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Review

Transmitting Pain and Itch Messages: A Contemporary View of the Spinal Cord Circuits that Generate Gate Control

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The original formulation of Gate Control Theory (GCT) proposed that the perception of pain produced by spinal cord signaling to the brain depends on a balance of activity generated in large (nonnociceptive)- and small (nociceptive)-diameter primary afferent fibers. The theory proposed that activation of the large-diameter afferent "closes" the gate by engaging a superficial dorsal horn interneuron that inhibits the firing of projection neurons. Activation of the nociceptors "opens" the gate through concomitant excitation of projection neurons and inhibition of the inhibitory interneurons. Sixty years after publication of the GCT, we are faced with an ever-growing list of morphologically and neurochemically distinct spinal cord interneurons. The present Review highlights the complexity of superficial dorsal horn circuitry and addresses the question whether the premises outlined in GCT still have relevance today. By examining the dorsal horn circuits that underlie the transmission of "pain" and "itch" messages, we also address the extent to which labeled lines can be incorporated into a contemporary view of GCT.

Introduction

In a recent review (Basbaum et al., 2009), we discussed the organization of primary afferents and highlighted several mechanisms through which tissue and nerve injury can produce a prolonged state of hypersensitivity. In the setting of injury, innocuous stimuli can now produce pain, a condition referred to as allodynia, and normally painful stimuli produce more intense pain (hyperalgesia). That review discussed several mechanisms of peripheral and central sensitization that contributes to chronic pain. Although the review indicated that many of the changes that occur in the spinal cord result from alterations in the function of dorsal horn interneurons, there was little discussion of the incredible heterogeneity of the interneurons. The present review has the interneurons as its focus, hopefully building upon several excellent reviews that appeared in the last few years (Ribeiro-da-Silva and De Koninck, 2009; Todd, 2010; Kuner, 2010).

Gate Control Theory Revisited

It has been almost 50 years since Melzack and Wall published their Gate Control Theory (GCT) of pain (Melzack and Wall, 1965), which proposed spinal cord circuits through which pain is generated and controlled (Figure 1). GCT postulated that the extent to which a particular stimulus or ongoing injury produced pain was not merely a function of the magnitude of activity generated in a population of "pain"-specific primary afferents and the brain's ability to read that input. Rather, GCT proposed that the brain monitors the activity generated by a complex, gated circuit that is located in the superficial dorsal horn of the spinal cord, a circuit that can be regulated by activity carried by nociceptive as well as nonnociceptive afferents (i.e., afferents that respond normally to innocuous stimuli). The heart of the gate of GCT consisted of a relatively simple circuit, with its literal and metaphorical key corresponding to an inhibitory interneuron in the substantia gelatinosa (SG), a region that corresponds to lamina II of the superficial dorsal horn. The gate of GCT closed when activity in large-diameter (nonnociceptive) afferents "turned on" an inhibitory SG interneuron, which in turn inhibited the spinal cord projection neurons that transmit the injury message to the brain. Furthermore, and what is often misunderstood or at least not recognized, is that GCT proposed that painful stimulation not only activates the projection neuron, but also turns the "key" in the opposite direction, i.e., noxious stimulation carried by the unmyelinated nociceptors open the gate, by inhibiting the SG interneuron. As all primary afferents are excitatory, the inhibitory action of these nociceptive afferents was presumed to involve complex interneuronal circuits in the dorsal horn, the final product of which was inhibition of the SG interneuron, increased transmission of the "pain" message to the brain, and thus increased pain.

Because there was remarkably limited knowledge of interneuron and projection neuron heterogeneity 50 years ago, the permutations that could be envisioned as to dorsal horn circuitry were also limited. Golgi studies demonstrated morphological varieties of interneurons (Gobel, 1975, 1978; Ramon y Cajal, 1909), but their function was unclear. Inhibitory GABAergic and glycinergic interneurons were appreciated and it was assumed that excitatory interneurons were glutamatergic, but there was almost no information on the circuits engaged by these interneurons or on their heterogeneity.

Almost 50 years after the publication of GCT, we are faced with an ever-growing list of morphologically and neurochemically distinct interneurons and projection neurons, but we are only beginning to assign function to these populations. In the present review, we hope to provide more than an encyclopedic version of interneuron and projection neurons' morphological and neurochemical heterogeneity. We do introduce the major neuronal

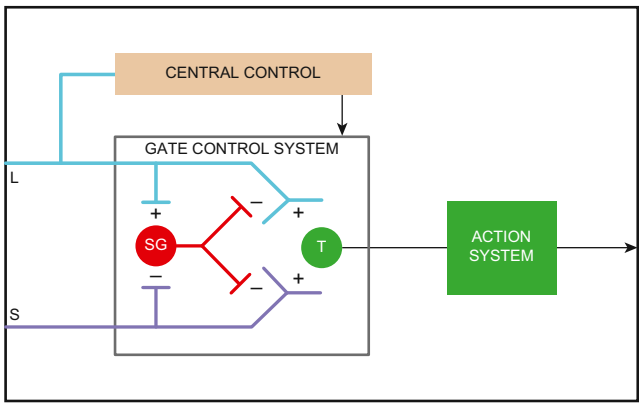

Figure 1. Gate Control Theory
The original formulation of Gate Control Theory proposed that spinal cord signaling to the brain (the "Action System") depended on the balance of activity of large (L)- and small (S)-diameter primary afferent fibers. The large fibers can excite output/projection cells (transmission [T]), but the large fibers also exert a feedforward inhibition of the T cells by concurrently activating inhibitory interneurons in the substantia gelatinosa (SG). The inhibition was presumed to involve presynaptic connections to the T cell; however, postsynaptic inhibition of the T cell by SG interneurons was not ruled out. The net effect of the large-diameter afferents is to "close" the gate. Key to the gate of GCT, however, is the action of small-diameter (presumptive) nociceptors, which not only directly activate the T cells and thus the "Action System" but also engage inhibitory circuits that reduce the activity of the SG inhibitory interneurons. This mechanism "opens" the gate. Finally, the schematic included an illustration of the engagement of supraspinal control systems (the Central Control loops) that can regulate the output of the spinal cord dorsal horn.

populations but then highlight information that addresses critical questions about the circuits that are relevant to the generation of pain and itch, both in the normal state and after injury, where chronic pain and itch occur. We have tried not to reiterate what was emphasized in our recent review. Although that review pointed out that many of the injury-associated changes that occur in the spinal cord dorsal horn result from alteration in the function of interneurons, notably changes in GABAergic inhibitory controls, we did not attempt to relate function with the heterogeneity of the interneuron and projection neurons. Relating form and neurochemistry to circuitry and function is the objective of the present review.

Anatomy

Primary Afferents: Nociceptive and Nonnociceptive Inputs to the Dorsal Horn

Primary afferent neurons of the dorsal root and trigeminal ganglia transmit sensory information from skin, muscle, joints, and viscera to the spinal cord and to its trigeminal homolog in the brainstem, respectively. Information from these regions is transmitted to the brain where a pain or itch perception is generated (Figure 2). The largest-diameter, myelinated (Aβ) primary afferents have a low mechanical threshold and transmit nonpainful, tactile, and proprioceptive information to the spinal cord. A subset of the smallest myelinated (Aδ) afferents also conveys innocuous mechanical inputs. Pain-provoking stimuli are carried by slowly conducting unmyelinated, C fiber nociceptors and by a subset of myelinated Aδ fibers. Itch is also generated by a subset of unmyelinated afferents (the pruritoceptors). Finally, there are two populations of unmyelinated afferents that respond to low-intensity (nonpainful) mechanical stimuli and that have been associated, in humans as well as mice, with the generation of pleasant touch sensations (Olausson et al., 2002; Vrontou et al., 2013). The larger population of low-threshold C mechanoreceptors is characterized by its expression of tyrosine hydroxylase and the VGLUT3 subtype of vesicular glutamate transporter (Li et al., 2011; Seal et al., 2009); the second, much smaller population expresses the MrgB4 subtype of GPCR (Vrontou et al., 2013).

