# CHAPTER 1
# Anatomy of pain pathways

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## Basic organisation of spinal pain pathways

Primary afferent axons belonging to the somatosensory system can respond to a range of mechanical, thermal and chemical stimuli. Many of these afferents are activated by stimuli that damage (or threaten to damage) tissues, and these are known as nociceptors. Primary afferents that innervate the limbs and the trunk enter the spinal cord through the dorsal roots and form excitatory (glutamatergic) synapses with neurons in the dorsal horn. The dorsal horn contains a large number of neurons, the great majority of which have axons that arborise locally and remain in the spinal cord; these are known as interneurons and are involved in the local processing of sensory information. In addition, the dorsal horn contains projection cells – that is, neurons with axons that enter the white matter and travel rostrally to the brain. The axons of these cells are grouped into a number of different ascending tracts. The final neuronal component consists of descending axons, which originate from cells in the brain (particularly the brainstem) and terminate diffusely within the dorsal horn.

Rexed (1952) divided the grey matter of the dorsal horn into six parallel laminae (numbered from dorsal to ventral), and this scheme is widely used, for example to describe the arborisation of primary afferents and the distribution of different populations of spinal neurons (Figure 1.1). The dorsal horn is somatotopically organised, the body being mapped in a bidimensional pattern onto the rostrocaudal and mediolateral axes. The other, dorsoventral dimension is arranged in a modality-specific pattern, as will be described later.

Fifty years ago, Melzack and Wall (1965) proposed that neurons within the superficial dorsal horn could 'gate' the inputs from nociceptors and thus modify the perception of pain. This theory attracted a great deal of interest, and there have been numerous attempts to unravel the neuronal circuitry that underlies pain processing in the spinal cord. It turns out that this circuitry is highly complex, and we still have only a limited understanding of it. Nonetheless, it is clear that this region is very important for modulating pain in both normal and pathological states. The superficial dorsal horn is also likely to provide important targets

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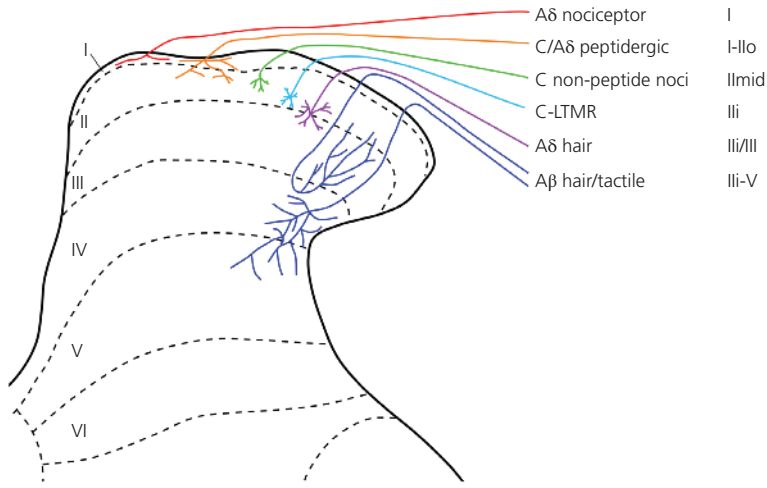


**Figure 1.1** Rexed's laminae and primary afferent inputs. The diagram shows the main areas targeted by the central arbors of different types of primary afferent, apart from proprioceptors. These have been superimposed on Rexed's laminar scheme, as applied to the rat dorsal horn. Note that Woodbury and Koerber (2003) have described another class of myelinated nociceptor, with axonal arbors that extend throughout laminae I–V, and this has not been included. Source: Todd, 2010. Reproduced with permission of Nature Publishing Group.

for new drugs designed to treat pain, as it contains a wealth of receptors and signalling molecules. This chapter will summarise some of the available information about the anatomy of spinal somatosensory pathways, placing particular emphasis on the organisation of neuronal populations and their synaptic connections.

## Primary afferent input to the dorsal horn

Primary afferents have their cell bodies in the dorsal root ganglia and an axon that bifurcates, sending one branch to a peripheral target and the other to the spinal cord. They can be classified according to a series of criteria. On the basis of their peripheral targets, they can be divided into afferents that innervate the skin, the muscles, the viscera and so on. Secondly, they can be characterised in terms of the strength and type of the adequate stimulus (e.g., hot/cold nociceptors, low-threshold mechanoreceptors). They also vary in axonal diameter – which is related to conduction velocity – and in whether or not they are myelinated: very fine afferents are unmyelinated and are known as C fibres, while the remainder can be divided into large-diameter (Aβ) and small-diameter (Aδ) myelinated fibres. Finally, there are various neurochemical markers, for example neuropeptides, that can be used to distinguish functional populations. These parameters are interrelated; for example, the majority of C and Aδ fibres are nociceptors or thermoreceptors, whereas most Aβs are low-threshold mechanoreceptors (LTMRs) that respond to touch or hair movement. However, this is not an absolute distinction and there are LTMRs among both the Aδ and the C fibre classes, while some nociceptors conduct action potentials in the Aβ range.

Although the focus of this chapter is on pain, the LTMRs are relevant because in many pathological pain states touch can elicit pain (tactile allodynia), and at least a part of this effect is mediated by the low-threshold afferents (Campbell et al., 1988).

The laminar distribution pattern of the main classes of primary afferent is shown in Figure 1.1.

## Termination of nociceptors within the dorsal horn

Myelinated nociceptors (mostly Aδ fibres) convey 'fast' pain, whereas the nociceptive C fibres underlie 'slow' pain. The central projections of Aδ nociceptors have been demonstrated through intra-axonal labelling techniques. Many of these fibres terminate in a compact distribution in lamina I and in the outermost part of lamina II, while some arborise diffusely throughout laminae I–V (Light and Perl, 1979, Woodbury and Koerber, 2003). The central projections of myelinated afferents have also been studied through bulk-labelling after the injection of cholera toxin B subunit (CTb) into peripheral nerves. However, although this technique reveals projections to lamina I, some Aδ afferents (e.g., those terminating throughout laminae I–V) are not labelled.

Because of the small size of C fibres, there have been few studies of the central projections of individual afferents of this type (Sugiura, Lee and Perl, 1986). Conveniently, there are neurochemical markers that can be used to recognise different functional populations. C nociceptors can be divided into those that contain neuropeptides – the peptidergic group – and those that do not – the non-peptidergic group. All of the peptidergic afferents appear to contain calcitonin gene-related peptide (CGRP), which is only found in primary afferents in the dorsal horn. In addition, they can express a variety of other peptides, including substance P. Such afferents, which innervate most tissues of the body (including the skin), can be identified in anatomical experiments through immunocytochemistry for CGRP, although this approach does not distinguish between C fibres and peptidergic Aδ nociceptors (Lawson, Crepps and Perl, 1997). Most peptidergic afferents project to lamina I and the outer part of lamina II (IIo), but some send branches to deeper laminae (III–V) (Todd, 2010).

The non-peptidergic C nociceptors have been identified by their ability to bind the plant lectin IB4, although this property is not restricted to the non-peptidergic C afferents. More recently they have been shown to express mas-related G protein-coupled receptor D (MRGD) (Zylka, Rice and Anderson, 2005). These afferents innervate the skin and terminate more superficially than cutaneous peptidergic fibres. Their central arbors occupy a narrow band in the middle part of lamina II.

## Central terminations of low threshold mechanoreceptors

Studies of intra-axonally injected Aβ afferents have shown that these terminate in the deep laminae, with specific patterns for the various types of tactile and hair follicle afferent. Low-threshold mechanoreceptive Aδ fibres correspond to down-hair (D-hair) afferents, and these have a more restricted projection, to the inner half of lamina II (IIi) and the dorsal part of lamina III. Recent studies have identified neurochemical/genetic markers for these afferents in the mouse, and these markers have confirmed the distribution patterns that were previously

reported from intra-axonal injection studies (Abraira and Ginty, 2013). Some C fibres respond to tactile stimuli (C-LTMRs), and these have been shown to terminate in lamina IIi (Seal et al., 2009).

## Vesicular glutamate transporters and primary afferents

All primary afferents use glutamate as their principal fast transmitter, and this is concentrated into synaptic vesicles by a family of three vesicular glutamate transporters (VGLUT1–3). These are differentially distributed among the primary afferents. All myelinated LTMRs express VGLUT1 and form the main source of VGLUT1 in the dorsal horn, although some originates from descending corticospinal axons (Todd et al., 2003). In contrast, Aδ nociceptors possess VGLUT2.

