

PART I

THE NEUROSCIENCE OF ARTICULAR PAIN

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SPINAL MECHANISMS CONTRIBUTING TO JOINT PAIN

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INTRODUCTION

Nociceptive input from the joint is processed in different types of spinal cord neurons. A proportion of these neurons are only activated by mechanical stimulation of the joint and other deep tissue (e.g., adjacent muscles). Other neurons are activated by mechanical stimulation of the joint, muscles, and skin. The majority of the neurons are wide dynamic range neurons (small responses to innocuous pressure to deep tissue and stronger and graded responses to noxious mechanical stimulation). Importantly, neurons with joint input show pronounced hyperexcitability during development of joint inflammation (enhanced responses to mechanical stimulation of the inflamed joint as well as to healthy adjacent deep structures, reduction of mechanical threshold in high threshold neurons, and expansion of the receptive field). Thus inflammation induces neuroplastic changes in the spinal cord, which alter nociceptive processing. This state of hyperexcitability is maintained during persistent

inflammation. The neurons are under strong control of descending inhibition, which increases during the acute phase of inflammation. Several transmitters and mediators contribute to the generation and maintenance of inflammation-induced spinal hyperexcitability including glutamate, substance P, neurokinin A, CGRP, and prostaglandins. The latter compounds show enhanced release and an altered release pattern during inflammation in the joint.

PAIN SENSATION IN THE JOINT

Sensory information from muscle and joint influences the motoric system and is involved in the sense of movement and position but usually this does not reach consciousness. The major conscious sensation in deep tissue such as joint and muscle is pain. In a normal joint, pain is most commonly elicited by twisting or hitting the joint. In awake humans, direct stimulation of fibrous structures with innocuous mechanical stimuli evoked pressure sensations. Pain was elicited when noxious mechanical, thermal, and chemical stimuli were applied to the fibrous structures such as ligaments and fibrous cartilage.^{1,2} No pain was elicited by stimulation of cartilage, and stimulation of normal synovial tissue rarely evoked pain.¹

Joint inflammation is characterized by hyperalgesia and persistent pain at rest that is usually dull and badly localized.^{1,3,4} Hyperalgesia means that the application of noxious stimuli causes stronger pain than normal, and that pain is even evoked by mechanical stimuli whose intensity is normally not sufficient to elicit pain (i.e., movements in the working range and gentle pressure, e.g., during palpation). This heightened pain sensitivity results from peripheral sensitization (increase of sensitivity of nociceptive primary afferent neurons⁵) and central sensitization (hyperexcitability of nociceptive neurons in the central nervous system).

Pain resulting from degenerative osteoarthritis (OA) shows similarities and differences to inflammatory arthritic pain. Osteoarthritic pain is usually localized to the joint with OA but it can be referred (e.g., hip OA may cause knee pain). It varies in intensity and is usually worsened by exercise (weight-bearing, movement) and relieved at rest. It is usually episodic but may be constantly present in advanced OA. A particular quality of OA pain is pain at night.⁶ The *site* of OA pain and the *nature* of OA pain are under discussion because the cartilage is not innervated⁷ and because there is a poor correlation between radiological signs (narrow joint space and osteophytes) and the occurrence of joint pain.⁶ Some recent studies used magnetic resonance imaging (MRI) and found that painful OA knee joints exhibit more MRI abnormalities than nonpainful OA joints. Pathological findings in MRI studies are synovial hypertrophy and synovial effusions as well as subchondral bone marrow edema lesions (which may increase intraosseal pressure).⁸ These data and the observation of inflammatory cells in the sublining tissue⁶ evoked a discussion to which

extent OA pain is evoked by inflammatory mechanisms that appear from time to time (possibly corresponding to painful episodes in chronic OA). At later stages, capsular fibrosis and muscle contracture around the joint may contribute to OA pain. Quite clearly, however, factors such as obesity, perceived helplessness, and other psychological factors influence OA pain as well.⁶

SPINAL CORD NEURONS THAT RESPOND TO MECHANICAL STIMULATION OF THE JOINT

The articular nerves supplying the knee or elbow joint of rat, cat, and monkey enter the spinal cord via several dorsal roots, thus projecting to several spinal segments. Due to the widely distributed projection area, joint afferents influence sensory neurons and reflex pathways in several spinal segments. Within the gray matter, knee joint afferents project to the superficial lamina I and to the deep laminae V–VII.⁷ Figure 1.1A shows the spinal termination fields of horseradish peroxidase-labeled knee joint afferents in the segment L7 in the cat spinal cord. Correspondingly, spinal cord neurons that are synaptically activated by joint afferents can be identified in the superficial and deep dorsal horn and also in the ventral horn.^{9,10}

Receptive Fields and Activation Thresholds of Neurons with Joint Input

In both cat and rat, mechanonociceptive inputs from the joint are processed in dorsal horn neurons that respond solely to mechanical stimulation of deep tissue, or in neurons that respond to mechanical stimulation of both deep tissue and the skin. Receptive fields of single sensory neurons (regions from which neurons can be activated) are usually not restricted to the joint but more extended. Figure 1.1C shows the receptive field of a spinal cord neuron with convergent inputs from skin, deep tissue, and the knee joint. The neuron was activated by pressure applied to the knee joint (capsule, ligaments) and also by compression of the quadriceps muscle in the thigh and the gastrocnemius–soleus muscle in the lower leg, and in addition it had a cutaneous receptive field at the paw. However, many neurons have receptive fields that are restricted to the deep tissue. Figure 1.1D shows the receptive field of a spinal cord neuron with a receptive field in the deep tissue of the leg and in the knee joint. Some neurons have bilateral receptive fields.⁷

Concerning mechanical thresholds, neurons are either nociceptive-specific (NS) or wide-dynamic-range (WDR) neurons. Nociceptive-specific neurons respond only to intense pressure and/or to painful movements such as forceful supination and pronation. These stimuli elicit pain. WDR neurons respond to both innocuous pressure and noxious pressure, encoding stimulus intensity by

