Dynorphin Acts as a Neuromodulator to Inhibit Itch in the Dorsal Horn of the Spinal Cord

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SUMMARY

Menthol and other counterstimuli relieve itch, resulting in an antipruritic state that persists for minutes to hours. However, the neural basis for this effect is unclear, and the underlying neuromodulatory mechanisms are unknown. Previous studies revealed that Bhlhb5−/− mice, which lack a specific population of spinal inhibitory interneurons (B5-I neurons), develop pathological itch. Here we characterize B5-I neurons and show that they belong to a neurochemically distinct subset. We provide cause-and-effect evidence that B5-I neurons inhibit itch and show that dynorphin, which is released from B5-I neurons, is a key neuromodulator of pruritus. Finally, we show that B5-I neurons are innervated by menthol-, capsaicin-, and mustard oil-responsive sensory neurons and are required for the inhibition of itch by menthol. These findings provide a cellular basis for the inhibition of itch by chemical counterstimuli and suggest that kappa opioids may be a broadly effective therapy for pathological itch.

INTRODUCTION

Itch, like pain, is an aversive sensation that warns us of potential threats to the body (Ross, 2011; Bautista et al., 2014). However, itch is a distinct sensation, characterized by the desire to scratch. Although scratching may remove irritants from the skin (providing at least transient relief from itch), it has the paradoxical effect of causing tissue damage that potentiates itch through release of inflammatory mediators. This pathological itch-scratch-itch cycle is a hallmark of chronic pruritus, which can be just as debilitating as chronic pain (Weisshaar and Dalgaard, 2009; Yosipovitch, 2008). Unfortunately, there are few therapeutic options for those that suffer from severe pathological itch. Whereas mu opioids such as morphine are highly effective for the treatment of pain, these drugs actually worsen itch (Ko and Naughton, 2000; Szarvas et al., 2003). Thus, there is a great need for better therapies to treat intractable pruritus.

One reason that itch has lagged behind pain in terms of effective therapies is because, until recently, we lacked a clear understanding of how itch is detected and encoded in the nervous system. However, over the last few years there has been much progress in this field. There is now good evidence that MrgprA3-expressing sensory neurons selectively mediate itch, even when activated by the classic algogen capsaicin (Han et al., 2013). It is very likely that these are not the only itch-selective fibers, since histamine-dependent itch appears to be mediated by a different subset of sensory neurons (Roberson et al., 2013). Next, itch seems to be relayed by at least two populations of spinal interneurons—those that express the Npra receptor and those that express the gastrin-releasing peptide receptor (GRPR)—before being conveyed to the brain where it is consciously perceived (Mishra and Hoon, 2013; Sun et al., 2009).

Menthol and other forms of counterstimulation, such as scratching, heat, cool, and noxious agents, provide relief of itch that begins almost instantaneously and lasts from minutes to hours (Ward et al., 1996; Yosipovitch et al., 2007; Bromm et al., 1995). This relief occurs even when the counterstimulus is applied at great distances from the source of itch sensation (Nilsson et al., 1997). Together, these psychophysical observations suggest that crossmodal inhibition occurs centrally, possibly within the spinal dorsal horn, where sensory information is first integrated and modulated (Todd, 2010). The instantaneous relief of itch...
experienced upon scratching is presumably mediated by a fast-acting neurotransmitter (Akiyama et al., 2011). In contrast, prolonged inhibition is thought to involve neuromodulators, but the nature of such neuromodulation remains elusive and the neural basis for inhibition of itch by counterstimuli is not known.

We previously generated a mouse model of pathological chronic itch through the constitutive deletion of Bhlhb5 (also known as Bhlhe22), a transcription factor that is transiently expressed in several neuronal subtypes during embryonic and early postnatal development (Ross et al., 2010, 2012). Through selective ablation, we provided strong evidence that the pathological itch in Bhlhb5 mutant mice was due to loss of Bhlhb5 in inhibitory neurons in the spinal dorsal horn. Using fate-mapping approaches, we found that Bhlhb5 mutant mice lack a subset of inhibitory neurons in laminae I and II (Ross et al., 2010). These findings suggested that Bhlhb5 is essential for the survival of a set of spinal inhibitory interneurons (termed B5-I neurons) that are required for normal itch sensation. However, the identity of B5-I neurons was not clear, and how they inhibit itch was not known.

Here we provide evidence that acute inhibition of B5-I inhibitory neurons results in elevated itch. We identify and characterize B5-I neurons, showing that they correspond to specific neurochemically defined populations and that they release the kappa opioid dynorphin. Our data suggest that kappa agonists act locally within the spinal cord to selectively reduce itch and not pain. We find that B5-I cells are directly innervated by primary afferents that respond to counterstimuli, such as heat and coolness, which relieve itch in humans. Moreover, we show that menthol inhibits itch in wild-type mice but does not do so in mice lacking B5-I neurons. Thus, B5-I neurons may mediate the inhibition of itch by chemical counterstimuli.

RESULTS

Acute Inhibition of B5-I Cells Results in Elevated Itch

We previously showed that Bhlhb5 is needed for survival of spinal inhibitory interneurons that are required for normal itch sensation (Ross et al., 2010). To more specifically identify these neurons, we performed coimmunostaining for Bhlhb5 and markers that define distinct populations of spinal interneurons. Bhlhb5 is transiently expressed in ~7% of neurons in the dorsal horn of mice from embryonic day 13.5 to postnatal day 10 (P10), so we performed these experiments using P4 mice. Consistent with our previous report (Ross et al., 2010), we found that three-quarters of Bhlhb5-expressing neurons in superficial dorsal horn (laminae I and II) are inhibitory, as shown by coexpression of Pax2 (Figure 1A). We refer to these Bhlhb5-expressing inhibitory interneurons as B5-I neurons.