Both peptidergic and non-peptidergic C nociceptors express VGLUT2 (Brumovsky, Watanabe and Hokfelt, 2007), but the level of protein detected with immunocytochemistry in their central terminals is generally very low. The C-LTMRs are unique, in that they express VGLUT3 (Seal et al., 2009).

## Receptors expressed by primary afferents

Primary afferents can express a wide variety of ligand-gated ionotropic, metabotropic and tyrosine kinase receptors (Todd and Koerber, 2012). For example, peptidergic and non-peptidergic C nociceptors have different growth factor receptors: trkA and RET, respectively. Glutamate receptors of the NMDA and AMPA type are widely expressed, as are $GABA_A$ and $GABA_B$ receptors, while non-peptidergic nociceptors possess purinergic $P2X_3$ receptors. There are several receptors for neuropeptides, including μ, δ and κ opioid receptors. In addition, TRP channels (TRPV1, TRPA1, TRPM8), which transduce thermal and chemical signals, are found on the peripheral and central terminals of certain afferents.

## Ultrastructure of primary afferent central terminals

While most central terminals of primary afferents form relatively simple synaptic arrangements, others form complex arrangements known as synaptic glomeruli (Ribeiro-da-Silva and Coimbra, 1982) (Figure 1.2). Two different types of synaptic glomerulus have been identified, and these involve the central terminals of non-peptidergic C nociceptors and Aδ D-hair afferents. Both types have a central axonal bouton (the primary afferent terminal), which is surrounded by dendritic profiles (mainly dendritic spines) and peripheral (GABAergic) axons (Todd, 1996). Some of the dendritic spines that originate from GABAergic interneurons also possess synaptic vesicles. Glomeruli are sites of complex synaptic interactions, in which the primary afferent bouton receives axoaxonic (inhibitory) synapses from the peripheral axons (and in some cases from vesicle-containing dendrites) and forms axodendritic (excitatory) synapses with the various dendritic profiles. There are also triadic synapses: for example, a peripheral axon can form a synapse with the central axon and also with a dendritic spine that receives a synapse from the central axon. Since all glomerular central axons are of primary afferent origin, they provide a convenient way of identifying primary afferent boutons in the dorsal horn. However, as stated above, many primary afferents have simpler endings, forming axodendritic synapses onto a small number of dendritic shafts or spines and receiving axoaxonic synapses.

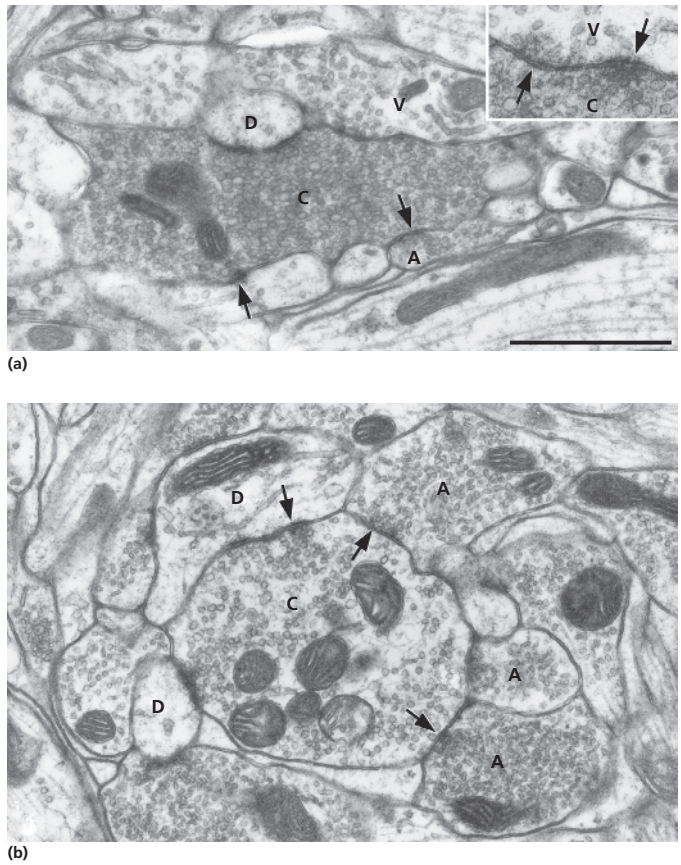


**Figure 1.2** Two types of synaptic glomerulus in the superficial dorsal horn of the rat. Some primary afferents form the central axons of synaptic glomeruli, and examples are shown here. **(a)** The central axon of a type I glomerulus (C). These are derived from non-peptidergic C nociceptors and are typically surrounded by vesicle-containing dendrites (V), dendrites that lack vesicles (D), and a single peripheral axon (A). The peripheral axon is presynaptic at an axoaxonic synapse with the central bouton. The central bouton forms synapses with both types of dendrite and can receive dendroaxonic synapses from the vesicle-containing dendrites. In some cases reciprocal axodendritic synapses are present, as shown in the inset. **(b)** The central ending of a type II glomerulus (C). These are thought to originate from Aδ D-hair endings, and are presynaptic to dendrites (D), most of which lack vesicles. These glomeruli are often surrounded by several peripheral axons (A), which are presynaptic to the C bouton and can form triadic synapses involving the C bouton and a dendrite. Arrows in both parts indicate synapses. Scale bar: 1 μm. Source: Todd, 1996. Reproduced with permission of John Wiley & Sons.

## Projection neurons and ascending tracts

Projection neurons are present in relatively large numbers in lamina I and are scattered throughout the deeper laminae of the dorsal horn (III–VI); but they are essentially absent from lamina II (Figure 1.3). The lamina I projection cells, together with many of those in laminae III–VI, have axons that cross the midline and enter the ventral or lateral funiculus on the opposite side, before ascending to the brain. The axons of these cells travel to several brain regions – including the thalamus, the periaqueductal grey matter (PAG), the lateral parabrachial area

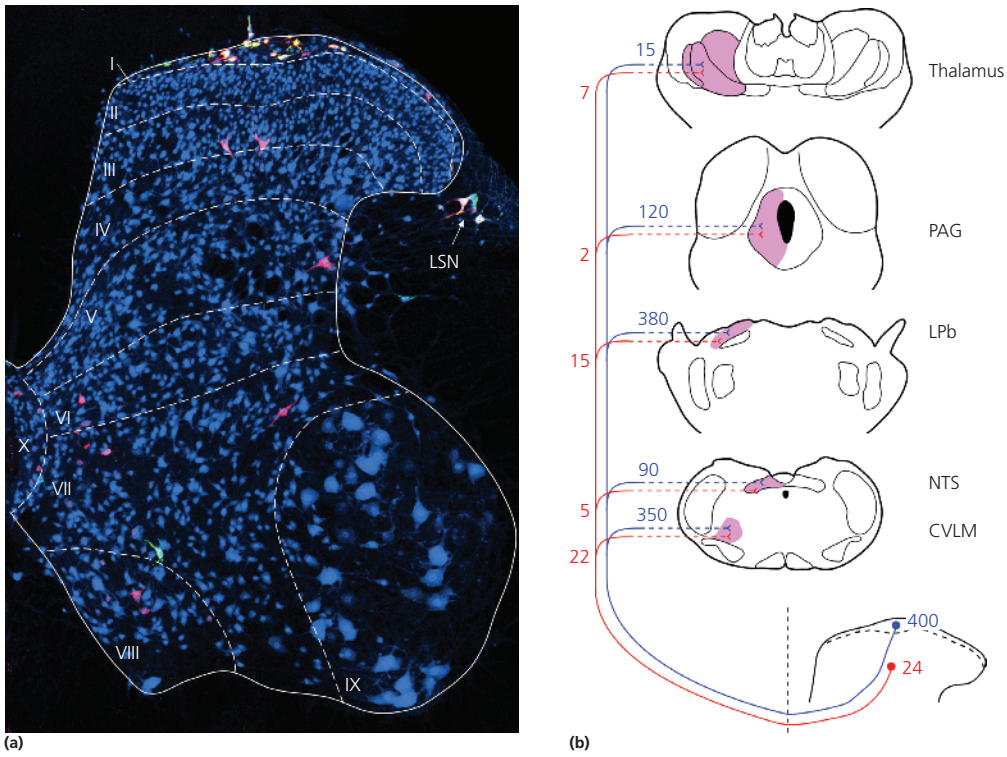


**Figure 1.3** Anterolateral tract projection neurons in the rat lumbar enlargement. **(a)** A transverse section from the L4 segment of a rat that had received injections of cholera toxin B subunit (CTb) into the caudal ventrolateral medulla (CVLM) and Fluorogold into the lateral parabrachial area (LPb). The section was immunostained to reveal CTb (red), Fluorogold (green) and the neuronal marker NeuN (blue). Tracer injections into these two sites can label virtually all of the projection neurons in lamina I, as well as scattered cells throughout the deep dorsal horn (laminae III–VI). Note that some cells have taken up both tracers, and therefore appear yellow. **(b)** Quantitative data from the rat lumbar spinal cord showing the approximate numbers of ALT projection neurons in laminae I and lII, and the numbers that can be retrogradely labelled from each target. There are approximately 400 ALT neurons in lamina I (~5 per cent of the total neurons in this laminae), and ~24 of these cells in lamina III. LSN = lateral spinal nucleus; PAG = periaqueductal grey matter; NTS = nucleus of the tractus solitarius. Source: In Handbook of Clinical Neurology, 3rd series: Pain, ed. by F. Cervero and T. S. Jensen, vol. 81. Reproduction with permission of Elsevier..