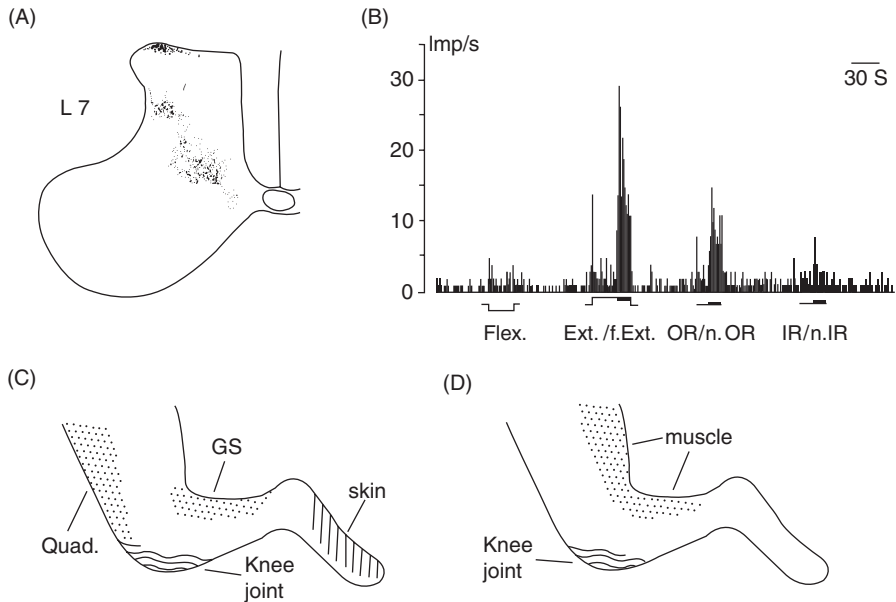


Figure 1.1. Spinal projection of primary afferent fibers of the knee joint and response properties of spinal cord neurons with input from the knee joint. (A) Spinal termination field of horseradish peroxidase-labeled primary afferent fibers of the posterior articular nerve of the knee joint in the gray matter of the segment L7 in the cat. (B) Responses of a wide dynamic range neuron with input from the knee joint to innocuous and noxious movements of the knee joint. The histograms show the number of action potentials per second that were elicited by the movements (bin width 1 s). Flex, flexion of the knee joint; Ext., extension of the knee joint; f.Ext, forced extension of the knee joint; OR, outward rotation of the knee joint (supination); n.OR, noxious outward rotation of the knee joint; IR, inward rotation of the knee joint (pronation); n.IR, noxious inward rotation of the knee joint. (C) Receptive field of a spinal cord neuron with input from the knee joint. This neuron was excited by pressure applied to the skin of the paw, the deep tissue of thigh (quadriceps muscle), and lower leg (gastrocnemius-soleus muscle) and the structures of the knee joint. (D) Receptive field of a spinal cord neuron that was only excited by pressure applied to deep tissue (muscles) and the knee joint. (Part A from Ref. 77; B–D from Ref. 10).

the frequency of action potentials. They may also be weakly activated by movements in the working range, but they show much stronger responses to painful movements. Figure 1.1B displays the response pattern of a WDR neuron with joint input. The neuron exhibited small responses to flexion, extension, and outward rotation (OR) of the knee in its physiological range, but pronounced responses were elicited by forced extension (f.Ext.) and by noxious outward rotation (n.OR) exceeding the working range of the joint. By and large, NS neurons have smaller receptive fields restricted to deep tissue in joint and muscle, and they do not have a receptive field in the skin.^{5,7}

Supraspinal and Spinal Projections of Spinal Neurons with Joint Input

Neurons with joint input project to different supraspinal sites (cerebellum, spinocervical nucleus, thalamus, reticular formation) or to intraspinal (segmental) interneurons and motoneurons.⁷ Ascending projections to the thalamus (in the spinothalamic tract) are important to activate the thalamocortical systems that generate the conscious pain sensation. In the cat, neurons were identified that have cell bodies in the ventral horn, belong to the spinoreticular tract, and are predominantly or exclusively excited by noxious stimulation of deep tissue.^{11,12} Segmental projections are important for the generation of motor and sympathetic reflexes. Spinal and supraspinal motor reflexes regulate movements and exert protective functions including flexor reflexes upon nociceptive stimulation.⁴ Noxious stimulation of joint afferents can evoke nociceptive withdrawal reflexes.^{7,13} During acute chemical stimulation of the knee and during inflammation in the joint, spinal motor reflexes are enhanced.^{13–15} Articular dysfunction and ligamentous strain may cause muscle spasms.¹⁶ However, there is some evidence that the reflex pattern of γ -motoneurons changes in the course of inflammation such that inhibitory reflexes are generated.¹⁵ The latter may create a new motoric balance and allow the leg with an inflamed knee to be kept in midposition. In midposition, the nociceptive outflow from the inflamed joint is at a minimum.

Inhibition by Heterotopic and Descending Inhibitory Systems

Neurons with joint input are inhibited by heterotopic noxious stimuli, in line with the concept of diffuse noxious inhibitory controls (DNICs). The latter means that painful stimulation at one site of the body may reduce the pain at another site of the body.¹⁷ In addition, most spinal cord neurons with joint input are tonically inhibited by descending inhibitory systems that keep the spinal cord under continuous control.^{18,19} The interruption of descending inhibition can lower the excitation threshold of spinal cord neurons for mechanical input from the knee, substantially increase the receptive fields of neurons, and cause (increased) ongoing discharges. Thus the response properties of neurons with joint input are controlled by the primary afferent input, by intrinsic properties of the spinal cord neurons, by local circuits, and by descending pathways.

