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Neuronal circuitry for pain processing in the dorsal horn

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Preface

Neurons in the spinal dorsal horn process sensory information, which is then transmitted to several brain regions, including those responsible for pain perception. The dorsal horn provides numerous potential targets for the development of novel analgesics, and is thought to undergo changes that contribute to the exaggerated pain felt after nerve injury and inflammation. Despite its obvious importance, we still know little about the neuronal circuits that process sensory information, mainly because of the heterogeneity of the various neuronal components that make up these circuits. Recent studies have begun to shed light on the neuronal organisation and circuitry of this complex region.

Dorsal horn neurons receive sensory information from primary afferents that innervate the skin and deeper tissues of the body and that respond to specific types of noxious and non-noxious stimuli. These afferents terminate in the dorsal horn with a distribution pattern that is determined by their sensory modality and the region of the body that they innervate. The incoming information is processed by complex circuits involving excitatory and inhibitory interneurons, and transmitted to projection neurons for relay to several brain areas. In addition, nociceptive information is conveyed

to the ventral horn and contributes to spinally-mediated nocifensive reflexes[1]. Activity at various points in these circuits can be modulated by axons that descend from the brainstem.

The balance between excitation and inhibition is critical for maintaining normal sensory function — blocking inhibitory transmission at spinal levels, for example, can lead to allodynia[1,2]. Indeed, changes in the function of these circuits have been implicated in the development and maintenance of inflammatory and neuropathic pain.

Despite the importance of the dorsal horn in normal sensory processing and in pathological conditions, we still know little about the neuronal circuits that link incoming primary afferents to projection neurons, which constitute its major output. The main reason for this is that the great diversity of dorsal horn neurons has made it difficult to develop a comprehensive classification scheme for either the interneurons or the projection cells. Without such a scheme it is not possible to establish the roles of different neurons within these circuits.

In this review I describe the basic neuronal components of the dorsal horn and what we know about the circuits in which they are involved, with particular emphasis on pathways that process nociceptive information. This description will be restricted to laminae I-III of Rexed[3] (Figure 1), as we know more about the organisation of this region than that of the deeper laminae. Moreover, this region includes the major termination zone of nociceptive primary afferents (laminae I and IIo). I also discuss changes that could underlie the abnormal sensations that arise following tissue inflammation and in cases of neuropathic pain. The review is based mainly on findings in the rat, as the majority of the relevant studies have been carried out in this species, but also includes information obtained from other species. For example, many recent studies have been carried out in the mouse, due to the increasing availability of animals in which genes have been knocked out, modified or used to drive expression of green fluorescent protein (GFP). In general, there seems to be a remarkable consistency in neuronal organisation across the species, although there are undoubtedly some differences[4], and it is important to bear these in mind when comparing data obtained from different species.

Neuronal components in laminae I-III

Primary afferents

Primary afferent axons can be classified by peripheral target (for example, cutaneous, articular or visceral afferents), conduction velocity (which is related to size and myelination), response properties (including sensory modality and the intensity of stimulus necessary to activate them) and neurochemical phenotype (such as peptide expression). These features are interrelated, as most large myelinated cutaneous (Aβ) afferents are low-threshold mechanoreceptors, responding to touch or hair movement, whereas the majority of fine myelinated (Aδ) and unmyelinated (C) fibres are nociceptors or thermoreceptors.

Primary afferents terminate with a specific distribution pattern that is determined by their functional class (Figure 1). In general, myelinated low-threshold mechanoreceptive afferents arborise in an area extending from lamina IIi-VI, whereas nociceptive and thermoreceptive Aδ and C afferents innervate lamina I and much of lamina II, except for its most ventral part. Recent studies have identified a group of cooling-specific C afferents that terminate in lamina I[5], as well as two possible candidates for low-threshold mechanoreceptive C fibres that project to lamina II[6,7]. Nociceptive C fibres can be divided into two major neurochemical groups: those that contain neuropeptides, such as substance P[8], and those that do not[9]. These two groups have distinctive termination zones within the superficial laminae. Non-peptidergic C fibres are mainly associated with the skin, where they innervate the epidermis[10], whereas peptidergic fibres innervate various other tissues as well as deeper regions of the skin[11-13]. Expression of Mas-related G-protein coupled receptor member D (MRGPRD), a sensory neuron-specific G protein-coupled receptor, has recently been shown to define a population of non-peptidergic nociceptive C fibres in the mouse[14]. They have axons that terminate peripherally, in the epidermis, and centrally, in a narrow band within lamina II. The differences in their peripheral and central distributions suggest that peptidergic and non-peptidergic nociceptive C fibres differ in function. This view is supported by a recent study in

which ablation of the MRGPRD afferents in adult mice resulted in a selective loss of sensitivity to noxious mechanical (but not thermal) stimuli[15].

Determining the relative proportions of primary afferents that belong to each of these classes is difficult. Most of the anatomical studies involving cell counts in dorsal root ganglia have not been corrected for the sampling bias that results from variation in cell sizes, and electrophysiological recordings from nerves are inevitably biased towards larger axons. However, it has been estimated that in the rat around 80% of cutaneous primary afferents are unmyelinated[16], and about half of the lumbar dorsal root ganglion cells that give rise to C fibres are peptidergic[17].

All primary afferents use glutamate as their major fast transmitter, and thus have an excitatory action on their postsynaptic targets. However, their ultrastructural appearance and synaptic arrangements differ. Those belonging to non-peptidergic C fibres and Aδ hair-follicle afferents can form the central axons of two different types of synaptic glomerulus, in which they are presynaptic to several dendrites and postsynaptic at axoaxonic synapses[18,19]. Central terminals of Aβ afferents and Aδ nociceptors generally form simpler synaptic arrangements, but again have axoaxonic synapses as well as being presynaptic at axodendritic synapses. By contrast, peptidergic primary afferents receive few axoaxonic synapses[20].

Descending inputs

Two descending monoamine-containing pathways project to the dorsal horn: a serotonergic system originating mainly in the nucleus raphe magnus, and a noradrenergic pathway from the locus coeruleus and other pontine regions. Both serotonergic and noradrenergic axons terminate diffusely throughout the dorsal horn, and although some form synapses, much of their action is mediated through volume transmission[21]. Another descending pathway that is likely to have a role in pain mechanisms consists of GABA (γ-aminobutyric acid)-ergic axons from the rostral ventromedial medulla that arborise widely in the dorsal horn[22] and synapse with lamina II interneurons[23].

Interneurons

The vast majority of neurons in laminae I-III have axons that remain in the spinal cord and arborise locally, and these are therefore defined as interneurons. They include virtually all of the neurons in lamina II, and most of those in laminae I and III.

Interneurons can be divided into two main classes: excitatory (glutamatergic) and inhibitory. The inhibitory interneurons use GABA and/or glycine as their main neurotransmitter(s), and their cell bodies can be identified with antibodies against these amino acids. In the rat, GABA is present in ~25%, 30% and 40% of neurons in laminae I, II and III, respectively[24]. Glycine is present at high levels in many lamina III neurons and some of those in laminae I-II, but within laminae I-III glycine immunostaining is largely restricted to GABA-containing cells[24,25]. This suggests that many inhibitory interneurons co-release GABA and glycine, whereas the others only release GABA. However, electrophysiological studies have identified synapses in this region that are purely glycinergic[26,27]. Although these may involve axons that originate from glycinergic neurons located outside laminae I-III, in many cases the lack of a GABAergic component probably results from the absence of $GABA_A$ receptors at these synapses. The axons of inhibitory interneurons can be identified with antibodies against the vesicular GABA transporter (VGAT, which also transports glycine), glutamate decarboxylase (GAD, the GABA-synthesizing enzyme), or the neuronal glycine transporter (GlyT2). These reveal a dense plexus of inhibitory axons in laminae I-III, most of which originate from local interneurons.

