The Ying and Yang of Pain: Protective Versus Damaging

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Introduction

From day-to-day experience (be it direct, for we feel it ourselves, or indirect, because of what we are told about someone else's experience) everyone knows what it feels like to be in pain. Localized, episodic injuries such as scraped elbows or knees or breaking a bone; toothaches, giving birth, heart attacks and headaches are all forms of acute pain, while migraines, cancer, and heart pain are examples of more permanent forms of pain. In all these cases, however, pain permeates our entire lives. It is easy to assume that this "perception" is the end of the story: 'pain-is-pain', and that is all there is to say about it. It clearly is not. In fact, the way in which people react to what they describe as something 'painful' has changed considerably over time. In the eighteenth and nineteenth centuries people believed that pain served a specific function [1]. It was seen as a message from God or Nature; its influence would perfect the spirit. 'Suffer in *this* life and you wouldn't suffer in the *next* one', was a common way of summarizing the prevalent beliefs at that time. Submitting to pain was required. This view could hardly be more removed from twentieth and twenty-first century understandings, where pain is regarded as an unremitting evil to be 'fought'.

One of the first researchers to offer a definition of what constitutes pain (and by extension, the stimuli likely to be responsible for this evoked pain) was Charles Sherrington. He stated that "harmfulness is the characteristic of the stimuli by which [the nerve endings] are provocable, for physiological reference therefore they are preferably termed nocicipient" [2]. A few years later Sherrington expanded his definition of a noxious stimulus as one with 'an intensity and quality sufficient to trigger a reflex withdrawal, autonomic responses, and pain' collectively representing what he called the 'nociceptive reaction'. In that work [3] he introduced the notion of a neural apparatus constituted by nociceptive nerves or nociceptors which were responsible for detecting noxious stimulus. That new term implied that pain was a specific sensation with its own sensory machinery and was directly contrary to the then widely accepted theories stating that pain resulted from either a central summation resulting from excessive sensory stimulation or that all nerve endings are similar and that particular (undefined) patterns of activity provoked by intense stimulation evoke pain. This divergence of opinions reflected the competition between the so called 'specificity' and 'pattern theories' of pain that

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somewhat define the state of pain sensory biology in much of the nineteenth and early twentieth centuries. By the 1960s and 1970s the debate reached a climax with two well-defined positions: on one hand, Ed Perl strongly argued that pain is mediated by specialized high-threshold nociceptor sensory neurons [4], while on the other hand, Pat Wall and Ron Melzack emphasized central processing as generating pain [5].

It is now clear that pain is not an either/or situation: ***nociceptors*** are the peripheral path to nociceptive pain, and altered central processing does contribute to pain hypersensitivity in patients. It is also certain that we can discard the notion that sensory specificity is somehow encoded by non-specialized primary sensory neurons.

Currently, the accepted view is that stimuli that are damaging or potentially damaging to the tissues are said to be noxious, and primary afferent neurons that respond only, or preferentially, to such stimuli are called ***nociceptors***. As quoted by Light and Perl, "Nociceptors are defined as primary afferent units that uniquely signal stimuli intense enough to cause damage to the tissue" [6]. Furthermore, we now know that nociceptors are pliant and modifiable, particularly in response to both injury of its axon and exposure to a large number of inflammatory influences. This intrinsic flexibility is central to their role as pain-generating units, as will be discussed later in the chapter.

Importantly, in their interaction with the environment, living organisms must recognize and react to harmful stimuli in order to avoid them. To achieve this, nociceptors have a high stimulation threshold and therefore normally respond only to stimuli of sufficient energy to potentially or actually damage tissue. This high threshold for nociceptor activation is often found to be significantly lowered in conditions leading to chronic and pathological pain.

As was previously mentioned, nociceptors are a type of primary afferent sensory neuron and a thorough description of these neurons is available [7]. In this chapter we merely skim through some of the basic aspects of nociceptors. To begin with, these neurons are pseudo-unipolar. That is, in their mature form they have only one process leaving the soma. This process is called the initial segment and it branches at its T junction into a peripherally and a centrally projecting process, where they synapse on nociceptive second order neurons. Their cell bodies are grouped to form dorsal root and trigeminal ganglia. Some nociceptors are thinly myelinated (Aδ-fibers) but most are unmyelinated [8], and these slowly conducting afferents represent the majority of sensory neurons in the peripheral nervous system. The nociceptors may respond to mechanical, thermal, and/or chemical stimuli; and they may project to skin, muscle, and blood vessels of the trunk and limbs or to visceral organs in the thorax and abdomen. If we link cellular morphology and function, we recognize that the nociceptor unit has four functional compartments: the peripheral terminal that transduces external stimuli and it is where action potentials initiate; the axon that conducts these action potentials; the cell body (or neuronal soma) that controls the identity and integrity of the neuron (and where most of the biosynthetic activity underlying neuronal plasticity takes place); and finally the central terminal which forms the pre-synaptic element of the first synapse in the sensory pathway in the central nervous system (CNS) (Fig. 20.1).

Remarkably, the nociceptor is also subjected to influences emerging from their innervations targets, nerves and also the spinal cord. These extrinsic signals contribute to the function and phenotype of the nociceptor and are added to the intrinsic properties of the nociceptor itself.

That nociceptors and the ability to sense pain are central to survival in normal individuals can be illustrated by the unfortunate patients carrying a mutation in TrkA, the receptor for nerve growth factor (NGF). These patients suffer from hereditary sensory and autonomic neuropathy type 4, in which because of the lack of a functional TrkA, nociceptors failed to survive [9]. This condition produces congenital pain hyposensitivity, causing the patients to burn and chew their tongues and lips, and as a result of undetected damage, lose the tips of their fingers and damage their joints. Clearly, ignorance of noxious stimuli is not bliss: this is the Yin side of pain, a necessary mechanism set to protect us from inflicting self-damage (either by action or omission) or a warning signal to alert us that something in our body is not right. Other examples in which there is an innate inability to sense pain not associated

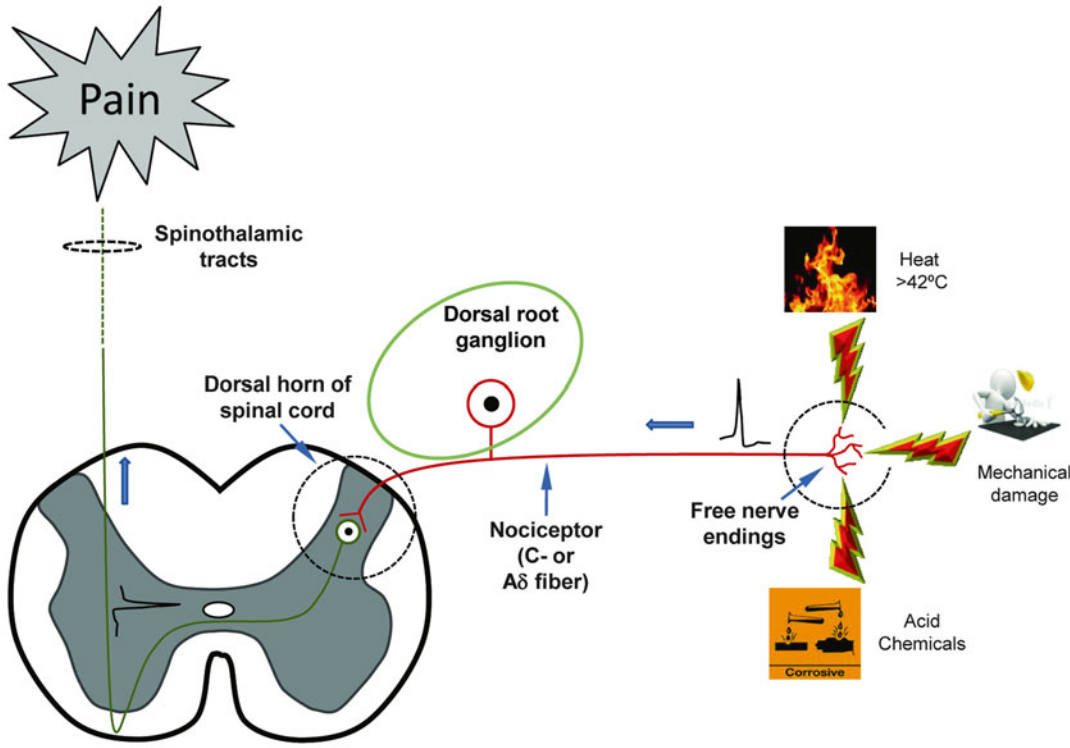

Fig. 20.1 Diagrammatic representation of a classic pain pathway. In this simplified representation only the commonest of noxious stimuli have been included (chemically-triggered irritation, burning and mechanical damage)

to nociceptor loss but rather to a lack of key molecular mediators of pain in these neurons, are those carrying mutations in voltage-dependent Na^+ channels such as SCN9A (the gene encoding the alpha subunit of Nav1.7 voltage-gated sodium channel) [10, 11] as well as SCN11A (which encodes for Nav1.9) [12–14].