Although glutamate is the major excitatory neurotransmitter of all primary afferents, the C nociceptors can also be subdivided into an NGF/TrkA-dependent, peptidergic population and a GDNF/cRet-dependent, nonpeptidergic subset (Julius and Basbaum, 2001; Snider and McMahon, 1998). Many of the peptidergic neurons express substance P and CGRP as well as the capsaicin receptor TRPV1, which confers heat pain sensitivity upon them. Other TRPV1-expressing sensory neurons appear to define a distinct population of pruritoceptors (Han et al., 2013; Imamachi et al., 2009; Liu et al., 2009). Although there is evidence for expression of gastrin-releasing peptide (GRP) by pruritoceptors (Sun and Chen, 2007), recent studies indicate that GRP may instead be concentrated in a subset of itch-relevant dorsal horn interneurons (Mishra and Hoon, 2013). The nonpeptide population of C fibers is defined anatomically by its binding of the lectin IB4 and its expression of the diverse Mrg family of GPCRs (Dong et al., 2001).

The peptidergic and nonpeptidergic subsets of afferents are also distinguished by their spinal cord targets. The peptidergic afferents project to the most superficial laminae of the dorsal horn (I and outer II); the nonpeptidergic afferents target inner lamina II. This differential targeting suggests that there are functional distinctions in the contribution of the peptidergic and nonpeptidergic sensory neurons. Indeed, based on behavioral analyses after selective ablation of the peptidergic (TRPV1-expressing) or nonpeptidergic (MrgprD-expressing) nociceptors and on the different central circuits that these populations engage (Braz et al., 2005), we and others (Cavanaugh et al., 2009; Mishra et al., 2011) concluded that there is significant functional specificity in the contribution of these afferents. Specifically, the ability to transmit painful heat information requires the TRPV1 population. In contrast, a component of mechanical, but not heat, pain sensitivity is transmitted by the MrgprD subset of IB4-binding nociceptors.

In contrast to the nociceptors, there is much more limited neurochemical classification of the nonnociceptive afferents. However, recent studies have demonstrated considerable heterogeneity of the myelinated afferents (Abraira and Ginty, 2013; Li et al., 2011). It is also of particular interest that the central projections of the different populations of low-threshold mechanoreceptive afferents (including the low-threshold C mechanoreceptors) project to the spinal cord dorsal horn in a columnar manner, with different populations targeting specific laminae. This pattern provides a modular substrate for the regulation of dorsal horn interneuron responsiveness via interactions between painful C and Aδ with nonpainful Aβ, Aδ, and low-threshold C fiber inputs. As described below, the interneurons of the dorsal horn provide the critical link between afferent input to these

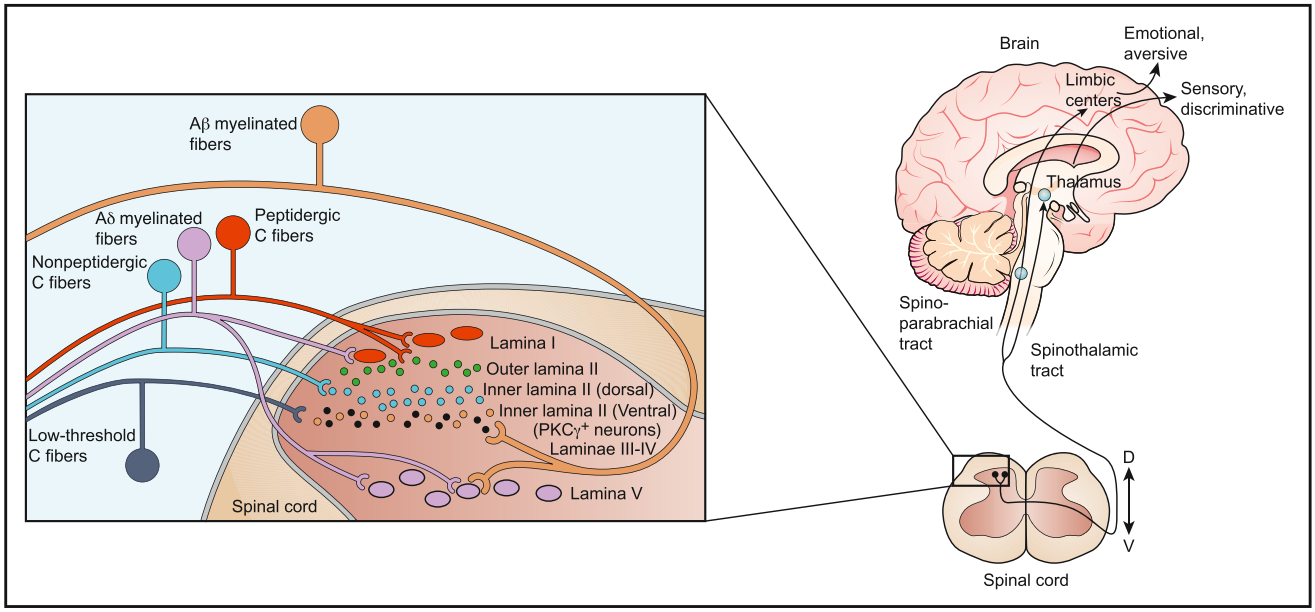

Figure 2. Spinal Cord Neuroanatomy: Inputs and Projections

The different populations of primary afferent fibers target different regions of the dorsal horn of the spinal cord, with the input from C nociceptors concentrated in the superficial dorsal horn (laminae I and II). The peptidergic, TRPV1-expressing subset of unmyelinated nociceptors targets laminae I and outer II; the non-peptidergic subset, many of which expresses Mrgpr GPCRs, targets the dorsalmost part of inner lamina II. The small myelinated Aδ nociceptors target both laminae I and V. The low-threshold C mechanoreceptors, in contrast, target neurons in the ventral part of inner lamina II, which contains many PKCγ-expressing populations of interneurons. The latter have been implicated in nerve injury-evoked mechanical allodynia provoked by stimulation of the large-diameter (Aβ) afferents, the terminals of which are concentrated in laminae III–V (see also Figure 5). The right side of the figure illustrates the major ascending pathways that derive from the spinal cord dorsal horn. Although there are multiple ascending pathways to various loci in the brainstem and thalamus, pathways that derive from projection neurons in laminae I and V predominate. The spinothalamic tract carries sensory-discriminative information to the contralateral thalamus. By contrast, information transmitted via the spinoparabrachial pathway to the parabrachial nuclei of the dorsolateral pons, engages limbic system circuits, including amygdala and insular cortex, and contributes to the affective/emotional component of the pain experience.

modules and to the projection neurons that carry information from the spinal cord to the brain.

Projection Neurons: Transmitting Information to the Brain

The extent to which a particular stimulus is perceived as painful depends on the brain's interpretation of the activity generated in the nociresponsive output cells of the spinal cord. Many projection neurons are found in the most superficial layer of the dorsal horn (lamina I) and here the majority of the neurons, at least in the uninjured animal, are nociceptive specific, i.e., only respond to painful stimulation. A subset of these neurons also responds to the pruritic agents, histamine and cowhage (Davidson et al., 2012). By contrast, the deeper dorsal horn (lamina V) contains the so-called wide dynamic range (WDR) projection neurons that respond to both noxious and innocuous stimulation. Other nociresponsive projection neurons are scattered throughout the ventral horn, but the fact that these neurons have very large and complex receptive fields taken together with their great sensitivity to general anesthetics, which significantly alters their properties, has made their analysis more difficult (Fields et al., 1977). Somewhat surprisingly, given their importance, projection neurons make up a remarkably small proportion of the total neuron count in the spinal cord, e.g., only 5% of lamina I neurons project beyond the cord (Spike et al., 2003).

Although the thalamus is the predominant target of spinal cord neurons in primates, the majority (~80%) of lamina I projection neurons in rodents terminate in the parabrachial nucleus (PB) of the dorsolateral brainstem. The PB, in turn, provides rapid access to forebrain limbic structures, including the amygdala and hypothalamus, which are presumed to contribute to the emotional quality of the pain experience. Other spinal cord projection neurons target the midbrain periaqueductal gray (PAG), reticular formation, hypothalamus, and thalamus (Bernard et al., 1995; Burstein et al., 1990; Todd et al., 2000) and many spinal cord projection neurons collateralize to multiple supraspinal sites (Al-Khater and Todd, 2009; Spike et al., 2003). As to neurochemistry, most PB-projecting neurons (~80%) express the neurokinin 1 receptor (NK1), which is targeted by substance P (SP)-expressing primary afferent nociceptors (Ding et al., 1995; Todd et al., 1998) as well as SP-expressing local interneurons. As these projection neurons express neither GABA nor glycine, they are presumed to be exclusively excitatory (Littlewood et al., 1995).