The somatostatin receptor sst2A is exclusive to inhibitory neurons in superficial dorsal horn and is found in ~50% of the inhibitory interneurons in this region (Polgár et al., 2013a, 2013b; Todd et al., 1998; Yasaka et al., 2010). To determine whether the B5-I neurons belonged to this subset, we used antibodies against Bhlhb5, Pax2, and sst2A. This immunostaining showed that the vast majority of B5-I neurons (~90%) coexpressed the somatostatin receptor sst2A (Figures 1A and S1A available online). Furthermore, when we recorded from spinal interneurons genetically labeled with the Bhlhb5-cre allele (Ross et al., 2010), half showed strong hyperpolarization in response to somatostatin (Figure 1B), confirming that B5-I neurons express functional sst2A receptors. Given the loss of B5-I neurons in Bhlhb5−/− mice, we reasoned that there would be a corresponding decrease in the number of sst2A-expressing neurons in these animals. As predicted, the number of sst2A-expressing neurons was reduced by two-thirds in Bhlhb5−/− mice, with no significant change in the number of sst2A-negative inhibitory neurons (Figures 1C and 1D). Thus, the vast majority of B5-I neurons belong to the subset of inhibitory spinal interneurons that express sst2A, and a large proportion of the sst2A-expressing population is missing in Bhlhb5−/− mice.

Since somatostatin inhibits neuronal activity and sst2A is the only somatostatin receptor that is expressed by dorsal horn neurons (see http://www.brain-map.org), the finding that B5-I neurons express sst2A allowed us to directly test the idea that B5-I neurons normally function to inhibit itch. This experiment was important because, although we had previously shown that loss of B5-I neurons during development is associated with abnormally elevated itch (Ross et al., 2010), the evidence was merely correlational. Specifically, it was not clear whether B5-I neurons function in the adult to inhibit itch, or whether B5-I neurons play a key developmental role in the formation of proper itch circuits. We hypothesized that if B5-I neurons normally function to inhibit itch, then acute inhibition of these neurons by somatostatin would increase itch sensitivity (Figure 1E). Indeed, upon intrathecal injection of the somatostatin analog octreotide, we observed vigorous scratching, biting, and licking behavior that was suggestive of itch (Figure 1F), consistent with previous reports (Seybold et al., 1982). This spontaneous behavior was dose dependent, with an immediate onset and a duration of approximately half an hour.

Because B5-I neurons account for the majority (two-thirds) of sst2A-expressing cells, the finding that acute treatment with octreotide results in elevated scratching behavior is consistent with the hypothesis that B5-I neurons inhibit itch. Nevertheless, it remained possible that the observed scratching behavior was due instead to the effect of octreotide on the one-third of sst2A-expressing neurons that are not B5-I neurons. We therefore tested the effect of octreotide on Bhlhb5−/− mice, which lack B5-I neurons. Specifically, we reasoned that, if octreotide-induced scratching is due to inhibition of B5-I neurons, this treatment would have no effect in mice that lack these cells. As predicted, we observed very little scratching behavior upon intrathecal injection of 100 nM octreotide in Bhlhb5−/− mice (Figure 1F). This finding strongly suggests that octreotide-induced itch behavior is due to inhibition of B5-I neurons.

To further assess whether the octreotide-induced scratching was due to elevated itch (rather than a nociceptive response or a grooming behavior), we tested the effect of octreotide on pruritogen-evoked itch. For these experiments, we selected a very low dose of octreotide that had no significant effect on its own (3 ng) and tested its effect on chloroquine-induced itch. We found that intrathecal octreotide significantly increased the amount of time that mice spent biting at the injection site in response to intradermal chloroquine (Figure 1G). In contrast, this dose of intrathecal octreotide had no effect on acute nociceptive reflexes, as measured by hindpaw withdrawal latency.
Figure 1. Acute Inhibition of B5-I Cells Results in Elevated Itch

(A) The inhibitory subset of Bhlhb5-expressing neurons in the superficial dorsal horn (B5-I neurons) coexpress sst2A. Spinal cord sections from P4 mice were immunostained to reveal Bhlhb5 (green), sst2A (red), and the inhibitory marker Pax2 (blue). The vast majority (~90%) of cells expressing Bhlhb5 and Pax2 in laminae I and II co-label with sst2A (arrows). A single confocal optical section through laminae I-IIo is shown. Scale bar, 20 μm.

(B) Outward current was observed upon application of somatostatin (SST, 1 μM) to 50% of cells with the Bhlhb5-cre allele. (Note that the Bhlhb5-cre allele also labels other populations including excitatory neurons, which do not respond to SST.)

(C) The number of sst2A-expressing neurons in dorsal horn is significantly diminished in Bhlhb5-/- mice. Spinal cord sections from 4- to 5-week-old wild-type (top) or Bhlhb5-/- (bottom) mice were immunostained to reveal sst2A (green) and Pax2 (magenta). Approximately half of the inhibitory (Pax2-expressing) neurons in laminae I and II express sst2A in wild-type mice (arrows), and these cells are dramatically reduced in Bhlhb5 mutant mice. A single optical section is shown. Scale bar, 50 μm.

(D) Quantification of (C). There is a significant reduction (*p < 0.05) in the number of sst2A-expressing neurons (sst2A +ve) in laminae I-II of the dorsal horn in Bhlhb5-/- relative to wild-type (WT), with no significant change (NS) in the number of Pax2-positive inhibitory interneurons that do not express sst2A (sst2A –ve). Data are represented as mean ± SD number of cells in laminae I-II per dorsal horn through 100 μm cord taken from L4 (n = 6 mice per genotype, 2 dorsal horns per mouse), analyzed by two-way ANOVA followed by pairwise comparison using the Holm-Sidak method.

(E) Schematic depicting inhibition of sst2A-expressing interneurons with the somatostatin analog octreotide, resulting in elevated itch.

(F) Intrathecal administration of octreotide (3, 10, 30, or 100 ng in 5 μl vehicle) dose dependently evoked spontaneous scratching behavior in wild-type mice (n = 6–8 mice/treatment). Bhlhb5-/- mice receiving 100 ng of octreotide intrathecally showed very little scratching response. Intrathecal injections were confirmed by coinjection of 10% methylene blue (n = 8 mice). Total scratching bouts (mean ± SEM) were measured over a 30 min observation period. One-way ANOVA was used to compare mean scratch bouts between treatment groups followed by Tukey’s post hoc test (* indicates significantly different than PBS, p < 0.05).