Finally, we must bear in mind that, beyond the events occurring at the cellular and molecular level, pain is a phenomenon that has a physical and a psychological dimension. In the specific case of chronic or maladaptive or pathological pain, these two aspects are characterized by the occurrence of vicious circles (understood as self-sustaining and self-preserving mechanisms that reinforce undesirable/uncomfortable behaviors).

The psychological vicious cycle begins with feelings of anger, anxiety, fear, etc. arising from the presence of pain (particularly if it is chronic, moderate to severe in intensity or if it is spontaneous and unpredictable). These feelings drive the individual to a bad, poor mood, which if prolonged in time, could lead to depression. In turn, depressive syndromes can accentuate the subjective side of pain, leading to increased pain perception (even when the intensity of the pain remains unchanged over time). This takes us back to the beginning and the cycle is then perpetuated unless the pain is effectively suppressed.

The physical vicious cycle typically begins when the person avoids doing physical activity because of his/her pain. The longer this avoidance goes on, the more deconditioning occurs. The lack of activity has several implications: the patient becomes less active, hence with lesser social life and growing isolation—this feeds back to the poor mood and the depression and also leads to further activity avoidance. Again, the longer this state lasts, the more difficult it becomes to return to physical activity, which nurtures a heightened level of physical discomfort and eventually causes more pain. This cycle is then closed, and links to the psychological one (see Fig. 20.2). That type of pathological pain is

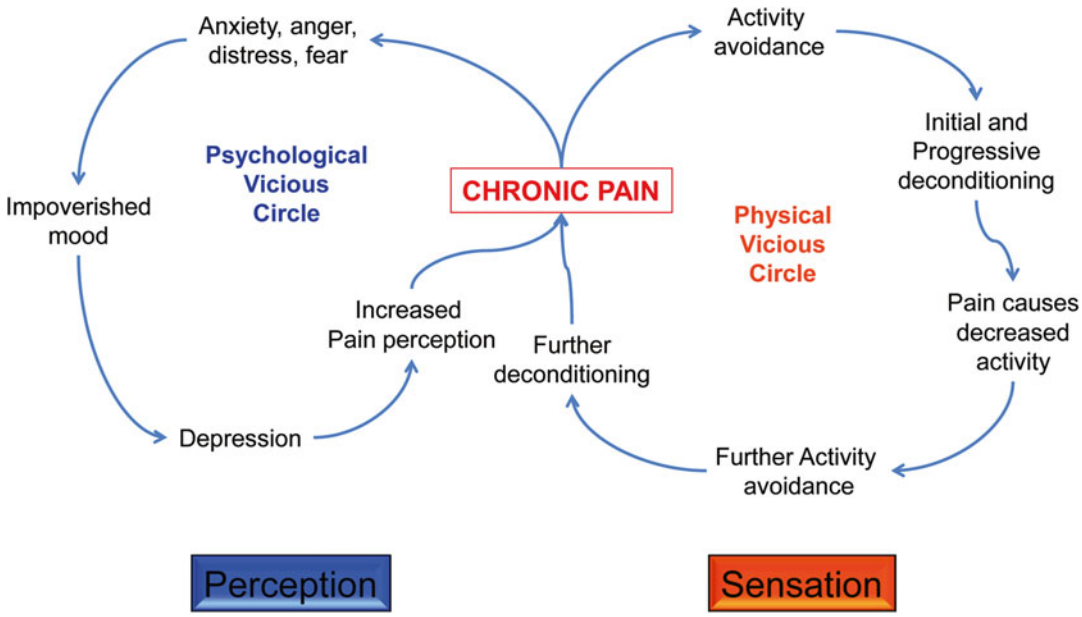

Fig. 20.2 The *vicious circles* of chronic pain. The flow diagram illustrates the likely sequence of events leading to depression and avoidance of physical activity, two of the most important side effects of chronic pain with a significant impact on patient's quality of life

seen as the Yang, the one that no longer serves the protective role of the warning system, and instead becomes a debilitating and hard to treat medical condition with significant clinical relevance [15, 16].

In summary, in this chapter we briefly present the main aspects of pain. First we present evidence on how nociceptors (as the mediators of pain) acquire their specialized molecular phenotype. Second, we comment on how they transduce noxious stimuli and transfer input to the CNS. Finally, we elaborate on how some of the adaptive and maladaptive functional and phenotypic changes that occur in them in conditions of inflammation and illness lead to spontaneous pain and pain hypersensitivity.

The Terminology of Pain

There are a number of key concepts in the field of pain and pain research that we need to define as best as we can so as to properly understand the extent of the problem and also to put into context many of the challenges that physicians (and other health professionals) face during diagnosis and treatment of pathological pain.

According to the *Online Etymology Dictionary*, the word pain probably originated from the Latin *poena*, meaning "punishment, penalty, retribution, indemnification" (in Late Latin also "torment, hardship, suffering") and from the Greek *poine*, that is "retribution, penalty, quit-money for spilled blood" and also possibly from PIE **kwei-* "to pay, atone, compensate". The earliest sense in English survives in the phrase *on pain of death*. The word pain also has a root in the Old French (eleventh century) *peine* "difficulty, woe, suffering, punishment, Hell's torments". *Pains* as in "great care taken (for some purpose)" is first recorded in the 1520s (in the singular in this sense, it is attested from c.1300). The first record of the term *pain-killer* dates from 1853. These concepts of pain as being essentially associated to the idea of hardship and punishment is in agreement with its endurance bringing spiritual elevation and purification, ideas that were predominant in Medieval and Modern times.

Contemporaneously, the International Association for the Study of Pain (IASP) set a

permanent committee in charge of periodically reviewing the definitions for a number of key terms. Here we pursue those definitions, which are presented as listed in the IASP website (http://www.iasp-pain.org/AM/Template.cfm?Section=Pain_Definitions) with some additional comments where it is pertinent to make clarifications or where debate is ongoing about the exact meaning of a term.

Pain: An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage.

First must note that pain is always subjective. Therefore, individuals report their perception as being painful, and they do so verbally. However, the *inability* to communicate verbally does not negate the possibility that an individual is experiencing pain and is in need of appropriate pain-relieving treatment.

We learn about the meaning of the word "pain" through experiences in early life. The episodes linked to the use of the term are normally related to injury and possible tissue damage. Accordingly, *pain is that experience we associate with actual or potential tissue damage.* It must be noted that although pain is unquestionably a sensation in a part or parts of the body, it is also always unpleasant and therefore also an emotional experience. In line with this argument, experiences which resemble pain but are not unpleasant, e.g., pricking, *should not be called pain*.

Unpleasant abnormal experiences (called dysesthesias and defined below) may also be called pain but are not necessarily so because, subjectively, they may not have the usual sensory qualities of pain. Many people report pain in the absence of tissue damage or any likely pathophysiological cause; usually this is attributable to psychological rather than physiological reasons. Unfortunately, there is usually no way to distinguish their experience from that due to actual tissue damage if we limit our investigations to the subjective report. The position of the IASP Committee is that if the patients regard their experience as pain, and if they report it in the same ways as pain caused by tissue damage, it should be accepted as pain. This definition clearly avoids tying pain to the stimulus causing it. Activity induced in nociceptors and nociceptive pathways by a noxious stimulus is not considered pain, which is always a psychological state.