There are no reliable immunocytochemical markers for the cell bodies of glutamatergic neurons, but it is likely that all dorsal horn neurons that are not immunoreactive for GABA or glycine are glutamatergic. Their axons can be identified by the presence of vesicular glutamate transporters (VGLUTs)[28], and excitatory interneurons in the dorsal horn express VGLUT2[28-30]. There are numerous VGLUT2-containing boutons in laminae I-III, most of which originate from local excitatory interneurons.

Although there have been several attempts to classify interneurons in laminae I-III, we still do not have a generally accepted scheme that covers all of these cells. Many studies have investigated morphology in the hope of defining specific classes, initially using the Golgi technique, and more recently, with single-cell labelling during electrophysiological recordings. Electrophysiological criteria for determining interneuron subtypes include synaptic inputs from different classes of primary afferent and firing patterns in response to injected current. Several patterns have been described, and among these the delayed, gap and reluctant firing patterns are thought to result from an A-type potassium current (I_A)[31,32], involving channels containing the Kv4.2 or Kv4.3 subunits[33,34], which suppresses neuronal excitability.

Lamina II interneurons have been studied extensively, and the most widely accepted classification scheme for these cells is that developed by Perl and colleagues[35-38], who combined whole-cell recording in slices from adult guinea pigs, mice and rats with subsequent morphological analysis of the neurons, which had been labelled with biocytin from the patch pipette. They identified four main classes: islet, central, vertical and radial cells, which differed in dendritic morphology (Figure 2). Two recent electrophysiological studies in slices of rat spinal cord used immunocytochemistry to identify the neurotransmitter phenotype of lamina II interneurons, allowing a direct comparison of morphology and function[29,30]. Together with data from other experimental approaches[36,39], these indicate that although there is a relationship between morphology and the neurotransmitter used by the cell, this relationship is not straightforward. Islet cells were invariably GABAergic, whereas radial and most vertical cells were glutamatergic, and central cells could be of either type. A further limitation of this classification scheme is that all morphological studies included a substantial population of 'unclassified' cells (typically ~30%)[27,29,30,35]. Although the firing pattern of lamina II interneurons shows little correlation with morphology[31], it is closely related to neurotransmitter, as I_A-type patterns were largely restricted to glutamatergic interneurons[30]. Consistent with this, immunostaining for the Kv4.2 and 4.3 subunits is mainly found on calretinin-expressing neurons[34], which are thought to be glutamatergic[40].

These findings raise the question of whether the morphological classes that have been identified in lamina II (islet, vertical, radial, central) represent genuine functional populations. Islet cells are readily recognisable, and invariably inhibitory, and these are likely to constitute a discrete class. Radial and most vertical cells are glutamatergic, and these may also correspond to distinct populations. However, this can only be confirmed after further analysis of their synaptic inputs and outputs. In particular, it will be important to determine whether the inputs and outputs are consistent within each of the two populations. For the remaining neurons (central, inhibitory vertical and unclassified cells), there may be further classes to be defined. Alternatively, they may represent morphologically heterogeneous populations of excitatory and inhibitory interneurons. The first interpretation is supported by the finding of small but distinct populations, such as inhibitory central cells that express GFP under the control of the prion promoter[36,41,42]. Further analysis of the roles of these different cells in the neuronal circuitry of the dorsal horn will be required before this question can be answered.

Our knowledge of the organisation of lamina I interneurons is even more limited. Although 3 morphological types of neuron (pyramidal, fusiform and multipolar) have been described[43,44], interpretation of these morphological findings is complicated by the presence of projection cells, which have often not been distinguished from the interneurons. Prescott and De Koninck[45] reported a clear correlation between morphology and firing pattern for a sample of small neurons (presumably interneurons[46]) in lamina I. However, we know little about the relation between either of these parameters and neurotransmitter phenotype for lamina I interneurons.

Elsewhere in the CNS, functional populations of neurons have been defined using a neurochemical approach based on expression of neuropeptides and various proteins. The dorsal horn contains a large array of potential neurochemical markers and this approach has therefore also been applied here[47-49]. Immunocytochemical studies have shown that certain neuropeptides (somatostatin, neurotensin, substance P and neurokinin B) are found exclusively in glutamatergic neurons, some (neuropeptide Y and galanin) in GABAergic neurons, whereas others (enkephalin

and dynorphin) are expressed by both types. Among other markers that have been investigated, the calcium-binding protein parvalbumin and the neuronal form of nitric oxide synthase (nNOS) are found in restricted populations of dorsal horn neurons, mainly those that are both GABAergic and glycinergic. In contrast, two other calcium-binding proteins (calbindin and calretinin) and the γ isoform of protein kinase C (PKCγ) are largely restricted to glutamatergic cells[40,47,50-53]. The large number of neurochemical markers in laminae I-III indicates the need for caution in using expression of individual neuropeptides or proteins to define populations. Some of these compounds are present in a relatively high proportion of neurons or are expressed by both excitatory and inhibitory interneurons, suggesting that they do not define discrete populations. On the other hand, certain markers show a restricted distribution and occur in non-overlapping groups (Figure 2), which may represent functional populations.

Projection neurons

Projection neurons are concentrated in lamina I and scattered throughout laminae III-VI, with very few present in lamina II at lumbar levels. Many dorsal horn projection neurons, including those in lamina I, have axons that cross the midline and travel rostrally in the contralateral white matter to terminate in various brainstem and thalamic nuclei, forming a collection of pathways that are thought to underlie pain and temperature perception. As a major component of this group of pathways originates from lamina I neurons, the projections of these cells have been analysed in detail (Figure 3). Retrograde tracing studies have investigated the numbers of neurons labelled from each brain region, the extent to which individual neurons project to more than one target and various anatomical and neurochemical features of the cells[54-60]. Anterograde tracing has been used to map the terminations of these ascending projections[61-64]. These two approaches have shown that the main supraspinal targets for lamina I projection neurons include the caudal ventrolateral medulla (CVLM), the nucleus of the solitary tract (NTS), the lateral parabrachial area (LPb), the periaqueductal grey matter (PAG), and certain nuclei in the thalamus[63]. There is extensive

colateralisation of axons, with some lamina I neurons projecting to at least 3 targets (LPb, PAG and thalamus[65]). Although most cells have only contralateral projections, some project bilaterally[58]. Quantitative retrograde tracing studies in the rat suggest that in the L4 segment, projection neurons constitute ~5% of lamina I neurons[58] (Figure 3). Of these, 95% project to LPb, around a third to PAG, a quarter to NTS, and <5% to the thalamus. The low number of lamina I spinothalamic cells is surprising, given the presumed importance of this pathway for pain perception. However, this seems to be a specific feature of the rat lumbar enlargement as lamina I spinothalamic neurons are far more numerous in the rat cervical enlargement[66,67] and in both enlargments of the cat and monkey[68,69]. The large number of supraspinal targets of lamina I projection cells suggests that they are involved in diverse functions, including the sensory-discriminative aspects of pain perception, as well as motivational/affective and autonomic components (Box 1).