(G) Pruritogen-induced itch behavior was significantly enhanced following intrathecal octreotide (3 ng). Chloroquine (100 μg) was injected intradermally in the calf 30 min after treatment with either octreotide (i.t.) or vehicle (PBS; i.t.). Itch behavior was defined as the cumulative amount of time spent biting/licking the injection site over 30 min. Data are represented as mean ± SEM (*p < 0.05, Student’s t test). Also see Figure S1 for the specificity of octreotide-mediated behavioral effects.
on a hot plate (Figure S1B). Furthermore, the effect of intrathecal octreotide was very likely mediated by spinal neurons (rather than the central terminals of primary afferents) since intradermal octreotide caused no itch-like behavior (Figure S1C). Together, these findings suggest that acute inhibition of B5-I neurons results in elevated itch.

**Neurochemical Identification of B5-I Neurons**

Sst2A-expressing inhibitory neurons in laminae I-II can be further subdivided based on the presence or absence of galanin and neuronal nitric oxide synthase (nNOS), which are expressed in mostly nonoverlapping subsets (Figure 2B; Iwagaki et al., 2013; Tiong et al., 2011). To investigate whether B5-I neurons constitute one or more of these subsets, we performed immunostaining experiments. These experiments revealed that virtually all (~95%) of the galanin-expressing cells coexpress Bhlhb5 and that these account for 78.1% (range: 72.8%–86.4%) of B5-I neurons (image from lamina I-Ii). Numerous nNOS-immunoreactive cells (magenta) as shown by the arrow (n = 3, image from lamina I). NPY seldom colocalized with Bhlhb5 (2.3% of NPY cells, range 1.1%–4.7%, n = 3; arrowheads illustrate cells that do not colocalize, lamina II). Images are single confocal optical sections from mice of indicated ages.

(B) Populations of inhibitory interneurons in laminae I-II of adult dorsal horn. Approximately 54% of these cells express sst2A (sst2A positive), and these can be further subdivided into classes based on expression of galanin (red), nNOS (blue), galanin and nNOS (magenta), or sst2A only (neither galanin nor nNOS; green). B5-I neurons belong to the classes that express galanin and/or nNOS.

(C) Single optical sections of laminae I-II from wild-type or Bhlhb5−/− mice reveal a dramatic loss of the sst2A-expressing cells that contain galanin (red, double-headed arrow) and nNOS (blue, arrows), while the sst2A-expressing cells that contain neither galanin nor nNOS (sst2A only) are still present (arrowheads). Scale bar, 20 μm.

(D) Quantification of (C) showing a significant reduction in cells expressing galanin (red), nNOS (blue), and galanin/nNOS (magenta). There was no significant change (NS) in the number of cells expressing sst2A alone (green). Data are represented as mean ± SD number of cells in laminae I-II per dorsal horn through 100 μm cord taken from L4 (n = 6 mice, genotype, 2 dorsal horns/mouse), analyzed by two-way ANOVA followed by pairwise comparison using the Holm-Sidak method (* indicates p < 0.05). See Figure S2 for large field views.

Figure 2. B5-I Neurons Belong to a Population that Expresses Galanin and/or nNOS

(A) The vast majority (95.4%, range 95.3%–95.7%, n = 3) of galanin-immunoreactive cells in laminae I-II (magenta) were Bhlhb5 immunoreactive (green; double arrow), and galanin-expressing cells accounted for 78.1% (range: 72.8%–86.4%) of B5-I neurons (image from lamina I-Ii). Numerous nNOS-immunoreactive cells (magenta) as shown by the arrow (n = 3, image from lamina I). NPY seldom colocalized with Bhlhb5 (2.3% of NPY cells, range 1.1%–4.7%, n = 3; arrowheads illustrate cells that do not colocalize, lamina II). Images are single confocal optical sections from mice of indicated ages.

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of sst2A-expressing cells that contained neither galanin nor nNOS did not differ significantly between wild-type and Bhlhb5−/− mice. This analysis of sst2A-expressing interneurons, together with the previous quantification of inhibitory neurons that lack sst2A (Figure 1D), suggest that the galanin and nNOS populations of inhibitory neurons are severely depleted in Bhlhb5−/− mice, whereas all other inhibitory populations are unchanged.

B5-I Neurons Express the Kappa Opioid Dynorphin

The loss of galanin cells in the Bhlhb5−/− mice was of particular interest because these cells also express the kappa opioid dynorphin (Bröhl et al., 2008; Sardella et al., 2011), and there is precedent for the idea that kappa opioids inhibit itch (Inan and Cowan, 2004; Ko et al., 2003; Togashi et al., 2002). We therefore confirmed that dynorphin is expressed in B5-I neurons by reacting spinal cords from P4 mice with antibodies against Bhlhb5 and the dynorphin precursor, preprodynorphin (PPD; Figure 3A). As expected, we found that virtually all dynorphin-expressing neurons in laminae I-II were Bhlhb5 immunoreactive. We also assessed the number of dynorphin-expressing inhibitory interneurons in adult Bhlhb5−/− animals with antibodies against either PPD or dynorphin B, a cleavage product of the full-length dynorphin peptide. These experiments revealed an almost complete loss of dynorphin-expressing inhibitory interneurons in Bhlhb5−/− mice (Figures 3B, 3C, S3B, S3A and S3C), consistent with the finding that galanin-expressing neurons are largely absent in these mice.

Kappa Opioids Inhibit Itch in Response to a Variety of Pruritogens

The finding that Bhlhb5−/− mice lack spinal inhibitory neurons that release dynorphin raised the possibility that B5-I neurons normally inhibit itch in part through activation of the kappa opioid receptor (KOR). As a first step to test this idea, we investigated the effect of kappa agonists nalfurafine and U-50,488 (Figure 4A; Morgan and Christie, 2011; Wikström et al., 2005; Williams et al., 2013) on acute pruritogen-evoked itch behavior. To investigate whether kappa agonists inhibit itch mediated by MrgprA3/C11-expressing afferents, we quantified scratch bouts following intradermal injection of chloroquine into the nape of the neck of mice that had been pretreated with either nalfurafine (20 μg/kg) or vehicle. We found that nalfurafine significantly reduced chloroquine-induced itch behavior (Figures 4B and 4C), consistent with previous findings (Inan and Cowan, 2004). Likewise, nalfurafine significantly attenuated SLIGRL-mediated itch (Figure 4D). To extend these observations, we tested the effect of U-50,488 (3 mg/kg), a molecularly distinct kappa opioid agonist and observed a similar inhibition of chloroquine- and SLIGRL-induced scratching (Figures 4B, 4C, and 4D). Thus, kappa agonists inhibit itch mediated by MrgprA3/C11-expressing sensory neurons.