(LPb), and some nuclei in the caudal brainstem. Individual cells can have axons that project to several of these targets, and the projection is often referred to as the 'anterolateral tract' (ALT). Quantitative studies in the rat have shown that there are around 400 ALT neurons in lamina I on each side of the L4 segment and that virtually all of these neurons project as far as the LPb, one third reaching the PAG. However, somewhat surprisingly, less than 5 per cent of those in the lumbar cord project to the thalamus. Spinothalamic lamina I neurons are much more common in the cervical enlargement of the rat and in both lumbar and cervical enlargements of the cat and of the monkey (Todd, 2010). The ALT cells in deeper laminae of the dorsal horn are less numerous. For example, there are around twenty of these cells in lamina III, but these have projection targets similar to those of the lamina I cells.

In the rat, around 80 per cent of lamina I projection neurons, as well as the ALT neurons in lamina III, express the neurokinin 1 receptor (NK1r). This is a target for substance P,

which is released from nociceptive primary afferents, and internalisation of the receptor following ligand binding can be seen after noxious stimulation (Mantyh et al., 1995). Not surprisingly, ablation of these cells with substance P conjugated to the cytotoxin saporin leads to a dramatic reduction in hyperalgesia in both inflammatory and neuropathic pain states (Nichols et al., 1999). Among the lamina I ALT cells that lack the NK1r we have identified a population of very large (giant) cells, which have an extremely high density of excitatory and inhibitory synapses that coat their cell bodies and dendrites (Polgár et al., 2008). These cells are very sparse (they represent about 3 per cent of all lamina I projection neurons) but have extensive dendritic trees that are widely distributed throughout the lamina.

Electrophysiological studies have demonstrated that virtually all lamina I projection neurons in the rat are activated by noxious stimuli, while some are also activated by innocuous mechanical or thermal stimuli (Andrew, 2009; Bester et al., 2000). In addition, some of these cells are likely to respond to pruritic stimuli. Most lamina I projection neurons have dendrites that remain within the lamina. Various morphological types (e.g. fusiform, pyramidal, multipolar) have been identified, and it has been suggested that these represent specific functional populations and that pyramidal cells in the cat respond to thermal, rather than noxious stimuli (Han, Zhang and Craig, 1998). However, this hypothesis remains controversial, since pyramidally shaped lamina I projection neurons in the rat express the transcription factor Fos in response to noxious stimuli (Todd et al., 2002). In addition, these cells account for around one third of lamina I projection cells in the rat and, as stated above, virtually all of these respond to noxious stimuli.

The ALT is thought to be responsible for the perception of various stimuli as pain or as itch, as well as for thermal sensation. However, it is not the only ascending tract to originate from the dorsal horn. The spinocervical tract and the postsynaptic dorsal column (PSDC) pathway both arise from cells located in the deep dorsal horn, mainly lamina IV (Abraira and Ginty, 2013; Brown, 1981). Much less is known about the functional role of either of these pathways.

## Dorsal horn interneurons

Interneurons account for around 95 per cent of the neurons in lamina I, virtually all of those in lamina II, and an unknown proportion of those in the deeper laminae (Todd, 2010). Numerous anatomical studies have been carried out on interneurons in laminae I–III, which were labelled either with the Golgi technique or during electrophysiological recording experiments carried out *in vivo* or *in vitro*. These studies have shown that the interneurons almost invariably give rise to axonal boutons within the same segment, often forming complex local axonal arbors. These arbors are generally found in the lamina that contains the cell body, but they often extend into adjacent laminae. However, it is also clear that many interneurons in laminae I–III also give off long intersegmental branches, although the function of the latter is not yet understood (Todd and Koerber, 2012).

There are undoubtedly several different functional populations of interneurons in this region, and these are likely to perform distinct tasks. It is very important to be able to define

these populations, so that we may be able to explore their locations in the synaptic circuits of the dorsal horn and to investigate their functions, for example by selectively ablating or silencing them.

## Classification of interneurons in laminae I–III

### Inhibitory and excitatory interneurons

A fundamental distinction can be made between inhibitory and excitatory interneurons. The inhibitory interneurons use GABA and/or glycine as their major fast transmitter(s), and both amino acids can be revealed in neuronal cell bodies with immunocytochemistry. Quantitative studies in rat and mouse have shown that GABA is present in around 25–30 per cent of neurons in laminae I–II and in around 40 per cent of neurons in lamina III (Polgar et al., 2013a). Since the main role of GABA is to act as an inhibitory transmitter, it is very likely that these cells are all GABAergic interneurons. Glycine is also enriched in some neurons; but, because glycine has other roles apart from neurotransmission, it is not certain that all glycine-enriched neurons are glycinergic (Zeilhofer et al., 2005). Nonetheless, the vast majority of glycine-enriched neurons in laminae I–III were also found to be GABA-immunoreactive. This is significant for two reasons: first, it indicates that essentially all inhibitory interneurons can be revealed with GABA antibodies; and, secondly, it suggests that many of these interneurons can co-release GABA and glycine. However, despite evidence that GABA and glycine are co-localised in both interneuron cell bodies and their axon terminals, it appears that co-transmission is relatively rare in the adult spinal cord, possibly because of differential distribution of the postsynaptic receptors (Zeilhofer, Wildner and Yevenes, 2012).

All of the neurons in this region that lack GABA immunoreactivity are likely to be excitatory, glutamatergic neurons. These neurons include projection cells (in laminae I and III), but most are excitatory interneurons. Although glutamate can be revealed with immunocytochemistry, this has not proved to be a useful technique for identifying cell bodies of glutamatergic neurons, presumably because metabolic (i.e. non-transmitter) levels of glutamate are relatively high and this makes it difficult to distinguish them from 'transmitter glutamate'. Until around ten years ago it was difficult to identify glutamatergic neurons in anatomical studies. However, the discovery of the VGLUTs has provided very useful markers for identifying these cells. Unfortunately the levels of VGLUTs in cell bodies are too low for immunocytochemical detection, although their mRNAs can be revealed with in situ hybridisation (Malet et al., 2013). VGLUTs are concentrated in axon terminals and, where axons of individual neurons can be identified, they can be tested for the presence of the different VGLUT proteins (Maxwell et al., 2007, Todd et al., 2003, Yasaka et al., 2010). The results of these different approaches have shown that VGLUT2 is the major vesicular transporter used by glutamatergic neurons in laminae I–III, although some neurons in lamina III express low levels of VGLUT3.

We know something about the roles of inhibitory interneurons from studies in which antagonists acting at $GABA_A$ or glycine receptors have been applied intrathecally (Yaksh, 1989). Although some GABAergic and glycinergic axons in the superficial dorsal horn originate in

the brainstem (Antal et al., 1996), most are thought to belong to local interneurons, and this means that the effects of intrathecal antagonists are most likely to result from blocking transmission by local inhibitory interneurons. Studies of this type have suggested that these neurons are involved in limiting the extent and severity of pain in response to noxious stimuli, in preventing spontaneous pain and in avoiding non-noxious stimuli (e.g., touch) from being perceived as painful (Sandkuhler, 2009). There is also evidence that one role of inhibitory interneurons in the superficial laminae is to suppress itch, for example when a counterstimulus such as scratching is applied (Ross et al., 2010).

Much less is known about the roles of excitatory interneurons, but these roles may include the transmission of primary afferent information across laminar borders. For example, it is thought that excitatory interneurons provide a polysynaptic pathway through which tactile afferents (which terminate in laminae IIi–VI) can activate nociceptive projection neurons in lamina I (Lu et al., 2013, Torsney and MacDermott, 2006). This pathway may normally be closed by local inhibitory mechanisms, but it could open up in pathological states, thus allowing these cells to acquire low-threshold inputs. This phenomenon may contribute to tactile allodynia.

