20 Spinal Microcircuits and the Regulation of Itch

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Itch is a somatosensory percept that is triggered by irritants at the skin's surface. However, the manner in which itch is coded in the nervous system remains almost completely unknown. Recent work has uncovered a key role of spinal interneurons in the modulation of itch. Here we discuss these recent discoveries in the context of our understanding of spinal microcircuitry, highlighting the possible roles of the dorsal horn in the processing of pruritic input.

While it is not known which specific subsets of primary afferents underlie itch, there is good evidence that the main receiving zone for these afferents is within laminae I and II of the spinal cord. For instance, itch sensation is only lost when the conduction of all fibers (including C-fibers) is blocked, implying that itch is mediated in large part by fine diameter fibers, which are known terminate in superficial laminae. In particular, many of these itch-mediating C-fibers are likely to be sensory afferents that express TrpV1 and/or TrpA1, and the primary afferents that express these channels have synaptic connections with lamina I and lamina II neurons (Yang et al. 1998; Nakatsuka et al. 2002; Kosugi et al. 2007; Shim et al. 2007; Imamachi et al. 2009; Uta et al. 2010; Patel et al. 2011; Wilson et al. 2011). As further evidence, neurons in the superficial dorsal horn, show fos induction upon intradermal injection of itch-evoking chemicals such as serotonin and SLIGRL as well as in a dry skin model of pruritus (Nojima et al. 2003, 2004; Akiyama et al. 2009a,b). Last, *in vivo* responses to various pruritogens such as histamine, serotonin, SLIGRL, and

chloroquine, as well as the response to mosquito allergy have been recorded from neurons in the superficial dorsal horn (Jinks and Carstens 2000, 2002; Akiyama et al. 2009a,b; Omori et al. 2009; Akiyama et al. 2012c). Together these findings suggest that neurons in the superficial dorsal horn receive the somatosensory input that gives rise to itch sensation.

Laminae I and II of the spinal cord contain numerous functional populations of neurons. However, projection neurons that convey information to the brain represent around one percent of the total number of neurons in this region (Todd 2010). Thus, the vast majority of neurons in the superficial dorsal horn are interneurons with local (and in some cases also long propriospinal) axonal projections, and these are involved in the processing of sensory information. Does the existence of a complex network of spinal interneurons imply that itch is decoded within the spinal cord? To what extent is itch modulated in the spinal cord? While we do not yet know the answers to these key questions, there are a number of important psychophysical experiments that are suggestive of the idea that itch is modulated by spinal microcircuits.

20.1 PUTATIVE SPINAL MICROCIRCUITS INVOLVED IN THE MODULATION OF ITCH

20.1.1 Inhibition of Itch by Counterstimuli

The idea that counterstimuli can relieve itch is familiar to anyone who has ever found himself scratching in response to a mosquito bite. In experimental settings, stimuli that have been shown to block histamine-induced itch include scratching, noxious heat and cold, pinprick, menthol, capsaicin, mustard oil, and cutaneous field stimulation (Bickford 1937; Graham et al. 1951; Bromm et al. 1995; Ward et al. 1996; Nilsson et al. 1997; Yosipovitch et al. 2007). What these stimuli have in common is that they robustly activate subsets of C-fibers. This idea has led to the suggestion that certain types of C-fiber input may inhibit itch. Importantly, counterstimuli can inhibit itch when delivered quite a significant distance from the itch itself—10 cm or more away (Bickford 1937). The finding that the inhibition of itch by counterstimuli occurs at such a large distance (larger than the receptive field of an individual primary afferent) suggests that this inhibition occurs through the integration of sensory input, potentially by local circuits at the level of the spinal cord.

Because all C-fibers release the excitatory neurotransmitter glutamate, the simplest explanation for how activity within excitatory C-fibers could result in the inhibition of itch within the spinal cord is via inhibitory spinal interneurons. According to this model, the activation of subsets of C-fibers in response to a counterstimulus such as scratching would not only activate scratch-sensitive (nociceptive) projection neurons but also activate inhibitory spinal interneurons that function to inhibit pruritoception and thereby reduce the perception of itch (Figure 20.1a).

In support of this idea, when Davidson et al. (2009) recorded from spinothalamic projection neurons, they found that scratching the skin resulted in the inhibition of histamine-evoked responses. Furthermore, scratching at a distance from the site of itch suppressed the firing rate of itch-responsive neurons in the superficial dorsal horn, indicating that mechanical inhibition of itch is evoked in an area that is wider