HYPEREXCITABILITY OF SPINAL CORD NEURONS DURING INFLAMMATION IN THE JOINT

Experimental Models of Joint Inflammation

As described in the Introduction, pain and hyperalgesia are usually elicited during inflammation of the joint. Hence experimental models have been used to

study neuronal mechanisms underlying these pain symptoms. Acute inflammation in the joint can be induced by the intra-articular injections of crystals such as urate and kaolin or by carrageenan. The injection of kaolin and carrageenan (K/C) into the joint produces an edema and granulocytic infiltration within 1–3 hours with a plateau after 4–6 hours. Awake animals show limping of the injected leg and enhanced sensitivity to pressure onto the joint. By contrast, the injection of Freund's complete adjuvant (FCA) into a single joint produces a monoarthritis that is present for 2–4 weeks. Usually the lesion is restricted to the injected joint, although bilateral effects are observed sometimes. Hyperalgesia (limping or guarding of the leg, enhanced sensitivity to pressure onto the joint) develops within a day, reaches a peak within 3 days, and is maintained to some degree up to several weeks. When FCA is injected at a high dose into the tail base or lymph node, a polyarthritis develops.⁷ More recently, other models such as collagen-induced polyarthritis²⁰ and antigen-induced monoarthritis^{21,22} are also being used in order to investigate inflammatory pain.

Generation of Spinal Hyperexcitability (Central Sensitization)

During the development of a K/C-induced inflammation in the joint, both NS and WDR neurons with joint input show within 1–3 hours enhanced responses to noxious stimuli applied to the inflamed joint. NS neurons exhibit a reduction in their mechanical threshold such that the application of innocuous stimuli to the inflamed joint is sufficient to excite the neurons. Figure 1.2A shows the generation of hyperexcitability in a spinal cord neuron with joint input. Initially, while the joint was normal, the neuron responded only to noxious pressure applied to the knee (and adjacent muscles in thigh and lower leg, Fig. 1.2B, left side). No responses were elicited by pressure onto the ankle and the paw. After injection of kaolin and carrageenan (K/C) into the knee joint, the responses to noxious compression of the knee increased markedly, and at a latency of about half an hour the neuron started also to respond to pressure applied to the ankle and the paw. Thus the receptive field expanded from the knee toward the paw (Fig. 1.2B, right side), and the previously high threshold neuron was then even activated by gentle innocuous pressure. The increased responses to stimuli applied to the inflamed joint result most likely from the enhanced synaptic input from afferent units that are sensitized during stimulation. However, the appearance of responses to stimulation of ankle and paw must result from a mechanism in the spinal cord because these regions were not inflamed. Thus nociceptive spinal cord neurons obviously develop a state of hyperexcitability in which the responsiveness to both inputs from inflamed and noninflamed areas is increased.^{23–26} The increased responses to stimulation of the inflamed area are thought to be the neuronal mechanism of primary hyperalgesia (hyperalgesia at the site of inflammation), whereas the increased responses to stimuli applied to healthy tissue are thought to be the neuronal

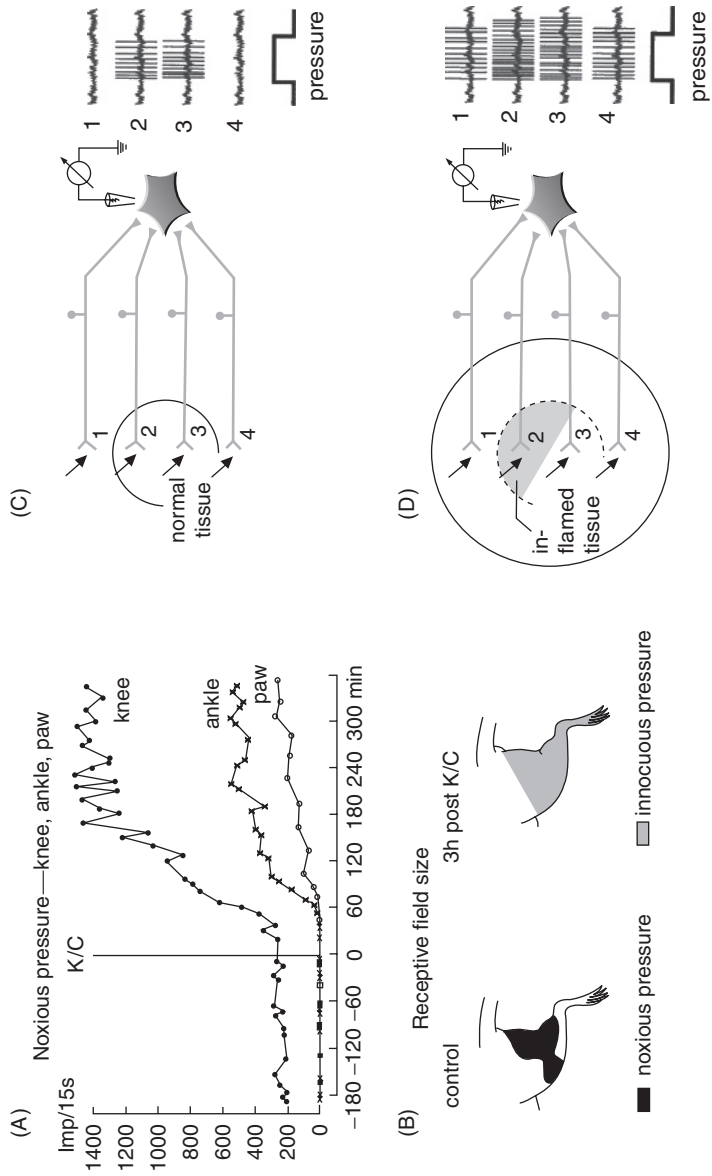


Figure 1.2. Development of inflammation-evoked hyperexcitability in a spinal cord neuron with input from the knee joint. (A) Histogram showing the responses (action potentials/response) of the neuron to noxious pressure applied to the knee joint, the ankle, and the paw before and after injection of kaolin and carrageenan (K/C) into the ipsilateral knee joint. (B) Receptive field (shaded area) of the neuron before (control) and during knee joint inflammation (3 h post K/C). (C,D) Model showing the responses and the receptive field of a spinal cord neuron before inflammation (C) and after development of hyperexcitability (D). Before inflammation the neuron was only excited by pressure to the initial receptive field (stimulation sites 2 and 3). After inflammation the neuron was activated from a larger area (stimulation sites 1–4). (Parts A and B from Ref. 25.)

mechanism underlying secondary hyperalgesia (hyperalgesia in healthy tissue adjacent to and remote from inflamed tissue).