Lamina I projection neurons can also be distinguished on morphological grounds, being multipolar, fusiform, or pyramidal (Cheunsuang and Morris, 2000; Han et al., 1998; Lima and Coimbra, 1989). The multipolar and fusiform neurons are largely NK1-receptor expressing (Almarestani et al., 2007) and are presumed to be nociceptive, whereas the pyramidal neurons, at least in the uninjured animal, do not express NK1 receptors and appear to be nonnociceptive (Polgár et al., 2008). The latter can be defined by their expression of other markers, notably, the sst2A subtype

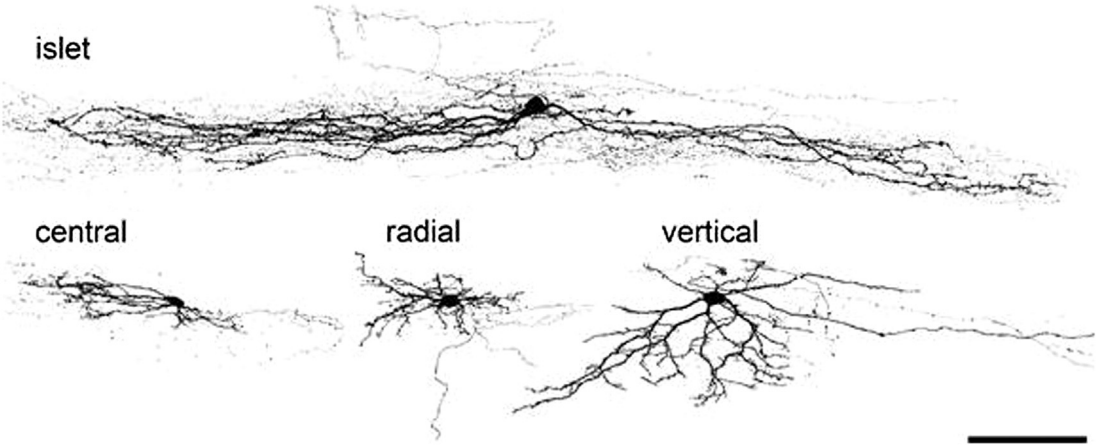

Figure 3. Morphology of Superficial Dorsal Horn Interneurons
Four morphologically distinct subsets of interneurons predominate in lamina II of the superficial dorsal horn. Islet cells have the longest dendritic arborization, extending long axons and dendrites in the rostrocaudal plane, largely within the lamina where their cell bodies reside. Central cells also have rostrocaudally directed intralaminar arborizations, but the extent of the arborization is less than that of the islet cells. Vertical cells have complex and ramified dendritic arbors that extend largely in the dorsoventral plane, crossing multiple laminae, where they can receive convergent inputs from different populations of primary afferents. Many of the vertical cell axons arborize dorsally in lamina I. Radial cells have a restricted dendritic arbor and a variably directed axon. Finally, whereas islets cells are exclusively inhibitory, vertical and radial cells are predominantly glutamatergic and thus excitatory. Both inhibitory and excitatory central cells have been described. (Image provided by Dr. Andrew Todd.)

of somatostatin receptor, the calcium binding protein, calbindin, and/or GluR4-containing AMPA receptors. Given their nonnociceptive properties, these latter neurons may receive inputs from the low-threshold C mechanoreceptors and/or from unmyelinated (probably TRPM8-expressing) afferents, which confers their responsiveness to innocuous cooling. Note however that peripheral inflammation induces de novo expression of the NK1 receptor in pyramidal neurons (Almarestani et al., 2009; Saeed and Ribeiro-da-Silva, 2013). Thus, it is likely that in the setting of tissue injury, nonnociceptive pyramidal neurons also respond to noxious stimuli.

Other projection neurons derive from NK1-expressing neurons in lamina III, and many of these have long dorsally directed dendrites that penetrate the superficial dorsal horn (laminae I and II) where they are targeted by SP-containing nociceptors (Brown et al., 1995). In contrast to the predominant nociceptive-specific properties of the lamina I projection neurons, the lamina III neurons (as well as others in lamina V) respond to both innocuous and noxious inputs. The innocuous input derives from large-diameter nonnociceptive Aβ afferents that terminate in laminae III–V (Naim et al., 1997).

Interneurons

One of the more confusing features of the GCT model is that the critical, presumptive GABAergic inhibitory interneurons of the substantia gelatinosa, which "closed the Gate," appeared to be directly (i.e., monosynaptically) excited and inhibited by large-diameter A fibers and unmyelinated C fibers, respectively. As all primary afferent fibers are excitatory, the model was clearly representing the integrative result of the activity of complex, but at that time completely unknown, interneuronal circuits in the superficial dorsal horn. Recent studies, however, have dramatically altered our understanding not only of the heterogeneity of the interneurons and their critical contribution to inhibitory control of "pain" transmission, but also to their essential contribution to the transmission of pain messages by projection neurons to the brain. In fact, a polysynaptic circuit through which an unmyelinated C fiber can inhibit overall GABAergic control of the dorsal horn output has been described (see "Dorsal Horn Circuitry").

Morphological Heterogeneity of the Interneurons

Patch-clamp studies have greatly extended the early Golgi studies of superficial dorsal horn interneuron classes. Grudt and Perl (2002) described four morphologically distinguishable interneurons: islet, central, radial, and vertical cells (Figure 3; Grudt and Perl, 2002). Islet and central cells have dendritic arbors that arborize in the rostrocaudal plane (more extensively in the case of the islet cell) and that are largely limited to the lamina in which the cell body resides. The vertical cell, which probably corresponds to the stalked cell described in earlier Golgi preparations (Gobel, 1975, 1978), has its cell body in outer lamina II, near the I/II border. The ventrally directed dendritic arbor of the vertical cell allows this neuron to integrate inputs from a column of functionally distinct (nonnociceptive and nociceptive) afferents and local interneurons (see next section). As the great majority of islet cells are GABAergic, with a subset of these co-expressing glycine, this class of interneuron is presumed to be exclusively inhibitory (Proudlock et al., 1993; Todd and McKenzie, 1989). In contrast, radial and vertical cells are predominantly glutamatergic and thus presumed to be excitatory. Central cells include both inhibitory and excitatory subsets (Maxwell et al., 2007; Yasaka et al., 2010).

Neurochemistry: Differentiation of Excitatory and Inhibitory Interneurons

The excitatory interneurons are heterogeneous and develop under the control of distinct transcription factor (Xu et al., 2008,

2013). The expression of the VGLUT2 vesicular glutamate transporter in many of these interneurons confirms their excitatory profile (Oliveira et al., 2003; Santos et al., 2009). However, in addition to glutamate, a host of neurochemical markers has defined distinct subpopulations of excitatory interneurons, including PKCγ (Polgár et al., 1999), the mu opioid receptor (MOR [Eckert et al., 2003; Kemp et al., 1996; Spike et al., 2002]), neurotensin (Todd et al., 1992), somatostatin (Todd et al., 2003), and neurokinin B (Polgár et al., 2006). Of particular functional interest to the development of selective pharmacological tools to regulate the firing of these interneurons is their differential expression of voltage-gated potassium channels (Marker et al., 2006) and subtypes of glutamate receptors (Albuquerque et al., 1999; Kyrozis et al., 1996). Finally, as noted above, recent studies defined a subset of presumptive excitatory interneurons that express GRP (Mishra and Hoon, 2013), a peptide implicated in the transmission of pruritoceptive (itch) messages from the periphery, and another population that expresses GRPR (Sun and Chen, 2007), which is targeted by GRP.