***Neuropathic pain*:** Pain caused by a lesion or disease of the somatosensory nervous system.

We must allude to the fact that neuropathic pain is a clinical description (and ***not*** a diagnosis) which requires the presence of a demonstrable lesion or a disease that satisfies the established neurological diagnostic criteria. *Lesion* is commonly used when diagnostic investigations (e.g., imaging, neurophysiology, biopsies, laboratory tests) reveal an abnormality or when there was obvious trauma. The term *disease* is typically used when the underlying cause of the lesion is known (e.g., stroke, vasculitis, diabetes mellitus, genetic abnormality). *Somatosensory* refers to information about the body per se including visceral organs, rather than information about the external world (e.g., vision, hearing, or olfaction). The presence of symptoms or signs (i.e., touch-evoked pain) alone does not justify the use of the term *neuropathic*. Some diseases, such as trigeminal neuralgia, are currently defined by their clinical presentation rather than by objective diagnostic testing. Other diagnoses such as postherpetic neuralgia are normally based on the clinical history of the patient. It is common when investigating possible neuropathic pain that diagnostic testing yield inconclusive or even inconsistent data. In such instances, clinical judgment is required to reduce the totality of findings in a patient into one putative diagnosis or concise group of diagnoses.

The main difference between ***central neuropathic pain*** and ***peripheral neuropathic pain*** is that the former is caused by a lesion or disease of the central somatosensory nervous system, while the latter is caused by a lesion or disease of the peripheral somatosensory nervous system.

In sharp contrast to **neuropathic pain,** ***nociceptive pain*** is pain that arises from actual or threatened damage to non-neural tissue and is a result of the activation of nociceptors. In fact, this term is used to describe pain occurring with a normally functioning somatosensory nervous system to contrast with the abnormal function

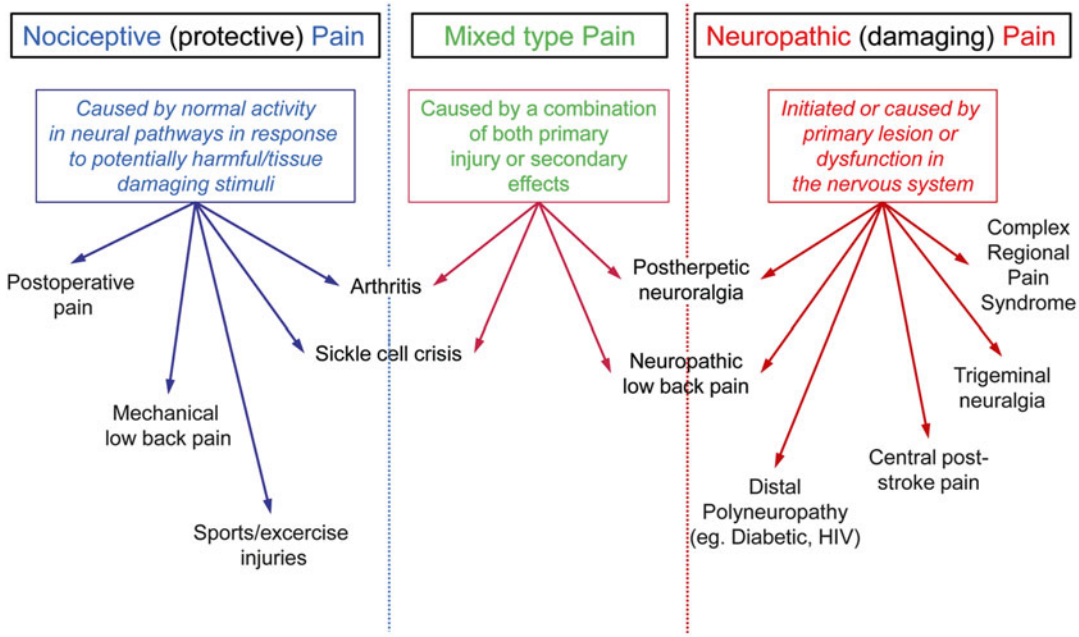

Fig. 20.3 Comparative definitions of nociceptive, neuropathic and mixed pain with examples of clinically relevant conditions typically classed into each type

seen in neuropathic pain. In other words, the protective, normally arising acute pain caused by a sudden injury for example, is called nociceptive while pain arising from an underlying malfunction or damage in the somatosensory system is termed as neuropathic (provided the damage has been diagnosed and established as the cause for the reported pain, usually chronic in duration). A patient may occasionally exhibit a combination of symptoms which in turn can be described as being partly neuropathic and partly nociceptive. Figure 20.3 summarizes what we have described and provides a few examples of each type of pain.

When the pain is located in the distribution of a single nerve or nerves, it is referred to as ***neuralgia***, while a demonstrable inflammation of a nerve or nerves is called ***neuritis***. Note that pain associated with inflammation of tissues other than nerves could be termed ***inflammatory pain***. However, this may lead to confusion because chronic inflammation resulting from conditions such as arthritis often causes pain syndromes that fit well within the definition of neuropathic pain. Furthermore, many clinical conditions that present with chronic pain are associated with ongoing chronic inflammation, which is believed to play a role in both, causing and maintaining this type of pathological pain.

Neuropathy is a disturbance of function or pathological change in a nerve: in one nerve, mononeuropathy; in several nerves, mononeuropathy multiplex; if diffuse and bilateral, polyneuropathy. Note that neuritis is a special case of neuropathy.

Patients occasionally present with a combination of symptoms which constitute a syndrome. Such is the case of ***causalgia***, a syndrome of sustained burning pain, allodynia, and hyperpathia after a traumatic nerve lesion, often combined with vasomotor and sudomotor dysfunction and later trophic changes. ***Hyperpathia***, on the other hand, is a painful syndrome characterized by an abnormally painful reaction to a stimulus, particularly a repetitive stimulus, presenting with an increased threshold and the pain is often explosive in character.

Additionally, there are signs or symptoms that are associated with the different types of clini-

cally relevant pain and need to be defined. They include allodynia, hyperalgesia, dysesthesia, hyperesthesia, hypoalgesia and paresthesia. Next, we briefly introduce each term, followed by its accepted IASP definition.

Allodynia is pain resulting from a stimulus that does not normally provoke pain. In this case, a stimulus that normally does not cause pain, leads to an unexpectedly painful response. This is a clinical term that does not imply a mechanism. Allodynia may be observed following application of different types of somatosensory stimuli to various other tissues. *Allo* means "other" in Greek and is a common prefix for medical conditions that diverge from the expected. *Odynia* is derived from the Greek word "odune" or "odyne" meaning "*pain*" which is used in "pleurodynia" and "coccydynia" and is similar in meaning to the root from which we derive words with *-algia* or *-algesia* in them.

The term *allodynia* was originally introduced to separate hyperalgesia from hyperesthesia, the conditions seen in patients with lesions of the nervous system where touch, light pressure, or moderate cold or warmth evoke pain when applied to apparently normal skin.

There are a number of potential problems with the definition of allodynia. First, how can we be certain that a strong pinch applied to the skin of a normal individual does not cause significant tissue damage, even in the short term? Can we consider that sensitized skin (e.g., sunburn) constitutes a sort of peripheral pain amplifier that can result in light tactile stimulation leading to pain? And yet, sunburned skin is technically normal skin that has transiently been affected by excessive solar irradiation that is unlikely to result in any permanent damage. These complicating factors are By "implicated" we mean that the definition of allodynia implies an abnormal pain processing being associated to abnormal tissue (or damaged tissue) whereas in the example of sunburn skin, the skin is physiologically normal.

It is also important to recognize that allodynia involves a change in the *quality* of a sensation, whether tactile, thermal, or any other sort. The original modality is normally non-painful, but the response it triggers is painful. Thus there is a loss of specificity of a sensory modality. By contrast, ***hyperalgesia*** (see later) represents an augmented response in a specific mode, that is, pain. In allodynia, the stimulus mode and the response mode differ, unlike the position with hyperalgesia.

Hyperalgesia is increased pain from a stimulus that normally provokes pain; in other words, hyperalgesia reflects increased pain on suprathreshold stimulation. As with allodynia, this is a clinical term that does not have any mechanistic implications (i.e., it does not convey information about the actual ontogeny of the phenomenon being described by the term). For pain evoked by stimuli that usually is not painful, the term *allodynia* is preferred (see above), while *hyperalgesia* is more appropriately used for cases with an increased response at a normal threshold, or at an increased threshold (e.g., in patients with neuropathy). It should also be recognized that with hyperalgesia the stimuli and the response are both in the same sensory mode. Current evidence suggests that hyperalgesia is a consequence of perturbation of the nociceptive system with concurrent peripheral or central sensitization, or both. However, it is important to distinguish between the clinical phenomena, which this definition emphasizes, as well as the interpretation, which may well change as knowledge advances.

Hyperesthesia is an increased sensitivity to stimulation, excluding the special senses. *Hyperesthesia* can refer to various modes of cutaneous sensibility including touch and thermal sensation without pain, as well as to pain. The word is used to indicate both *diminished* threshold to any stimulus and an *increased* response to stimuli that are normally recognized. In this sense, *hyperesthesia* includes both allodynia and hyperalgesia, but the more specific terms should be used wherever they are applicable.