## Morphological classification

Elsewhere in the central nervous system (CNS), the morphology of neurons has often allowed them to be assigned to distinct populations; this approach has therefore been used extensively in the spinal dorsal horn. Most anatomical studies have concentrated on lamina II and, and while the early reports were based on Golgi staining, more recent investigations have examined neurons that were labelled during electrophysiological recording. The most widely accepted classification scheme was that developed by Grudt and Perl (2002), who identified four main morphological types of lamina II neuron: islet, vertical, radial and central cells (Figure 1.4a). Islet cells have dendritic trees that are highly elongated in the rostrocaudal axis; vertical cells generally have a dorsally located soma and a cone-shaped, ventrally directed dendritic arborisation; radial cells have short, radiating dendrites; while central cells are somewhat similar to islet cells but have much more restricted dendritic trees. However, although these cells have been identified in several other studies, a limitation of this approach is that many cells (typically ~30 per cent) cannot be assigned to any of these classes.

Yasaka et al. (2010) recorded from a large sample of lamina II neurons and related the morphology of the recorded neurons to their transmitter phenotype by immunostaining for VGLUT2 and for the vesicular GABA transporter (VGAT) in sections that contained the axons of these cells. They found that all islet cells were inhibitory – that is, had VGAT-immunoreactive axons – while radial cells and most vertical cells were excitatory. However, cells that were classified as central could be either excitatory or inhibitory interneurons; and there were many ‘unclassified’ cells among both transmitter types. These results suggest that, while certain functional populations can be identified on the basis of morphological criteria, many cells cannot be classified in this way. In addition, care is needed when using this approach: for example, some cells that resemble glutamatergic vertical cells are in fact inhibitory interneurons (Maxwell et al., 2007, Yasaka et al., 2010).

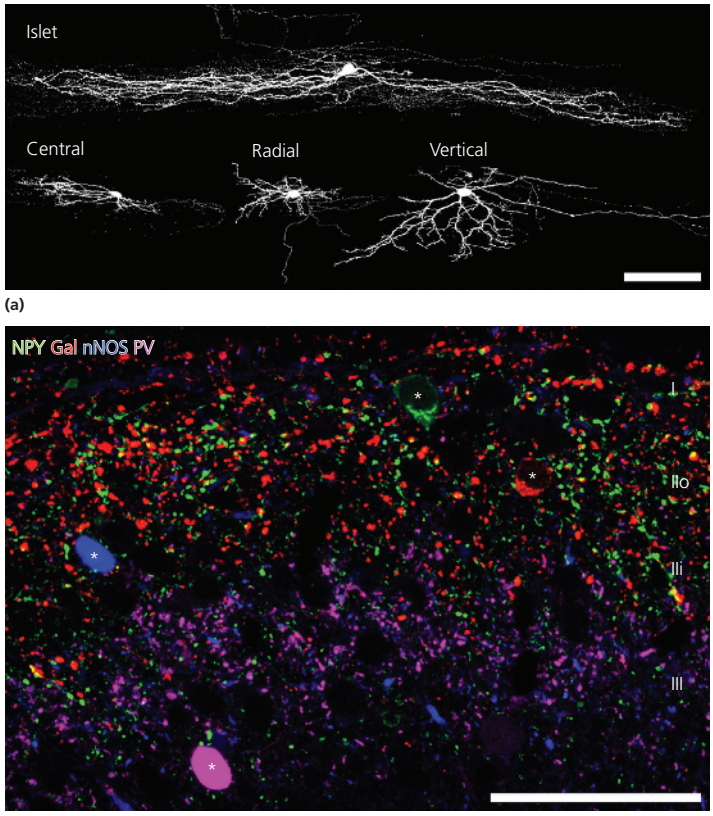


**Figure 1.4** Schemes for classifying interneurons in the superficial dorsal horn. **(a)** Confocal images of four lamina II neurons, recorded in parasagittal spinal cord slices from young adult rats. Neurobiotin in the pipette allowed labelling with fluorescent avidin after whole-cell recording. The cells correspond to the four main classes recognised by Grudt and Perl (2002). **(b)** Four non-overlapping populations can be recognised among the inhibitory interneurons in laminae I–III of the rat dorsal horn on the basis of expression of neuropeptide Y (NPY), galanin (Gal), neuronal nitric oxide synthase (nNOS) and parvalbumin (PV). This confocal image shows a single optical plane through a transverse section of rat lumbar spinal cord that had been reacted with antibodies to each of these substances. A single cell of each type is present, and these cells are indicated through asterisks. Approximate positions of laminae are shown. Scale bars: 100 μm (a), 50 μm (b). Sources: (a) Yasaka 2010.

## Neurochemical classification

Neurons in laminae I–III of the dorsal horn express a wide variety of potential neurochemical markers: neuropeptides, neuropeptide receptors, calcium-binding proteins and miscellaneous other proteins – for example, neuronal nitric oxide synthase (nNOS) and the γ isoform of protein kinase C (PKCγ). It has been found that many of these substances are restricted to limited numbers of neurons with distinctive laminar distributions, and in some cases to either excitatory or inhibitory interneurons (Todd and Koerber, 2012). For example, among the neuropeptides, it has been reported that somatostatin, neurotensin, substance P, gastrin-releasing

**Table 1.1** Expression of various neuropeptides and proteins by interneurons in laminae I–III of the dorsal horn. Some of the neuropeptides and proteins that are selectively distributed among inhibitory and excitatory interneurons in laminae I–III. Most of these are described in the text. The neurokinin 3 (NK3) receptor is expressed by specific types of inhibitory and excitatory interneuron, while the NPY Y1 is found on certain types of excitatory interneuron: neuronal nitric oxide synthase (nNOS) and protein kinase Cγ (PKCγ).

| | Inhibitory (GABAergic) | Excitatory (glutamatergic) |
|---|---|---|
| **Neuropeptides** | Neuropeptide Y<br>Galanin<br>Enkephalin<br>Dynorphin | Somatostatin<br>Neurotensin<br>Neurokinin B<br>Substance P<br>Gastrin-releasing peptide<br>Enkephalin<br>Dynorphin |
| **Neuropeptide receptors** | sst2A<br>NK3 | NK1<br>NK3<br>MOR-1<br>NPY Y1 |
| **Other proteins** | nNOS<br>Parvalbumin | nNOS<br>Calbindin<br>Calretinin<br>Parvalbumin<br>PKCγ |

peptide and neurokinin B (NKB) are expressed by excitatory interneurons, neuropeptide Y (NPY) and galanin by inhibitory interneurons, and the opioid peptides dynorphin and enkephalin by both types (see Table 1.1). An additional complication here is that some of these peptides (substance P, somatostatin and galanin) are also present in peptidergic primary afferents. However, since all peptidergic afferents are thought to contain CGRP in the rat, this feature can be used to distinguish the axons of these interneurons from primary afferents in this species.

## Neurochemical populations among the inhibitory interneurons

We have recently identified four non-overlapping populations among the inhibitory interneurons in laminae I–III of the rat, on the basis of expression of NPY, galanin, nNOS and parvalbumin (Polgar et al., 2013b) (Figure 1.4b). The galanin cells also contain the opioid peptide dynorphin, although this is additionally expressed by some excitatory interneurons in the dorsal horn (Sardella et al., 2011). Between them, these four populations account for at least half of the inhibitory interneurons in laminae I–II and for a smaller proportion of those in lamina III. A similar pattern has been observed in the mouse, except that in that case there is a moderate degree of overlap between nNOS and galanin/dynorphin populations (Iwagaki et al., 2013). There are differences in the distribution of these cell types, since the galanin/dynorphin-containing cells are concentrated in laminae I–IIo and the parvalbumin cells in laminae IIi–III, while the other two classes are present throughout laminae I–III.

We found that there were differences among these populations in their responses to noxious stimulation, as determined by expression of the transcription factor Fos (Hunt, Pini and Evan, 1987) or by the phosphorylation of extracellular signal-regulated kinases (ERKs) (Ji et al., 1999). Many of the galanin and NPY-expressing cells responded to noxious heat, as well as to the intradermal injection of capsaicin or formalin (Polgar et al., 2013b). Although nNOS-containing cells up-regulated Fos after noxious heat or formalin injection, they did not do so after injection of capsaicin. In contrast, the parvalbumin-positive cells were not apparently activated by any of these noxious stimuli.