In some respects, the inhibitory interneurons of the superficial dorsal horn are not as heterogeneous. As noted above, the islet cell is the predominant inhibitory interneuron in the dorsal horn. All inhibitory interneurons use GABA as their neurotransmitter and many of these coexpress glycine. On the other hand, although GABA and glycine are stored and can be released from the same synaptic vesicle, it is the differential expression of GABA and glycine receptors that determines which inhibitory neurotransmitter predominates. The inhibitory interneurons can also be divided on other neurochemical grounds. Thus, double-label studies using antibodies directed against glutamic acid decarboxylase (GAD67), one of the enzymes required for the synthesis of GABA, identified subpopulations of GABAergic neurons that express neuropeptide Y (NPY), galanin (GAL), parvalbumin (PV), or neuronal nitric oxide synthase (nNOS). These populations do not overlap and account for ~65% of inhibitory interneurons in lamina I and ~50% in lamina II (Sardella et al., 2011). More recently, subsets of inhibitory interneurons have been defined by the different transcription factors that regulate their development (Ross et al., 2010; Wildner et al., 2013).

Of interest, and possibly relevant to the question of modality-specific spinal cord circuits, is the fact that these distinct populations of inhibitory interneurons differ, at least in the rat, in their responses to painful stimulation. For example, Polgár et al. recently reported that mechanical, thermal, and chemical noxious inputs activate a large number of GAL- and NPY-expressing neurons (monitored by phosphorylation of extracellular signal-regulated kinase [ERK]) but very few nNOS-expressing inhibitory interneurons (Polgár et al., 2013). Furthermore, there is evidence that the sst2A somatostatin receptor is expressed by virtually all GAL-positive and nNOS-positive neurons but few NPY-expressing and none of the PV-positive neurons. Given that somatostatin is pronociceptive, and indeed directly activates the lamina I projection neurons (Gamboa-Esteves et al., 2004), it follows that concurrent engagement of inhibitory circuits by somatostatin must somehow lead to disinhibition of the output cells. Importantly, none of the noxious stimuli tested induced activity-dependent neuronal markers in PV neurons, which is in agreement with the suggestion that these cells primarily receive innocuous inputs from low-threshold primary afferents (Hughes et al., 2012). Conceivably the latter circuit corresponds to the pathway through which large-diameter afferents "close the Gate" of GCT (see also Daniele and MacDermott, 2009).

Dorsal Horn Circuitry: From Afferents to the Projection Neurons

The dorsal horn of the spinal cord is not merely a "relay station" where primary afferents engage the projection neurons. Rather it is the locus of incredible integration, where sensory information is subjected to local and supraspinally derived excitatory and inhibitory regulation. Understanding how these controls are exerted clearly requires a detailed circuit map of the connectivity between primary afferents and the projection neurons.

Not long after the discovery of the lamina I contribution to pain processing (Christensen and Perl, 1970), dual recording studies in primate dorsal horn provided evidence for the direction of information flow to the output cells. Price et al. used concurrent recording of neighboring neurons in laminae I and outer II and concluded that information flowed from interneurons in lamina II to projection neurons in lamina I (Price et al., 1979). As Golgi studies as well as morphological characterization of intracellularly recorded neurons had identified a population of stalked cells, with a cell body at the I-II border and an axon that arborizes in lamina I (Bennett et al., 1980; Gobel, 1975; Gobel et al., 1980; Ross et al., 2010), it was proposed that nociceptive inputs activate stalked cells and that these in turn engage the projection cells. Contemporary electrophysiological studies also combined paired recording from morphologically characterized neurons or used laser-scanning photostimulation and identified a modular organization of circuits consisting of specific combinations of interneurons (Kato et al., 2009; Kosugi et al., 2013; Lu and Perl, 2003, 2005; Wu et al., 2010; Yasaka et al., 2007).

A summary of these observations (Figure 4) reveals that central cells in inner lamina II receive an excitatory input from C nociceptors and in turn excite vertical cells of outer lamina II. The vertical cells, which receive monosynaptic primary afferent input from both nociceptive C and Aδ fibers, in turn establish excitatory connections with both interneurons and projection neurons of lamina I (including the NK1-expressing population). The latter, of course, also receive direct nociceptive C and Aδ input. Thus, noxious stimuli directly engage the projection cells and, through a dorsally directed circuit, provide additional feedforward excitatory drive to the projection cells. Presumably, loss of the excitatory interneurons that generate this "backup" excitatory drive to the projection cells underlies the dramatically reduced pain behavioral phenotype that we recently described in mice with a deletion of the gene that encodes the testicular orphan nuclear receptor (TR4 [Wang et al., 2013]). Note that there are additional sources of excitatory drive generated within the interneuronal circuitry, including intralaminar excitatory connections to the central cells from radial and or other central cells (Kato et al., 2009). Finally, nonnociceptive Aβ inputs that target deeper populations of excitatory interneurons (in laminae III and IV) can also engage the lamina I projection neurons through the same

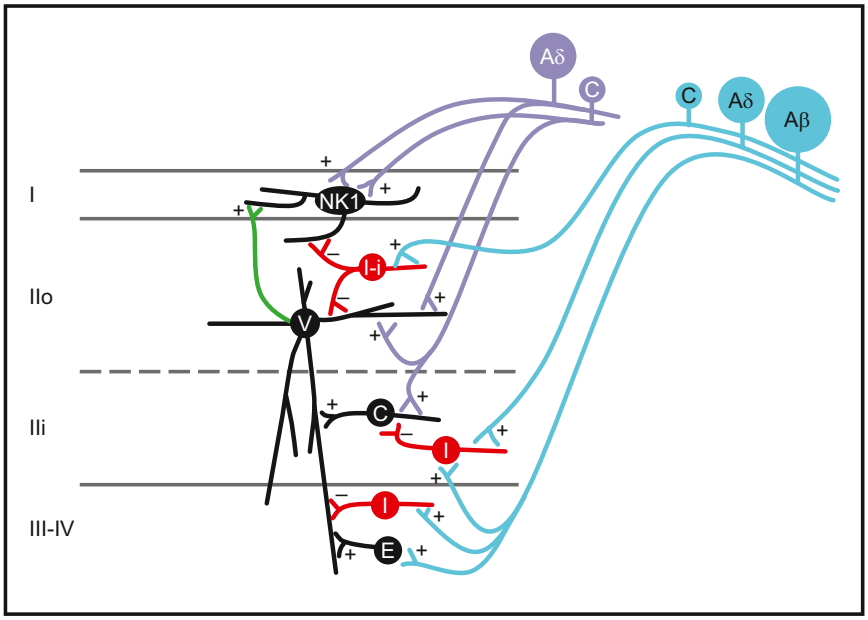

Figure 4. Primary Afferent-Derived Excitatory and Inhibitory Drive to the Spinal Cord Dorsal Horn

Ventral-to-dorsal excitatory activation circuits: nociceptive C and Aδ fibers (dark blue) directly activate vertical cells (V) of lamina IIo and these, in turn, excite NK1-expressing projection neurons of lamina I (green axon). In addition, nociceptive C fibers activate central cells (C) of lamina IIi, and the central cells directly contact, and probably excite, vertical cells in lamina IIo. Of course, projection neurons of lamina I also receive direct nociceptive C and Aδ input. Finally, nonnociceptive Aβ fibers (light blue) project to deeper laminae (III–IV), where they establish monosynaptic connections with local excitatory interneurons (E). The latter directly activate vertical cells. These dorsally directed circuits are the route through which both noxious and innocuous primary afferent input can engage the projection neurons of lamina I. As described in Figure 5, in the setting of nerve injury, additional routes of Aβ engagement of the projection neurons are uncovered. Feedforward inhibitory control of superficial dorsal horn circuits: these excitatory circuits are subject to profound inhibitory controls (red neurons). The majority of the inhibitory neurons (I) in lamina II are islet cells (I-i) and these can be directly engaged by input from low-threshold, mechanoreceptive C fibers (light blue). The islet cells in turn establish monosynaptic inhibitory connections with both vertical cells of lamina IIo and NK1 receptor-expressing projection neurons of lamina I. Low-threshold Aδ (D-hair; light blue) and Aβ fibers also directly engage inhibitory interneurons in laminae IIi–IV. The latter in turn exert inhibitory control of a variety of excitatory interneurons, including vertical (V) and central (C) cells. The Aβ-to-inhibitory cell circuit presumably underlies the circuit through which the "Gate" of Gate Control Theory can be closed. Figure 5 illustrates the neurochemical identity of critical elements in this circuit.

ventral-to-dorsal circuits. Conceivably, this Aβ input contributes to the WDR properties of some lamina I neurons (the majority of which are nociceptive specific) and to the mechanical allodynia that develops after tissue or nerve injury (Hylden et al., 1987, 1989).