Figure 1.2C,D shows the working hypothesis of how these changes are produced. When the tissue is normal, the neuron is only excited by stimuli applied to the restricted receptive field (circle in Fig. 1.2C) but not by stimuli applied to adjacent areas. When an inflammation develops in the receptive field (shaded area, Fig. 1.2D), primary afferents in this region are sensitized and they induce a process of spinal sensitization. When the spinal neuron is hyperexcitable, it shows stronger responses to stimuli applied to the original receptive field (stimulation sites 2 and 3), and in addition the neuron responds to inputs that are normally too weak to excite the neuron above threshold (stimulation sites 1 and 4). Hence the receptive field expands (Fig. 1.2D). The spinal “functional connection” between knee and paw and the change of synaptic effectiveness during inflammation was also shown in a recent study in which field potentials in the spinal cord were recorded. Electrical stimulation of the posterior articular nerve (PAN) of the knee joint evoked typical field potentials in lumbar spinal segments. The elicited N2 and N3 waves (generated by synaptic activation of dorsal horn neurons by thin myelinated PAN afferents) became gradually increased after induction of a local inflammation in the paw by capsaicin.²⁷ These data show that a disease process in an area may change synaptic processing from adjacent and even remote areas.

Central sensitization can persist during chronic inflammation. In rats with unilateral arthritis²⁸ as well as in rats suffering from chronic polyarthritis,²⁹ spinal cord neurons with joint input appear on average more sensitive and have expanded receptive fields. During chronic FCA-induced inflammation in the knee joint, secondary hyperalgesia at the ankle can also last several weeks, and for a long time this hypersensitivity is associated with enhanced responses of spinal cord neurons to A and C fiber inputs.³⁰

Interestingly, the stimulation of primary afferents from deep tissue (muscle and joint) evokes more prolonged facilitation of a nociceptive flexor reflex than stimulation of cutaneous afferents,¹³ and capsaicin injection into deep tissue elicits more prolonged hyperalgesia than injection of capsaicin into the skin,³¹ suggesting that deep input is particularly able to induce long-term changes in the nociceptive system. However, spinal sensitization is counteracted to some extent by inhibitory influences. Descending inhibition¹⁹ as well as heterotopic inhibitory influences (see above) are increased during inflammation,³² at least in early stages.

While spinal cord recordings can only be done in experimental studies, human studies lend support to the concept of central sensitization. In humans it is possible to map areas of referred pain upon noxious stimulation at a restricted site. When a noxious stimulus, (e.g., intramuscular injection of 6% NaCl) is applied to a muscle, the area in which pain is felt extends far beyond the stimulation site. Interestingly, such areas were found to be significantly larger during pathological conditions such as osteoarthritis.³³ The enlargement of painful areas may correspond to the expansion of receptive fields of spinal

cord neurons. By and large, the described neuronal changes in the spinal cord are likely to account for deep referred pain and secondary hyperalgesia that are induced in humans by noxious stimulation of deep tissue.³⁴ Based on this paradigm, numerous pathological conditions in humans such as inflammation and osteoarthritis seem to be associated with central sensitization, suggesting that the spinal cord is indeed in a state of hyperexcitability.

MOLECULAR MECHANISMS OF SPINAL SENSITIZATION

In general, the process of spinal sensitization depends on several preconditions. First, nociceptive spinal cord neurons have the potential for activity-dependent neuroplastic changes. For example, repetitive electrical stimulation of C fibers at the same current can induce wind-up of the synaptic responses to electrical nerve stimulation (a short-lived increase of responsiveness, however, not outlasting the stimulation protocol)³⁵ or a long-term potentiation (a persistent increase of synaptic responses to electrical stimulation outlasting the conditioning stimulus).³⁶ Second, both an increase of excitatory mechanism as well as a reduction of inhibition (e.g., by apoptosis of inhibitory interneurons under neuropathic conditions) may contribute to central sensitization.³⁷ Third, both neurons and glial cells may be involved in enhanced neuronal excitability.^{20,38,39}

In the case of inflammation, peripheral nociceptive fibers play a key role in triggering the process of spinal sensitization. During developing inflammation, numerous nociceptive mechanosensitive joint afferents are sensitized to mechanical stimulation such that innocuous stimuli (palpation of the joint and movements in the working range) become sufficient to evoke action potentials. In addition, initially mechanoinensitive nociceptive afferents are rendered mechanosensitive and contribute to the input into the spinal cord upon stimulation of the inflamed joint.^{5,37} Numerous inflammatory mediators including classical inflammatory mediators such as bradykinin and prostaglandins,⁵ as well as the cytokines interleukin-6⁴⁰ and TNF- α ²¹, have the potential to sensitize joint afferents for mechanical stimuli. As a consequence of peripheral sensitization, the intraspinal release of glutamate (the main transmitter of nociceptive afferents),⁴¹ the neuropeptides substance P, neurokinin A, and CGRP (cotransmitters in primary afferents and interneurons), and spinal prostaglandins is enhanced, and these mediators are involved in the generation (and maintenance) of spinal hyperexcitability.

Excitatory Amino Acids (Glutamate)

As mentioned, glutamate is the major transmitter in the synaptic activation of spinal cord neurons with joint input. On the postsynaptic site, glutamate activates *N*-methyl-D-aspartate (NMDA) receptors and non-NMDA receptors.