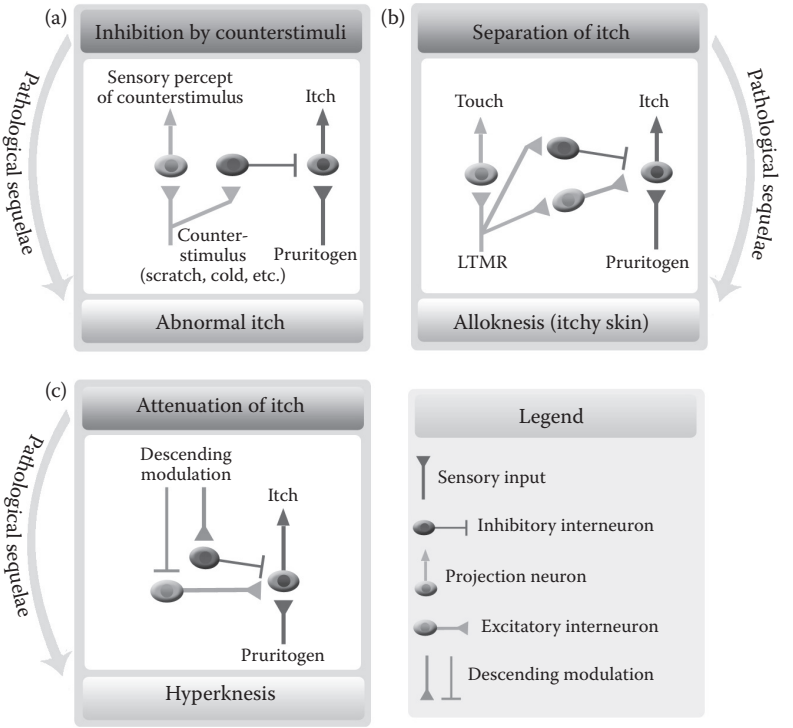

FIGURE 20.1 (See color insert.) Putative spinal microcircuits involved in the modulation of itch. (a) Counterstimuli such as scratching and cold may inhibit itch by activating inhibitory interneurons (purple) that function to inhibit itch within the spinal cord. (b) Though itch and touch are normally separate, there may be spinal neural circuits that allow the coupling of these sensations. Under normal circumstances, inhibition (purple) may predominate. However, following strong pruritogenic input, the synaptic connections may be reweighted such that excitation (green) predominates and the activation of low threshold mechanoreceptors (LTMRs) results in alloknesis. (c) There are likely to be multiple mechanisms that are involved in the attenuation of itch. Some of these likely include descending modulation that may act directly on projection neurons (not shown) or via excitatory (green) or inhibitory (purple) interneurons in the spinal cord to inhibit itch and prevent hyperknesis. These conceptual models are based in part on ideas described in a review by Sandhulker (2009) on the role of inhibition within the nociceptive system.

than the site of the itch itself (Akiyama et al. 2012b). Electrophysiological data also suggest that inhibitory input is widely distributed (Kato et al. 2004, 2011). *In vivo* patch clamp recording of neurons in the dorsal horn reveals that mechanical stimulation of the skin evokes IPSCs (Narikawa et al. 2000). Together these studies hinted at possible circuitry underlying the inhibition of itch by counterstimuli such as scratching, but the specific neurons, mediators, and pathways involved remained unclear.

Recently, we identified a subset of spinal inhibitory neurons that may be involved in mediating the inhibition of itch. This work began when we discovered that mice lacking the transcription factor *Bhlhb5* develop self-inflicted skin lesions due to

excessive licking and scratching (Ross et al. 2010). Because this behavior was suggestive of abnormal itch, we tested the response of these mice to pruritogens and found that *Bhlhb5* mutant mice showed significantly elevated scratching responses compared to littermate controls.

To understand which neurons are responsible for this elevated itch phenotype, we generated a conditional *Bhlhb5* knockout mouse, allowing us to selectively ablate *Bhlhb5* in specific regions of the nervous system. When we ablated *Bhlhb5* in either the forebrain or in primary sensory neurons, the resulting mice were completely normal with respect to itch. However, we found that loss of *Bhlhb5* within inhibitory neurons of the spinal cord was sufficient for elevated itch (Ross et al. 2010). Finally, to investigate what happens to these spinal interneurons in the absence of *Bhlhb5*, we generated a *Bhlhb5*-cre knockout mouse, thereby allowing us to follow the fate of *Bhlhb5*-expressing cells throughout the life of the animal. Using this approach, we discovered that *Bhlhb5* is selectively required for the survival of specific neurons in the dorsal horn—without *Bhlhb5*, these neurons undergo apoptosis during embryonic development. Together, these findings suggest that *Bhlhb5* is required for the survival of a subset of spinal inhibitory interneurons that function to inhibit itch (Ross et al. 2010). We and others have speculated that these neurons might be involved in mediating the inhibition of itch by counterstimuli (Ross 2011; Ma 2010; Lagerstrom et al. 2010; Liu et al. 2010; Patel and Dong 2010). However, while this remains an attractive hypothesis, there is as of yet no direct evidence for this idea.