Interestingly, the predominantly ventral-to-dorsal excitatory drive to the lamina I projection cells is paralleled by a dorsal-to-ventral excitatory drive to projection neurons in deeper laminae, notably the WDR neurons of lamina V. Our own studies have shown that lamina II neurons that receive inputs from a subpopulation of Nav1.8-expressing, nonpeptidergic afferents relay information to lamina V neurons (Braz et al., 2005). This view of information flow is, in fact, consonant with the early studies of Wall (Wall, 1967), who proposed that the receptive field properties of lamina V WDR neurons results from convergence of input from more dorsally located neurons. There is also evidence that activation of lamina V neurons is indirectly influenced via lamina I-derived spinal-brainstem-spinal connections (Choi et al., 2012; Kim et al., 2009).

Figure 4 further illustrates that the "pain" transmission neurons of lamina I are also subject to profound feedforward inhibitory controls (red neurons). For example, the lamina II central, and probably also the radial cells, receives an intralaminar-derived inhibitory input from islet cells. The inhibitory input to lamina I neurons, in contrast, derives predominantly from islet cell inhibition of vertical cells. There may be some direct islet cell inhibition on dendrites of projection neurons; however, as the great majority of projection neurons have dendrites restricted to lamina I, most of the islet cell inhibition is presumed to be indirect. It is also likely that some of the descending monoaminergic inhibitory control of spinal cord pain transmission neurons is mediated via connections with these inhibitory islet cells (Lu and Perl, 2007; Yasaka et al., 2010). And as postulated by GCT, Aβ afferents, via inhibitory interneurons in lamina IIi through IV, can exert profound and direct inhibitory control of lamina V pain transmission neurons, as well as an indirect inhibition through effects on vertical cells.

What is particularly difficult to unravel is the consequence to the islet cell of activity of the nociceptive, TRPV1-expressing C and Aδ primary afferent fibers (Zheng et al., 2010). For example, while a low-threshold C fiber mechanoreceptive input to the islet cell could contribute to the inhibitory effects of innocuous mechanical stimulation (Choi et al., 2012; Grudt and Perl, 2002; Yasaka et al., 2007), the nociceptive C fiber input to the islet is, at first glance, paradoxical. Specifically, existence of this connection implies that noxious stimuli engage inhibitory circuits that can reduce pain. That painful stimuli can reduce pain is not a new idea (Le Bars, 2002) and, in fact, Nakatsuka et al. (2005) reported that primary afferent-derived substance P can activate GABAergic inhibitory circuitry in the dorsal horn. It is more likely, however, that because islet cells can inhibit other inhibitory interneurons (Kato et al., 2007; Zheng et al., 2010), disinhibitory circuitry comes into play in the ultimate transmission of the "pain" message to the projection neurons. Of course, this disinhibitory circuit that is triggered by noxious stimulation is precisely one that was promulgated in the GCT, i.e., it is the circuit through which C fiber stimulation "opens" the gate.

Labeled Lines: The Controversy Continues

Gate Control Theory challenged a prevailing view, epitomized by the now famous Descartes illustration of a "pain" pathway, namely that there are labeled lines that transmit physiologically

distinct information from the periphery to the brain. Recent functional and anatomical studies, however, indicate that at least at the level of the primary afferent fiber, there are labeled lines for specific somatosensory modalities (noxious heat, cold, noxious mechanical stimulation, and pruritogens). As noted above, intrathecal capsaicin-induced ablation of TRPV1-expressing afferents produces heat-specific behavioral deficits in mice without affecting mechanical thresholds, whereas genetic ablation of the MrgprD subset of nonpeptidergic afferents results in a selective decrease of mechanical pain sensitivity, without altering responses to noxious heat (Cavanaugh et al., 2009). Given the polymodal properties of the great majority of unmyelinated C fibers (Bessou and Perl, 1969; Jankowski et al., 2012; Tominaga et al., 1998), this dichotomy was unexpected. A subsequent in vivo study extended these findings by showing that the TRPV1 and MrgprD subsets of afferents only contribute to the noxious heat and mechanical responsiveness, respectively, of polymodal dorsal horn neurons (Zhang et al., 2013).

There is also evidence for a differential expression of ''analgesic'' receptors on these different afferent populations. Specifically, the MOR, which binds morphine, is concentrated in peptidergic neurons that also express substance P. In contrast, the delta opioid receptor (DOR) is expressed in nonpeptidergic and myelinated afferents (Scherrer et al., 2009). Although this opioid receptor distinction countered the longstanding view that the DOR is concentrated in the peptidergic population (Guan et al., 2005; Riedl et al., 2009), our studies indicate that those conclusions were based on antibodies that were not selective for the DOR. More importantly, perhaps, and consistent with the differential distribution of MOR and DOR in primary afferents, our studies revealed a selective control of heat and mechanical pain responsiveness after intrathecal injection of MOR and DOR agonists, respectively (Scherrer et al., 2009).

With the exception of interneurons associated with the generation of itch, however, few interneuron populations have been linked to a single pain modality (e.g., painful heat or mechanical stimulation). In fact, the great majority of dorsal horn interneurons are also polymodal, making it difficult to determine the particular perceptual modality evoked by their activation. On the other hand, when the afferent-spinal cord circuitry is examined from the perspective of neurochemically defined subsets of afferents, then distinct and functionally specific neural circuits, possibly representing labeled lines, can be demonstrated. For example, using a spinal cord slice preparation, Zheng and colleagues described differential projections of TRPV1- and TRPM8-expressing primary afferents to distinct populations of inhibitory interneurons in the superficial dorsal horn (Zheng et al., 2010). Specifically, TRPV1 (i.e., heat-responsive) and TRPM8 (cold-responsive) unmyelinated afferents converge upon an inhibitory interneuron that is distinct from the traditional islet cell interneuron, which only receives TRPV1 input. Interestingly, as these two populations of inhibitory interneurons are mutually inhibitory, the authors suggested that this differential engagement of inhibitory circuitry by modality-specific afferents could underlie effects of cool stimuli on heat pain processing and vice versa (McCoy et al., 2013).

Other studies also documented selective targeting of subpopulations of interneurons by neurochemically distinct afferents. For example, Zylka and colleagues reported that the MrgprD subset of afferents activates all subtypes of excitatory interneurons but may not directly engage the islet cell inhibitory population (Wang and Zylka, 2009). Similarly, TRPA1-expressing primary afferents, which are a subset of the nociceptive TRPV1 population and are implicated in the responsiveness to a wide variety of irritants, including mustard oil and the pruritogen, chloroquine (Wilson et al., 2011), selectively innervate vertical and radial cells (Uta et al., 2010), but not islet or central cells, raising the possibility that these morphologically distinct excitatory interneurons are part of a circuit that is dedicated to a specific pain and/or itch modality.

Specific Functional Associations of Neurochemically Distinct Dorsal Horn Neurons/Glia

NK1 Receptor-Expressing Projection Neurons: Where Specificity Breaks Down?

As noted above, up to 80% of the lamina I projection neurons express the NK1 receptor and these neurons unquestionably contribute to the transmission of pain messages to the brain. For example, noxious thermal stimuli induce expression of Fos in neurons that express the NK1 receptor, and release of SP in the dorsal horn after peripheral noxious stimulation induces internalization of the NK1 receptor in lamina I neurons (Mantyh et al., 1995; Trafton et al., 1999). On the other hand, genetic deletion of either SP (Cao et al., 1998) or of the NK1 receptor (De Felipe et al., 1998) has limited effects on nociceptive processing. That finding is consistent with the reports that NK1 receptor antagonists reduce but do not eliminate pain in animals and have proven disappointing in clinical studies (Borsook et al., 2012). It follows that other inputs to the lamina I neurons are essential, including that from glutamatergic nociceptors and excitatory interneurons in lamina II (Wang et al., 2013). Most importantly, selective ablation of neurons that express the NK1 receptor using substance P-saporin-based toxins reduces thermal hyperalgesia and mechanical allodynia produced by capsaicin, inflammation, and nerve injury (Mantyh et al., 1997; Nichols et al., 1999; Wiley et al., 2007). Interestingly, baseline pain responses (e.g., to capsaicin) are preserved in these animals, which indicates that there are other projection neurons that contribute to acute pain sensitivity, including non-NK1 receptor-expressing neurons of lamina I and deep dorsal horn neurons. Importantly, those studies used reflex responsiveness to monitor residual ''pain'' processing after ablation of the NK1 receptor-expressing neurons. In other words, it is possible that the reflex occurred but that the rat, in fact, did not ''experience'' the noxious stimulus as painful.