Quantitative studies have shown that around 50 per cent of the inhibitory interneurons in laminae I-II possess the somatostatin receptor $sst_{2A}$. This appears to be the only receptor for somatostatin that is expressed by dorsal horn neurons; and it is virtually restricted to the inhibitory cells, which makes it a very convenient marker. In addition, there is evidence that the receptor is functional, since the application of somatostatin results in hyperpolarisation of these cells (Iwagaki et al., 2013, Yasaka et al., 2010). Interestingly, in both rat and mouse, nearly all of the galanin/dynorphin and nNOS-containing inhibitory interneurons were found to be included in the $sst_{2A}$-expressing population, while most NPY- and all parvalbumin-containing cells lacked $sst_{2A}$.

Two further pieces of evidence suggest distinct functions for these neurochemical classes of inhibitory interneurons. Hughes et al. (2012) have shown that parvalbumin-immunoreactive axons, presumably derived from the parvalbumin-containing inhibitory interneurons in laminae II–III, are closely associated with the central terminals of myelinated low-threshold mechanoreceptive afferents, with which they form axoaxonic synapses. Since the parvalbumin cells appear to receive inputs from the same types of afferent, their role may be to generate feedback presynaptic inhibition, and they are likely to be involved in maintaining tactile acuity. Ross et al. (2010) have reported that mice lacking the transcription factor Bhlhb5 develop exaggerated itch, but an almost completely normal pain phenotype. This was shown to be associated with loss of certain inhibitory interneurons from the superficial dorsal horn. We have recently found that the cells that are absent in the Bhlhb5 knock-out mouse correspond to the nNOS- and galanin/dynorphin-expressing populations, which between them account for around two thirds of $sst_{2A}$-expressing cells and for around one third of all inhibitory interneurons (Kardon et al., 2014). This loss appeared to be selective, since the remaining one third of $sst_{2A}$-expressing cells were still present and the numbers of $sst_{2A}$-negative inhibitory interneurons were unchanged. This suggests that either or both of the nNOS- and galanin/dynorphin-populations of inhibitory interneurons are involved in the suppression of itch. Since both types can be activated by noxious stimuli (Polgar et al., 2013b), both may be responsible for the scratch-mediated inhibition of itch.

Although in some cases individual neurochemical markers can be used to define discrete neuronal populations, caution is needed in interpreting immunocytochemical results. For example, both nNOS and dynorphin are found in some excitatory interneurons as well as in inhibitory cells. However, in each case the expression of $sst_{2A}$ can be used to distinguish between these cell types, as the receptor is present on virtually all the inhibitory cells but on none of the excitatory ones. It is likely that in many cases a combinatorial approach will be necessary in order to identify populations of interneurons in this region.

### Neurochemical populations among the excitatory interneurons

Considerably less is known about the organisation of neurochemical populations among the excitatory interneurons. Many of those in lamina II contain somatostatin: these are distributed throughout the lamina and include some vertical and radial cells (Yasaka et al., 2010). Two different peptides, neurokinin B and neurotensin, are found in mutually exclusive populations of excitatory interneurons in lamina IIi–III. It is known that there is overlap between the somatostatin cells and those that contain NKB or enkephalin, but not between the somatostatin and neurotensin cells. Another peptide– gastrin-releasing peptide (GRP) – is found in presumed excitatory interneurons in lamina II, and these are thought to be involved in itch pathways (Mishra and Hoon, 2013). However, the relation of this group to the other populations mentioned above is not yet known.

Although the NK1r is present on many ALT projection neurons in laminae I and III, it is also expressed by excitatory interneurons, particularly in lamina I. Antibodies directed against the μ-opioid receptor MOR-1 label a distinctive population of excitatory interneurons in the middle part of lamina II, but little is known about the extent to which these cells overlap with the peptide-containing classes described above.

Another protein that has been investigated in several studies is PKCγ, which is expressed by around 30 per cent of neurons in lamina IIi (Peirs et al., 2014), together with scattered cells dorsal and ventral to this. These neurons, which are innervated by myelinated low-threshold mechanoreceptors, have been implicated in the development of neuropathic pain (Lu et al., 2013, Malmberg et al., 1997). It is known that the PKCγ cells overlap extensively with those that express somatostatin, neurotensin or NKB.

Two calcium-binding proteins are present in large numbers of cells in laminae I–III, and most of these are excitatory interneurons. Some overlap between peptide-containing and calbindin-containing cells has been demonstrated.

## Descending pathways

The superficial dorsal horn receives descending inputs from various brain areas such as the cortex, the ventromedial medulla (including the raphe nuclei), and the locus coeruleus and surrounding areas of the pons. There has been particular interest in the role of descending monoaminergic systems, because there is much evidence that these play a role in modulating pain transmission at the spinal level. The serotoninergic pathway originates in the nucleus raphe magnus and terminates throughout the dorsal horn, but at particularly high density in laminae I–IIo. Norepinephrinergic axons from the locus coeruleus have a generally similar pattern of termination. Because the monoamine transmitters are thought to act through volume transmission, it is probably more important to know about the distribution of their receptors than about exactly where the axons terminate. However, although several monoamine receptors have been identified in the dorsal horn, we still know relatively little about their expression by specific cell types.

It has been shown that some of the descending axons originating in the ventromedial medulla are GABAergic and/or glycinergic (Antal et al., 1996), but the functions of this pathway are not yet known.

## Neuronal circuits

Information about the anatomical organisation of synaptic circuits in the dorsal horn has come from two main approaches. Immunocytochemical studies have been able to demonstrate contacts (and in many cases synapses) between axons belonging to one neurochemically defined population and the dendrites or cell bodies of retrogradely labelled projection neurons or of interneuron populations. Electrophysiological studies involving paired recordings have revealed functional synapses between different cell types. Interestingly, with the latter approach it has been reported that only around 10 per cent of randomly selected pairs of interneurons are synaptically linked, which suggests that there may be fairly selective patterns of synaptic connectivity in this region (Lu and Perl, 2003). The following sections discuss synaptic inputs to projection neurons, interneurons and primary afferent terminals. A diagram summarising some of these synaptic circuits is shown in Figure 1.5.

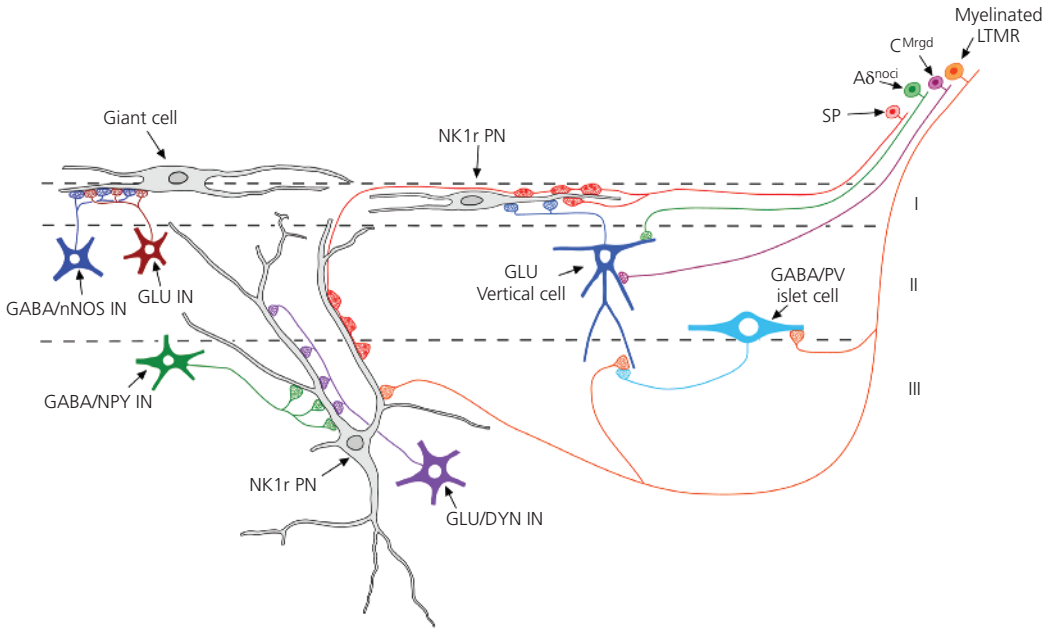


**Figure 1.5** Neuronal circuits in the superficial dorsal horn. This diagram shows some of the synaptic connections that have been identified in laminae I–III of the rodent dorsal horn. Three ALT projection neurons are indicated: a lamina I giant cell and projection neurons (PN) in laminae I and III that express the neurokinin 1 receptor (NK1r). Both lamina I and lamina III NK1r-expressing cells receive numerous synapses from peptidergic primary afferents that contain subtance P (SP), and these account for around a half of their excitatory synaptic input. The lamina I NK1r+ PN receives excitatory synapses from glutamatergic (GLU) vertical cells in lamina II, which are thought to be innervated by Aδ nociceptors (Aδnoci), non-peptidergic C fibre nociceptors (CMrgd) and myelinated low-threshold mechanoreceptors (LTMR). The myelinated LTMRs also innervate GABAergic islet cells that contain parvalbumin (PV), and they receive axoaxonic synapses from these interneurons. The lamina III PNs are selectively innervated by two distinct classes of interneuron: inhibitory cells that express neuropeptide Y (NPY) and excitatory (glutamatergic) cells that express dynorphin. The giant lamina I projection neurons seem to receive little or no direct primary afferent input but are densely innervated by excitatory and inhibitory interneurons. Many of the latter contain neuronal nitric oxide synthase (nNOS).