The activation of non-NMDA receptors leads to basic excitation of neurons. By contrast, the activation of NMDA receptors leads to a calcium influx into neurons and causes processes of neuronal plasticity such as long-term changes of responses in many neuronal circuits. The ionophoretic application of antagonists at AMPA/kainate (non-NMDA) receptors close to neurons with joint input reduced the responses to innocuous and noxious pressure, whereas the application of NMDA receptor antagonists reduced only the responses to noxious mechanical stimulation. Thus, in our hands, NMDA receptors are only activated by noxious stimulation.²⁵

The ionophoretic application of NMDA antagonists at AMPA/kainate and NMDA receptors to spinal cord neurons as well as systemic application of NMDA antagonists prevents the development of inflammation-evoked spinal hyperexcitability.²⁵ Figure 1.3 shows the effect of ketamine, an antagonist at NMDA receptors. In six control neurons without ketamine, the induction of inflammation in the knee joint by injection of kaolin/carrageenan (K/C) caused increases of the responses to noxious pressure applied to the injected knee and the noninjected ankle. When ketamine was administered before and during induction of inflammation, the development of inflammation in the knee joint did not cause changes of responses as long as the antagonist was applied (Fig. 1.3C,D). Importantly, antagonists at both receptor types can reduce responses of the neurons to mechanical stimulation of the joint also after inflammation is established, and this is even seen in a chronic model of inflammation.^{25,42} Thus glutamate receptors play a key role in the generation and maintenance of inflammation-evoked spinal hyperexcitability even in the long-term range. In addition, NMDA receptors are involved in the regulation of spinal NOS isoforms during monoarthritis. During monoarthritis, the expression of nNOS, iNOS, and eNOS in the dorsal horn was increased, and ketamine reduced nNOS expression and increased iNOS and eNOS expression.⁴³ However, the functional consequences of these changes are to be determined.

Neuropeptides

Numerous joint afferents contain the neuropeptides substance P, neurokinin A, and CGRP that are coexpressed with glutamate. Noxious compression, but not innocuous compression, of the normal joint enhances the intraspinal release of these peptides above baseline.⁴⁴ This pattern of release changes when the joint is inflamed. During acute inflammation, release of neuropeptides occurs when the joint is stimulated at innocuous intensity. Thus under inflammatory conditions, a “cocktail” of transmitters and/or modulators is released in the spinal cord, which changes synaptic processing.^{45–47} As a further indicator of spinal release of substance P during arthritis, movements of an arthritic joint was found to induce internalization of the neurokinin 1 receptor.⁴⁸ The

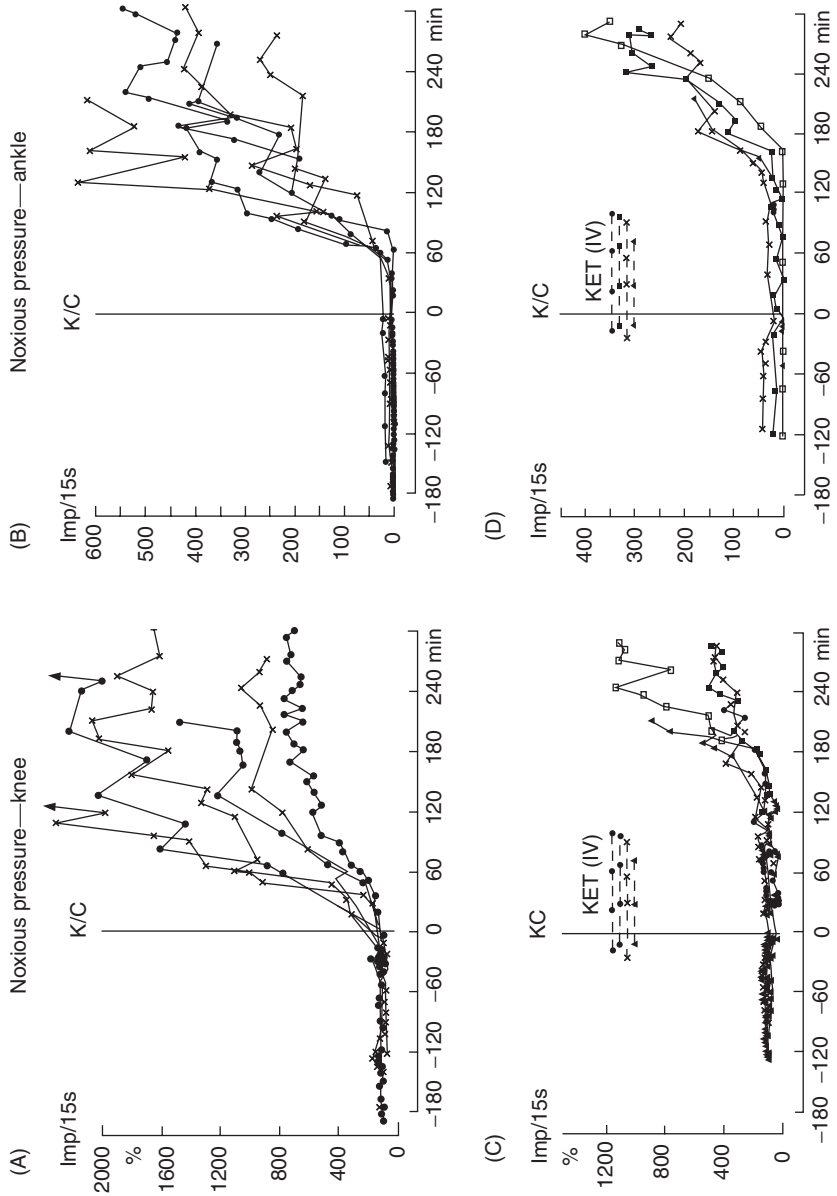


Figure 1.3. Blockade of the development of hyperexcitability in spinal cord neurons by intravenous (IV) administration of the NMDA receptor antagonist ketamine. (A,B) Changes of the responses of spinal cord neurons to noxious pressure applied to the knee joint and the ankle during development of inflammation in the knee joint after the injection of K/C into the ipsilateral knee joint. (C,D) Same experimental approach as in (A) and (B), but in these experiments ketamine was given IV during induction and in the initial period of inflammation in the knee joint. (From Ref. 25).