In addition to the possible role of a subset of *Bhlhb5*-expressing inhibitory interneurons in the inhibition of itch by counterstimuli, there are likely to be numerous other mechanisms working in parallel. Evidence for this idea comes from Akiyama et al. (2011) who performed *in vivo* recordings from spontaneously active neurons in the superficial dorsal horn in a dry skin model of itch. Dry skin is known to drive spontaneous activity in itch-responsive primary sensory fibers, which would presumably provide tonic drive to itch circuits within the cord. Because neurons in the dorsal horn are normally silent in the absence of a stimulus, the spontaneously active neurons in the dorsal horn of mice with dry skin are likely to be involved in mediating itch. In the mouse model, cutaneous scratching inhibited these spontaneously active neurons, consistent with the idea that these neurons may be involved in integrating input to mediate the inhibition of itch by scratching. Importantly, scratch-evoked inhibition of the spontaneous activity of such neurons was reduced upon blockade of either GABA or glycine receptors, implicating both types of inhibitory neurotransmitter in the inhibition of itch. Furthermore, cervical transection to block descending inhibition also attenuated scratch-evoked inhibition. These findings suggest that multiple mechanisms are involved in the inhibition of itch.

When we scratch an itch, the relief is almost instantaneous, implying that neurotransmitters acting on a timescale of milliseconds are likely involved. However, there is also strong evidence that fast-acting inhibitory neurotransmission is not the only mechanism at play. Lewis et al. (1927) found that, following the electrical stimulation of the skin, histamine was no longer able to elicit itch. This state, which Bickford (1937) termed an antipruritic state, can be achieved when the counterstimulus is delivered either before or after the pruritogen and can last for minutes or even hours (Graham et al. 1951). For instance, brief noxious heating of the skin caused

a 50% decrease in perceived itch that lasted more than 30 min (Ward et al. 1996). Analogously, cutaneous field stimulation, which causes a burning and pricking sensation, abolishes itch for up to four hours (Nilsson et al. 1997). These long-lasting effects suggest that, in addition to fast-acting neurotransmitters, there may also be neuromodulatory mechanisms involved in the inhibition of itch by counterstimuli. Though little is known about the neuromodulators of itch, there is good evidence that the mu opioid receptor is involved. In particular, morphine-induced scratching was recently shown to be mediated by a specific isoform of the mu opioid receptor that interacts functionally with gastrin-releasing peptide receptor (GRPR) (Liu et al. 2011).

20.1.2 Separation of Itch

In one of the early studies on the psychophysical properties of itch, Bickford (1937) noted that the skin surrounding a site of itching (caused by a gnat bite) was abnormally sensitive to rubbing. Indeed, rubbing of the surrounding skin elicited abnormal itch. This common phenomenon is often referred to as itchy skin, and it has been studied in the context of both histamine- and cowhage-induced itch (Graham et al. 1951; Simone et al. 1991). Because itchy skin is not the norm but rather a state that can be elicited under specific circumstances, this implies that touch and itch are normally subserved by largely separate neural circuits that have the capacity to influence each other (Figure 20.1b).

Important insight into these neural circuits came from Simone et al. (1991), who performed a quantitative assessment of itchy skin and noted commonalities between this phenomenon and allodynia. In light of this similarity, they proposed the term alloknesis to describe itch in response to innocuous mechanical stimulation. One key implication of this idea is that allodynia and alloknesis may be mediated by parallel neural circuits. Indeed, when an aversive stimulus causes pain, the pain is accompanied by allodynia, whereas when an aversive stimulus causes itch, the itch is accompanied by alloknesis. Thus, perhaps the strong activation of pruritoceptors causes alloknesis just as strong activation of nociceptors causes allodynia.

What types of primary afferents mediate alloknesis? Handwerker (1992) proposed that light-touch evoked itch was likely mediated by A-delta fibers. However, the observation by Bickford (1937) that itchy skin is only *completely* lost at the same time that pain is lost implies that some C-fibers must also be capable of eliciting alloknesis. In this regard, C-low threshold mechanoreceptors may be a good candidate.

Evidence for the existence of spinal circuits that mediate alloknesis comes from *in vivo* recordings of spinothalamic neurons. When Andrew and Craig (2001) investigated the response properties of putative itch-mediating projection neurons, they found that those that responded to histamine application were not responsive to mechanical stimuli, at least not initially. However, once the skin was treated with histamine, these neurons became sensitized such that light mechanical stimulation was now sufficient to trigger a response. This finding suggests that alloknesis is mediated by neural circuits in the spinal cord.

Though the neural basis of alloknesis is poorly understood, some of the underlying mechanisms of its nociceptive counterpart have been elucidated, and this insight

into the mechanisms of allodynia may be revealing. Noxious and innocuous inputs are mainly conveyed to distinct laminae, but it is clear that polysynaptic connections exist that connect low threshold input in deeper laminae to noxious input in superficial laminae (Takazawa and MacDermott 2010). Although the transmission across this polysynaptic circuit is normally silenced by inhibitory interneurons, sustained noxious inputs are thought to cause allodynia through the release of this inhibition. Recently, one of the underlying mechanisms for this loss of inhibition has been elucidated in detail. Specifically, when noxious C-fiber input into the dorsal horn is abnormally sustained, this strong activity in nociceptors results in the release of endocannabinoids from the postsynaptic target, which act upon nearby inhibitory neurons to inhibit neurotransmitter release (Pernia-Andrade et al. 2009). As a consequence, endocannabinoid-mediated disinhibition allows low threshold input to activate pain circuits in the superficial dorsal horn, resulting in allodynia. Whether endocannabinoids are likewise involved in mediating alloknesis is not known.