## Synaptic inputs to projection neurons

### Inputs from primary afferents

The large NK1r-expressing ALT projection neurons in laminae I and III are densely innervated by substance P-containing primary afferents, and the latter account for over half of the glutamatergic synapses on these cells (Polgár, Al Ghamdi and Todd, 2010, Baseer et al., 2012). These cells therefore receive a powerful direct synaptic input from peptidergic nociceptors, although the peripheral targets of the afferents (e.g., skin, viscera) are not yet known. Since the lamina III cells have extensive dendritic trees that pass through several laminae, they could in principle be innervated by a variety of different types of primary afferent. However, while they receive some synapses from myelinated LTMRs, they seem to have little or no input from non-peptidergic C nociceptors (Todd, 2010).

Unlike these two groups of NK1r-expressing ALT cells, the giant lamina I projection neurons seem to receive little if any monosynaptic primary afferent input (Polgár et al., 2008).

### Inputs from interneurons

The NK1r-expressing ALT cells in laminae I and III, and the giant lamina I cells, all receive numerous synapses from VGLUT2-immunoreactive boutons, and most of these probably originate from local excitatory interneurons. Some of those on the lamina I projection cells are presumably derived from the axons of lamina II vertical cells, which are known to innervate these cells (Cordero-Erausquin et al., 2009, Lu and Perl, 2005). We have recently shown that nearly 60 per cent of the non-primary glutamatergic synapses on the lamina III projection neurons contain preprodynorphin, and are therefore likely to be derived from local dynorphin-containing excitatory interneurons (Baseer et al., 2012). The fact that preprodynorphin is found in between 5 and 7 per cent of the VGLUT2$^+$ boutons in this region indicates a highly selective targeting of the projection cells by these interneurons.

There is also evidence that inhibitory interneurons innervate the projection cells in a very selective manner (Todd, 2010). The lamina III ALT cells are densely innervated by axons that contain NPY and GABA: over 30 per cent of the GABAergic boutons that synapse with these cells are NPY-immunoreactive, by comparison with less than 15 per cent among the general population of GABAergic boutons in laminae I–II. In contrast, the giant lamina I projection neurons are preferentially innervated by inhibitory interneurons that express nNOS.

## Synaptic inputs to interneurons

### Inputs from primary afferents

Electrophysiological studies in slice preparations have demonstrated that vertical and radial cells in lamina II can receive monosynaptic input from Aδ and C fibres and that this includes input from C fibres that possess TRPV1 and TRPA1 (Grudt and Perl, 2002, Yasaka et al., 2007, Uta et al., 2010). Although some of the myelinated primary afferent input to vertical cells is from Aδ nociceptors, it is also likely that these cells are innervated by myelinated LTMRs, because their dendrites often extend ventrally into the region where these afferents terminate. In support of this suggestion, we have recently found numerous contacts involving the

central terminals of myelinated low-threshold afferents and dendritic spines of vertical cells in laminae IIi–III (Yasaka et al., 2014). Electrophysiological studies have also shown that islet cells receive monosynaptic input from C fibres but not from myelinated primary afferents. PKCγ-expressing excitatory interneurons in lamina IIi are directly innervated by Aβ primary afferents, which are presumably LTMRs (Lu et al., 2013).

### Inputs from other interneurons

The interconnections between interneurons in lamina II have been examined in a series of elegant experiments in which pairs of neurons have been recorded simultaneously in spinal cord slices (Lu et al., 2013, Lu and Perl, 2003, 2005, Zheng, Lu and Perl, 2010), and these studies have identified a number of consistent patterns. Islet cells were found to be synaptically connected to another population of inhibitory interneurons, defined by expression of green fluorescent protein (GFP) under the control of the prion promoter (PrP-GFP mouse line). In some cases this connection was reciprocal: either cell could cause an inhibitory postsynaptic current (IPSC) in the other. It has since been shown that the PrP-GFP cells correspond to those that contain nNOS and/or galanin (Iwagaki et al., 2013). It was reported that these two types of inhibitory interneuron were presynaptic to different classes of excitatory interneuron in lamina II: the PrP-GFP cells to vertical cells, and islet cells to a class defined as 'transient central' cells. An excitatory circuit was identified in which transient central cells were presynaptic to vertical cells, which in turn innervated lamina I projection neurons. Finally it was shown that transient central cells could receive from Aβ afferents a disynaptic input that was transmitted by PKCγ cells in lamina IIi.

While these studies have provided remarkable insights into the synaptic connections between interneurons, some caution is needed when interpreting their findings. First, it is not known whether the populations described in these studies are really homogeneous. Secondly, it is very likely that each class of interneuron receives input from, and provides output to, several other neuronal populations. This means that attempting to track the flow of information through circuits involving several neurons that are connected in series becomes very difficult.

## Synaptic inputs to primary afferents

Primary afferent boutons often receive axoaxonic synapses from GABAergic neurons that mediate presynaptic inhibition. Various types of afferent differ in the extent to which they receive axoaxonic synapses, and also in the source of these synapses. For example, peptidergic afferents have few if any axoaxonic synapses, while the latter are found in moderate numbers on non-peptidergic C nociceptors (Figure 1.2a) and in large numbers on myelinated LTMRs, in particular on the central terminals of Aδ D-hair afferents (Ribeiro-da-Silva, Tagari and Cuello, 1989, Ribeiro-da-Silva and Coimbra, 1982, Maxwell and Rethelyi, 1987) (Figure 1.2b). The cells giving rise to axoaxonic synapses on C fibres and Aδ D-hair afferents are likely to belong to different populations, as axons belonging to the former are enriched with GABA but not with glycine, while axons belonging to the latter often contain high levels of both amino acids (Todd, 1996). Hughes et al. (2012) have recently identified the parvalbumin-containing inhibitory interneurons (which correspond to a subset

of the islet cells) as a source of axoaxonic synapses on the central terminals of the Aδ D-hair afferents, since around 80 per cent of parvalbumin axons in lamina IIi were associated with these afferents.

## Future directions

Although our understanding of the neuronal organisation and synaptic circuitry of the dorsal horn has increased dramatically over recent years, there are still major gaps in our knowledge.

Classification of neurons into functional populations is an essential prerequisite for unravelling neuronal circuits, and some progress has been made in the identification of neurochemical populations among the inhibitory interneurons. This scheme needs to be expanded to include those cells that have not already been assigned to classes. For the excitatory interneurons in laminae I–III, it is clear that there are numerous neurochemical markers that are expressed by subsets of these cells, and future studies will need to clarify their interrelationships, for example by looking for mutually exclusive expression patterns. In addition, this information will have to be related to the morphological classes that have been identified among the excitatory interneurons (e.g. vertical and radial cells). Since many of these markers can be detected in axon terminals, it should be possible to identify patterns of the synaptic input that goes from excitatory interneurons to different classes of projection neuron and interneuron. It will also be important to define discrete populations among the projection neurons in lamina I, for example to identify those that are responsible for conveying different types of stimulus that are perceived as itch (Davidson et al., 2007).

While some progress has been made in defining synaptic circuitry, a vast amount remains to be done. Much of what we know concerns the synaptic inputs to projection neurons, which are relatively easy to investigate with conventional anatomical techniques, as they generally have few dendritic spines and a high density of synapses on their dendritic shafts and cell bodies. We need far more information on the synaptic inputs to interneurons, but this will depend on our ability to define functional populations among these cells. It will also be vital to have quantitative data in order to assess the relative strengths of different synaptic inputs. Another important outstanding issue is that of the origin of the axoaxonic synapses on nociceptors. It appears that the presynaptic inhibition of primary afferents is performed by distinct populations of inhibitory interneurons in the spinal cord (Hughes et al., 2005, Hughes et al., 2012). Identifying those that innervate non-peptidergic C nociceptors could reveal novel targets for analgesic drugs and may provide insight into pathological pain states.

