands*

| | Mu | Delta | Kappa | ORL-1 |
|---|---|---|---|---|
| Endogenous agonist | Beta-endorphin<br>Endomorphin | Met-enkephalin<br>Leu-enkephalin | Dynorphin $A_{(1-8)}$<br>Dynorphin $A_{(1-13)}$<br>Dynorphin B | Nociceptin/OFQ |
| Other | Morphine | DPDPE | U50488H | – |
| Agonists | DAMGO | DSTBULET | | |
| Antagonists | Naloxone | Naltrindole<br>Naloxone | Naloxone<br>Nor-BNI | $Phe^1\psi(CH2NH)$<br>$Gly^2NC_{(1-13)}NH_2$ |

*Abbreviations:* DAMGO, [D-Ala2, N-Me-Phe4, Gly5-ol] enkephalin; DPDPE, [D-Pen2, D-Pen5]-enkephalin; DSTBULET, Tyr-D-Ser(OtBu)-Gly-Phe-Leu-Thr; OFQ, orphanin FQ.

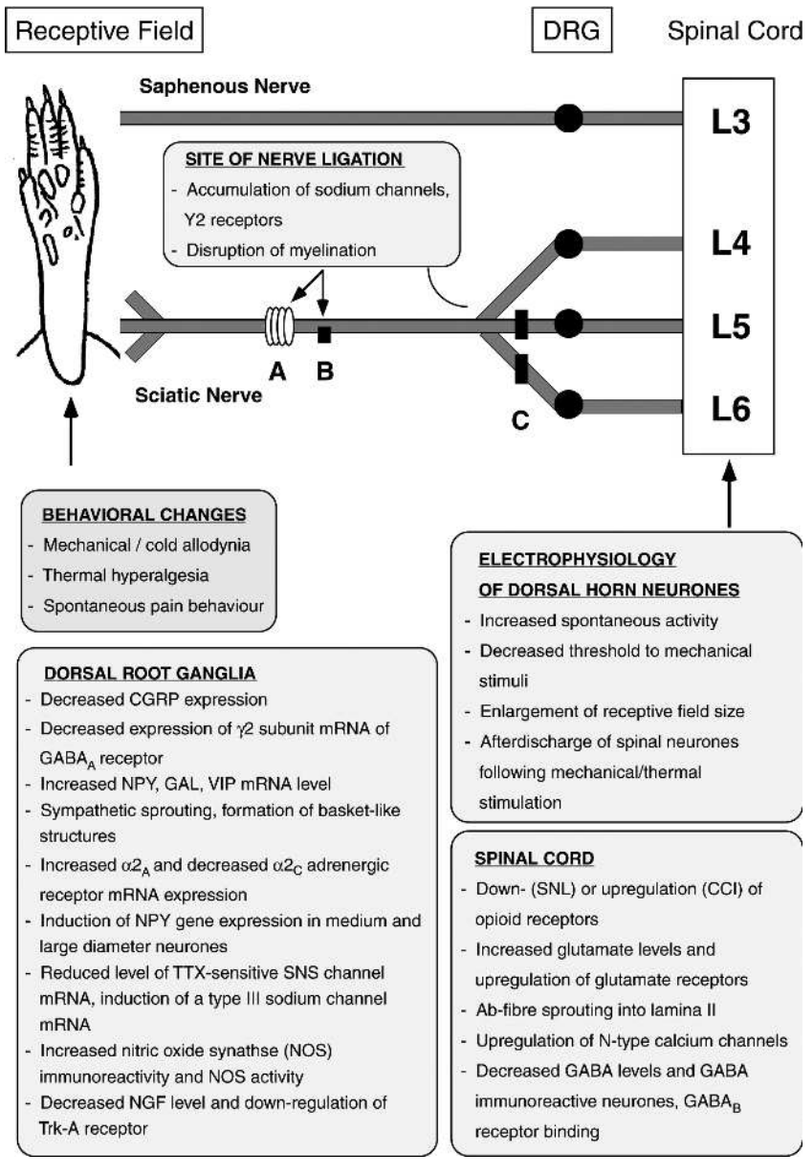


Fig. 1.4. An overview of some of the changes that occur after injury to a peripheral nerve together with the types of animal models used to study the mechanisms of neuropathy. A: CCI model, B: PSTL model, C: selective SNL model.