Dysesthesia is an unpleasant abnormal sensation (often painful) while ***paresthesia*** is just an abnormal sensation (e.g., tingling under the skin), in both cases regardless of whether these sensations are spontaneous or evoked. From these definitions it is clear that hyperalgesia and allodynia are both special cases of dysesthesia. It may also be true that dysesthesia is a particular form of paresthesia, however,the reverse is not true.

Hypoalgesia is a diminished pain in response to a normally painful stimulus. This term refers only to the occurrence of relatively less pain in response to stimulation that normally causes pain. On the other hand, ***hypoesthesia*** refers to the case of diminished sensitivity to stimulation that is normally painful.

Finally, ***analgesia*** is understood to be absence of pain in response to stimulation which would normally be painful. As with allodynia, the stimulus is defined by its usual subjective effects.

Some aspects of pain are relevant to the understanding of the mechanisms underlying its occurrence. They suggest what physiological parameters are likely to be affected at the cellular level, and are therefore, useful to guide research efforts to what is causing pain. In this category, we encounter concepts such as ***pain threshold***, defined as the minimum intensity of a stimulus that is perceived as painful. Although this definition is accurate, in practice the *threshold* itself is really the experience of the patient, whereas the *intensity measured* is an external event. It has been a common mistake for many pain researchers to define the threshold in terms of the stimulus, which should be avoided. Nonetheless, the threshold stimulus can be recognized as such and measured. In psychophysics for example, thresholds are defined as the level at which 50 % of stimuli are recognized. In that case, the pain threshold would be the level at which 50 % of stimuli would be recognized as painful. We should keep in mind that the stimulus is not pain and it cannot be a measure of pain. Other subjective experience of the individual is the ***pain tolerance level***, that is, the maximum intensity of a pain-producing stimulus that a subject is willing to accept in a given situation. As with the pain threshold, it is not a measure of pain. However, both measurements are important because lowered pain thresholds or tolerance levels suggest changes in the excitability of the nociceptors, which are likely to involve, for example, alterations in the electrical properties of the neuronal membrane. Hence their importance as tools to gain insights into the mechanisms underlying pain must be pointed out.

There has been extensive research into the ability of nociceptors to exhibit increased responsiveness to their normal input, and/or recruitment of a response to normally sub-threshold inputs. This complex phenomenon is called ***sensitization*** and can include a lowered threshold and an increase in supra-threshold responses. Spontaneous discharges and increases in receptive field size can also occur. This is a neurophysiological term that can only be applied when both input and output of the neural system under study are known (e.g., by controlling the stimulus and measuring the neural event). Clinically, sensitization can only be inferred indirectly from phenomena such as hyperalgesia or allodynia. It has been shown that these sensations involve a degree of increased nociceptive responsiveness to external stimuli. If the sensitization affects the function of central neurons only while peripheral neurons function normally, we refer to ***central sensitization***. When what we observe is an increased responsiveness and reduced threshold of nociceptive neurons in the periphery to the stimulation of their receptive fields, we are observing ***peripheral sensitization***.

Up to this point we have used terms such as "nociceptor" or "nociceptive" without properly defining them. For that matter we have not as yet clearly stated what we understand by the term ***nociception.*** The simplest possible definition states that nociception is the neural process of encoding noxious stimuli. Note, however, that this definition does not necessarily imply pain sensation. As a matter of fact, consequences of encoding may be autonomic (e.g., elevated blood pressure) or behavioral (e.g., motor withdrawal reflex or more complex nocifensive behavior). The former is not usually linked to pain per se. Having said this, we need to establish the difference between a nociceptive neuron and a nociceptor. In general, a ***nociceptive neuron*** is a central or peripheral neuron in the somatosensory nervous system that is capable of encoding noxious stimuli. Notice that this definition does not explicitly state any specific physiological properties for these neurons, and therefore can be applied to a number of neuronal types not necessarily linked to pain sensation. This is not true for nociceptors,

whose function is to encode and transduce noxious stimuli, and to do so normally in response to high stimulation thresholds.

The Nociceptor

In all clinically relevant situations, and at the core of pain sensation, lies the specialized neuron we call nociceptor (reviewed in detail by [17]). As excitable cells with receptive fields projecting to the external as well as the internal environment of the organism, they possess a large number of receptors acting as sensors. In turn, the activation of these receptors leads to signalling events terminating in a number of possible targets: the nucleus, where they promote synthesis of new proteins; the proteins of the cell membrane involved in controlling neuronal excitability, e.g., ion channels or even other receptors, also membrane bound, that can be sensitized to react to the presence of small quantities of certain chemicals; and so on. We must add to this cellular complexity that a given subtype of nociceptor (e.g., mechanoceptive, thermoceptive, polymodal, etc.) carries its own, specific complement of receptors and effectors. These phenotypical markers are quite often used to functionally and histochemically define a nociceptor . Note that sensory ganglia contain more than one type of nociceptor. Finally, there is the added complication of the temporal dimension of the neuronal responses to noxious stimuli: some are quite prompt, such as those triggered by burning of the nerve endings of the skin, while other influences work over extended periods of time, such as chronic underlying inflammation or axonal degeneration resulting from serious ongoing medical conditions such as diabetes. It seems obvious that more than one mechanism must be at work at the cellular and physiological levels for nociception to be accurate and reliable. It is also not surprising that being so complex and involving such a large number of molecular players, it can go wrong fairly often.

In the next sections we will briefly discuss some of the main aspects of the cellular biology and physiology of nociceptors and their link to pain. However, this is not intended as a full review and neither is it an in-depth description of the topics: it is only a guide to point out the most salient and active areas of interest and research in the field with some mechanistic insights and future perspectives.

The Nociceptors: Its Development and Maturation

Nociceptors develop from those neural crest stem cells that migrate from the dorsal part of the neural tube and form late during neurogenesis, whereas neurons born earlier become proprioceptors or low-threshold mechanoceptors [18–20]. All newly formed embryonic nociceptors express TrkA, the NGF receptor [20]. However, the transcription factors that determine nociceptor cell fate remain poorly understood. Differentiation of most TrkA+ neurons depends on the pro-neural transcription factor Neurogenin1 (Ngn1) [21, 22]. However, Ngn1 activity is not specific for nociceptors—it is also required for formation/differentiation of TrkB+ and TrkC+ cells, which eventually mature to become low-threshold mechanoceptors [21, 22]. However, the Runx1 runt domain transcription factor is expressed exclusively in TrkA+ neurons at early embryonic stages [23–26] but because its expression is initiated some time after the onset of TrkA expression, it is unlikely to be involved in early nociceptor cell fate determination [23]. Here it is important to mention that 1) some large, myelinated, fast conducting TrkA+ neurons become Aβ-nociceptors in adulthood, but are likely to have been TrkA- early in embryonic life; and 2) it is now clear that there is a degree of phenotypical switch in the dependence on trophic factors and hence its receptors (TrkA, TrkB, TrkC, etc.) happening between late embryonic and early postnatal stages [27–30]. It is also important to bear in mind that following sensory neurogenesis, potential nociceptors undergo two distinct differentiation pathways leading to the formation of two main classes of nociceptors: peptidergic and non-peptidergic nociceptors. These two sets of nociceptors express distinct repertoires of ion channels and receptors and innervate distinct

peripheral and central targets [31–33]. This topic is briefly discussed in more detail in the next section, followed by a more in-depth description of the main differences existing between peptigergic and non-peptidergic nociceptors.