An important corollary of the conclusion drawn from the SP-saporin studies is that the same population of output cells appears to transmit the chronic pain message generated in the setting of both tissue (inflammatory) and nerve injury, as well as the itch that is evoked by pruritogens (Carstens et al., 2010). It is possible that there are functionally distinct subsets of NK1 receptor-expressing lamina I neurons or that a differential code generated in the same population of neurons underlies distinct pain and itch percepts generated under tissue and nerve injury conditions.

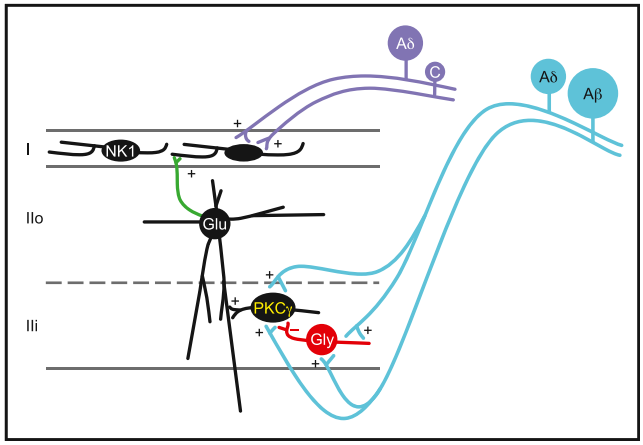

Figure 5. Glycinergic Inhibitory Control and Neuropathic Pain
Disruption of glycinergic inhibitory controls contributes to the development of nerve injury-induced pain hypersensitivity: low-threshold Aβ and possibly Aδ (light blue) inputs directly activate PKCγ-expressing interneurons of lamina II and these interneurons in turn activate projection neurons of lamina I, probably via polysynaptic pathways involving glutamatergic (Glu) excitatory vertical cells of lamina IIo. Under normal conditions, this excitatory drive is tonically inhibited by glycinergic interneurons, and the inhibition can be enhanced by activity of low-threshold afferents. In conditions in which this tonic glycinergic inhibitory control is disrupted (e.g., after peripheral nerve injury), primary afferent-derived innocuous inputs can gain access to the pain transmission circuitry of the superficial dorsal horn, leading to mechanical allodynia and thermal hyperalgesia.

PKCγ Interneurons: A Contemporary Gate Control Circuit?

As excitatory interneurons are under strong inhibitory control (Yasaka et al., 2007), any disruption of these controls will enhance the response to noxious stimuli, leading to hyperalgesia, and can result in nociceptive circuits being engaged by innocuous stimuli. This is the condition referred to as allodynia (Coderre, 2009). In our previous review, we discussed several general mechanisms through which central sensitization occurs in the setting of injury. Here we highlight several specific examples in which distinct subpopulations of dorsal horn interneurons (or glia) have been implicated. For example, Figure 5 schematizes the results from Miraucourt and colleagues, who reported that intracisternal (CSF) injection of the glycine receptor antagonist strychnine induced a mechanical allodynia of the face of the rat, as well as light brush-induced Fos expression in interneurons and lamina I projection neurons of the trigeminal nucleus caudalis (Miraucourt et al., 2007). The latter region is the homolog of the spinal cord dorsal horn. Among the interneurons were many PKCγ-expressing cells of inner lamina II, presumptive excitatory interneurons that receive inputs from low-threshold mechanoreceptors (Neumann et al., 2008) and that we previously implicated in nerve injury-induced mechanical hypersensitivity (Malmberg et al., 1997). Importantly, injection of a PKCγ antagonist not only reduced the strychnine-induced mechanical allodynia but also the brush-induced Fos. The authors concluded that there is a tonic glycinergic inhibition of the PKCγ interneurons and when that inhibition is lifted, low-threshold mechanical input carried by Aβ afferents can gain access to the pain transmission circuitry of the superficial dorsal horn. Whether the release of the PKCγ-regulated excitatory drive is exerted directly on the projection neurons could not be determined.

Differential Contribution of GABAergic and Glycinergic Inhibitory Controls

Administration of GABA and glycine elicits powerful antinociception under most circumstances (Dirig and Yaksh, 1995; Zeilhofer et al., 2012) and, conversely, pharmacological blockade of both glycinergic and GABAergic neurotransmission induces many behavioral signs of hypersensitivity and pain (Sherman and Loomis, 1995; Sivilotti and Woolf, 1994; Yaksh, 1999). Other studies indicate that even though the great majority of inhibitory glycinergic neurons in the superficial dorsal horn corelease GABA, injury-induced biochemical changes in the spinal cord can selectively influence the postsynaptic consequences of glycine corelease. Of particular interest is the report that inflammation-induced spinal cord release of prostaglandins facilitates pain transmission through a blockade of glycinergic inhibitory controls (Ahmadi et al., 2002). Specifically, release of prostaglandin E2, perhaps from primary afferent-stimulated astrocytes, acts on PGE_2 receptors and this, in turn, can counteract the inhibitory action of glycine via its binding to the GlyR3 subtype of strychnine-sensitive glycine receptors (Figure 6A).

The lack of effect of PGE_2 on GABAergic function in the setting of tissue injury and inflammation contrasts sharply with the profound reduction of GABAergic inhibitory controls that occurs in the setting of peripheral nerve injury. It remains to be determined, however, whether the reduction of spinal GABAergic tone is a consequence of decreased GABA release (Lever et al., 2003), downregulation of GABA, GAD, or pre- and postsynaptic GABA receptors (Castro-Lopes et al., 1993; Eaton et al., 1998; Fukuoka et al., 1998; Ibuki et al., 1997; Polgár et al., 2004), or even a loss of GABAergic interneurons (Moore et al., 2002; Scholz et al., 2005; Sugimoto et al., 1990), although the latter is more controversial (Polgár et al., 2003). Regardless of the mechanism, the latter changes are presumed to underlie the mechanical allodynia and thermal hyperalgesia characteristic of the neuropathic pain condition. It follows that therapeutic approaches targeting those changes have the potential to be disease modifying. Indeed, our own studies demonstrate that transplant-mediated recapitulation of the spinal cord GABAergic circuitry that is altered by a peripheral nerve injury completely reverses the injury-induced mechanical hypersensitivity characteristic of the animal model of neuropathic pain (Bráz et al., 2012).