Recently, Akiyama et al. (2012a) developed a mouse model of alloknesis. In this model, following the intradermal injection of a pruritogen, nearby mechanical stimulation with fine von Frey filaments elicits a scratching response. Importantly, this effect persists for hours, long after scratching due to the pruritogen itself has passed. This mouse model is an important step forward because it will allow us to investigate the precise neural mechanisms underlying alloknesis.

20.1.3 Attenuation of Itch

Drawing on the parallels between itch and pain, there are likely to be multiple mechanisms that can modulate itch, ensuring the proper response level to a pruritogen. Some of these mechanisms are likely to be mediated in part by descending pathways that may act to limit itch (Figure 20.1c). This idea is supported by experiments in which the electrical stimulation of the periaqueductal gray (a key region for descending control) was found to suppress the activity of neurons in the dorsal horn to an intradermal injection of histamine (Carstens 1997). Furthermore, in a dry skin model, cervical transection reduced the effect of scratching on activity of spontaneously active neurons in the dorsal horn (Akiyama et al. 2011). These observations suggest that descending input from supraspinal areas such as midbrain periaqueductal gray and the rostral ventromedial medulla may inhibit pruritoception, just as they inhibit nociception (Yoshimura and Furue 2006; Heinricher et al. 2009).

One of the key mediators of this descending inhibition is likely to be noradrenaline acting through α_2-adrenoceptors. There is anecdotal evidence that postherpetic itch can be relieved by intrathecal delivery of the α_2-agonist, clonidine (Elkersh et al. 2003). Likewise in mice, both serotonin and mosquito allergy mediated itch were inhibited by intrathecal clonidine, whereas intracisternal injection had no effect (Gotoh et al. 2011a,b,c). Furthermore, these effects were blocked by yohimbine, an α_2-adrenoceptor antagonist. These studies suggest that spinal neurons are the target of tonic inhibition via a descending noradrenergic system through stimulation of α_2-adrenoceptors. Noradrenaline is known to act both at presynaptic sites and postsynaptic sites (Sonohata et al. 2004), but the specific targets of its antipruritic effect are not known.

20.2 ROLE OF GRPR NEURONS AND ITCH

One of the most exciting recent discoveries in the field of itch is the key role of a specific population of neurons—those that express GRPR. When GRPR-expressing neurons were ablated using a bombesin-saporin conjugate, the scratching responses to a variety of pruritogens were almost completely blocked, whereas the responses to painful stimuli were normal (Sun et al. 2009). This important finding suggests that GRPR neurons are absolutely required for itch; yet little else is known about them.

Within the dorsal horn, GRPR-expressing neurons are located predominantly within lamina I, which contains the majority of projection neurons. However, GRPR-expressing neurons are likely distinct from projection neurons because the expression of neurokinin 1 receptor (NK1r) (which is present on ~80% of anterolateral tract [ALT] neurons) was not affected by bombesin-saporin (Sun et al. 2009). Furthermore, projection neurons tend to have large cells bodies, whereas GRPR-expressing neurons appear to be small in size. Thus, GRPR-expressing neurons are likely to be a population of (presumably excitatory) spinal interneurons. If so, this raises the interesting possibility that itch input is relayed through a class of spinal interneuron before being transmitted through projection cells to the brain (Figure 20.2).

In addition to the essential role of GRPR-expressing neurons, it appears that the receptor itself is required for normal itch. Mice with a constitutive loss of GRPR show a significant reduction in their response to SLIGRL, chloroquine, and compound 48/80 (Sun and Chen 2007). Furthermore, the abnormal itch that is seen upon loss of VGLUT2 in subsets of primary sensory neurons is rescued in compound mutant mice that are also lacking GRPR (Lagerstrom et al. 2010). This genetic evidence is complemented by pharmacological manipulations showing that GRPR agonists cause scratching, whereas treatment with a GRPR antagonist reduces (but does not eliminate) pruritogen-evoked responses (Sun and Chen 2007).