Segregation of Peptidergic Versus Non-peptidergic Nociceptors

During the perinatal and postnatal period, about one half of developing nociceptors switch off TrkA expression and start expressing Ret, the transmembrane signaling component of the receptor for glial cell-derived neurotrophic factor (GDNF) and other GDNF-related growth factors. The neurons undergoing this phenotypic switch in their trophic factor dependence become the so-called non-peptidergic nociceptors, most of which (>95 %) bind isolectin B4 (IB4+). The remaining nociceptors retain TrkA (a few also co-express Ret) and develop into the peptidergic class of nociceptors that do not bind IB4 and express the calcitonin gene-related peptides (CGRP) and substance P (SP) [34, 35]. The dynamic expression of Runx1 appears to be an important participant in this process [23, 24, 26]. Early embryonic nociceptors share a similar molecular identity, co-expressing both TrkA and Runx1 [23]. During the period when nociceptor segregation occurs, cells from Runx1 persist as nonpeptidergic neurons. Conditional knockout of Runx1 in the dorsal root ganglia (DRG) transforms these nonpeptidergic cells to a TrkA+ CGRP+ identity, and in this situation most nociceptors develop into peptidergic nociceptors [23, 26]. Conversely, constitutive expression of Runx1 in all nociceptors is sufficient to suppress embryonic peptidergic differentiation [24]. Runx1 also coordinates afferent targeting to the spinal cord; in mice that lack Runx1 prospective IB4+ non-peptidergic afferents adopt the projection pattern typical of peptidergic afferents [23]. These observations suggest that persistent Runx1 expression promotes the Ret+ nonpeptidergic cell fate, whereas loss of Runx1 is essential for peptidergic differentiation. Several studies have suggested that Runx1 and TrkA/Ret signaling pathways form a complex interaction loop for establishing nonpeptidergic nociceptor cell fate [36, 37]. TrkA-signaling is required to activate Ret, partly it appears by maintaining Runx1 expression at perinatal stages [36]. However, despite progress in teasing out the determinants of nociceptor specification, several issues remain to be resolved. Because both TrkA and Ret are required for afferents to innervate peripheral targets [36, 38], a loss of either TrkA or Ret signalling prevents nociceptors from receiving other target derived signals. Consequently, it is not known if TrkA signalling directly or indirectly controls expression of Runx1, and Ret, or if Ret signalling is directly involved in TrkA expression suppression. Additionally, while TrkA signalling is required to maintain Runx1 expression at embryonic stages, Runx1 expression is extinguished from TrkA+ peptidergic nociceptors during perinatal/postnatal development, therefore, we need to determine whether TrkA signaling switches from activating to suppressing Runx1 expression at different developmental stages or if a peripheral innervation defect in the absence of TrkA signalling indirectly extinguishes Runx1 expression. A further problem is that the intrinsic transcription factors that establish peptidergic nociceptor cell fate still remain elusive. Runx1 can, therefore, exert opposing activities depending on the cellular context. It will be extremely interesting to establish if changes in context-dependent transcriptional activities contribute to the phenotypic switches in nociceptors that occur in pathological conditions.

Subpopulations of Nociceptors

It is frequently stated that IB4 binding and TrkA expression define separate subpopulations of small, putative, nociceptive neurons. In the DRG, most small neurons (defined as those neurons showing slow action potential conduction velocity and a total cell area at the level of the nucleus of <400 μm^2 in the rat—this cell area is different in other species). These small neurons express

either TrkA or bind IB4 or both. It is therefore worth comparing these two populations. IB4 is a lectin from the plant *Griffonia simplicifolia* that binds to β-D-galactose residues in glycoconjugates on the cell surface and Golgi apparatus of small, neurofilament–poor DRG neurons in a variety of species [39]. That there is some degree of co-localization between TrkA expression and IB4 binding [40, 41] was confirmed with intracellular recording and dye injection studies in rat DRGs. These showed [1] that a third of C-fiber neurons were positive for both TrkA and IB4, with a tendency for reciprocal staining intensities for these two markers [2]. Most nociceptors strongly expressed TrkA or IB4 binding sites [3]. IB4 binding sites were present on C-fiber but not A-fiber nociceptive neurons, whereas TrkA expression was in both C- and A-fiber nociceptors [4]. Some weak positive labelling for TrkA and IB4 was seen in some D hair units. TrkA-, and not IB4-, positive neurons express SP and CGRP, as we stated in the previous section. Other differences between these neurons include projection of TrkA-positive neurons to laminae I and IIouter and IB4-positive neurons mainly to IIinner [42] of the dorsal horn [39] Compared with IB4-negative small neurons, IB4-positive neurons have longer duration action potentials and a smaller noxious heat-activated current [1], and the tetrodotoxin-resistant (TTXR) Na^+ channel subunit Nav1.9 is preferentially expressed in IB4-positive cells [43]. It has also been shown that IB4-positive neurons selectively express the K^+ leak channel TREK2, causing these neurons to be more hyperpolarized than IB4-negative nociceptors, and preventing these neurons from firing spontaneously [44], which is assumed to be the underlying cause for spontaneous pain [45, 46]. In summary, A-fiber nociceptors express TrkA but not IB4 binding sites, while most C-fiber nociceptors express one or the other, or both of these (Fig. 20.4). Apart from the NGF and GDNF dependence of TrkA expressing and IB4 binding neurons respectively, the functional differences between these groups of nociceptors are still relatively poorly understood.

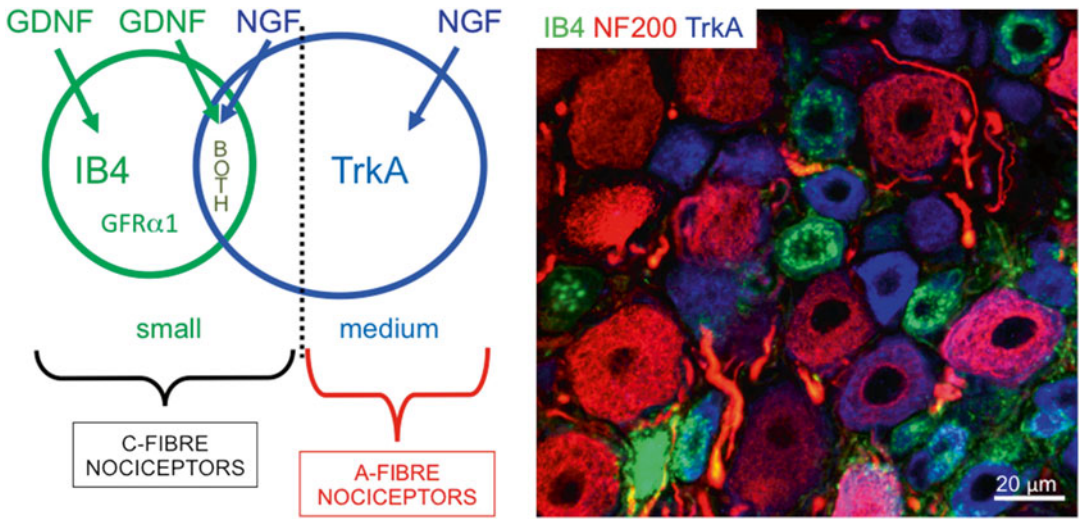

Fig. 20.4 Nociceptors are classified according to cell size (small, medium) and phenotype (binding of isolectin B4 or expression of the NGF receptor, TrkA) into C-fibre and A-fibre nociceptors. Note that IB4 binding small nociceptors express the GDNF-receptor GFR α 1. The right side panel shows triple immunofluorescence staining of a section of L5 normal rat DRG. Note the heterogeneity of subpopulations present in this small section of tissue. Neurons labelled with Neurofilament of 200 kDa (NF200, in *red*) are large and myelinated. Of these, a few express TrkA (in *blue*) which defines them as Aβ-nociceptors. Small neurons are either stained for TrkA or bind isolectin B4 (IB4, *green*) or rarely for both. All of these are nociceptors, unmyelinated and putative C-fiber neurons

Physiological Bases of Neuronal Excitability and Its Consequences for Pain

The mature nociceptor expresses dozens of ion channels and receptors, and the correct establishment of their expression is essential for nociceptors to detect specific noxious stimuli. There are two notable features about the developmental control of sensory channels/receptor expression. First, many sensory channels/receptors are expressed in only a partially overlapping or mutually exclusive manner, including TRP class thermal/chemical receptors and Mrg class G protein-coupled receptors [33, 47–49]. Second, the emergence of individual sensory channels/receptors is subject to complex temporal control. For example, expression of three TRP channels, TRPV1, TRPM8, and TRPA1, is initiated at E12.5, E16.5, and P0, respectively, while TRPA1 expression in peptidergic nociceptors is established at P0 and non-peptidergic nociceptors at P14, respectively [49]. Albeit of considerable interest in basic research, these complex developmental processes are beyond the scope of this chapter and have already been thoroughly reviewed in the literature [17].

As stated above, the mature nociceptor expresses a large number of ion channels and other associated receptors. These ion channels are carried through the cell membrane ion currents that are responsible for the excitability of the neuron. They play a pivotal role as direct or indirect controllers of or contributors to action potential generation and propagation along the nerve fibers. It is therefore important for us to look at the growing body of knowledge accumulated about these ion channels and to discuss the implications that their electrophysiological properties have for pain physiology normally and in models of chronic pain.