Lamina I projection neurons are not a homogeneous group, and there have been several attempts to classify them into discrete populations. These have been based on physiological properties[43,70-72], and various anatomical features (morphology and receptor expression)[43,56-59]. Electrophysiological recording studies in the monkey, cat and rat *in vivo* have shown that most lamina I projection neurons respond to noxious stimuli, although a few are activated by innocuous cooling[43,70,71,73,74]. The neurokinin 1 receptor (NK1R), which is expressed by many lamina I neurons and is the main target for substance P, has attracted considerable interest because its expression is restricted to dorsal horn neurons that are activated by noxious stimuli[75], and ablation of NK1R-expressing cells with a substance P–saporin conjugate prevents the development of hyperalgesia in models of neuropathic and inflammatory pain[76,77]. Around 80% of lamina I projection neurons in the rat show NK1R-immunoreactivity[58,59,66], and although the receptor is also found on many excitatory interneurons[78], its expression level is much lower than in projection neurons[46]. It is therefore likely that the effects of substance P–saporin result from loss of projection neurons in the superficial dorsal horn.

Dendrites of lamina I projection neurons generally remain within the lamina, and based on their orientation and branching pattern, the cells have been classified into fusiform, pyramidal and multipolar types[58,60,68,69,79]. Results of *in vivo* electrophysiological studies in the cat[43] and a combination of tract-tracing and immunocytochemistry in the monkey[79] and rat[60] suggest that morphology is related to function, such that pyramidal cells lack NK1Rs and respond to innocuous cooling. However, we have found that different morphological types of lamina I projection neurons in the rat have a similar percentage of NK1R-immunoreactive cells, and that following noxious stimulation the proportion that expresses Fos protein (a marker for neuronal activation) is the same for NK1R-positive cells of each morphological type[80]. Although these results suggest that somatodendritic shape does not distinguish functional classes of NK1R-expressing lamina I projection neurons, we have recently found evidence for two distinct populations based on soma size[81]. Large NK1R-positive projection cells had clusters of synaptic AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptors (AMPARs) that contained the GluA4 subunit, whereas small cells possessed GluA1-containing AMPARs. In addition, the density of glutamatergic axodendritic synapses was 3-4 times higher on the large cells.

Among the lamina I projection neurons that lack NK1Rs, there is a population of very large multipolar neurons that can be recognised by the dense inhibitory and excitatory synaptic input to their somata and dendrites[82,83] (Figure 3c). These "giant cells", which express Fos following noxious stimulation[82,83], are included among the Waldeyer-type cells that others have reported[3]. They are very sparse, since there are only ~10 per side in the rat L4 segment, constituting around 2-3% of the lamina I projection neurons.

Although many lamina I neurons project to more than one brain region, there is evidence that certain types preferentially innervate particular targets, since both the large NK1R-expressing neurons and the giant cells are over-represented among the spinothalamic population[65,82].

Another type of projection neuron that provides an output for the superficial dorsal horn consists of large NK1R-immunoreactive cells with somata in lamina III and extensive dendritic trees that

penetrate as far dorsally as lamina I[84] (Figure 3d). These cells have similar supraspinal targets to the lamina I projection cells, and immunocytochemical studies have shown that they contain phosphorylated extracellular signal-regulated kinases (ERKs, another activity-dependent marker) following various types of noxious stimulation[85].

Neuronal circuits in laminae I-III

The neuronal components of the dorsal horn are interconnected by highly complex synaptic circuits. For example, it is likely that most (if not all) dorsal horn neurons receive synaptic input from primary afferents and from both excitatory and inhibitory interneurons. However, the specific types and relative strengths of these inputs differs between neuronal populations. Our knowledge of how these circuits are organised is still limited, but having defined at least some of the neuronal populations, we are now in a position to investigate the circuits in which they are involved. Some of the circuits that have been identified are shown in Figure 4.

Inputs to projection neurons

Although interneurons are the major postsynaptic target for primary afferents, there are direct synaptic connections between primary afferents and projection cells. Combined immunocyto-chemical and tract-tracing studies have demonstrated that NK1R-expressing projection neurons in lamina I and lamina III are densely innervated by peptidergic primary afferents, most of which contain substance P[80,84], and for the lamina I cells this constitutes approximately half of their glutamatergic input[81]. This approach has also revealed that although dendrites of the lamina III NK1R-expressing projection neurons arborise throughout the dorsal horn, they receive only a moderate input from myelinated (low-threshold) afferents in deeper laminae[86] and are not innervated by non-peptidergic IB4-binding C fibres[87]. These cells are therefore likely to have wide-dynamic range receptive fields dominated by nociceptive inputs[85]. Unlike NK1R-expressing projection neurons, the giant lamina I projection neurons receive little direct synaptic input from

primary afferents[82], and so their responses to noxious stimuli are presumably mediated through excitatory interneurons. Whole-cell patch-clamp recordings have shown that some lamina I projection neurons receive monosynaptic input from Aδ nociceptive afferents[35,72], but it is not known whether this arrangement is selective for particular types of cell.

Certain specific patterns of innervation from inhibitory interneurons to projection neurons have been identified in anatomical studies. The lamina III NK1R-expressing cells receive numerous synapses from GABAergic boutons that contain neuropeptide Y (NPY)[88], which presumably originate from local inhibitory interneurons. They receive few contacts from another population of inhibitory interneurons: those that contain nNOS and GABA. By contrast, the giant lamina I cells are often densely innervated by nNOS-containing GABAergic boutons, which provide around one quarter of their GABAergic input.

Less is known about the types of excitatory interneuron that innervate projection cells. Lu and Perl[38] have carried out patch-clamp recordings from pairs of neurons and showed that glutamatergic vertical cells in lamina II that were innervated by Aδ primary afferents, provide synaptic inputs to lamina I projection neurons expressing NK1Rs. The giant lamina I neurons receive a dense synaptic input from VGLUT2-immunoreactive boutons that are thought to be derived from excitatory interneurons[82], although the laminar location and morphology of these interneurons is not yet known.

Inputs to interneurons

The primary afferent inputs to different types of lamina II interneuron have been investigated in patch-clamp recordings from spinal cord slices. Islet cells and most central cells are innervated mainly or exclusively by C fibres, whereas vertical and radial cells can receive monosynaptic inputs from both C and Aδ afferents[27,35-38,41]. With this approach it is difficult to identify the classes of C and Aδ fibre that innervate each cell type, however, Uta *et al.*[89] reported that C fibres expressing both transient receptor potential A1 (TRPA1) and transient receptor potential vanilloid 1 (TRPV1)

are presynaptic to vertical and radial cells, but not to islet and central cells in lamina II. Another recent study has demonstrated that specific classes of C fibre innervate different types of lamina II interneuron, based on a comparison of the latencies of monosynaptic primary afferent inputs to pairs of synaptically coupled cells[41]. PKCγ is expressed by a morphologically diverse group of excitatory interneurons with cell bodies in lamina IIi and III[52], and at least some of these cells are innervated by low-threshold mechanoreceptive myelinated afferents[90,91].