Intriguingly, GRPR appears to play a greater role for histamine-independent itch than histamine-dependent itch. In fact, GRPR mutant mice show completely normal itch behavior in response to histamine (see supplemental data in Sun et al. [2009]). Furthermore, while bombesin-saporin significantly lowered the number of fos-positive cells in dorsal horn after stimulation with histamine and chloroquine, the reduction of fos expression was greater for chloroquine than for histamine (Han et al. 2012). Finally, systemic or intrathecal injection of a GRPR antagonist attenuated itch evoked by chloroquine and SLIGRL, but not that evoked by BAM8-22 or histamine (Akiyama et al. 2012c). These findings imply that GRPR is not necessarily involved in all types of itch. Intriguingly, neuromedin B and bombesin (two peptides that are related to gastrin-releasing peptide [GRP]) also cause itch and, importantly, their effects appear to be mediated by the neuromedin B receptor (NMBR) rather than GRPR (Su and Ko 2011). Thus, it is possible that different bombesin-like peptides are involved in distinct types of itch.

The key role of GRPR signaling in certain types of itch, together with the apparent expression of GRP in subsets of sensory neurons, raised the possibility that GRP released from primary afferents might mediate itch (Sun et al. 2009). However, this interpretation remains a matter of controversy. Part of this controversy is based on experiments using *in situ* hybridization, which show that spinal interneurons (rather

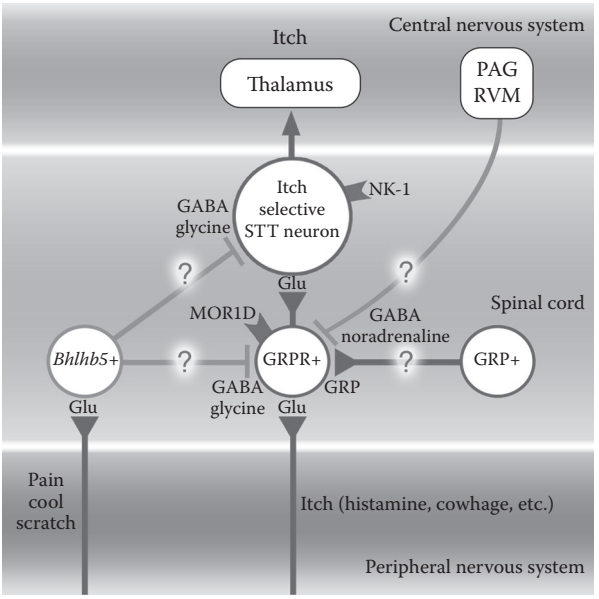

FIGURE 20.2 **(See color insert.)** Speculative model of the spinal microcircuits of itch. Itch appears to be mediated at least in part by an unknown subset of NK-1-expressing, itch selective spinothalamic tract (STT) neurons. GRPR-expressing neurons, which are likely interneurons, also appear to be required for itch and might therefore be involved in the transmission of itch between primary sensory neurons and projection neurons. Though the origin of GRP that activates GRPR remains controversial, one possibility is that spinal interneurons are an important source (shown); alternatively, GRP may come from primary sensory afferents that also release glutamate (not shown). *Bhlhb5* is transiently expressed in a subset of inhibitory interneurons that appear to inhibit itch, though their specific target is unknown. These inhibitory neurons are well positioned to mediate the inhibition of itch by counterstimuli, as illustrated.

than primary sensory neurons) are the major source of GRP mRNA (Fleming et al. 2012). Moreover, the idea that GRP released from sensory neurons mediates itch has recently been challenged by a new study that performed patch clamp recordings from neurons in the superficial dorsal horn that respond to GRP. Using this approach, Koga et al. (2011) showed that activation of GRP-responsive neurons by primary afferents is mediated by glutamate, not GRP. Thus, while there is abundant evidence that GRPR plays a key role in pruritus, more research is required to settle the controversy of how GRP and its receptor fit within the neural circuits of itch.

20.3 BRIEF OVERVIEW OF NEURONAL ORGANIZATION AND CIRCUITRY WITHIN THE DORSAL HORN

While the exact nature of spinal circuits that modulate itch remains a matter of speculation, it is clear that itch input is processed by a highly complex network of neurons in the dorsal horn. The main limitation to our understanding of the circuitry

has been the difficulty of defining functional populations among the diverse array of dorsal horn neurons. Without a satisfactory scheme for classifying these cells, it is impossible to define their inputs and outputs, and therefore their functions within these circuits. However, recent work has begun to shed light on some aspects of the neuronal organization and circuitry.

The dorsal horn consists of a continuous column of gray matter on either side of the midline that extends throughout the length of the spinal cord and merges with the spinal trigeminal nucleus in the medulla. The neural circuits in this region are made up from four basic components: (1) primary afferent axons which provide sensory (including pruritic) input; (2) axons descending from the brain that can modulate transmission of this input; (3) projection neurons, with axons that travel to the brain and represent the major output from the region; and (4) interneurons, which form the great majority of dorsal horn neurons and have axons that remain within the spinal cord (Todd 2010).

20.3.1 Projection Neurons

The main ascending pathway that is responsible for perception of itch is the ALT. As evidence of this idea, severing of the ALT results in the complete loss of itch sensation (as well as pain). This tract consists of neurons that are concentrated in lamina I and scattered throughout the deeper laminae (III-VI) of the dorsal horn, and it is not yet clear which of these projection neurons are involved in itch sensation. However, the finding of lamina I ALT neurons that respond to pruritic stimuli (Andrew and Craig 2001; Davidson et al. 2009) implicates a subset of ALT cells in this lamina.