One advantage of the neurochemical approach is that it provides genetic and pharmacological targets that can be used to investigate the functions of specific neuronal populations. For example, the intrathecal administration of saporin conjugated to peptides can be used to destroy cells that express the peptide receptors. If these receptors are associated with particular neuronal populations, the resulting behavioural outcomes can provide insight into the function of these cells (Wiley and Lappi, 2003). The neurochemical approach can also identify genetic markers that can be used, for example, to activate cells with the help of optogenetics or to silence or ablate them with toxins (Johansson et al., 2010).

## References


Abraira, V. E. and Ginty, D. D. 2013. The sensory neurons of touch. *Neuron* 79: 618–39.

Andrew, D. 2009. Sensitization of lamina I spinoparabrachial neurons parallels heat hyperalgesia in the chronic constriction injury model of neuropathic pain. *Journal of Physiology* 587: 2005–17.

Antal, M., Petko, M., Polgár, E., Heizmann, C. W. and Storm-Mathisen, J. 1996. Direct evidence of an extensive GABAergic innervation of the spinal dorsal horn by fibres descending from the rostral ventromedial medulla. *Neuroscience* 73: 509–18.

Baseer, N., Polgár, E., Watanabe, M., Furuta, T., Kaneko, T. and Todd, A. J. 2012. Projection neurons in lamina III of the rat spinal cord are selectively innervated by local dynorphin-containing excitatory neurons. *Journal of Neuroscience* 32: 11854–63.

Bester, H., Chapman, V., Besson, J. M. and Bernard, J. F. 2000. Physiological properties of the lamina I spinoparabrachial neurons in the rat. *Journal of Neurophysiology* 83: 2239–59.

Brown, A. G. 1981. *Organization in the Spinal Cord: The Anatomy and Physiology of Identified Neurons*. Berlin: Springer.

Brumovsky, P., Watanabe, M. and Hokfelt, T. 2007. Expression of the vesicular glutamate transporters-1 and -2 in adult mouse dorsal root ganglia and spinal cord and their regulation by nerve injury. *Neuroscience* 147: 469–90.

Campbell, J. N., Raja, S. N., Meyer, R. A. and Mackinnon, S. E. 1988. Myelinated afferents signal the hyperalgesia associated with nerve injury. *Pain* 32: 89–94.

Cordero-Erausquin, M., Allard, S., Dolique, T., Bachand, K., Ribeiro-Da-Silva, A. and de Koninck, Y. 2009. Dorsal horn neurons presynaptic to lamina I spinoparabrachial neurons revealed by transynaptic labeling. *Journal of Comparative Neurology* 517: 601–15.

Davidson, S., Zhang, X., Yoon, C. H., Khasabov, S. G., Simone, D. A. and Giesler, G. J., Jr. 2007. The itch-producing agents histamine and cowhage activate separate populations of primate spinothalamic tract neurons. *Journal of Neuroscience* 27: 10007–14.

Grudt, T. J. and Perl, E. R. 2002. Correlations between neuronal morphology and electrophysiological features in the rodent superficial dorsal horn. *Journal of Physiology* 540: 189–207.

Han, Z. S., Zhang, E. T. and Craig, A. D. 1998. Nociceptive and thermoreceptive lamina I neurons are anatomically distinct. *Nature Neuroscience* 1: 218–25.

Hughes, D. I., Sikander, S., Kinnon, C. M., Boyle, K. A., Watanabe, M., Callister, R. J. and Graham, B. A. 2012. Morphological, neurochemical and electrophysiological features of parvalbumin-expressing cells: A likely source of axo-axonic inputs in the mouse spinal dorsal horn. *Journal of Physiology* 590: 3927–51.

Hughes, D. I., Mackie, M., Nagy, G. G., Riddell, J. S., Maxwell, D. J., Szabo, G., Erdelyi, F., Veress, G., Szucs, P., Antal, M. and Todd, A. J. 2005. P boutons in lamina IX of the rodent spinal cord express high levels of glutamic acid decarboxylase-65 and originate from cells in deep medial dorsal horn. *Proceedings of the National Academy of Sciences of the United States of America* 102: 9038–43.

Hunt, S. P., Pini, A. and Evan, G. 1987. Induction of c-fos-like protein in spinal cord neurons following sensory stimulation. *Nature* 328: 632–4.

Iwagaki, N., Garzillo, F., Polgár, E., Riddell, J. S. and Todd, A. J. 2013. Neurochemical characterisation of lamina II inhibitory interneurons that express GFP in the PrP-GFP mouse. *Molecular Pain* 9: 56. doi: 10.1186/1744-8069-9-56.

Ji, R. R., Baba, H., Brenner, G. J. and Woolf, C. J. 1999. Nociceptive-specific activation of ERK in spinal neurons contributes to pain hypersensitivity. *Nature Neuroscience* 2: 1114–9.

Johansson, T., Broll, I., Frenz, T., Hemmers, S., Becher, B., Zeilhofer, H. U. and Buch, T. 2010. Building a zoo of mice for genetic analyses: A comprehensive protocol for the rapid generation of BAC transgenic mice. *Genesis* 48: 264–80.

Kardon, A., Polgár, E., Hachisuka, E., Snyder, L., Cameron, D., Savage, S., Cai, X, Karnup, S., Fan, C., Hemenway, G. et al. 2014. Dynorphin acts as a neuromodulator to inhibit itch in the dorsal horn of the spinal cord. *Neuron* 82(3): 573–586.

Lawson, S. N., Crepps, B. A. and Perl, E. R. 1997. Relationship of substance P to afferent characteristics of dorsal root ganglion neurones in guinea-pig. *Journal of Physiology* 505 (Part 1), 177–91.

Light, A. R. and Perl, E. R. 1979. Spinal termination of functionally identified primary afferent neurons with slowly conducting myelinated fibers. *Journal of Comparative Neurology* 186: 133–50.

Lu, Y. and Perl, E. R. 2003. A specific inhibitory pathway between substantia gelatinosa neurons receiving direct C-fiber input. *Journal of Neuroscience* 23: 8752–8.

Lu, Y. and Perl, E. R. 2005. Modular organization of excitatory circuits between neurons of the spinal superficial dorsal horn (laminae I and II). *Journal of Neuroscience* 25: 3900–7.

Lu, Y., Dong, H., Gao, Y., Gong, Y., Ren, Y., Gu, N., Zhou, S., Xia, N., Sun, Y. Y., Ji, R. R. and Xiong, L. 2013. A feed-forward spinal cord glycinergic neural circuit gates mechanical allodynia. *Journal of Clinical Investigation* 123: 4050–62.

Malet, M., Vieytes, C. A., Lundgren, K. H., Seal, R. P., Tomasella, E., Seroogy, K. B., Hokfelt, T., Gebhart, G. F. and Brumovsky, P. R. 2013. Transcript expression of vesicular glutamate transporters in lumbar dorsal root ganglia and the spinal cord of mice: Effects of peripheral axotomy or hindpaw inflammation. *Neuroscience* 248C, 95–111.

Malmberg, A. B., Chen, C., Tonegawa, S. and Basbaum, A. I. 1997. Preserved acute pain and reduced neuropathic pain in mice lacking PKCgamma. *Science* 278: 279–83.

Mantyh, P. W., Demaster, E., Malhotra, A., Ghilardi, J. R., Rogers, S. D., Mantyh, C. R., Liu, H., Basbaum, A. I., Vigna, S. R., Maggio, J. E., et al. 1995. Receptor endocytosis and dendrite reshaping in spinal neurons after somatosensory stimulation. *Science* 268: 1629–32.

Maxwell, D. J. and Rethelyi, M. 1987. Ultrastructure and synaptic connections of cutaneous afferent fibres in the spinal cord. *Trends in Neurosciences* 10: 117–23.

Maxwell, D. J., Belle, M. D., Cheunsuang, O., Stewart, A. and Morris, R. 2007. Morphology of inhibitory and excitatory interneurons in superficial laminae of the rat dorsal horn. *Journal of Physiology* 584: 521–33.

Melzack, R. and Wall, P. D. 1965. Pain mechanisms: A new theory. *Science* 150: 971–9.

Mishra, S. K. and Hoon, M. A. 2013. The cells and circuitry for itch responses in mice. *Science* 340: 968–71.

Nichols, M. L., Allen, B. J., Rogers, S. D., Ghilardi, J. R., Honore, P., Luger, N. M., Finke, M. P., Li, J., Lappi, D. A., Simone, D. A. and Mantyh, P. W. 1999. Transmission of chronic nociception by spinal neurons expressing the substance P receptor. *Science* 286: 1558–61.