Recent studies have revealed that histamine-induced itch is different than chloroquine-induced itch in that it is mediated by a distinct subset of primary afferents (Roberson et al., 2013). We therefore tested the effect of KOR agonists on histamine-induced itch and found that scratching behavior was significantly reduced by nalfurafine, as previously reported (Togashi et al., 2002), as well as by U-50,488 (Figure 4E). Similarly, our experiments revealed that both nalfurafine and U-50,488 significantly reduced serotonin-induced itch (Figure 4F). Finally, we investigated a dry skin model of itch that develops following daily topical application of acetone/ether followed by water (AEW) (Akiyama et al., 2010b; Miyamoto et al., 2002). Both kappa agonists significantly reduced spontaneous scratching behavior produced by AEW treatment (Figure 4G). Importantly, neither U-50,488 (3 mg/kg) nor nalfurafine (20 μg/kg) had any significant effect on rotarod performance, indicating that their effects were not due to motor impairment (Figure S4A). Thus, KOR agonists significantly abate various types of pruritus, including histamine-dependent and histamine-independent itch.
These findings raised the possibility that decreased kappa opioid signaling, due to loss of dynorphin-expressing spinal interneurons, contributes to the abnormally elevated itch in Bhlhb5−/− mice. Thus, we reasoned that exogenous KOR agonists would relieve abnormal itch in these animals. As observed previously, we found that intradermal injection of chloroquine caused significantly more scratching in Bhlhb5−/− mice relative to littermate controls (Figure 4H; Ross et al., 2010). Importantly, pretreatment with nalfurafine almost completely abrogated scratching behavior in Bhlhb5−/− mice, consistent with the idea that abnormally elevated itch responses in these mice are partly due to decreased kappa tone in spinal cord (Figure 4H).
A question that remained unclear was whether the elevated itch in Bhlhb5−/− mice was simply due to the loss of dynorphin, or whether the absence of fast synaptic inhibition from B5-I neurons was also involved. To test whether constitutive loss of dynorphin was sufficient for abnormally elevated itch, we analyzed itch in mice lacking the dynorphin precursor PPD. We found that PPD−/− mice and their wild-type littermates showed no difference in pruritogen-induced itch behavior (Figure 4I). This observation suggests that the abnormally elevated itch observed in Bhlhb5−/− mice is not due to loss of spinal dynorphin alone, hinting at a key role for GABA and/or glycine in the inhibition of itch by B5-I neurons.

A consequence of the loss of B5-I neurons in Bhlhb5−/− mice is that these mice develop spontaneous skin lesions due to severe pathological itch. To test whether treatment with KOR agonists might provide therapeutic relief for neuropathic itch, we tested these drugs on Bhlhb5−/− mice with pruritic skin lesions. Systemic treatment with either U-50,488 or nalfurafine significantly reduced the amount of time Bhlhb5−/− mice spent biting and/or licking the site of lesion by 33% ± 14% and 40% ± 22%, respectively (Figures S4B and S4C), suggesting that kappa opioids have therapeutic potential for neuropathic itch conditions.

Kappa Opioids Are Selective for Itch
Because of the key role of mu opioids in inhibition of pain, numerous groups have assessed the potential role of KOR agonists as analgesics (Kivell and Prisinzano, 2010; Vanderah, 2010). While KOR agonists were found to be analgesic in some acute, inflammatory, and neuropathic pain tests, their analgesic efficacy at doses that do not affect motor coordination remains unclear (Leighton et al., 1988; Stevens and Yaksh, 1986). We therefore wondered whether a concentration sufficient to inhibit itch (i.e., 20 µg/kg of nalfurafine) is selective for pruritoception rather than nociception. To address this question, we used the cheek model (Figures 5A and 5B), in which pruritic agents elicit scratching with the hindlimb, whereas nociceptive substances
cause wiping with the forepaw (Shimada and LaMotte, 2008; Akiyama et al., 2010a). As expected, intradermal injection of chloroquine into the cheek induced robust hindlimb-mediated scratching with minimal wiping behavior, indicative of itch. Systemic pretreatment with nalfurafine led to an almost complete suppression of scratching, with no significant effect on wiping behavior (Figures 5C and 5D), in accordance with the idea that kappa agonists inhibit itch.

Next, to investigate the effect of kappa agonists on nociception, we injected capsaicin into the cheek. This treatment evoked intense site-directed wiping with little scratching, in keeping with the idea that pain is the predominant sensation elicited by capsaicin. Importantly, capsaicin-induced wiping was not affected by pretreatment with nalfurafine (Figure 5F), suggesting that nociceptive responses were unaffected by kappa opioid signaling. In contrast, the modest scratching in response to capsaicin was almost completely abolished following treatment with nalfurafine (Figure 5E). These results suggest that kappa opioid agonists, at least at low doses, can selectively inhibit itch with no effect on pain.

**KOR Agonists and Antagonists Bidirectionally Modulate Itch at the Spinal Cord Interface**

The finding that systemic kappa opioids inhibit itch, together with our discovery that Bhlhb5−/− mice lack dynorphin-expressing spinal interneurons, raised the possibility that endogenous dynorphin and exogenous kappa opioids modulate itch through common neural circuits in the spinal cord. To test the idea that the inhibition of itch by kappa opioids is due, at least in part, to activation of spinal KORs, we manipulated KOR signaling in the spinal cord through intrathecal delivery of KOR agonists. Since intrathecal injections allowed us to target dermatomes L3–L5 (corresponding to the hindlimbs), itch behavior was assessed using the calf model (LaMotte et al., 2011), in which injection of a pruritic agent into the skin elicits a biting response (Figure 6A). Importantly, we found that intrathecal administration of either U-50,488 (10 μg) or nalfurafine (40 ng) to the lumbar spinal cord significantly reduced chloroquine-evoked biting (Figure 6B). These findings suggest that activation of KORs in the spinal cord is sufficient to inhibit itch.