expression of substance P and of its (neurokinin 1) receptor was increased in the superficial dorsal horn during monoarthritis.⁴⁸

Excitatory neuropeptides facilitate the responses of spinal cord neurons. The effect of substance P is shown in Figure 1.4A,B. The WDR neuron in Figure 1.4 showed graded responses to innocuous and noxious pressure applied to the knee joint. A short ionophoretic application of substance P to the spinal cord neuron caused reversible increases of ongoing discharges and responses to mechanical stimulation. In the NS neuron in Figure 1.4B, substance P caused an increase of responses to noxious pressure and a small response to innocuous pressure and to ankle stimulation.

Ionophoretic application of antagonists at neurokinin 1, neurokinin 2, and CGRP receptors attenuates the development of inflammation-evoked hyperexcitability. Figure 1.4C shows the effect of CP96,345, an antagonist at the neurokinin 1 receptor, on the development of inflammation-evoked hyperexcitability. Compared to control neurons (top graph, induction of inflammation in the absence of the antagonist), the neurons treated with spinal administration of the neurokinin 1 receptor antagonist showed a smaller increase of their responses after induction of inflammation (middle graph). The inactive enantiomer, CP96,344, did not attenuate the magnitude of inflammation-evoked hyperexcitability (bottom graph). The antagonists also reduce hyperexcitability when it is established.⁴⁹⁻⁵¹ Probably, the activation of these peptide receptors enhances the sensitivity of glutamatergic synaptic transmission.⁵² However, it is important to point out that the antagonists at neuropeptide receptors are less antinociceptive than antagonists at glutamate receptors.

Prostaglandins

Spinal prostaglandins (PGs) are synthesized in dorsal root ganglion (DRG) neurons and in the spinal cord by both cyclooxygenases (COX) 1 and 2. PGE₂ receptors are located on primary afferent neurons and on spinal cord neurons, indicating that PGs can act presynaptically (influencing the release of synaptic mediators) and postsynaptically (influencing excitability).⁵³ During inflammation in the joint, there is a tonic release of PGE₂ within the dorsal and ventral horn.⁵⁴ This is likely to result from an upregulation of spinal COX-2 that is already increased at 3 hours after induction of knee joint inflammation (Fig. 1.5A,B). The application of PGE₂ to the spinal cord surface facilitates the responses of spinal cord neurons to mechanical stimulation of the joint, and the pattern of effects is similar to that observed during peripheral inflammation. When the COX inhibitor indomethacin was applied to the spinal cord before inflammation, the development of hyperexcitability was significantly attenuated compared to control rats in which only vehicle was applied to the spinal cord (Fig. 1.5C), indicating that spinal PGs are involved in the generation of inflammation-evoked spinal hyperexcitability.⁵⁵ Interestingly, however, the application of indomethacin to the spinal cord, after knee inflammation and

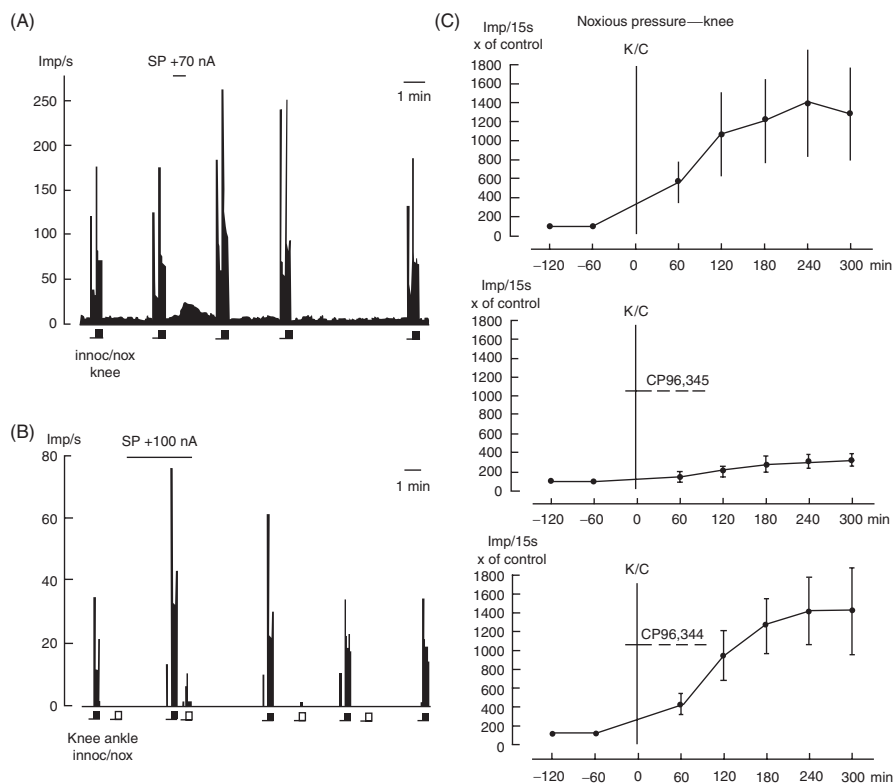


Figure 1.4. Effect of substance P on the responses of spinal cord neurons to mechanical stimulation of the knee joint and effect of a neurokinin 1 receptor antagonist on the development of inflammation-evoked hyperexcitability of spinal cord neurons. (A, B). The histograms (action potentials/second) show that ionophoretic application of substance P at 70 nA or 100 nA enhances responses to mechanical stimulation. In the neuron in (A), substance P also caused enhanced ongoing discharges. (C) Development of inflammation-evoked hyperexcitability in spinal cord neurons (responses to noxious pressure) in the absence of the antagonist (top), in the presence of the neurokinin 1 receptor antagonist CP96,345 at the spinal cord neurons (middle), and in the presence of the inactive enantiomer CP96,344 of the neurokinin 1 receptor antagonist (bottom). Parts A and B from Ref. 44; C from part Ref. 49.