Axons in the ALT cross the midline and ascend in the ventrolateral white matter to reach several brain regions, including the thalamus, periaqueductal gray matter, lateral parabrachial area, and various nuclei within the medulla. Individual neurons within this pathway typically send their axons to several of these brain targets (Todd 2010). Many of the lamina I ALT neurons also give rise to local axon collaterals within the spinal cord, although the postsynaptic targets of these are not yet known (Szucs et al. 2010).

The majority of ALT neurons in lamina I, together with a distinctive population of large ALT cells in lamina III, express the NK1r, the main receptor for substance P (Todd et al. 2000). Both types are densely innervated by substance P-containing primary afferents (Naim et al. 1997; Todd et al. 2002), which provide approximately half of the excitatory synapses on these cells (Baseer et al. 2012; Polgar et al. 2010). The lamina III cells also receive a few synapses from myelinated low-threshold mechanoreceptive (LTM) primary afferents (Naim et al. 1998). The importance of these NK1r-expressing projection neurons has been demonstrated by selectively ablating them *in vivo* with intrathecal injections of substance P conjugated to saporin, which prevents the development of hyperalgesia (Mantyh et al. 1997) and may also lead to the loss of some forms of itch (Carstens et al. 2010). Although NK1rs are also expressed by many excitatory interneurons in the superficial laminae, this is generally at a much lower level than that seen on the projection neurons (Al Ghamdi et al. 2009), and it is therefore likely that the effects of substance P-saporin result from loss of NK1r-expressing ALT cells in laminae I and III.

Not all lamina I ALT cells express the NK1r, and among those that do not, we have identified a population of giant cells that are characterized by their very high density of synapses from both inhibitory and excitatory interneurons, but little if any primary afferent input (Polgar et al. 2008; Puskar et al. 2001). Whether this intriguing population plays a role in itch is not yet known.

20.3.2 Interneurons

Within the lumbar enlargement, virtually all of the neurons in lamina II and around 95% of those in lamina I have axons that remain in the spinal cord and are therefore classified as interneurons. However, although these cells all give rise to local axonal arbors (Grudt and Perl 2002; Maxwell et al. 2007; Yasaka et al. 2007), many of them also have axons that travel for several segments within the spinal cord (Bice and Beal 1997a,b). Many small neurons in laminae I-II in the cervical enlargement can be retrogradely labeled from the medulla (Lima et al. 1991), and these may correspond to the lumbar interneurons with long propriospinal axons. Interneurons in the superficial dorsal horn are therefore not only involved in local circuits. However, the functional significance of these long intersegmental pathways in itch is still poorly understood.

Interneurons can be divided into two broad classes: inhibitory cells that use GABA and/or glycine as their fast transmitter, and excitatory (glutamatergic) neurons. Immunocytochemical studies suggest that 25% of the neurons in lamina I and 30% of those in lamina II are GABAergic inhibitory interneurons, with some using glycine as a cotransmitter (Polgar et al. 2003; Todd and Sullivan 1990). However, even though glycine and GABA are likely to be coreleased at synapses formed by these cells, the selective distribution of postsynaptic receptors may mean that some of their synapses are purely glycinergic (Keller et al. 2001). Although there are no reliable immunocytochemical markers for the cell bodies of glutamatergic interneurons, their axons express vesicular glutamate transporter 2 (VGLUT2), which can be used to identify these cells in combined electrophysiological/anatomical studies (Maxwell et al. 2007; Yasaka et al. 2010). Because projection neurons make up ~5% of the neurons in lamina I, but are largely absent from lamina II, excitatory interneurons presumably account for around 70% of the neurons in both laminae.

Both excitatory and inhibitory interneuron classes are made up from several functionally distinct populations. For example, it has been suggested that inhibitory interneurons have a number of different antinociceptive roles (Sandkuhler 2009) and also mediate the inhibition of itch by noxious counterstimulation (Ross et al. 2010). These tasks are probably performed by different cell types. In addition, while most inhibitory interneurons mediate postsynaptic inhibition through axodendritic or axosomatic synapses on dorsal horn neurons, some presynaptically inhibit primary afferents via axo-axonic synapses (Hughes et al. 2012). Considerable effort has therefore gone into trying to identify interneuron populations within the superficial dorsal horn (Graham et al. 2007; Todd 2010).