Na^+ Currents and Channels

Voltage-gated sodium channels (called Nav) are essential for generation and conduction of action potentials. The currents are subdivided into tetrodotoxin-sensitive (TTXS) and TTXR. The channels designated Nav1.1, 1.2, 1.3, 1.6, and 1.7 are TTXS, while Nav1.5, 1.8, and 1.9 are TTXR. Three of these proteins, Nav1.8 and Nav1.9 (both TTXR), and Nav1.7 (TTXS) are preferentially (but not exclusively) expressed in small DRG neurons. Described next were the main known properties of these channels and how their function and/or expression are altered in models of inflammation and nerve injury.

Nav1.8 (Also Called SNS/PN3)

Nav1.8 is expressed in rats in small- to medium-sized DRG neurons [50] most of which are nociceptive [51]. Nav1.8 gives rise to a TTXR Na^+ current that is believed to contribute the majority of the inward Na^+ current in action potentials of small DRG neurons and it is most likely responsible for the TTXR current at nociceptive receptor terminals [52, 53]. Its electrophysiological properties probably contribute to properties of nociceptive neurons, including its high activation threshold of about −35 mV. Consider that normal resting potential in C-fiber nociceptors is ~55 mV and therefore a threshold of −35 mV implies that a neuron must be made 20 mV more positive in order to fire an action potential. This is a significant change in membrane potential and it suggests that such a nociceptor will not fire easily unless the intensity of the stimuli is high enough to be registered as a threat or it is noxious. The slow kinetics of Nav1.8 give rise to long-duration action potentials, and contributes to the large action potential overshoot, and its rapid repriming. All this may enable firing even in depolarized fibers [50–52, 54]. In essence, the slow inactivation of Nav1.8 means that these channels may be able to sustain repetitive firing even at depolarized membrane potentials. That is why they are thought to underlie spontaneous pain which requires ongoing firing in C-fiber neurons at very slow rates [45].

Inflammation and Nerve Injury

Several inflammatory mediators can acutely (minutes to hours) decrease the activation threshold, and/or increase the kinetics or magnitude of the TTXR Na^+ current (presumed to be Nav1.8

related) [55] and may contribute to acute hypersensitivity at nerve terminals. In the longer term, Nav1.8 mRNA and TTXR Na^+ current in small DRG neurons and in cutaneous fibers are upregulated during most studies on inflammation [56, 57]. In some models of neuropathic pain, such as 7-day axotomy of the L5 nerve, TTXR current density and Nav1.8 mRNA and protein are decreased [58]. This reduction in Nav1.8 may explain why axotomized C-fiber neurons are incapable of action potential firing despite being relatively depolarized [44].

Nav1.9

Nav1.9 is also known as NaN/SNS2. Similarly to Nav1.8, Nav1.9 gives rise to a TTXR current. Nav1.9 immunoreactivity is mostly present Sin small DRG neurons [59, 60], preferentially in those with IB4 binding [43] (and therefore, non-peptidergic) and along C-fibers and at nodes of Ranvier of thinly myelinated fibers [61]. It gives rise to a persistent, depolarizing TTXR Na^+ current in small DRG neurons as a result of its low activation threshold and ultra-slow inactivation [62], thus it is likely to influence membrane excitability, although the magnitude and mode of this effects remain poorly understood. Interestingly, GDNF, but not NGF, upregulates Nav1.9 mRNA [43]. This is in agreement with GDNF being the main trophic factor required to maintain the phenotype of IB4-binding small C-nociceptors in vivo [41]. Interestingly, Nav1.9 expression is linearly and positively correlated with TREK2 expression (a K^+ leak channel, see below) in DRG neurons, which suggests that both channels are part of the same control mechanism of neuronal excitability in IB4-binding neurons [44].

Inflammation and Nerve Injury

Nav1.9 mRNA in DRG neurons is increased after *inflammation* [59], after exogenous GDNF administration [43], and it is also activated by the application of a cocktail of pro-inflammatory mediators [63]. *Axotomy* induces a decrease in Nav1.9 mRNA and protein in the DRG [43, 58, 59] that is reversed in IB4-positive neurons by exogenous GDNF [43]. A lack of function mutation of the gene encoding for Nav1.9 in humans (SCN11A) has recently been reported [12, 14]. Patients with this rare mutation experience lack of pain, and are prone to suffer extensive burns. This highlights the importance of the protective role of acute pain which prevents us from suffering life-threatening injuries.

Nav1.7 (or PN1)

Nav1.7 is expressed more highly in small rather than large DRG neurons [64] despite the fact that its mRNA is present in neurons of all sizes [65]. It is thought to carry much of the TTXS inward current in action potentials in small neurons [66]. Nav1.7 protein is present in fibers and terminals of cultured DRG neurons [67]. Its slow inactivation, combined with its low activation threshold (closer to −50 mV) [66], may be important in the generation of receptor potentials and contributing to the generation of action potentials. NGF causes a long-lasting (weeks) increase in Nav1.7 protein in DRG neurons in vivo [68].

Nav1.7 expression drops substantially after axotomy while essentially remaining unchanged after acute cutaneous inflammation [44]. The role of this channel in pain is normally emphasized by the finding that a lack-of-function mutation of its gene derives a lack of pain sensitivity and the consequent exposure to injury (e.g., breaking bones). This seems to be a genetic trait that is inherited [10, 69, 70]. It has also been shown that a scorpion toxin causes a gain of function of Nav1.7 leading to pain hypersensitivity [71].

It is important to note that certain types of pain (e.g., oxaliplatin-induced neuropathy), do not require either Nav1.7 or Nav1.8 [72]. Thus, proper patient stratification and accurate diagnosis is essential to correctly treat chronic pain using modulators of Na^+ channel function.

Other Na^+ Subunits

mRNAs of several other TTXS subunits are more abundant in medium and large neurons. These include Nav1.1, 1.2, and 1.6 and Nav2.2 (NaG) [73]. Following *axotomy*, the appearance of a more rapidly repriming TTXS current is thought to contribute to hyperexcitability in axotomized small DRG neurons in vitro [74, 75]. This has been ascribed to increased expression of Nav1.3 (also known as brain type III), but roles for other TTXS channels have not been ruled out.

Na^+ Channel β Subunits

Na^+ channel β subunits may interact with the cytoskeleton or extracellular matrix and play roles in Na^+ channel trafficking within cells and insertion into membrane, thought to be mediated by annexins and the auxiliary protein p11 [76–78]. When co-expressed with β subunits, subunits can alter the kinetics, peak current, and/or voltage dependencies of β subunits [79].

K^+ Currents and Channels

K^+ channels are central to the control of resting membrane potential, after-hyperpolarization, and firing frequency and they influence adaptation. They tend to increase membrane potential stability (i.e., less likely to oscillate), and at least some of the K^+ channels that contribute to long duration after-hyperpolarizations may prove to be more highly expressed in nociceptors. Despite much work in this field in recent years, there is still a lack of complete understanding of two main questions. 1) Which K^+ channels are expressed by different functional subpopulations of primary afferent neurons, and 2) How these channels work together to maintain membrane potential stability and to provide re-polarization/after-hyperpolarization in firing nociceptors [80, 81].

Voltage-Gated K^+ Currents and Kv Channels

The two main groups of calcium-insensitive voltage-gated K^+ currents are the depolarization-activated delayed rectifier (IKv) and fast transient (IA) currents. The protein subunits of the channels that underlie these currents are the Kv subunits.

Delayed Rectifier Currents

Delayed rectifier currents (also called IKv) serve to rapidly terminate the action potential in the soma and inhibit repetitive firing in myelinated axons [82]. They are particularly prominent in some large cutaneous afferent neurons [83], but are also present in small DRG neurons [84].

Fast Transient (A-Type) K^+ Currents

Fast transient K (IA) currents tend to clamp resting potential at hyperpolarized voltages until they inactivate, thus prolonging the after-hyperpolarization and slowing/preventing repetitive discharges [85], IA currents are present in both large cutaneous afferents and small DRG neurons [84] but are more prominent in slowly conducting afferents [86]. IA can be subdivided into rapidly (fast IA) and slowly [87] inactivating types [88]. Slow IA is particularly prominent in small DRG neurons with TTXR action potentials (see [88]), and may therefore contribute to the broad after-hyperpolarizations of nociceptive neurons.