spinal hyperexcitability are established, did not reduce enhanced responses of spinal cord neurons to mechanical stimulation of the inflamed knee joint,⁵⁵ thus raising the question of whether the continuous presence of PGE₂ is required for the maintenance of inflammation-evoked spinal hyperexcitability. By contrast, after systemic application, indomethacin reduced the responses of spinal cord neurons, showing that indomethacin still acted outside the spinal cord.⁵⁵

Further support for a differential role of PGE₂ in the generation and maintenance of spinal hyperexcitability came from studies on the spinal effect

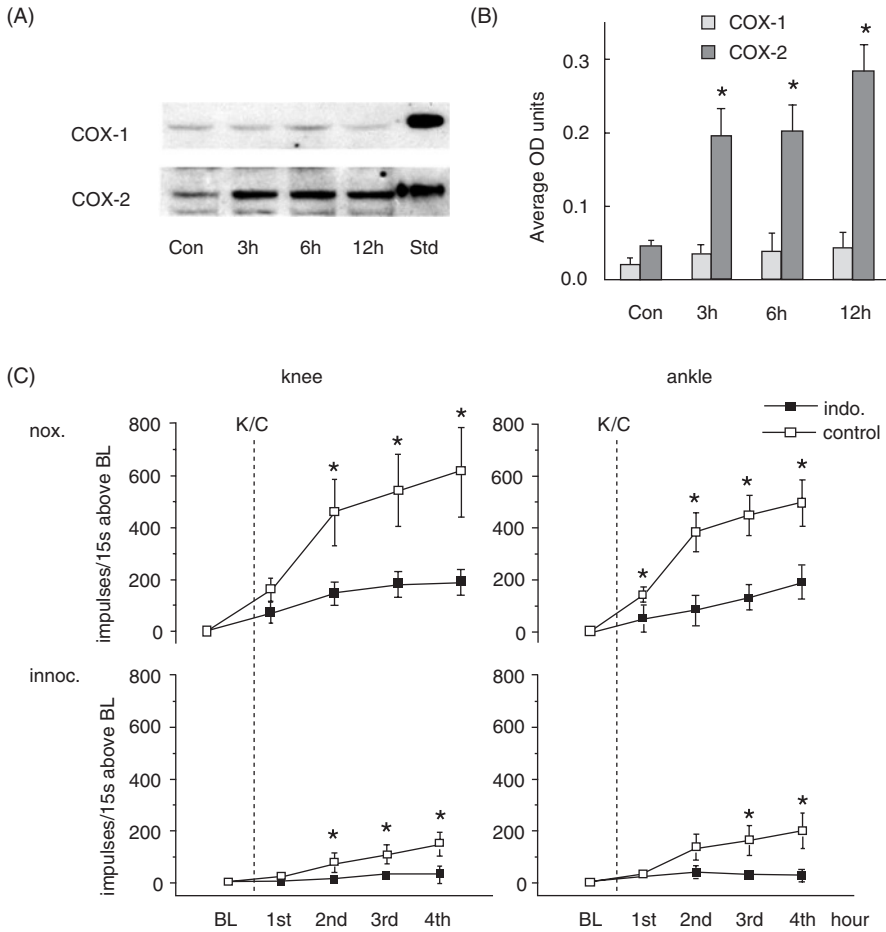


Figure 1.5. Upregulation of cyclooxygenase 2 in the spinal cord during inflammation in the knee joint and effect of spinal administration of indomethacin on the development of inflammation-evoked hyperexcitability of spinal cord neurones. (A,B). During inflammation in the joint, mainly spinal cyclooxygenase 2 (COX-2) shows an increase. (C) The spinal application of indomethacin, a blocker of COX-1 and COX-2, attenuates spinal hyperexcitability. Open squares show the inflammation-evoked changes of responses after kaolin/carrageenan injection in control neurons; filled squares show the changes of the responses during development of inflammation after topical administration of indomethacin to the spinal cord. Top graphs show responses to noxious pressure, bottom graphs responses to innocuous pressure. (Parts A and B from Ref. 54; Part C from Ref. 55).

of EP receptor agonists⁵⁶ and on the inhibition of the transcription factor NF κ B in the spinal cord.⁵⁷ The enhancement of responses of spinal cord neurons to mechanical stimulation of the normal knee joint by spinal PGE₂ was mimicked by the spinal application of agonists at the EP1 receptor (which enhances calcium influx in neurons), and by agonists at the EP2 and EP4 receptors (which activate G_s proteins and adenylyl cyclases). However, after inflammation and spinal hyperexcitability were established, only the EP1 receptor agonist further increased responses to mechanical stimulation of the inflamed knee, whereas the EP2 and EP4 agonists did not influence neuronal responses. On the other hand, spinal application of an agonist at the EP3 receptor (most isoforms are coupled to G_i proteins and reduce cAMP levels) had no influence on neuronal responses when the joint was normal but reduced the responses to mechanical stimulation of the knee when it was spinally applied during established inflammation.⁵⁶ Thus the status of the spinal cord may determine which EP receptor agonist causes an effect upon spinal application, and the level of cAMP could be an important molecular factor.