The most widely accepted classification scheme is that developed by Grudt and Perl (2002), who combined an electrophysiological recording of lamina II neurons in spinal cord slices from hamsters with anatomical reconstruction of their dendritic and axonal

trees. They identified four main classes: islet cells, with dendrites that were highly elongated in the rostrocaudal axis, central cells (which resembled islet cells but had far shorter dendritic trees), vertical cells, with a dorsally located soma and ventrally directed dendrites, and radial cells, with short radiating dendrites (Figure 20.3). Subsequent studies have identified cells with similar morphology in other species (Heinke et al. 2004; Maxwell et al. 2007; Yasaka et al. 2007, 2010; Wang and Zylka 2009), but a major limitation of this scheme is that in most of these studies many of the recorded cells (typically ~30%) did not fit into any of these classes.

An alternative approach has been to identify cells that show various firing patterns during injection of depolarizing current pulses (Heinke et al. 2004; Maxwell et al. 2007; Wang and Zylka 2009; Yasaka et al. 2007, 2010). These patterns include tonic, initial bursting, single-spike, reluctant, delayed, and gap-firing and depend on the expression of specific ion channels (Graham et al. 2007). Recent studies have related morphological and physiological properties of lamina II interneurons to their neurotransmitter phenotype (Maxwell et al. 2007; Yasaka et al. 2010). This approach has shown that all islet cells are inhibitory, while radial cells and most vertical cells are excitatory, and this is consistent with physiological results obtained from paired recordings from neurons in spinal cord slices (Lu and Perl 2003, 2005). However, both inhibitory and excitatory interneuron classes include some central cells as well as other cells that are morphologically diverse. Interestingly, the reluctant, delayed and gap firing patterns, which are associated with A-type potassium (I_A) currents, are largely restricted to excitatory interneurons (Yasaka et al. 2010).

Another way of classifying interneurons involves using the rich array of neurochemical markers (neuropeptides, receptors, and other proteins) that are expressed in the superficial dorsal horn (Table 20.1). Some of these are restricted to either inhibitory or excitatory interneurons, while others are found among both types (Todd and Koerber 2005). It is important to note that a single marker may not define

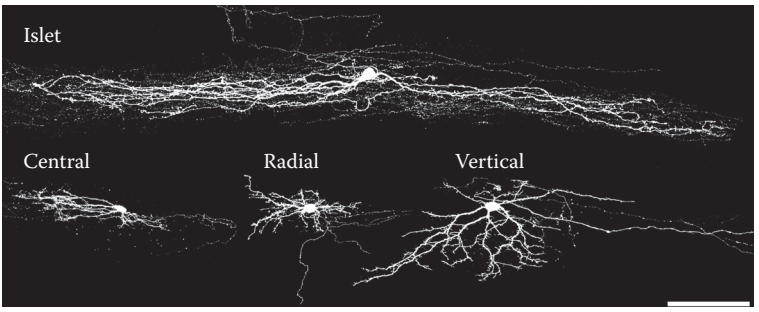

FIGURE 20.3 Morphological types of lamina II interneurons. Confocal images of four lamina II interneurons labeled with neurobiotin following whole-cell patch-clamp recording in a sagittal slice from an adult rat (Yasaka et al. 2010). These correspond to the four main morphological classes identified by Grudt and Perl (2002). The dendritic trees of islet cells are highly elongated along the rostrocaudal axis, with little medio-lateral or dorso-ventral spread. Central cells are similar, but with much smaller dendritic trees. Radial cells have short radiating dendrites. Vertical cells have a dendritic tree that fans ventrally from a dorsally located cell body.

TABLE 20.1
Expression of Various Neuropeptides, Receptors, and Other Proteins by Interneurons in the Superficial Dorsal Horn

	Inhibitory (GABAergic)	Excitatory (Glutamatergic)
Neuropeptides	NPY	Somatostatin
	Galanin	Neurotensin
	Enkephalin	Neurokinin B
	Dynorphin	Substance P
		Enkephalin
		Dynorphin
Neuropeptide receptors	sst_{2A}	NK1
	NK3	NK3
		MOR-1
		NPY-Y1
Calcium binding proteins	Parvalbumin	Calbindin
		Calretinin
Other proteins	nNOS	nNOS
	ChAT	PKCγ

Note: ChAT, choline acetyltransferase; MOR-1, μ-opioid receptor 1; NK1, neurokinin 1; NK3 neurokinin 3; nNOS, neuronal form of nitric oxide synthase; NPY, neuropeptide Y; PKC-γ, protein kinase C-γ; sst_{2A}, somatostatin receptor 2A.

a specific population, and that combinations of markers may be more useful. For example, dynorphin is expressed by both excitatory and inhibitory interneurons, and the inhibitory cells also contain galanin (Sardella et al. 2011; Brohl et al. 2008). The presence of galanin can therefore be used to distinguish these two populations. We have recently shown that neuropeptide Y (NPY), galanin, neuronal nitric oxide synthase (nNOS), and parvalbumin are present in nonoverlapping populations of inhibitory interneurons, and that between them these account for around half of the inhibitory interneurons in laminae I-II in the rat (Sardella et al. 2011; Tiong et al. 2011). We also know that there are differences between the postsynaptic targets for these different populations. Specifically, some NPY neurons preferentially target the NK1r-expressing ALT cells in lamina III (Polgar et al. 1999; Polgar et al. 2011), nNOS-containing inhibitory interneurons innervate the giant lamina I projection neurons (Puskar et al. 2001), while axons of the parvalbumin cells form axoaxonic synapses on the central terminals of myelinated LTM afferent (Hughes et al. 2012). The parvalbumin neurons correspond to a subset of islet cells (Antal et al. 1990) and because they are also innervated by low-threshold afferents (Hughes et al. 2012), they are presumably involved in regulation of tactile input. Because most (if not all) ALT neurons in laminae I and III respond to noxious stimulation, NPY and nNOS cells are likely to be involved in limiting the transmission of pain signals through the dorsal horn.