In DRG neurons IKv and slow IA are both sensitive to dendrotoxin, but fast IA is not [88]. Kv1.1 and 1.2 are associated with the delayed rectifier, whereas Kv1.4 is associated with fast IA [89, 90], and it has been suggested that Kv1.1/1.2 associated with Kv1.4 (dendrotoxin insensitive) may give rise to the slow IA in DRG neurons [88, 89], Kv1.1, 1.2, 1.3, 1.4, 1.5, and 1.6 and Kv_2.1 are all expressed in DRG neurons; of these, Kv1.1 and 1.2 are more abundant and highly expressed in medium- to large-sized neurons, and Kv_2.1 is also more highly expressed in medium to large neurons, whereas Kv1.4 is more highly expressed in small neurons [88, 89, 91]. Kv1.1 has recently been shown to play a central role in mechanosensation [92].

M Currents

M currents (I_M) are voltage and time dependent, noninactivating, and activated at negative voltages (beginning at approximately −70 mV). Their inhibition by acetylcholine or other agents leads to increased neuronal excitability [93]. I_M are associated with KCNQ2/3 and −5 subunits [93–95], all members of the six transmembrane superfamily that are distantly related to Kv channels. I_M as well as KCNQ2, −3, and −5 have been detected in both small and large DRG neurons [94, 96]. Blocking of I_M with linopirdine causes increased firing in response to current injection in small DRG neurons, indicating that the I_M may normally act as a brake to firing in these neurons. After cutaneous inflammation, there is inhibition

of I_M leading to depolarization and exacerbated firing [97]. More recently, I_M present in nociceptors (probably including peptidergic and non-peptidergic C-neurons) have been attributed to the expression of Kv7.2 [97, 98] and Kv7.5 [8, 99], although evidence is contradictory and requires further study.

Inwardly Rectifying K^+ Channels

Inwardly rectifying K^+ (Kir) channel [100] current contributes to the resting membrane potential in a number of cell types, including DRG neurons, where Kir current is mostly in medium-size neurons [101]. Little is known about Kir-related channel subunits in DRGs. However, there is evidence that paclitaxel reduces the expression of Kir1.1 and Kir3.2 in sensory neurons, leading to neuropathic pain and nociceptor hiperexcitability [102]. Kir3.2 has also been involved in the response to opioids [103]. Other Kir channels (2.1, 2.2 and 2.3) have been proposed as key controllers of the pacemaker activity of lamina I spinal cord neurons part of the pain circuit [104].

Ca^{2+}-Activated K^+ Currents

Ca^{2+}-activated K^+ currents (IKCa) are of three types, related to BK [87], IK, and SK channels (big, intermediate, and small conductance channels, respectively), all activated by elevated intracellular Ca^{2+}; BK is also voltage dependent. Functionally BK is associated with after-hyperpolarizations that develop rapidly and decay in 10–100 ms, and SK with slower after-hyperpolarizations that may last for seconds and limit firing frequency [105, 106]. Either or both of these could therefore contribute to the long after-hyperpolarization in nociceptors, although this remains to be established. BK and SK currents are found in DRG neurons [107–109], with BK currents observed in two thirds of small DRG neurons. Immunoreactivity for SK1 and IK1 channels was found in many human and rat DRG neurons [110]. A related channel, SLACK (sequence like a Ca^{2+}-activated K^+), is expressed in rat DRG neurons [111]. SLACK has a conductance similar to that of BK, with which it can interact to generate an I-KCa channel (different from IK1). Interestingly, SLACK (and its partner, SLICK) is required for the depolarization of afterpotential in medium DRG neurons [112]. Protein kinase A induced internatilization of SLACK causes neuronal hyperexcitability [113]. It has been demonstrated that a reduced SLACK expression leads to increased thermal and mechanical sensitivity, a process regulated by the chloride channel TMEM16C [114].

Background K^+ Channels

The greater part of the time-independent resting conductance in a variety of neurons is contributed by background K^+ channels. They are two pore domain (called K2P), homo- or heterodimeric channels. They are mainly responsible for setting resting membrane potential (Em) and are constitutively open at rest, generally voltage independent, and respond to a number of different factors [115, 116]. Thus, through setting Em, K2P channels strongly influence neuronal excitability and firing [117, 118].

The K2P channels are encoded by the K2P (originally termed KCNK) family of genes; 15 distinct isoforms have been cloned, with 12 apparently functional [119]. K2Ps are grouped and named in families according to their functional properties: TWIK, weak inward rectifiers; THIK, halothane inhibited; TREK, lipid, stretch and temperature activated; TASK, acid inhibited; TALK, alkaline activated; and TRESK, Ca^{2+} activated [119–121]. Some (e.g., TRESK) are inhibited by arachidonic acid while others (e.g.,TRAAK, TREK2) are activated by it and also by G-protein coupled receptor agonists [122]. Thus, far from being passive, K2P channels are acutely modulated by ligands or environmental factors, resulting in altered leak K^+ current, and thus altered Em.

mRNA studies have found high levels of TRESK, and variable levels of TRAAK, TREK1, TREK2, TWIK1 and TWIK2 in rodents (rat or mouse) DRGs [123, 124].

There is growing evidence that K2P channels are implicated in nociception and pain. TREK1 is colocalized with TRPV1 in some nociceptive DRG neurons and its mRNA expression is reduced after inflammation in neurons innervating the colon [125]. TREK1 knockout also reduces inflammation-induced mechanical and

thermal hyperalgesia [126]. TRESK knockout enhances DRG neuron excitability [127], and a dominant negative TRESK mutation is implicated in migraine [128]. In 2006, Kang and Kim showed that at 37 °C, TREK2 contributed ~69 % of the K^+ standing current (responsible for the majority of Em) in a third of small sized cultured neonatal rat DRG neurons; TRESK, expressed in all sizes of DRG neurons [124] contributed 16 % and TREK1 12 % of the total K^+ leak current recorded in these neurons. The relatively high TREK2 mRNA in rat DRGs [129] supports TREK2 contributing substantially to Em in adult DRG neurons. We recently demonstrated that TREK2 hyperpolarizes IB4-binding C-nociceptors and limits pathological spontaneous pain. Similar TREK2 distributions in small DRG neurons of several species suggest that the role(s) of TREK2 may be widespread [44].

Axotomy and K^+ Channels

Changes in the in vivo electrophysiological properties of different subpopulations of DRG neurons after axotomy suggest altered (mostly decreased) K^+ channel expression or activation. Decreases in IK-immunoreactivity and fast IA occur after axotomy in large cutaneous afferent neurons [130] and in the former in small neurons [88]. Reductions in expression of Kv1.1, 1.2, and 1.4 and Kv2.1 have all been reported [88, 89, 91]. Overexpression of Kv1.2 impairs axotomy-induced neuropathic pain in rats [131]. Kv2 channels dysfunction after axotomy enhances sensory neuron responsiveness to stimuli exacerbating pain [132].

Reverse transcription–polymerase chain reaction showed increased KCNQ−2, −3, and −5 in both large with retigabine resulted in decreased electrophysiologic and behavioral changes in a model of neuropathic pain [94]. A reduction of K(ATP) currents after axotomy in both small and large DRG neurons has also been reported [133].

Interestingly, IK(Ca) is reduced after axotomy in DRG neurons a finding linked to increased neuronal excitability and possibly pain [134].

Inflammation and K^+ Channels

After acute (2- to 3-h) inflammation, activation of KCNQ/I_M with retigabine resulted in animals putting increased weight on the inflamed foot [94, 96]. K^+ leak channels (K2P) mRNA levels have also been shown to change as a result of CFA-induced cutaneous inflammation for 1 or 4 days. Some of these changes in mRNA (for TASK1 and TASK3) were correlated with spontaneous foot lifting, a measure of spontaneous pain [129]. However, most studies suggest that changes in expression resulting from inflammation may occur bilaterally, and affects DRG neurons projecting to sites not directly affected by cutaneous inflammation. This is believed to be caused by circulating cytokines and hormones whose release into the bloodstream is triggered by local inflammation, making it a systemic event [135, 136]. It is often referred to as global effects and may explain why underlying clinical conditions associated with chronic inflammation have widespread pain symptoms that can encompass multiple organs and systems, and not only those directly affected by the ongoing inflammatory process.