A key question is the identity of the cellular targets for kappa opioids within the spinal cord. Though the central processing of itch is not clearly understood, recent work has suggested that itch information is sequentially relayed by at least two types of spinal interneurons (Npra-expressing neurons followed by GRPR-expressing neurons) before being transmitted to the brain (Mishra and Hoon, 2013). We therefore investigated whether kappa opioids act upstream or downstream of GRPR-expressing neurons by testing the effect of nalfurafine on GRP-mediated itch. Intrathecal injection of GRP caused robust scratching that was significantly reduced by nalfurafine (Figure 6C). This finding suggests that kappa agonists mediate their effect (either directly or indirectly) on GRPR-expressing neurons, or on neurons downstream of GRP activation in the spinal cord.

Next, we reasoned that if B5-I neurons normally release dynorphin to inhibit itch, then blocking endogenous KOR signaling in the dorsal horn might result in elevated itch. To test this idea, we investigated whether treatment with the KOR antago-

nists norbinaltorphimine (norBNI) or 5'-guanidinonaltrindole (5’GNTI; Figure 6D) could trigger an enhanced response to chloroquine in the calf. We found that chloroquine-induced biting was significantly increased by intrathecal norBNI. Likewise treatment with 5’GNTI intrathecally increased the amount of chloroquine-induced biting relative to control (Figure 6E). The finding that blocking KOR signaling increases itch response to chloroquine suggests that endogenous spinal dynorphin normally functions to dampen itch. Together, these results show that modulating opioid tone in the spinal cord can bidirectionally alter itch sensitivity—increasing kappa opioid signaling causes decreased itch, whereas decreasing kappa opioid signaling results in increased itch.

**B5-I Neurons Mediate Inhibition of Itch by Chemical Counterstimuli**

In light of the finding that B5-I neurons function to inhibit itch, we wished to characterize these cells in more detail. We performed patch-clamp recordings from lamina II neurons genetically labeled with the Bhlhb5-cre allele (Figure 7A). Since this allele labels a somewhat broader population than those that we define as B5-I neurons, we used hyperpolarization in response to somatostatin to confirm that we were recording from B5-I neurons. Four basic firing patterns can be identified in lamina II interneurons in response to injection of depolarizing current: tonic, delayed, phasic/transient, and single spiking (Graham et al., 2007; Heinke et al., 2004; Ruscheweyh and Sandkühler, 2002). We found that the majority (29 out of 34) of B5-I neurons showed tonic firing (Figures 7B and S5A) and may therefore function as integrators. Neurons can be classified based on morphology and previous studies have described several types including vertical, islet, central, and radial, although many cells cannot be classified according to this scheme (Grudt and Perl, 2002; Yasaka et al., 2007, 2010). To determine whether B5-I neurons belonged to any of these subsets, we reconstructed B5-I neurons. Though B5-I neurons did not fit strictly into a single class, the majority were either central or unclassified, with axons and dendrites mainly restricted to lamina II (Figure 7C). Thus, B5-I neurons are likely to be involved in integrating sensory input within the substantia gelatinosa.

One of the hallmarks of itch is that it is relieved by a variety of counterstimuli, such as scratching, noxious chemicals, or menthol (Bromm et al., 1995; Ward et al., 1996; Yosipovich et al., 2007). While the neural basis for this phenomenon is unknown, it has been suggested that counterstimuli reduce itch through activation of spinal inhibitory interneurons (Akiyama et al., 2011; Ma, 2010; Patel and Dong, 2010; Ross, 2011; Baustista et al., 2014). Based on our findings, B5-I neurons seemed well positioned to mediate the inhibition of itch by counterstimuli. If so, we reasoned that they would receive input (either directly or indirectly) from primary afferents that mediate the counterstimuli. Capsaicin, mustard oil, and menthol activate discrete subsets of primary afferents (those that express TrpV1, TrpA1, and TrpM8, respectively). Since topical treatment with any of these substances can inhibit itch, we tested whether B5-I neurons receive input from primary afferents that express TrpV1, TrpA1, or TrpM8 (Figure 7D). Upon application of capsaicin to depolarize TrpV1-expressing afferents, we saw a significant increase...
in the frequency of spontaneous excitatory postsynaptic currents (sEPSCs) in 80% (4 of 5) of B5-I neurons, with an average 7.8-fold increase in EPSC frequency (Figures 7E and 7F). Moreover, a significant increase in mEPSC frequency was likewise observed in the presence of tetrodotoxin (TTX) to block action potential propagation, suggesting that B5-I neurons receive direct input from capsaicin-sensitive sensory neurons (Figure S5B). Similarly, allyl isothiocyanate, a key component of mustard oil, resulted in increased sEPSC frequency in 86% (6 out of 7) B5-I neurons, with an average increase of 3.3-fold (Figures 7G and 7H). Finally we observed that sEPSC frequency was also significantly increased 2.5-fold by menthol in 90% (9 out of 10) of B5-I neurons (Figures 7I and 7J). Once again, the increase in mEPSC frequency in response to capsaicin, mustard oil, and menthol was also observed in the presence of tetrodotoxin (TTX) to block action potentials, suggesting that TrpA1- and TrpM8-expressing afferents directly innervate B5-I neurons (Figures S5B, S5C, and S5D).

The finding that B5-I neurons receive direct input from sensory neurons that respond to capsaicin, mustard oil, and menthol is consistent with the idea that B5-I neurons mediate the inhibition of itch by chemical counterstimuli. To directly test this possibility, we developed a mouse model of inhibition of itch by menthol. When wild-type mice were treated with 8% menthol (topically) on the cheek, this caused a significant reduction in subsequent chloroquine-induced scratching. In contrast, Bhlhb5−/− mice showed no significant inhibition of itch by menthol (Figure 8A). These findings suggest that B5-I neurons are required for the inhibition of itch by menthol (Figure 8B).

DISCUSSION

While our everyday experience that itch is relieved by counterstimulation indicates that itch is under inhibitory control, the neural basis for this phenomenon has remained obscure and neuromodulators of itch have not been identified. Here we begin to shed...
light on this issue by identifying a neuronal subtype in the spinal cord—B5-I neurons—that inhibits itch. We discover that B5-I neurons correspond to specific neurochemical populations and show that they are the major source of the endogenous kappa opioid dynorphin in the dorsal horn. Our data suggest that kappa opioids selectively inhibit itch without affecting pain. Indeed, modulation of kappa opioid tone in the spinal cord can bidirectionally control itch sensitivity, implying that dynorphin acts as a neuromodulator. Finally, we demonstrate that B5-I neurons mediate the inhibition of itch by chemical counterstimuli (Figure 8B).