Microglial-Neuronal Interactions

In addition to the hyperexcitability arising from nerve injury-induced disruption of islet cell inhibitory control (see Figure 6B), De Koninck and colleagues provided evidence that microglia are critical intermediaries to the consequences of nerve injury (Coull et al., 2005). Specifically, the authors proposed that ATP release from injured primary afferents activates dorsal horn microglia, leading to the synthesis and release of brain-derived neurotrophic factor (BDNF) from the activated microglia. BDNF, via an action on TrkB receptors expressed by lamina I neurons, downregulates expression of the potassium-chloride exporter KCC2 (Figure 6B). This event, in turn, leads to a shift in the chloride gradient of lamina I neurons (Coull

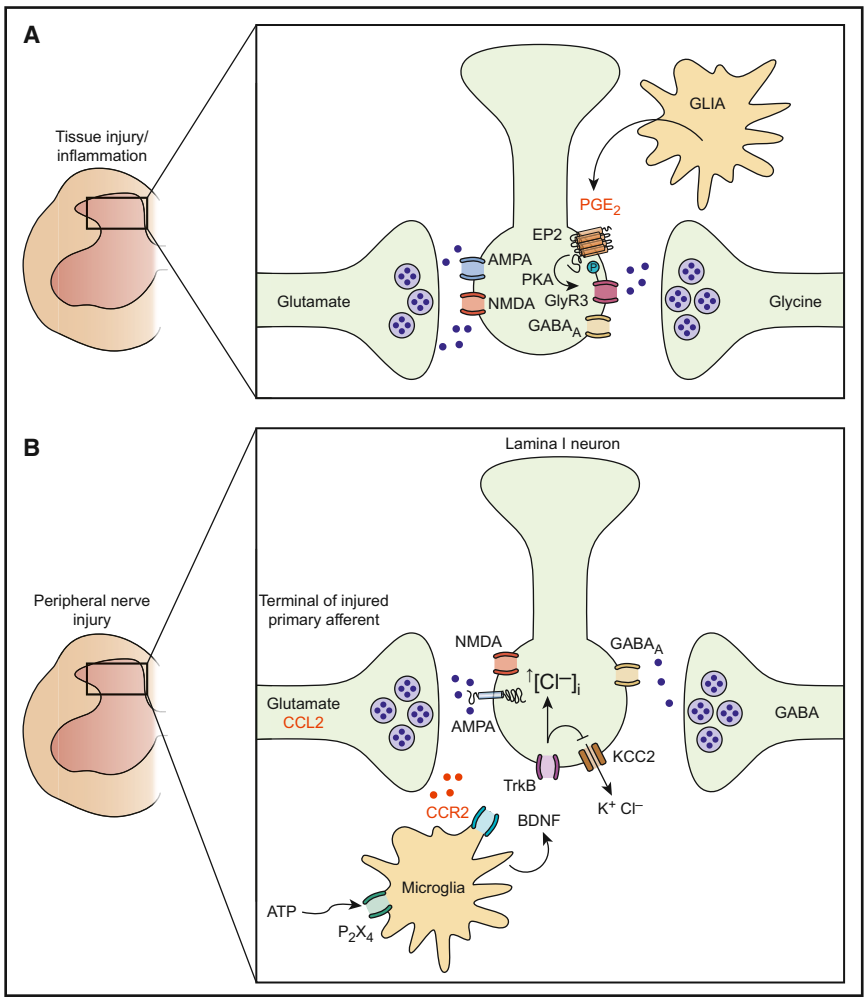

Figure 6. PGE2-Glycinergic Interactions and Inflammatory Pain

(A) PGE2-glycinergic interactions and inflammatory pain. Prostaglandins induce hypersensitivity in the setting of inflammation by regulating glycinergic inhibitory circuits: peripheral inflammation evokes cyclooxygenase (Cox)-dependent release into the superficial dorsal horn of prostaglandin E2 (PGE_2), probably from astrocytes and/or microglia. The PGE_2 binds to neuronal EP2 receptors expressed by many interneurons, including those under inhibitory glycinergic control. Through a cAMP-dependent phosphorylation of the GlyR3 subunit of glycine receptors, the PGE_2 reduces the effects of glycine on what are presumed to be excitatory interneurons that ultimately engage neurons in lamina I. The net result is that innocuous stimuli can provoke pain (allodynia) and noxious stimuli exacerbate pain (hyperalgesia) in the setting of tissue injury. In contrast to the effects of PGE_2 on glycinergic signaling, there is no influence on GABAergic signaling, even though GABA and glycine are coreleased from many superficial dorsal horn inhibitory interneurons.

(B) Microglial-neuronal interactions and neuropathic pain. Microglial-neuronal interactions disrupt GABAergic inhibitory control: peripheral nerve injury activates spinal cord microglia, which in turn release numerous molecules that enhance the excitability of spinal cord neurons. Some studies indicate that chemokine ligand 2 (CCL2), which is upregulated in and released from injured primary sensory neurons, binds to the microglial chemokine receptor 2 (CCR2) to induce microglial activation. Nerve injury-induced release of ATP, via an action on microglial P2X4 receptors, also activates the microglia. The latter release brain-derived neurotrophic factor (BDNF), which, via an action on neuronal TrkB receptors, downregulates the neuronal potassium-chloride cotransporter KCC2. The net result is a reduced chloride gradient that alters the magnitude of any GABAergic inhibitory input to the neuron. In this setting, there may be ongoing pain, mechanical allodynia, and thermal hyperalgesia.

et al., 2003). As a result, GABAergic inhibitory controls are greatly reduced and may even become excitatory. More recently, Smith and colleagues provided evidence that the mechanisms underlying the pronociceptive/central sensitization contribution of nerve injury-induced BDNF release from microglia are far more complex (Lu et al., 2009). Specifically, these authors reported that prior nerve injury or bath application of BDNF not only decreased synaptic excitation of inhibitory "tonic islet/central" neurons recorded from spinal cord slices, but also increased the activity of presumptive excitatory radial interneurons.

The BDNF studies focused on the influence of the microglia on inhibitory controls. There is also considerable evidence that microglia can be activated by primary afferent-derived cytokines and that the microglia, in turn, trigger release of pronociceptive molecules. For example, neutralization of CCL2, a chemokine released by primary afferents after nerve injury prevents microglial activation and inhibits neuropathic pain (Thacker et al., 2009). Mice deficient for CCR2, the receptor for CCL2, also display impaired responses in the formalin test and do not develop mechanical allodynia in a neuropathic pain model (Abbadie et al., 2003). Dorsal horn astrocytes are yet another source of nerve injury-induced CCL2, which underscores the diversity of the mechanisms through which neuropathic pain can be generated. Finally, a very recent report indicated that nerve injury can also trigger a cyclooxygenase-1-derived synthesis of prostaglandins in microglia (Kanda et al., 2013). Missing from the great majority of these studies, however, is a specific characterization of the interneurons influenced by these nerve injury-induced biochemical changes in microglia. Clearly, as Smith and colleagues have shown, the ultimate consequence of these changes depends on the particular circuits in which the target neurons are engaged.

Pain versus Itch: More Questions about Labeled Lines

As described above, we recently reported that despite the existence of polymodal nociceptors, selective ablation studies indicate that the consequences of activity of subsets of these afferents appear selective for a particular modality. Thus, the TRPV1 population is required for noxious heat, the MrgprD subset of nonpeptidergic C fibers for mechanical pain, and TRPM8-expressing afferents for cold (Knowlton et al., 2013). More recently, Dong and colleagues reported that a subset of MrgprD afferents also transmits the pruritic messages generated by β-alanine (Liu

et al., 2012) and that a distinct subset of TRPV1-expressing nociceptors, namely those that coexpress the MrgprA3 GPCR, is also an essential driver of itch, but not pain (Han et al., 2013). One presumes that the remaining, much larger population of TRPV1 nociceptors is the pain-relevant afferent population, although discrete subsets of the remaining TRPV1 population may carry pruritogen-selective information (Imamachi et al., 2009). Although Dong used Fos expression to monitor the central consequences of selective activation of the MrgprA3 line, it could not be concluded that the neurons engaged by that afferent line are not activated by noxious, pain-producing stimuli.

On the other hand, and as noted above, it is not at all clear whether the functionality of the peripheral labeled line is retained at the level of spinal circuitry. Evidence in favor of labeled line at the spinal cord level comes from studies demonstrating a unique population of lamina I neurons that respond to innocuous cooling, but not to painful thermal or mechanical stimulation (Han et al., 1998). Other studies comparing circuits that process itch and pain also argue strongly that separate interneuronal circuits are engaged by pain- and itch-producing stimuli. For example, Chen and colleagues demonstrated that ablation of gastrin-releasing peptide receptor (GRPR)-expressing neurons in the superficial dorsal horn abolishes scratching (indicative of itch) in response to a wide variety of pruritogens. Importantly, the authors found no effect of GRPR neuron deletion on pain behaviors. This finding suggested that there are separate pain and itch circuits engaged by pain- and itch-producing afferents. Chen and colleagues argue strongly that the afferent input to the GRPR neurons derives from unmyelinated GRP-expressing peptidergic afferents that coexpress substance P (Liu et al., 2014; Sun et al., 2009; Zhao et al., 2013). Other studies suggest that natriuretic polypeptide B (NPPB) is the critical neuropeptide transmitter of primary afferent pruritoceptors and that GRP is, in fact, expressed by yet another distinct population of superficial dorsal horn excitatory interneurons (Figure 7; Mishra and Hoon, 2013). The latter result is consistent with our finding that loss of GRP mRNA-expressing interneurons in the superficial dorsal horn of the TR4 mutant mouse is associated with a complete loss of pruritogen-induced itch (Wang et al., 2013).