Electrophysiological studies have also begun to reveal the arrangement of synaptic circuits that link different types of lamina II interneurons. Paired recordings suggest a low number of synaptic connections between interneurons[37,92], but have identified certain specific circuits, with islet cells forming GABAergic synapses onto central cells, and central cells forming glutamatergic synapses onto vertical cells[37,38]. Analysis of primary afferent-evoked inhibitory input to lamina II neurons suggest a complex pattern in which each type receives synapses from more than one class of inhibitory interneuron[27].

Inputs to primary afferents

Most primary afferent terminals throughout the spinal cord receive axoaxonic synapses, which are the substrate for GABAergic presynaptic inhibition. As axoaxonic synapses on Ia muscle afferents in the ventral horn originate from a single population of GABAergic neurons[93], it is likely that presynaptic inhibitory inputs to primary afferents in the dorsal horn originate from specific types of interneuron. Although GABA is found in all boutons that are presynaptic to primary afferents, most of those associated with Aδ and Aβ hair-follicle afferents are enriched with glycine, whereas those that synapse on non-peptidergic C fibres are not[94,95], providing evidence that they originate from different interneurons. However, the identity of GABAergic neurons that give rise to axoaxonic synapses with primary afferents is not yet known.

Volume transmission

Both neuropeptides and monoamines act through volume transmission, and their actions are therefore determined largely by the distribution of the corresponding receptors. The cellular distribution of several neuropeptide receptors in laminae I-III has been identified. As noted above, the NK1R is found on projection neurons in laminae I and III, and at a lower level on excitatory interneurons, particularly in lamina I[46,78]. Release of substance P following noxious stimulation activates (and internalises) NK1Rs on these cells[96], giving rise to slow inward currents[38]. Immunocytochemical studies have shown that other peptide receptors are also differentially distributed in the dorsal horn. The NK3R (the main target for neurokinin B) is present on many lamina II neurons, including both excitatory and inhibitory cells[97,98]. The somatostatin receptor type 2A (SSTR2A) is found exclusively on inhibitory interneurons in laminae I-II[30,99], the μ-opioid receptor (OPRM) on excitatory interneurons[100] and the NPY receptor type 1 (NPY1R) on several populations, including excitatory interneurons and projection cells[101,102]. Both OPRMs and NPY1Rs are also found on the central terminals of nociceptive primary afferents.

Monoamines act through a variety of receptors, including α_1 and α_2 adrenergic and serotonin (5-hydroxytryptamine) 1A ($5HT_{1A}$) and $5HT_3$ receptors. There is relatively little information about the neurons that express these receptors, although the effects of noradrenaline and 5-HT on different types of neuron have been described. Most cells hyperpolarise in response to both monoamines, although some islet and central cells are depolarised by 5-HT and some GABAergic neurons (including islet cells) by noradrenaline[103-105]. In addition, the monoamines can suppress primary afferent inputs to superficial dorsal horn neurons through a presynaptic mechanism[104].

Mechanisms of hyperalgesia/allodynia

Both tissue inflammation and nerve injury can result in abnormal pain sensations, including hyperalgesia, allodynia and spontaneous pain. Several changes in the spinal cord that could underlie these phenomena have been proposed, and these have recently been reviewed[106]. They include a

reduction of GABAergic and/or glycinergic inhibitory neurotransmission (disinhibition), development of long-term potentiation (LTP), intrinsic plasticity of dorsal horn neurons, and alterations in the properties of low-threshold mechanoreceptive Aβ afferents. Evidence relating to each of these mechanisms is considered below.

Disinhibition

Inhibitory interneurons have various functions, and disinhibition could contribute to allodynia, hyperalgesia and spontaneous pain[106]. For example, tactile allodynia may result from a loss of inhibition of excitatory interneurons that convey low-threshold mechanoreceptive inputs to lamina I projection neurons[107], leading to a mis-coding of the information by cells that normally only detect painful stimuli.

Several mechanisms involving the release of GABA and/or glycine[108] or their post-synaptic actions[109], could lead to disinhibition and these have been investigated in models of neuropathic pain in rodents (Box 2). Moore *et al.*[108] found a reduction in the proportion of lamina II neurons with primary afferent-evoked inhibitory postsynaptic currents following chronic constriction injury (CCI) or spared nerve injury (SNI). As these injuries are associated with allodynia and hyperalgesia, this supports the view that disinhibition contributes to these symptoms. Early studies reported a substantial (90-100%) loss of GABA-immunoreactive neurons from the dorsal horn following CCI that was maximal at around 2 weeks after surgery[110,111]. Because there was some recovery of immunoreactivity, it was assumed that the loss of GABA-immunostaining was at least partly due to down-regulation rather than to neuronal death. However, cell death in the dorsal horn does occur following nerve injury[108,112-115], and it has been reported that at least some of the affected cells are neurons. This has led to the suggestion that death of inhibitory interneurons following nerve injury may contribute to neuropathic pain[108,110-114]. However, using stereological methods to produce unbiased counts, we have found that following CCI there is no loss of neurons[116] and no alteration in the proportion that are GABA- or glycine-immunoreactive[24], even though the animals developed

clear signs of thermal hyperalgesia. In addition, although there was apoptosis in the dorsal horn in the SNI model, this affected microglia rather than neurons, and the number of neurons in laminae I-III did not change despite the development of tactile allodynia[115]. Although we cannot rule out the possibility that there is substantial death of inhibitory interneurons in some neuropathic models, these findings suggest that neuronal death is not necessary for the development of neuropathic pain[24,115,116]. It is difficult to explain the discrepancies in immunostaining for GABA and glycine between different studies[24,110,111], although these probably reflect the difficulty of retaining the amino acids during perfusion fixation[24].

Depletion of GABA (or glycine) from axons of inhibitory interneurons could lead to disinhibition and there is evidence that one isoform of GAD is down-regulated after CCI or SNI[108]. However, using a quantitative immunogold method, we have found that following SNI the level of GABA in GABAergic boutons in laminae I-II does not differ between the denervated and intact sides[117].

Although the number of $GABA_A$ receptors does not seem to be altered in neuropathic models[108,117], it is possible that GABA becomes less effective due to an alteration of the anion gradient across neuronal membranes, resulting from downregulation of the potassium-chloride co-transporter KCC2. This would have the effect of diminishing (and even reversing) the depolarising actions of GABA and glycine[109]. Although this is an attractive hypothesis, it does not explain why spinal administration of $GABA_A$ receptor agonists reduces tactile allodynia in neuropathic pain models[118,119].

Alternative mechanisms that could lead to disinhibition following nerve injury include alteration of the inputs to, or intrinsic properties of, inhibitory interneurons[106]. It has been reported that in the mouse, the firing patterns of a subset of GABAergic lamina II neurons, as well as the proportion of these cells activated by primary afferents, do not change after nerve injury[120]. However, more subtle alterations in the strength of primary afferent inputs to inhibitory interneurons may contribute to neuropathic pain, as different types of interneuron receive synapses from specific classes of C

fibre[41,89], and these may differ in the extent to which they lose their synaptic connections after peripheral axotomy[121,122].