Peirs, C., Patil, S., Bouali-Benazzouz, R., Artola, A., Landry, M. and Dallel, R. 2014. Protein kinase C gamma interneurons in the rat medullary dorsal horn: Distribution and synaptic inputs to these neurons, and subcellular localization of the enzyme. *Journal of Comparative Neurology* 522: 393–413.

Polgár, E., Al Ghamdi, K. S. and Todd, A. J. 2010. Two populations of neurokinin 1 receptor-expressing projection neurons in lamina I of the rat spinal cord that differ in AMPA receptor subunit composition and density of excitatory synaptic input. *Neuroscience* 167: 1192–204.

Polgár, E., Durrieux, C., Hughes, D. I. and Todd, A. J. 2013. A quantitative study of inhibitory interneurons in laminae i-iii of the mouse spinal dorsal horn. *PLoS One* 8: e78309.

Polgár, E., Al-Khater, K. M., Shehab, S., Watanabe, M. and Todd, A. J. 2008. Large projection neurons in lamina I of the rat spinal cord that lack the neurokinin 1 receptor are densely innervated by VGLUT2-containing axons and possess GluR4-containing AMPA receptors. *Journal of Neuroscience* 28: 13150–60.

Polgár, E., Sardella, T. C., Tiong, S. Y., Locke, S., Watanabe, M. and Todd, A. J. 2013. Functional differences between neurochemically defined populations of inhibitory interneurons in the rat spinal dorsal horn. *Pain* 154: 2606–15.

Rexed, B. 1952. The cytoarchitectonic organization of the spinal cord in the cat. *Journal of Comparative Neurology* 96: 414–95.

Ribeiro-Da-Silva, A. and Coimbra, A. 1982. Two types of synaptic glomeruli and their distribution in laminae I–III of the rat spinal cord. *Journal of Comparative Neurology* 209: 176–86.

Ribeiro-Da-Silva, A., Tagari, P. and Cuello, A. C. 1989. Morphological characterization of substance P-like immunoreactive glomeruli in the superficial dorsal horn of the rat spinal cord and trigeminal subnucleus caudalis: A quantitative study. *Journal of Comparative Neurology* 281: 497–15.

Ross, S. E., Mardinly, A. R., Mccord, A. E., Zurawski, J., Cohen, S., Jung, C., Hu, L., Mok, S. I., Shah, A., Savner, E. M., Tolias, C., Corfas, R., Chen, S., Inquimbert, P., Xu, Y., Mcinnes, R. R., Rice, F. L., Corfas, G., Ma, Q., Woolf, C. J. and Greenberg, M. E. 2010. Loss of inhibitory interneurons in the dorsal spinal cord and elevated itch in Bhlhb5 mutant mice. *Neuron* 65: 886–98.

Sandkuhler, J. 2009. Models and mechanisms of hyperalgesia and allodynia. *Physiological Reviews* 89: 707–58.

Sardella, T. C., Polgár, E., Garzillo, F., Furuta, T., Kaneko, T., Watanabe, M. and Todd, A. J. 2011. Dynorphin is expressed primarily by GABAergic neurons that contain galanin in the rat dorsal horn. *Molecular Pain* 7: 76. doi: 10.1186/1744-8069-7-76.

Seal, R. P., Wang, X., Guan, Y., Raja, S. N., Woodbury, C. J., Basbaum, A. I. and Edwards, R. H. 2009. Injury-induced mechanical hypersensitivity requires C-low threshold mechanoreceptors. *Nature* 462: 651–5.

Sugiura, Y., Lee, C. L. and Perl, E. R. 1986. Central projections of identified, unmyelinated (C) afferent fibers innervating mammalian skin. *Science* 234: 358–61.

Todd, A. J. 1996. GABA and glycine in synaptic glomeruli of the rat spinal dorsal horn. *European Journal of Neuroscience* 8: 2492–8.

Todd, A. J. 2006. Spinal cord processing: Anatomy and neurochemistry of the dorsal horn. In *Handbook of Clinical Neurology*, 3rd series: *Pain*, ed. by F. Cervero and T. S. Jensen, vol. 81. Elsevier: New York, pp. 61–76.

Todd, A. J. 2010. Neuronal circuitry for pain processing in the dorsal horn. *Nature Reviews of Neuroscience* 11: 823–36.

Todd, A. J. and Koerber, H. R. 2012. Neuroanatomical substrates of spinal nociception. In *Wall and Melzack's Textbook of Pain*, ed. by S. McMahon, M. Koltzenburg, I. Tracey, and D. C. Turk, 6th edn. Edinburgh: Elsevier, pp. 73–90.

Todd, A. J., Puskar, Z., Spike, R. C., Hughes, C., Watt, C. and Forrest, L. 2002. Projection neurons in lamina I of rat spinal cord with the neurokinin 1 receptor are selectively innervated by substance P-containing afferents and respond to noxious stimulation. *Journal of Neuroscience* 22: 4103–13.

Todd, A. J., Hughes, D. I., Polgar, E., Nagy, G. G., Mackie, M., Ottersen, O. P. and Maxwell, D. J. 2003. The expression of vesicular glutamate transporters VGLUT1 and VGLUT2 in neurochemically defined axonal populations in the rat spinal cord with emphasis on the dorsal horn. *European Journal of Neuroscience* 17: 13–27.

Torsney, C. and MacDermott, A. B. 2006. Disinhibition opens the gate to pathological pain signaling in superficial neurokinin 1 receptor-expressing neurons in rat spinal cord. *Journal of Neuroscience* 26: 1833–43.

Uta, D., Furue, H., Pickering, A. E., Rashid, M. H., Mizuguchi-Takase, H., Katafuchi, T., Imoto, K. and Yoshimura, M. 2010. TRPA1-expressing primary afferents synapse with a morphologically identified subclass of substantia gelatinosa neurons in the adult rat spinal cord. *European Journal of Neuroscience* 31: 1960–73.

Wiley, R. G. and Lappi, D. A. 2003. Targeted toxins in pain. *Advanced Drug Delivery Reviews* 55: 1043–54.

Woodbury, C. J. and Koerber, H. R. 2003. Widespread projections from myelinated nociceptors throughout the substantia gelatinosa provide novel insights into neonatal hypersensitivity. *Journal of Neuroscience* 23: 601–10.

Yaksh, T. L. 1989. Behavioral and autonomic correlates of the tactile evoked allodynia produced by spinal glycine inhibition: Effects of modulatory receptor systems and excitatory amino acid antagonists. *Pain* 37: 111–23.

Yasaka, T., Tiong, S. Y. X., Hughes, D. I., Riddell, J. S. and Todd, A. J. 2010. Populations of inhibitory and excitatory interneurons in lamina II of the adult rat spinal dorsal horn revealed by a combined electrophysiological and anatomical approach. *Pain* 151: 475–488.

Yasaka, T., Tiong, S. Y. X., Polgár, E., Wanabe, M., Kumamoto, E., Riddell, J. S. and Todd, A. J. 2014. A putative relay circuit providing low-threshold mechanoreceptive input to lamina I projection neurons via vertical cells in lamina II of the rat dorsal horn. *Molecular Pain* 10: 3. doi: 10.1186/1744-8069-10-3.

Yasaka, T., Kato, G., Furue, H., Rashid, M. H., Sonohata, M., Tamae, A., Murata, Y., Masuko, S. and Yoshimura, M. 2007. Cell-type-specific excitatory and inhibitory circuits involving primary afferents in the substantia gelatinosa of the rat spinal dorsal horn in vitro. *Journal of Physiology* 581: 603–18.

Zeilhofer, H. U., Wildner, H. and Yevenes, G. E. 2012. Fast synaptic inhibition in spinal sensory processing and pain control. *Physiological Reviews* 92: 193–235.

Zeilhofer, H. U., Studler, B., Arabadzisz, D., Schweizer, C., Ahmadi, S., Layh, B., Bosl, M. R. and Fritschy, J. M. 2005. Glycinergic neurons expressing enhanced green fluorescent protein in bacterial artificial chromosome transgenic mice. *Journal of Comparative Neurology* 482: 123–41.

Zheng, J., Lu, Y. and Perl, E. R. 2010. Inhibitory neurones of the spinal substantia gelatinosa mediate interaction of signals from primary afferents. *Journal of Physiology* 588: 2065–75.

Zylka, M. J., Rice, F. L. and Anderson, D. J. 2005. Topographically distinct epidermal nociceptive circuits revealed by axonal tracers targeted to Mrgprd. *Neuron* 45: 17–25.