Hyperpolarization-Activated Currents and Channels

The H current (Ih, also called the funny current when first described in the heart, and therefore also termed If) is a hyperpolarization-activated, time and voltage-dependent, non-selective cation current. When activated this current causes depolarization of the membrane, reducing afterhyperpolarization duration, increasing firing frequency and decreasing adaptation [137, 138]. An Ih is prominent in most or all large DRG neurons but in fewer small neurons [86, 101, 139]. The channels that give rise to Ih are made up of HCN (Hyperpolarization-activated cyclic nucleotide-gated channel) protein subunits, four isoforms (HCN1 through HCN4) of which have been cloned [138, 140, 141]. In DRG neurons there is strong expression of HCN1 mRNA in all large- to

medium-diameter and most small-diameter DRG neurons, lower expression of HCN2 mRNA in approximately 80 % of large and approximately 60 % of small neurons, and low or undetectable levels of HCN3 and HCN4. HCN1 through HCN3 proteins are concentrated at the membrane especially of large neurons [142, 143]. In vivo, rat DRG neurons express HCN1 and HCN2, with the latter being more abundant in C and Aβ-nociceptors and remarkably high in muscle spindle afferents [144]. Detailed kinetic analysis of Ih in vivo shows that in most neuronal subtypes, Ih is made up of heteromeric HCN1 + HCN2 channels [145, 146] as described for DRG neurons [139]. There is growing evidence for involvement of HCN channels in chronic pain [147–149]. It has recently been shown that HCN2 expressed in small, putative C-neurons in mice is important to sustain chronic, inflammatory pain [150, 151]. However, expression of HCN2 is also altered in medium and large neurons after inflammation [144, 152] which suggests a more complex mechanism for the involvement of HCN2 in chronic pain. *Nerve injury* causes increased Ih in large-diameter neurons dissociated in vitro, and ZD 7288 (a specific Ih blocker) blocks ectopic discharge in axotomized A-afferent fibers [142]. Ivabradine (an approved Ih blocker with similar affinity for all 4 HCN isoforms used in the treatment of cardiac arrhythmias) has been studied as a potential treatment for inflammatory pain linked to changes in the expression of HCN2 [153–155]. However, the lack of selectivity of the drug and its tendency to reduce the heart rate in normal individuals may limit its clinical usefulness.

Ca^{2+} Currents and Channels

Ca^{2+} has crucial roles as a second messenger (therefore it is involved in signal transduction), in transmitter release (mediated by Ca^{2+} influx at presynaptic terminals), and in inhibiting firing by activation of IKCa. Also, the inflection seen on the falling phase of some of the broader (longer duration) action potentials in DRG neurons is partly due to an inward Ca^{2+} current. Based on electrophysiological and pharmacological criteria, several voltage-gated Ca^{2+} currents have been found and described in DRG neurons. These include L (nimodipine-sensitive, high voltage activated), T [156], and N (intermediate properties) [157]. Additional information on the properties of these currents can be seen in the review by Catterall [158]). Other currents are expressed in some DRG neurons; these are the P- type (sensitive to inhibition by low doses of ω-agatoxin IVA), the Q-type (blocked selectively by ω-conotoxin MVIIC) and a toxin-resistant fraction that has been termed R-type Ca^{2+} current [156, 159, 160]. Their amplitudes differ in neurons of different sizes, with relatively large L-type and N-type and smaller T-type currents in small cells, larger T- but little L- and N-type currents in medium-sized neurons, and little T-type current in large neurons [161]. T-type Ca^{2+} channels (Cav3.2) are thought to be necessary for the normal mechanosensitivity mediated by Aδ-fiber D hair LTMs [162]. Additionally, L- and N- but not T-type currents cause substance P release from isolated DRG neurons [163]. The secretory activity of some DRG neurons has been taken as an indication that these neurons behave physiologically like small neuroendocrine units. Importantly, Ca^{2+} currents can be modulated by a variety of agonists. For example, activation of δ-opioid receptor II on cultured early postnatal rat DRG neurons reduced N-, L-, P-, and Q- but not R-type currents [164], and 5-HT inhibits Ca^{2+} currents in small DRG neurons probably via 5-HT1A receptors [165]. Reports of expression include the following channel subunits demonstrated both immunocytochemically and by in situ hybridization (current type associated with the subunit in parentheses): Cav2.1 (P/Q), Cav2.2 (N), Cav1.2 and Cav1.3 (L), and Cav2.3 (R) [158, 166–168].

Nerve Injury and Future Treatments

There is currently substantial interest in calcium channels as new targets to treat neuropathic and inflammatory pain [167–169]. For instance, in relation to nerve damage it is now known that the T-type current in medium-sized neurons, as well as all Ca^{2+} currents decrease 10 days after CCI (chronic constriction injury) of the sciatic nerve [170]. Furthermore, the α2δ1subunit is upregulated [143] after various types of nerve injury [171, 172]. Additionally, regulation of α2δ1function and expression has been proposed

as a major contributor to mechanical and thermal hyperexcitability. This may be an important site of action of the analgesic gabapentin [173–175]. There are expectations that a synthetic peptide called ziconotide will be the first in a new class of neurological drugs: the N-type Calcium Channel Blockers, or NCCB. This drug (based on a snail toxin) had a novel mechanism of action and acts as a non-opioid analgesic. This feature gives it the potential to play a valuable role in treatment regimens for severe chronic pain [176]. However, N-type calcium channels are widespread throughout the body and preliminary clinical data suggests that ziconotide may be far too toxic to be used orally.

Conclusions

It is now well known that changes in the electrical properties of the neuronal membrane in DRG neurons underlie the changes in excitability associated with both acute and chronic pain, albeit the changes are different in nature depending of the type of pain involved. A good example of this is the demonstration that the rate of spontaneous firing in C-fiber nociceptors is directly and significantly related to the amount of spontaneous foot lifting in rats after cutaneous inflammation or partial nerve injury [45, 46]. This behavior is used as a marker for spontaneous pain. It decreases in animals in which C-fiber nociceptors express higher levels of the protective channel TREK2 (which exerts a hyperpolarizing influence on their membrane potentials) [44]. Altered expression of HCN channels is also associated with spontaneous firing in C- and Aδ-fibre neurons [152]. These are just but a few examples of the interplay between ion channels, excitability and pain.

To put the importance of the ion channels and their role as regulators of neuronal excitability into perspective, there has been a recent report of changes in C- and A-nociceptors, and Aα/β--cutaneous LTMs that are consistent with the uninjured neuron hypothesis [46]. These changes could contribute to different aspects of peripheral neuropathic pain as follows: spontaneous firing in C- and A-nociceptors to spontaneous burning and sharp-shooting pain, respectively; spontaneous firing in Aα/β-cutaneous LTMs to paresthesias. Finally, if decreased A-nociceptor electrical thresholds contribute to sensory hypersensitivity, they would result in greater evoked pain (hyperalgesia and/or allodynia).

Future Perspectives

The field of pain, and our understanding of its causes, has advanced a great deal since the time of Sherrington. From a historical perspective, we have moved from perceiving it as a test of faith that ought to be endured to the present notion that in itself pain acts as a warning that prevents us from harm unless it becomes maladaptive, persistent and therefore, pathological. This in turn imposed the need for treatments that can either suppress or at least provide temporary relief for pain. In the process of developing therapeutically effective ways of treating pain, knowledge about the nociceptor cell and its projections and integration to the CNS has been gained. We now know a lot about the molecular and cellular bases of how the sensation of pain is detected, transduced, transmitted and eventually, perceived by the individuals. This knowledge has been key to developing pharmacological tools that target specific receptors, ion channels, or signaling pathways that are involved in the genesis and maintenance of pain (be it acute or chronic).

Future treatments should be aimed at taking into account the complex temporal and spatial dynamic of the nociceptor and its molecular players. It is the sum of their expression patterns in specialized neuronal subpopulations plus their regulation by multiple endogenous and exogenous factors (ranging from hormones and cytokines to cold and pressure) that ultimately determines what type of pain we feel, its threshold and duration, as well as its intensity and physical location.

The new generation of pain treatments will most certainly target the cell machinery that synthesizes, assembles, and sorts ion channels and pain receptors to the cell membrane and nerve terminals. It should also contemplate the key role played by trophic factors and genetic determinants in the phenotype of primary sensory neu-

rons, a field of active research which has still to produce a useable drug, despite promising starting points such as monoclonal humanized anti-NGF proteins and others.

Finally, innate protective mechanisms against pain should be preserved and even stimulated as a more natural way of achieving clinically relevant results with the bare minimum of secondary, adverse effects. This will most certainly be achieved by combining selective pharmacological tools with a more holistic therapeutic approach including concomitant physical and psychological therapies.

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