LTP

Two different forms of LTP have been identified in NK1R-expressing lamina I projection neurons following dorsal root stimulation at C fibre strength, and these are thought to operate through Ca^{2+}-dependent postsynaptic mechanisms[123,124]. One form, induced by high-frequency (100 Hz) stimulation, was restricted to neurons retrogradely labelled from LPb[123], whereas the other, induced only by low-frequency (2 Hz) stimulation, was seen in cells labelled from PAG[124]. The difference between these two projection targets is difficult to interpret because virtually all spino-PAG lamina I neurons are included in the spino-parabrachial population[58]. As NK1R-expressing projection neurons have an important role in the development of chronic pain states[76,77], LTP of their nociceptive inputs may play a significant part in this phenomenon[106].

Hippocampal LTP is thought to involve insertion of AMPARs containing either GluA1 or GluA4 subunits into glutamatergic synapses, leading to increased postsynaptic responses to glutamate[125,126]. AMPAR insertion may contribute to LTP in lamina I projection neurons, because phosphorylation of GluA1 has been detected at glutamatergic synapses in lamina I following acute noxious stimulation[127]. Considering the recent finding that NK1R-immunoreactive lamina I projection neurons differ in AMPAR subunit composition — with large cells expressing GluA4 and the smaller ones GluA1[81] — an intriguing possibility is that this expression pattern underlies the two different forms of LTP[123,124].

AMPAR insertion and phosphorylation also occur at synapses in lamina II[127,128], which is consistent with the finding of LTP in interneurons[129]. It is difficult to predict the effects of LTP that involves interneurons on behaviour, and these effects would depend on the extent to which LTP occurred in excitatory and/or inhibitory cells.

Intrinsic plasticity

This term refers to changes in the excitability of neurons. One proposed mechanism for intrinsic plasticity in the superficial dorsal horn involves phosphorylation of Kv4.2[33], a downstream target for ERKs, which are activated in many neurons following noxious stimulation[130]. This would result in a reduction of I_A currents, leading to an increase in excitability. Kv4.2 is highly expressed in lamina II, but not lamina I[34], and as firing patterns associated with I_A currents are largely restricted to excitatory interneurons in lamina II[30], this mechanism is likely to be specific for these cells. Increasing transmission in excitatory interneurons could enhance activation of lamina I projection cells through polysynaptic pathways, contributing to the hyperalgesia arising in inflammatory pain states. In addition, many excitatory interneurons contain somatostatin, and the resulting increase in the release of this peptide may hyperpolarise nearby inhibitory interneurons, thus having a disinhibitory action[30,99].

Changes affecting Aβ primary afferents

Abnormal functioning of low-threshold mechanoreceptive Aβ afferents could contribute to tactile allodynia in neuropathic or inflammatory states if these afferents gain access to pathways that are normally nociceptive-specific. One study reported that central terminals of Aβ fibres sprouted dorsally into laminae I-IIo after peripheral axotomy[131]. Following injection of the B subunit of cholera toxin (CTb) (which is normally transported only by myelinated afferents) into an intact peripheral nerve, labelling was excluded from lamina IIo. However, when CTb was injected into a chronically transected nerve, it was transported to all dorsal horn laminae, and this was thought to result from sprouting of myelinated afferents. Additional evidence for this mechanism came from the finding that intra-axonally labelled Aβ afferents had terminals that entered lamina I in nerve-injured, but not naïve animals[131]. However, subsequent studies demonstrated that CTb is taken up and transported by injured C fibres, and this probably accounts for the novel labelling in lamina IIo seen after nerve injury[132,133]. It was also reported that there was no difference in the arborisation

patterns of intra-axonally-labelled Aβ afferents after peripheral nerve injury[91,134]. As some myelinated nociceptors with axonal arbors that resemble those of low-threshold afferents but that extend into lamina I, have been seen in normal animals[134], these nociceptors may account for the Aβ afferents with axons entering laminae I-IIo that were observed after nerve injury[131].

Expression of substance P by primary afferents is normally restricted to nociceptors[8], but it has been reported that there is a 'phenotypic switch', resulting in synthesis of the peptide by Aβ afferents (which are presumed to be low-threshold mechanoreceptors) following inflammation and nerve injury[135,136]. If substance P is released in substantial amounts from their central terminals it could activate NK1Rs on dorsal horn neurons, thus contributing to touch-evoked pain. Although it has been reported that substance P is released in the dorsal horn following electrical stimulation of Aβ afferents in rats that have undergone spinal nerve ligation (SNL)[137] (Box 2), we were unable to detect the peptide with immunocytochemistry in central terminals of injured myelinated afferents following various types of nerve injury (SNT, SNL or CCI) (Box 2)[138]. In addition, electrical stimulation of Aβ afferents in nerve injured rats did not lead to NK1R internalisation on dorsal horn neurons[138]. This suggests that release of substance P from low-threshold mechanoreceptive Aβ afferents does not contribute to allodynia in these models. It remains to be seen whether substance P is released by Aβ afferents in inflammatory pain states.

Transmission of polysynaptic inputs from Aβ afferents to neurons in the superficial laminae is increased in inflammatory and neuropathic pain states[139-142], and as this can also occur following application of $GABA_A$ and glycine receptor antagonists[107,140], it may result from disinhibition. One proposed circuit involves excitatory interneurons in lamina III with monosynaptic Aβ input and axons that enter the superficial dorsal horn[140]. However, most lamina III neurons seem to have axons that do not arborise extensively in lamina II[143], and an alternative possibility is that Aβ afferents activate ventral dendrites of lamina II vertical cells, which often enter lamina III[27,35].

Conclusions and future directions

Although our knowledge of dorsal horn circuitry is still limited, recent studies have begun to reveal some of the synaptic connections linking primary afferents, interneurons and projection cells, as well as the receptors and ion channels expressed on the different components of these circuits. Further information of this type should allow us to identify novel molecular targets for drugs to combat pain, as well as to identify (and ultimately prevent) changes that occur in the dorsal horn in chronic pain states. Establishing the pattern of expression of receptors and channels on different neuronal types will be essential, as the effect of manipulating these will depend on whether they are expressed by inhibitory or excitatory cells[31].

Future studies will need to refine our classification of each of the neuronal components in the dorsal horn. For example, although the distinction between non-peptidergic and peptidergic C fibres has revealed important differences in their peripheral and central distribution, this distinction does not take into account the various physiological classes that have been identified.

A major obstacle to progress remains the difficulty in recognising distinct populations of interneurons. Even in lamina II — the most extensively studied region — a substantial proportion of interneurons remain unclassified[27,29,30,35], and two morphological groups (vertical and central cells) include both excitatory and inhibitory cells[29,30]. Recent *in vitro* studies have begun to correlate electrophysiological and morphological properties with neurotransmitter type for these cells[29,30], but we will need to integrate this information with data on receptive field properties from *in vivo* studies, and with the more detailed neurochemical classification described above[48]. The availability of mice in which GFP is expressed in neurochemically-defined neuronal populations should allow targeted recording from these cells, and thus reveal their physiological properties and synaptic connections. Similar approaches are needed to identify populations of interneurons in other laminae, in order to allow us to identify their roles in dorsal horn circuits.