Dorsal Horn Interneurons

Inhibitory interneurons, which use GABA and/or glycine, account for 25%–30% of neurons in laminae I-II (Polgár et al., 2003, 2013b) and are thought to perform several distinct roles in sensory processing (Hughes et al., 2012; Ross, 2011; Sandkühler, 2009). To understand how these cells modulate somatosensory input, it is essential to distinguish different functional populations among them (Graham et al., 2007; Todd, 2010). The most widely accepted scheme for classifying superficial dorsal horn interneurons was developed by Grudt and Perl (2002), who identified four main groups, based largely on morphological criteria. However, though others have used this scheme, ~30% of neurons in these studies could not be classified based on morphology (Heinke et al., 2004; Maxwell et al., 2007; Yasaka et al., 2007, 2010). Moreover, with the exception of islet cells, inhibitory neurons are morphologically diverse (Yasaka et al., 2010). Thus, morphology does not appear to be particularly useful for defining inhibitory interneuron subpopulations.

An alternative approach uses the wide array of neuropeptides, receptors, and other proteins that are differentially expressed by dorsal horn neurons (Polgár et al., 2013a, 2013b). We previously found that ~50% of inhibitory cells in laminae I-II express sst2A, and these can be further subdivided into subpopulations that contain galanin and/or nNOS (which constitute ~60% of the sst2A-expressing cells and therefore approximately one-third of all the inhibitory neurons). The galanin cells coexpress PPD and are the major source of dynorphin in the superficial laminae (Brodh et al., 2008; Sardella et al., 2011). In addition, we identified two other nonoverlapping groups among inhibitory interneurons that lack sst2A (NPY- and parvalbumin-expressing cells). There is already evidence that these neurochemical classes differ, both in their responses to noxious stimuli and in their postsynaptic targets (Todd, 2010; Hughes et al., 2012; Polgár et al., 2013b). The present results provide further evidence in support of this classification scheme, since Bhlhb5−/− mice show a loss of

(E–J) Presynaptic effect of 2 μM capsaicin (E), 100 μM allyl isothiocyanate (mustard oil) (G), and 500 μM menthol (I). The majority of B5-I neurons showed a significant increase in sEPSC frequency after bath application of capsaicin (80%; F), mustard oil (86%; H), and menthol (90%; J). See Figure S5 for further electrophysiological analysis of B5-I neurons.
inhibitory interneurons that is apparently restricted to neurochemically defined populations.

**Identification and Characterization of B5-I Neurons**

We find that B5-I neurons correspond to two (mostly nonoverlapping) subpopulations—those that coexpress galanin and dynorphin and those that express nNOS. The subpopulation of B5-I neurons that expresses galanin/dynorphin likely uses GABA as its fast transmitter (Simmons et al., 1995), whereas the B5-I neurons that express nNOS are thought to release GABA and glycine (Spike et al., 1993). Since relief of itch by counterstimuli begins almost instantaneously, we favor the idea that this component is mediated by fast-acting inhibitory transmitters. In contrast, dynorphin, which modulates neuronal activity via G protein-coupled receptors, may underlie prolonged suppression of itch.

A key finding from our study is that the loss of B5-I neurons (which results in an almost complete absence of dynorphin in the spinal cord) has a different phenotypic outcome than loss of dynorphin alone. Thus, Bhlhb5−/− mice show dramatically elevated itch, whereas PPD−/− mice display normal itch sensitivity. This distinction implies that an organism can compensate for the loss of dynorphin, but not for the loss of dynorphin-expressing neurons in the dorsal horn. We speculate that neuromodulatory mechanisms may be particularly amenable to homeostatic compensation (Doi and Ramirez, 2010). In keeping with this idea, mice lacking either enkephalin or the mu opioid receptor have subtle pain phenotypes (König et al., 1996; Matthes et al., 1996), despite the fact that mu opioids are among the most effective analgesics. Adaptation also occurs in response to chronic opioid overexposure, as shown by the tolerance observed in humans and animal models following long-term treatment with opioid analogues (Morgan and Christie, 2011; Williams et al., 2013). These examples underscore the idea that the nervous system is robust in its ability to adjust neural circuit function over time when opioid neuromodulatory function is abnormal. In contrast, neural circuits in the dorsal horn are unable to normalize itch sensitivity when B5-I neurons are lacking, emphasizing the fundamental requirement of this neuronal subtype for the normal manifestation of itch.

**B5-I Cells Mediate the Inhibition of Itch by Counterstimuli**

The idea that spinal interneurons are involved in sharpening acuity between sensory modalities has been proposed by us and others (Ma, 2010; Ross, 2011; Zheng et al., 2010; Prescott et al., 2014). In keeping with this idea, conditional loss of VGLUT2 in subsets of primary afferents resulted in mice showing decreased nociceptive responses but heightened pruritic responses, suggesting a role for inhibitory neurons in the suppression of itch by noxious input (Lagerström et al., 2010; Liu et al., 2010). However, the specific identity of spinal interneurons that mediate this type of inhibition was unknown. The B5-I neurons that we describe here are well suited for this role since they receive direct input from primary afferents that are known to suppress itch. In addition, we now provide direct evidence that B5-I neurons suppress itch, since acute inhibition of these cells results in spontaneous scratching. Finally, we show that, whereas menthol inhibits itch in wild-type mice, it does not do so in mice that lack B5-I neurons. Together, these data suggest that B5-I neurons mediate the inhibition of itch by menthol and likely other chemical counterstimuli.

Our findings also suggest that specific neuromodulators may be involved in selectively tuning different types of somatosensory input. This has strong precedent elsewhere in the nervous system, where kappa and mu opioid receptors have distinct (and often opposing) neuromodulatory roles. In the limbic system, mu opioids are euphoric while kappa opioids are dysphoric (Pfeiffer et al., 1986; Schlaepfer et al., 1998). In the hypothalamus, mu and kappa opioids have opposing effects on body temperature (Xin et al., 1997). Indeed, mu and kappa receptor-expressing neurons have been found to inhibit one another directly, thereby mediating the mutually antagonistic effects in modulation of pain by the nucleus raphe magnus (Pan et al., 1997). Now parallels are beginning to emerge in the spinal cord, where mu agonists specifically target nociception, and kappa agonists, as we show here, selectively inhibit itch.