There is also evidence that excitatory interneurons have specific postsynaptic targets. We have recently shown that over half of the input from excitatory interneurons to lamina III NK1r$^+$ ALT cells comes from the neurons that express dynorphin (Baseer et al. 2012), while Lu and Perl (2005) have shown that lamina II vertical cells, which are innervated by small myelinated (Aδ) primary afferents, are presynaptic to NK1r-expressing lamina I projection neurons. These circuits are illustrated in Figure 20.4. This circuit diagram is clearly far from complete, since interneurons are shown as having only a single type of postsynaptic target, while in reality they will presumably innervate a number of different neuronal types. Also, several classes of neuron are not shown in this diagram because we know little about their synaptic connections. However, it reflects the fact that neuronal circuits in the dorsal

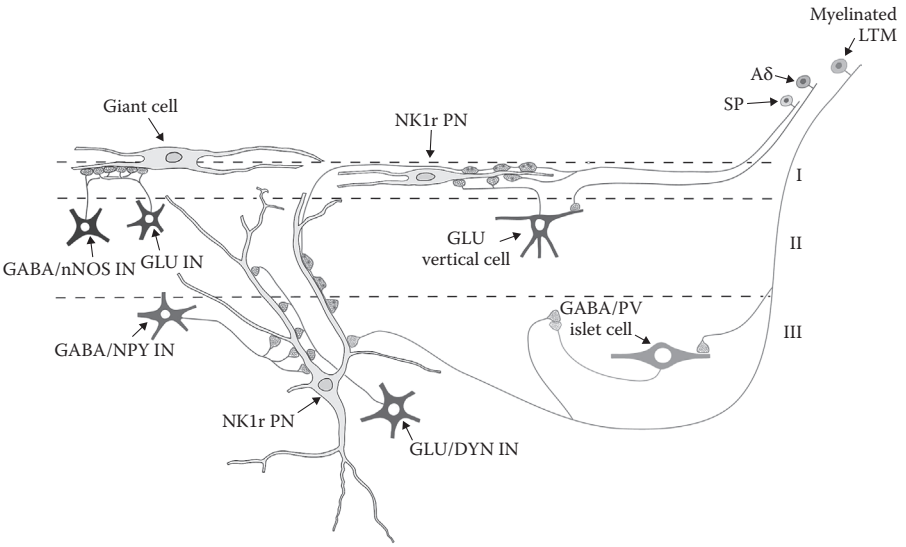

FIGURE 20.4 **(See color insert.)** A diagram showing some of the synaptic connections that have been identified in the rodent superficial dorsal horn. Three projection neurons are shown in grey. One of these is a lamina I giant cell, while the other two are NK1r-expressing projection neurons (PN) with their cell bodies in laminae I and III. Note that the lamina III cell has prominent dorsal dendrites that enter the superficial laminae. Both types of NK1r-expressing PN are densely innervated by substance P-containing primary afferents, while the lamina III cells also receive an input from myelinated low threshold mechanoreceptive (LTM) afferents. The giant cells receive dense synaptic input from GABAergic inhibitory interneurons, many of which contain neuronal nitric oxide synthase (GABA/nNOS), and from glutamatergic excitatory interneurons of unknown origin (GLU IN). The lamina III NK1r PN is densely innervated by NPY-containing GABAergic interneurons and dynorphin (DYN)-containing glutamatergic interneurons. Glutamatergic vertical cells, which receive monosynaptic input from small myelinated (Aδ) afferents are presynaptic to the lamina I NK1r PNs. Parvalbumin-containing GABAergic islet cells (GABA/PV) receive direct input from myelinated LTM afferents and their axons form axoaxonic synapses with the same type of afferent. For further information, see text.

horn do appear to be arranged in a selective (although highly complex) way, and it provides a basis from which a more complete picture will emerge.

Although we are just at the early stages of identifying and classifying the distinct neuronal populations found within the dorsal horn and understanding their function, progress is being made at a rapid pace. The emerging picture is that itch is likely to be modulated by numerous types of spinal interneurons, which have well-organized patterns of input and output, thus forming highly specialized circuits. Delineating these spinal microcircuits is an important first step in understanding how itch is coded and may eventually lead to therapies that provide some much needed relief for chronic itch.

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