For projection neurons in laminae I and III, it will be necessary to correlate the various types identified in *in vivo* physiological studies[43,70,71] with anatomical classifications based on

morphology and receptor expression[81-83], and to extend our knowledge of the synaptic inputs that they receive from primary afferents and interneurons. We will also need to fill in the substantial gaps in our understanding of the organisation, synaptic circuitry and expression of receptors and channels of projection neurons in other laminae, as many of these cells are activated by noxious stimuli and are likely to have important roles in pain perception.

Boxes and Figure legends

Box 1 **Brain areas that receive direct inputs from the superficial dorsal horn**

Axons of projection neurons in laminae I and III target several brain regions. Many of these are interconnected, and therefore also receive indirect nociceptive inputs from the superficial dorsal horn. The major targets are:

- the caudal ventrolateral medulla (CVLM), which gives rise to a descending projection to the dorsal horn and may integrate nociceptive and cardiovascular responses[144].
- the nucleus of the solitary tract (NTS), a region that also receives cardio-respiratory inputs and has a role in the reflex tachycardia that results from noxious stimulation[145].
- the lateral parabrachial area (LPb), which is the major target for lamina I projection neurons in the rat. The nuclei that receive input from the superficial dorsal horn project to forebrain areas such as the amygdala and hypothalamus[146]. Through these connections, the spinoparabrachial pathway is likely to be involved in emotional (including aversive) and autonomic components of pain.
- the periaqueductal grey matter (PAG), which is involved in organising strategies for coping with stressors (including pain) and is one of the central sites of action of analgesics[147]. Through its projections to other brainstem areas (including the rostral ventromedial medulla), it plays an important part in descending modulation of dorsal horn circuits.
- the thalamus. Several thalamic nuclei receive inputs from the superficial dorsal horn. In the rat, these include the ventral posterolateral (VPL) nucleus (which is reciprocally connected to the primary somatosensory cortex), the posterior group (the connections of which are poorly understood for the rat[63]) and the posterior triangular nucleus (which projects to the second somatosensory area and insular cortex[148]). Considering their projection areas, these pathways are likely to contribute to both sensory-discriminative and affective-motivational aspects of pain (through somatosensory and insular cortex, respectively).

Box 2 **Animal models of neuropathic pain**

Several different types of peripheral nerve injury have been performed to provide rodent models for neuropathic pain states seen in humans. A very brief account of those referred to in this article is given here. Complete transection of the sciatic nerve (SNT) provides a convenient way of denervating a large part of the lumbar dorsal horn, and results in a substantial area of anaesthesia in the peripheral territory of the nerve, with a corresponding motor disturbance. In many cases animals show a variable degree of autotomy (self-mutilation of the denervated paw), and it has been suggested that this results from spontaneous pain[149]. There may also be changes in mechanical and thermal responsiveness in the skin area that is innervated by the intact saphenous nerve[150].

As partial nerve injuries in humans are more likely to cause allodynia and hyperalgesia than complete nerve transection, several models that cause incomplete damage to the sciatic nerve or to the spinal nerves that give rise to it, have been developed.

- The chronic constriction injury (CCI) model is produced by loosely ligating the sciatic nerve[151]. The constriction results from intra-neural oedema secondary to occlusion of vessels within the nerve, and leads to a preferential loss of myelinated afferents. Rats exposed to CCI develop a robust thermal hyperalgesia, together with signs of cold and tactile allodynia, and mechanical hyperalgesia. These symptoms develop over the first post-operative week, peak during the second week and gradually disappear, by around 8 weeks.
- For the spinal nerve ligation (SNL) model[152], tight ligatures are placed around the L5 and L6 spinal nerves before these join the lumbosacral plexus. Animals exposed to SNL rapidly develop signs of tactile allodynia, which appear within the first post-operative day and last for several months. In addition, they show a thermal hyperalgesia that starts during the first week and lasts for around one month.
- Spared nerve injury (SNI)[150] involves tight ligation and distal transection of the two main branches of the sciatic nerve, the tibial and common peroneal, leaving the remaining sural

branch intact. Like the SNL model, this also results in a rapidly developing and prolonged tactile allodynia in the affected hindlimb, but without any change in thermal responsiveness.

Figure 1 **Laminar organisation of the dorsal horn and primary afferent inputs**

Rexed[3] divided the grey matter of the cat dorsal horn into a series of parallel laminae based on variations in the size and packing density of neurons, and this scheme has since been applied to other species. **a |** A transverse section of rat mid-lumbar spinal cord that is immunostained using an antibody (NeuN) that specifically labels neurons. Laminar boundaries are indicated by the dashed lines. Laminae I and II (also known as the marginal zone and substantia gelatinosa, respectively) constitute the superficial dorsal horn, and are characterised by the presence of numerous small neurons. Lamina II can be divided into outer (IIo) and inner (IIi) parts, with the latter having a somewhat lower density of neurons. Image is modified, with permission, from REF. 157. **b |** Primary afferents arborise within the dorsal horn in an orderly way: a laminar termination pattern based on fibre diameter and function is superimposed on a somatotopic distribution that determines mediolateral and rostrocaudal location. The central terminations of the major primary afferent types (excluding proprioceptors) are shown. In the 1970s and 1980s a series of intra-axonal labelling studies revealed the projections of different types of myelinated afferents[153-155]. These showed that Aβ tactile and hair afferents end mainly in laminae III-VI with some extension into lamina IIi, the precise arrangement depending on their function[153]. Aδ hair-follicle afferents arborise on either side of the lamina II/III border, whereas Aδ nociceptors end mainly in lamina I, with some giving branches to laminae V and X[154]. More recent studies have identified myelinated nociceptors with conduction velocities in the Aβ range that arborise throughout laminae I-V[134] (not shown). Peptidergic primary afferents (which also include some Aδ nociceptors[8]) arborise mainly in lamina

I and IIo, with some fibres penetrating more deeply, whereas most non-peptidergic C fibres form a band that occupies the central part of lamina II[156].

Figure 2 **Classification of lamina II interneurons**

Various experimental approaches have been used to classify interneurons, including somatodendritic morphology, firing pattern in response to injected current, and neurochemistry. Examples of these are illustrated. **a |** Confocal images of 4 lamina II neurons labelled with Neurobiotin following whole cell patch-clamp recording[30]. The cells are seen in sagittal sections and correspond to each of the main classes identified by Grudt and Perl[35]. Islet cells have dendritic trees that are elongated (>400 µm) in the rostrocaudal axis, with little dorsoventral or mediolateral spread. Central cells are similar, but have much shorter dendritic trees (<400 µm long)[27]. Radial cells have compact dendritic trees with primary dendrites radiating in several directions. Vertical cells typically have a dorsally placed soma and dendrites that fan out ventrally, often occupying a conical shape. Immunocytochemical staining of axons with antibodies against the vesicular glutamate transporter VGLUT2 and the vesicular GABA (γ-aminobutyric acid) transporter VGAT revealed that the islet cell was GABAergic ($VGAT^+$), whereas the other 3 cells in this figure were glutamatergic ($VGLUT2^+$) (not shown). Scale bar = 100 µm. **b** | Firing patterns in response to 1 sec pulses of injected current in 3 different lamina II neurons[30]. Values at the left of each trace indicate initial membrane voltage or current before application of the pulses, and the arrow shows the 'gap' seen after the initial spike in gap-firing neurons. The gap and delayed firing pattern, which are thought to result from the presence of I_A currents, were associated with most excitatory (18/22), but few inhibitory (2/23) cells[30]. **c** | This image shows a confocal optical slice through part of lamina II in a section immunostained to reveal neuropeptide Y (NPY, green), the neuronal form of nitric oxide synthase (nNOS, red) and parvalbumin (PV, blue), compounds that are present in the dorsal

horn and found in non-overlapping groups[50] (A.J. Todd and E. Polgár, unpublished data). A single cell body containing each compound is visible. Scale bar = 10 µm.