The activation of COX-2 depends on the activation of the transcription factor nuclear factor- κ B (NF κ B). In unstimulated tissue, NF κ B is bound in the cytoplasm to I κ B α and I κ B β , which prevent it from entering the nucleus. After stimulation, I κ B kinase (IKK) phosphorylates I κ B and causes its degradation, thus allowing the unbound NF κ B to enter the nucleus. Hence IKK inhibitors reduce NF κ B-mediated effects.^{58,59} Recent reports indicate a role of spinal NF κ B activation in spinal mechanisms of nociception.^{60,61} A study on spinal mechanisms of joint nociception showed that spinal application of a specific IKK inhibitor before and early during development of inflammation totally prevented spinal hyperexcitability during developing joint inflammation, suggesting an important role of spinal NF κ B in this process. However, during established inflammation, the IKK inhibitor did not reduce the responses of neurons to mechanical stimulation of the inflamed knee within 2.5 hours after spinal administration, thus suggesting that spinal hyperexcitability is not maintained by continuous NF κ B activation.⁵⁷ The pattern of effect of the IKK inhibitor is similar to that of indomethacin (see above), and because NF κ B inhibitors prevent the upregulation of spinal cyclooxygenases,^{60,61} these data collectively suggest spinal PGE₂ is mainly important for the generation of inflammation-evoked spinal hyperexcitability but not for its maintenance.

It should be noted, however, that other prostaglandins are also synthesized in the spinal cord. The other major prostaglandin in the central nervous system including the spinal cord is PGD₂.⁶² Electrophysiological recordings from spinal cord neurons with knee input showed that topical application of PGD₂ to the spinal cord at a high dose may cause a sensitization of spinal cord neurons for mechanical stimulation of the normal joint similar to PGE₂. This effect may result from synaptic facilitation due to an increase of the spinal release of substance P and CGRP from primary afferent neurons.⁶³⁻⁶⁵ However, under conditions of joint inflammation, PGD₂ dose-dependently reduced responses of spinal cord neurons to stimulation of the inflamed knee joint, and

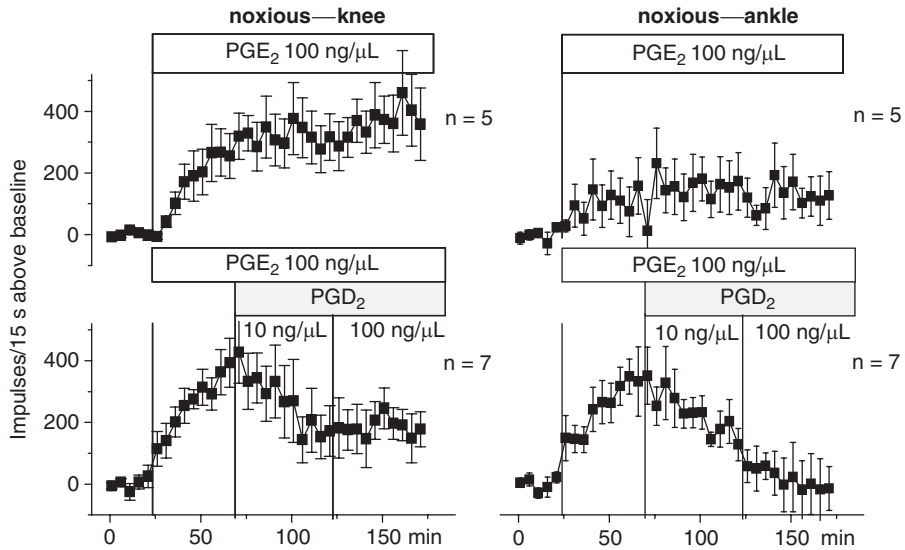


Figure 1.6. Effects of the coadministration of PGE₂ and PGD₂ on responses to mechanical stimulation of the normal knee and ankle joint. Increase of responses to noxious stimulation of the knee and ankle over predrug baseline (set to 0) by PGE₂ alone (top) and reduction of increased responses by coapplication of PGD₂ at two doses (bottom). Each symbol shows the average response of neurons at the time points indicated. (From Ref. 66).

spinal application of an antagonist at the DP1 receptor increased responses to stimulation of the inflamed knee.⁶⁶ Indeed, PGD₂ can reduce the enhanced discharges evoked by PGE₂.⁶⁶ This is shown in Figure 1.6. The spinal application of PGE₂ alone caused a pronounced and persistent facilitation of responses to noxious pressure onto the normal knee and ankle (Fig. 1.6, top). When instead PGD₂ and PGE₂ were coadministered starting 50 min after previous application of PGE₂ alone, responses to noxious stimulation were significantly reduced (Fig. 1.6, bottom). Thus PGD₂ seems to have potentially two opposite actions in the spinal cord depending on the state of the spinal cord. The reduction of enhanced responses by PGD₂ is in line with data showing that PGD₂ may have neuroprotective effects in the brain,⁶⁷ via DP1 receptors.⁶⁸ Possibly this inhibitory action is caused by activation of DP1 receptors on inhibitory spinal interneurons.⁶⁹

CONCLUSION

The investigation of the spinal mechanisms of pain including joint pain is an active area of research, and it is very likely that further important spinal

mechanisms of joint pain will be elucidated in the next few years. Of potential interest could be the role of glia cells, which produce a number of mediators including cytokines.^{39,70}

While the spinal cord is an important site where neuronal plasticity such as central sensitization occurs, the conscious pain response is produced in the thalamocortical system. The thalamus and cortex contain nociceptive neurons that are activated by nociceptive deep input from muscle and joint. Most of these neurons have convergent inputs from skin and deep tissue, but small proportions of neurons respond only to noxious stimulation of muscle and tendon.^{71–73} There is evidence that during arthritis neuroplastic changes are also induced at the thalamocortical level.^{74–76} It is unknown whether these alterations mirror the altered spinal processing or whether additional elements of neuroplasticity are generated in the thalamus and cortex. In any case, the working of the nociceptive system is substantially modified under clinical pain conditions, and it is a continuous challenge to identify suitable target sites for pain therapy.

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