**Kappa Opioid Agonists as a Treatment for Pruritus**

Pruritus is one of the most common adverse effects following spinal administration of mu opioid agonists, affecting >50% of patients receiving epidural morphine (Kjellberg and Tramèr,
stated. To generate age-matched wild-type and on 4- to 5-week-old mice that did not have skin lesions, unless otherwise Bhlhb5 mated. Immunocytochemistry was performed using standard protocols. The anti-Immunocytochemistry۔

**EXPERIMENTAL PROCEDURES**

**Animals and Behavioral Experiments**

Most behavioral tests were carried out on 6- to 8-week-old male C57Bl/6 mice. Experiments that involved Bhlhb5−/− mice (Ross et al., 2010) were performed on 4- to 5-week-old mice that did not have skin lesions, unless otherwise stated. To generate age-matched wild-type and Bhlhb5−/− mice, we harem mated Bhlhb5−/− mice and wild-type and Bhlhb5−/− offspring from the resulting litters were used. In all experiments, the observer was blind to genotype and/or treatment. The use of animals was approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh and/or the Ethical Review Process Applications Panel of the University of Glasgow. Experiments performed in A.J.T.’s lab were in accordance with the UK Animals (Scientific Procedures) Act 1986. Further details are provided in the Supplemental Experimental Procedures.

**Immunocytochemistry**

Immunocytochemistry was performed using standard protocols. The antibodies used in the study are listed in Table S1, and details of image analysis are given in the Supplemental Experimental Procedures.

**Electrophysiology**

Laminectomies were performed on 4- to 6-week-old mice was performed, and the spinal cord was excited to prepare parasagittal or transverse slices. We defined neurons as being sensitive to a particular drug when the synaptic response was altered by more than ±50%. Biocytin-filled cells were reconstructed with Neuruloida (MicroBrightField). Further details are provided in the Supplemental Experimental Procedures.

**SUPPLEMENTAL INFORMATION**

Supplemental Information includes Supplemental Experimental Procedures, five figures, and one table and can be found with this article online at http://dx.doi.org/10.1016/j.neuron.2014.02.046.

**AUTHOR CONTRIBUTIONS**

A.P.K., X.C., C.R.F., G.M.H., C.S.B., and E.S.S. performed and analyzed behavioral experiments with supervision from S.E.R. E.P., D.C., and S.S. performed and analyzed immunohistochemical experiments with supervision from A.J.T. J.H., L.M.S., and S.K. performed and analyzed electrophysiological experiments with supervision from H.R.K. and S.E.R. H.N., C.S., M.W., T.F., and T.K. contributed reagents. A.P.K., E.P., J.H., L.M.S., A.J.T., and S.E.R. wrote the paper.

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**REFERENCES**

Abboud, T.K., Lee, K., Zhu, J., Reyes, A., Afrasiabi, A., Mantilla, M., Steffens, Z., and Chai, M. (1990). Prophylactic oral naltrexone with intrathecal morphine for cesarean section: effects on adverse reactions and analgesia. Anesth. Analg. 71, 367–370.

Akiyama, T., Carstens, M.I., and Carstens, E. (2010a). Differential itch- and pain-related behavioral responses and mu-opioid modulation in mice. Acta Derm. Venereol. 90, 575–581.

Akiyama, T., Carstens, M.I., and Carstens, E. (2010b). Spontaneous itch in the absence of hyperalgesia in a mouse hindpaw dry skin model. Neurosci. Lett. 484, 62–65.

Akiyama, T., Iodi Carstens, M., and Carstens, E. (2011). Transmitters and pathways mediating inhibition of spinal itch-signaling neurons by scratching and other counterstimuli. PLoS ONE 6, e22665.

Arvidsson, U., Riedl, M., Chakrabarti, S., Vulchanova, L., Lee, J.H., Nakano, A.H., Lin, X., Loh, H.H., Law, P.Y., Wessendorf, M.W., et al. (1995). The kappa-opioid receptor is primarily postsynaptic: combined immunohistochemical localization of the receptor and endogenous opioids. Proc. Natl. Acad. Sci. USA 92, 5062–5066.

Bautista, D.M., Wilson, S.R., and Hoon, M.A. (2014). Why we scratch an itch: the molecules, cells and circuits of itch. Nat. Neurosci. 17, 175–182.

Bohnì D., Streihle, M., Wende, H., Hori, K., Bormuth, I., Nave, K.A., Muller, T., and Birchmeier, C. (2008). A transcriptional network coordinately determines

2001). Naltrexone, a mu opioid receptor antagonist, is commonly coadministered to reduce the intensity of pruritus, but its use is limited due to its antianalgesic effects (Abboud et al., 1990). Interestingly, nalbuphine, a mixed kappa opioid agonist/mu opioid antagonist, is extremely effective in reducing postoperative pruritus (Liao et al., 2011). Unlike naltrexone, nalbuphine does not significantly antagonize the analgesic effects of epidural morphine. This suggests that the ability of nalbuphine to reduce morphine-induced itch is largely due to its KOR agonist activity. Consistent with this, KOR agonists have been shown to reduce morphine-induced itch in monkey (Ko et al., 2003). These findings raise the possibility that coadministration of mu and kappa opioids (or the use of agonists with affinities for both receptors) may offer pain relief without causing itch.

An important question raised by our study is the identity of the dorsal horn neurons that respond to kappa opioids. KORs have been detected on some neurons in laminae I-II (Arvidsson et al., 1995), and approximately 15% of lamina II neurons are hyperpolarized by kappa opioids (Eckert and Light, 2002; Peckys and Landwehrmeyer, 1999). While we do not yet know the identity of these cells, our finding that GRP-evoked itch is attenuated by nalfurafine is consistent with the idea that kappa opioids directly inhibit GRPR-expressing spinal interneurons. Alternatively, kappa opioids may act downstream, targeting as-yet-undefined interneurons or projection neurons that mediate itch. Identifying the dorsal horn neuronal subtype(s) that express the KOR will be of great interest, as these cells may represent a point of convergence between neural circuits mediating itch and those responsible for inhibition of itch by counterstimuli.