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Figure 3 **Projection neurons**

a | This image shows a transverse section through the contralateral side of the L4 segment of a rat that received injections of two retrograde tracers: Fluorogold into the lateral parabrachial area (LPb) and cholera toxin B subunit (CTb) into the caudal ventrolateral medulla (CVLM). The section was immunostained for CTb (red), Fluorogold (green) and NeuN (blue). Injection of tracers into both of these regions has been previously shown to label virtually all lamina I projection neurons[59]. Cells retrogradely labelled from the LPb appear green, those labelled from the CVLM are red, and those that received label from both sites are yellow. Note the high density of double-labelled cells in lamina I, but that even here they are outnumbered by other neurons (blue). Lamina II contains virtually no projection cells at lumbar levels, and there are scattered retrogradely labelled cells in deeper laminae and in the lateral spinal nucleus (LSN). **b |** A summary of quantitative data from a series of studies of projection neurons in the L4 segment of the rat spinal cord[58,59,65-67]. These data suggest that there are approximately 400 projection neurons in lamina I (~5% of the total neuronal population in this lamina[116]) (blue numbers). Most of these can be retrogradely labelled following injection of tracer into the LPb or CVLM, with smaller numbers projecting to the other sites. Studies involving paired injections indicate that virtually all lamina I cells that are retrogradely labelled following tracer injection into thalamus, periaqueductal grey matter (PAG) or nucleus of the solitary tract (NTS) also project to LPb. Note that as spinal projections to the CVLM terminate near the main bundle of ascending axons, retrograde labelling from this region may include cells labelled through uptake of tracer into fibres of passage, rather than from axon terminals. The numbers of neurokinin 1 receptor (NK1R)-expressing projection neurons in laminae III-IV that can be labelled from each site are also shown (red numbers). There are around 24 of these cells per side

in the L4 segment (corresponding to ~0.1% of the neurons in this lamina[116]). **c |** A horizontal section through lamina I scanned to reveal NK1R (green) and the glycine receptor-associated protein gephyrin (magenta). Two NK1R-positive neurons and a giant lamina I cell (outlined by gephyrin puncta) are visible. Although retrograde labelling was not performed, all lamina I neurons of this size are known to be projection cells[46,83]. **d |** An example of a NK1R-expressing lamina III projection neuron in a sagittal section. The cell is retrogradely labelled with CTb (magenta) that had been injected into the LPb. Note the extensive dorsal dendrites that are labelled with the NK1R antibody (green), and can be followed into lamina I. Several retrogradely labelled lamina I cells are also visible in this field. Scale bars = 100 µm (a,d), 50 µm (c).
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Fig. 4 **Neuronal circuits involving projection neurons**

a | A diagram showing some of the synaptic circuits identified in laminae I-III. Three types of projection neuron (PN) are shown: a neurokinin 1 receptor (NK1R)-expressing cell in lamina I (NK1R PN), an NK1R-expressing cell in lamina III, and a giant lamina I neuron[82,83]. Both types of NK1R-expressing projection neuron are densely innervated by substance P-containing primary afferents (SP)[80,84], and the lamina III neurons also have an input from myelinated low threshold mechanoreceptive (LTM) afferents[86]. The lamina III NK1R cells receive a substantial input from GABAergic interneurons that contain neuropeptide Y (GABA/NPY IN)[88], whereas inhibitory interneurons that contain neuronal nitric oxide synthase (GABA/nNOS IN) innervate the giant lamina I cells[83]. These cells receive a high density of synapses from vesicular glutamate transporter 2-containing boutons derived from unknown populations of glutamatergic interneuron (?GLU IN)[82]. NK1R-expressing lamina I projection neurons also receive an input from glutamatergic vertical

cells (GLU Vertical cell), which are innervated by glutamatergic central cells (GLU Central cell)[38]. The primary afferents that synapse onto vertical cells include Aδ fibres[38], as well as C fibres that express both transient receptor potential A1 (TRPA1) and transient receptor potential vanilloid 1 (TRPV1)[89]. **b** and **c** show examples of synaptic inputs to projection neurons. **b |** A lamina III NK1R-positive projection neuron (blue), here seen in sagittal section, receives many contacts from NPY-containing boutons (red). The lower images show part of this region (boxed area) scanned to reveal gephyrin (green), NK1R and NPY. Gephyrin puncta (arrowheads) indicate the presence of synapses. **c |** The upper image (horizontal section) shows lamina I neurons labelled with Fluorogold from the lateral parabrachial area. The lower images show part of a dendrite of the cell marked with the asterisk (corresponding to the boxed area). The left image has been scanned to reveal GluA2 (blue) and GluA4 (green) subunits of the AMPA ((α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptors, together with Fluorogold (white). On the right, the same field has been scanned for GluA2, GluA4 and calcitonin gene-related peptide (CGRP, red). The high density of CGRP contacts on the dendrite indicate that this is likely to be a NK1R-expressing cell[80,82]. The presence of puncta containing GluA2 and GluA4 at sites where CGRP boutons are in contact (arrowheads) indicates that these are forming synapses. Scale bars: main images = 25 μm, lower images = 5 μm.

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Glossary

Allodynia	Pain following a normally non-painful tactile or thermal stimulus.
Delayed firing pattern	A response to injected depolarising current in which a neuron generates action potentials after a delay.
Gap firing pattern	A response to injected depolarising current in which an initial action potential is followed by a long inter-spike interval and then regular firing.
Hyperalgesia	Exaggerated pain in response to a noxious stimulus.
Laminae of Rexed	A system of 10 layers, described by Rexed, to divide the grey matter in the spinal cord.
Locus coeruleus	The major source of noradrenergic axons to the spinal cord.
Long-term potentiation (LTP)	A form of synaptic plasticity that results in a long-lasting increase in the strength of synaptic transmission.
Nucleus raphe magnus	The main source of descending serotonergic axons that innervate the dorsal horn.
Neuropathic pain	Pain resulting from pathology of the nervous system. Most commonly this is caused by conditions affecting peripheral nerves.
Nociceptive information	Stimuli through which we perceive damage (or potential damage) caused to the body by excessive heat, cold or physical injury for example.
Nocifensive reflex	A protective reflex generated in response to a damaging (or potentially damaging) stimulus
Reluctant firing pattern	This term is used to describe neurons that are resistant to action potential firing during injection of depolarising current.
Rostral ventromedial	

medulla A region of the brainstem that includes the nucleus raphe magnus and gives rise to many descending axons that innervate the dorsal horn.

Synaptic glomerulus A complex structure in which a central axonal bouton (of primary afferent origin) is in synaptic contact with several surrounding profiles, including dendrites and peripheral axons. Peripheral axons are GABAergic and are presynaptic to the central bouton. Some of the dendrites may contain vesicles and be presynaptic to other dendrites or the central bouton.