Taken together with the apparently strict association of the GRP and GRPR population with the generation of itch, these studies suggest that there exists an excitatory, labeled line for itch. Furthermore, Ross et al. observed spontaneous itch and exacerbated responses to the most common pruritogens in mice with a deletion of the transcription factor Bhlhb5 (Ross et al., 2010). The behavioral phenotype was associated with an almost 25% loss of GABAergic interneurons in the superficial dorsal horn. Importantly, these mice did not display exaggerated pain responsiveness, suggesting that a different population of inhibitory neurons regulates the pain circuits. On the other hand, as many superficial dorsal horn neurons respond to both itch- and pain-producing stimuli, it is possible that activity of the GRPR neurons is necessary for evoking behavioral manifestations of itch but dispensable for inducing pain behaviors. Redundant pain transmission circuits may compensate for loss of the GRPR interneuron contribution.

The question remains whether there are subsets of projection neurons (e.g., in laminae I or V) that only transmit itch messages.

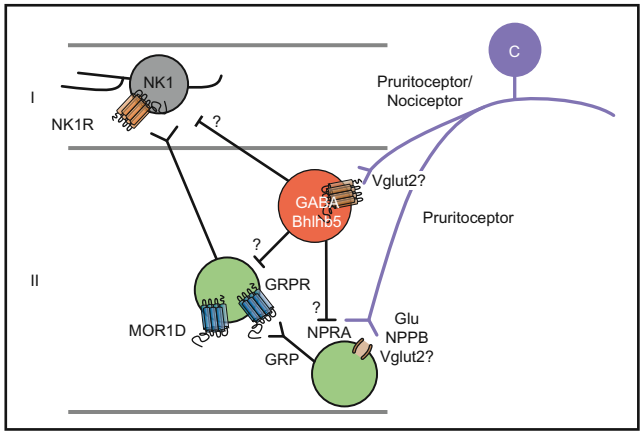

Figure 7. A Labeled Line for the Transmission of Itch Messages
Several subpopulations of primary afferent fiber (pruritoceptors) transmit messages provoked by a host of itch-producing stimuli that engage the afferent directly or indirectly. To what extent itch- and pain-producing afferents (nociceptors) overlap is unclear. Activation of the pruritoceptors evokes the release of natriuretic polypeptide B (NPPB) in the superficial dorsal horn. The receptor for NPPB, natriuretic peptide receptor A (NPRA) is expressed by presumptive excitatory interneurons of the superficial dorsal horn, and these cells in turn release gastrin-releasing peptide (GRP) and engage another interneuron population, namely one that expresses GRPR, the receptor for GRP. Whether GRPR-expressing cells project directly to the brain or signal locally to other projection neurons in the spinal cord is unclear, but our recent findings (Wang et al., 2013) suggest that the GRPR cells are interneurons. As noted in the discussion, another view holds that GRP, in fact, derives predominantly from pruritoceptors, not from dorsal horn interneurons (Zhao et al., 2013). Whether the "pain-responsive" NK1 receptor-expressing projection neurons also transmit itch messages to the brain (e.g., via inputs from GRPR cells) is also unclear. Finally, this figure illustrates that a subset of Bhlhb5-dependent inhibitory interneurons regulates the output of superficial dorsal horn itch circuits, as do primary afferent neurons that express the VGLUT2 subtype of vesicular glutamate transporter (Lagerström et al., 2010; Liu et al., 2010; Scherrer et al., 2010). Loss of either Bhlhb5 or of primary afferent-derived VGLUT2 generates mice with an exaggerated itch phenotype but limited effects on pain processing.

Although there is some limited evidence for differential central targeting of projection neurons that respond to different pruritogens (Davidson et al., 2012), based on the very polymodal (pain and itch responsiveness) properties of projection neurons, and indeed of almost all interneurons, it seems more likely that there is convergence of pain and itch messages to the NK1 receptor-expressing projection neurons. Indeed substance P-saporin-induced deletion of the NK1 receptor-expressing neurons in the rat dorsal horn significantly reduces behaviors indicative of both pain and itch (Carstens et al., 2010). Furthermore, based on our studies in the TR4 mutant mice, in which the great majority of GRPR as well as GRP dorsal horn neurons were lost, with no change in the number of projection neurons, we concluded that the GRPR interneurons must communicate with the projection neuron in order to send itch signals to the brain. Apparently, polymodal interneurons that respond to pain-producing algogens and itch-producing pruritogens converge upon projection neurons that are also polymodal. Unless there are subsets of polymodal projection neurons that engage pain- versus itch-relevant supraspinal targets, the perceptual distinction between pain and itch must result from a different firing code generated in the projection neurons in response to algogens and pruritogens. In this

model, some critical neuronal population in the brain presumably "reads" the patterns of activity generated by different dorsal horn circuits, resulting in a perception of pain or itch.

Conclusions

Despite the tremendous progress in our understanding of the heterogeneity of the functional circuits that populate the dorsal horn, much remains to be learned. With respect to the question of specificity versus patterning of nociceptive (pain) and pruritoceptive (itch) information, it is clearly critical to determine the extent to which there is convergence or segregation of the information processed by the different interneuron pools described above. It is also critical to determine whether selective activation of these different projection neuron populations by different modalities of pain and itch stimuli engages different brain regions. Such questions could be addressed through a combination of, for example, optogenetic or designer receptors exclusively activated by designer drugs (DREADDs) technology (Armbruster et al., 2007) and forebrain imaging in the same animals. Of course, there is also very limited information about the contribution to pain and itch processing of the other spinal cord projection systems, including the lateral spinal nucleus (Burstein et al., 1990; Menetrey and Basbaum, 1987) and the neurons of the deeper dorsal horn. The latter region includes the wide dynamic range neurons of lamina V (Price et al., 2003), which unquestionably transmit nociceptive and probably pruritoceptive information to higher centers.

Future studies will undoubtedly reveal additional neurochemical and functional features of the major subclasses of dorsal horn interneuron, which will demonstrate even greater heterogeneity. Indeed, as noted above, although there are several subtypes of GABAergic islet cell interneuron, the functional significance of these subtypes (e.g., NPY-, NOS-expressing) is unclear. A recent analysis, for example, indicates that a subset of dynorphin-expressing GABAergic interneurons is critical to the regulation of itch and, in fact, is the population lost in the Bhlhb5 mutant mouse that has exaggerated itch (Kardon et al., 2014). To what extent there is comparable heterogeneity of the excitatory interneuron population is also unclear. As the vertical cells appear critical to providing feedforward excitatory drive to the projection neurons, it will be of great interest to address their diversity. Again, even though these interneurons appear to be polymodal, as are almost all projection neurons, these interneurons could still engage different output systems depending on the firing patterns that they generate in response to the different stimuli.

Because our previous reviews focused extensively on the mechanisms that operate in the setting of injury and that underlie pathological conditions of mechanical hypersensivity and ongoing pain, in the present review we have focused primarily on the circuit organization of the normal spinal cord. Of course, there is considerable evidence that these different interneuronal populations are profoundly influenced by the changes in afferent drive that occur when there is persistent inflammation or frank nerve damage. Some cells may die, others may make new connections by sprouting of axons, and, without question, there are profound molecular changes that alter the responsiveness of these interneuron populations to both noxious and innocuous inputs. Finally, we have barely touched on the diversity of the cell surface receptors that populate the neurons in the dorsal horn, including traditional excitatory and inhibitory neurotransmitter receptors, the subtypes of which can be overwhelming. We have also ignored the contribution of neurotrophin receptors, cytokine and chemokine receptors, and the incredibly diverse downstream signaling pathways that can be triggered in these different interneuron populations. A better understanding of the diversity of these biochemical pathways and of the circuits in which they participate will undoubtedly direct the development of novel pharmacotherapies for the treatment of pain and itch, treatments that are hopefully devoid of the common adverse side effects of the current therapies.

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