TRPA1 Transient receptor potential A1. A nonselective cation channel that is activated by cold and by various chemical irritants (including mustard oil), and is expressed by certain nociceptive primary afferents (a subset of those that express TRPV1).

TRPV1 Transient receptor potential V1. A nonselective cation channel that can be activated by various noxious stimuli (including heat and application of capsaicin) and is expressed by many nociceptive primary afferents.

Volume transmission Release of a neurotransmitter at non-synaptic sites, whereby the neurotransmitter diffuses through the extracellular space to activate nearby receptors.

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a

b

I

II

III

IV

V

VI

X

VII

VIII

IX

Aδ nociceptors I

C/Aδ peptidergic I-IIo

C non-peptide II

Aδ hair-follicle IIi/III

Aβ hair/tactile IIi-V

islet

central

radial

vertical

a

tonic

gap

delayed

-69 mV

-85 mV

-73 mV

-22 pA

-45 pA

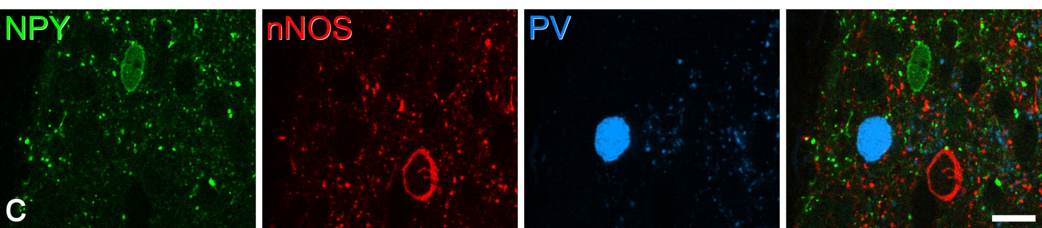
7 pA

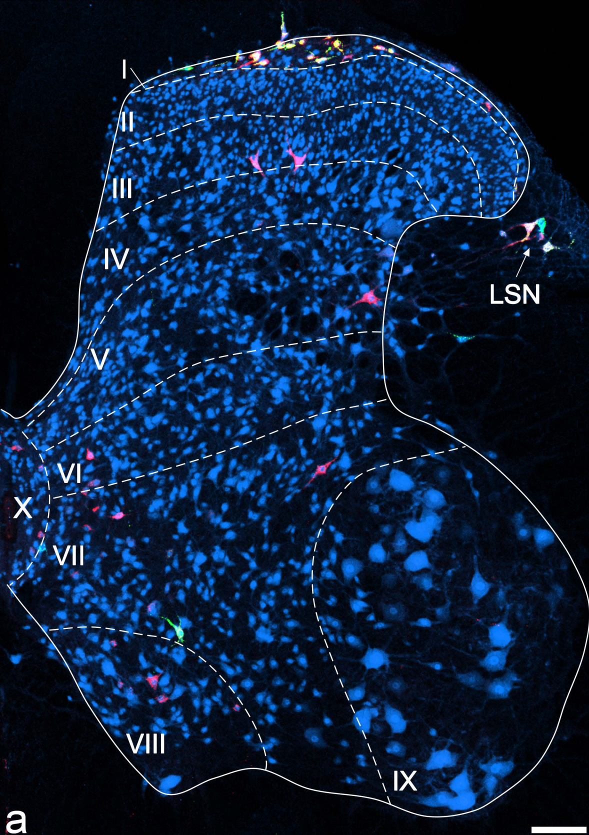
40 mV

124 pA

400 ms

b




I
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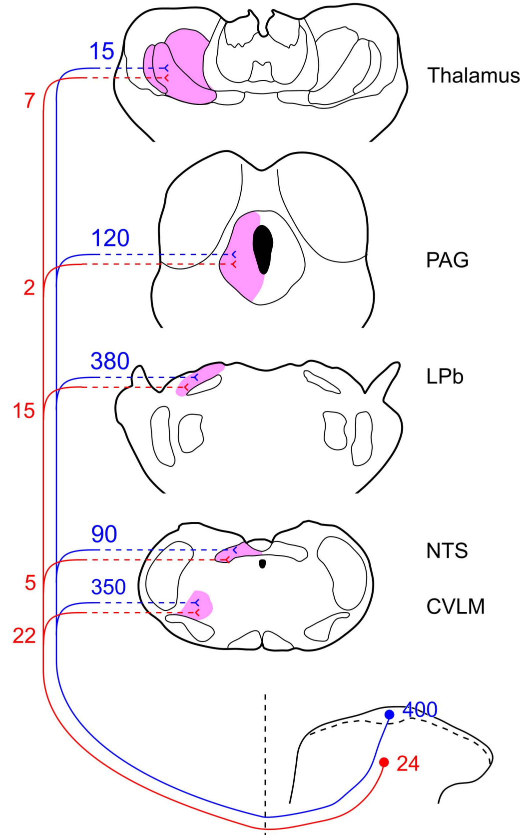
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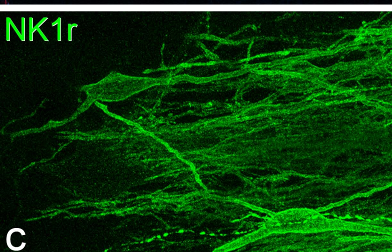
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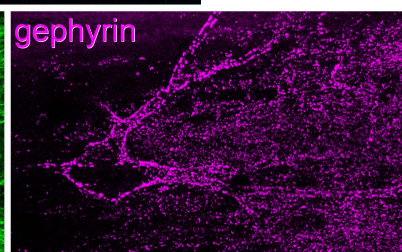
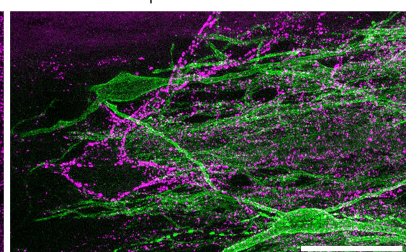
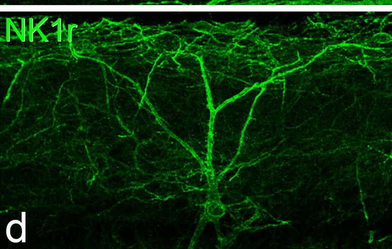
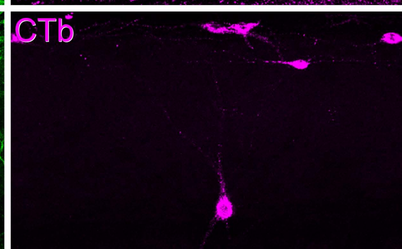

gephyrin




NK1r
d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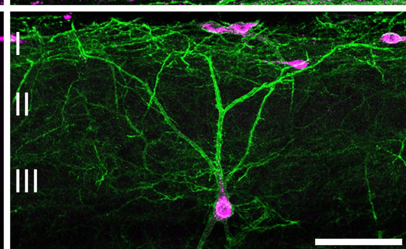
I
II
III

a

myelinated LTM
$C^{TRPA1/TRPV1}$
Aδ
SP
giant cell
NK1r PN
I
II
III
GABA/nNOS IN
?GLU IN
GLU Central cell
GLU Vertical cell
GABA/NPY IN
NK1r PN