Several clinical trials have shown that nalfurafine is effective in reducing itch in patients with chronic renal failure (Kumagai et al., 2012; Wikström et al., 2005). Furthermore, nalfurafine is well tolerated, and dysphoria is not reported even after 1 year of treatment. Our study provides insight into the mechanism through which kappa agonists inhibit itch, raising the possibility that this class of drugs may be broadly applicable as antipruritics. Thus, kappa agonists may have therapeutic potential for the treatment of pruritus resulting from a wide range of dermatological and systemic diseases.
transmitter and peptidergic fates in the dorsal spinal cord. Dev. Biol. 322, 381–393.

Bromb, B., Scharain, E., Darso, U., and Ring, J. (1995). Effects of menthol and cold on histamine-induced itch and skin reactions in man. Neurosci. Lett. 187, 157–160.

Doi, A., and Ramirez, J.M. (2010). State-dependent interactions between excitatory neuromodulators in the neuronal control of breathing. J. Neurosci. 30, 8251–8262.

Eckert, W.A., 3rd, and Light, A.R. (2002). Hyperpolarization of substantia gelatinosa neurons evoked by mu-, kappa-, delta 1-, and delta 2-selective opioids. J. Pain 3, 115–125.

Graham, B.A., Bricta, A.M., and Callister, R.J. (2004). Kappa opioid agonists suppress chloroquine-induced pruritus evoked by subcutaneous or intrathecal administration in the rat. Br. J. Pharmacol. 142, 825–831.

Hughes, D.I., Sikander, S., Kinnon, C.M., Boyle, K.A., Watanabe, M., Callister, R.J., and Graham, B.A. (2012). Morphological, neurochemical and electrophysiological features in the rodent superficial dorsal horn. J. Physiol. 569, 189–207.

Han, L., Ma, C., Liu, O., Weng, H.J., Cui, Y., Tang, Z., Kim, Y., Nie, H., Qu, L., Patel, K.N., et al. (2013). A subpopulation of nociceptors specifically linked to itch. Nat. Neurosci. 16, 174–182.

Heinke, B., Ruscheweyh, R., Forsthuber, L., Wunderbaldinger, G., and Ruscheweyh, R. (2004). Physiological, biochemical and morphological properties of a subgroup of GABAergic spinal lamina II neurons identified by expression of green fluorescent protein in mice. J. Physiol. 560, 249–266.

Hughes, D.I., Sikander, S., Kinnon, C.M., Boyle, K.A., Watanabe, M., Callister, R.J., and Graham, B.A. (2012). Morphological, biochemical and electrophysiological features of parvalbumin-expressing cells: a likely source of axo-axonic inputs in the mouse spinal dorsal horn. J. Physiol. 590, 3927–3951.

Inan, S., and Cowan, A. (2004). Kappa opioid agonists suppress chloroquine-induced scratching in mice. Eur. J. Pharmacol. 502, 233–237.

Iwagaki, N., Garzillo, F., Polgar, E., Riddell, J.S., and Todd, A.J. (2013). Neurochemical characterisation of lamina II inhibitory interneurons that express GFP in the PrP-GFP mouse. Mol. Pain 9, 56.

Kivel, B., and Prisinzano, T.E. (2010). Kappa opioids and the modulation of pain. Psychopharmacology (Berl.) 210, 109–119.

Kjellberg, F., and Tramer, M.R. (2001). Pharmacological control of opioid-induced pruritus: a quantitative systematic review of randomized trials. Eur. J. Anaesthesiol. 18, 346–357.

Ko, M.C., and Naughton, N.N. (2000). An experimental itch model in monkeys: characterization of intrathecal morphine-induced scratching and antinoiception. Anesthesiology 92, 795–805.

Ko, M.C., Lee, H., Song, M.S., Sobczyk-Kojirio, K., Mosberg, H.I., Kishioka, S., Woods, J.H., and Naughton, N.N. (2003). Activation of kappa-opioid receptors inhibits pruritus evoked by subcutaneous or intrathecal administration of morphine in monkeys. J. Pharmacol. Exp. Ther. 305, 173–179.

König, M., Zimmer, A.M., Steiner, H., Holmes, P.V., Crawley, J.N., Brownstein, M.J., and Zimmer, A. (1996). Pain responses, anxiety and aggression in mice deficient in pre-proenkephalin. Nature 383, 535–538.

Kumagai, H., Ebata, T., Takamori, K., Miyasato, K., Muramatsu, T., Nakamoto, H., Kurihara, M., Yanagita, T., and Suzuki, H. (2012). Efficacy and safety of a k agonist for managing intractable pruritus in dialysis patients. Am. J. Nephrol. 36, 175–183.

Lagerström, M.C., Rogoz, K., Abrahamsen, B., Persson, E., Reinius, B., Nordenanker, K., Olund, C., Smith, C., Mendez, J.A., Chen, Z.F., et al. (2016). VGLUT2-dependent sensory neurons in the TRPV1 population regulate pain and itch. Neuron 68, 529–542.

LaMotte, R.H., Shimada, S.G., and Sikand, P. (2011). Mouse models of acute, chemical itch and pain in humans. Exp. Dermatol. 20, 778–782.

Leighton, G.E., Rodriguez, R.E., Hill, R.G., and Hughes, J. (1988). kappa-Opioid agonists produce antinociception after i.v. and i.c.v. but not intrathecal administration in the rat. Br. J. Pharmacol. 93, 553–560.

Liao, C.C., Chang, C.S., Tseng, C.H., Sheen, M.J., Tsai, S.C., Chang, Y.L., and Wong, S.Y. (2011). Efficacy of intramuscular nalbuphine versus diphenhydramine for the prevention of epidual morphine-induced pruritus after cesarean delivery. Chang Gung Med. J. 34, 172–178.

Liu, Y., Abdel Samad, O., Zhang, L., Duan, B., Tong, Q., Lopes, C., Ji, R.R., Lowell, B.B., and Ma, Q. (2010). VGLUT2-dependent glutamate release from nociceptors is required to sense pain and suppress itch. Neuron 68, 543–556.

Loacker, S., Sayyah, M., Wittmann, W., Herzog, H., and Schwarzer, C. (2007). Endogenous dynorphin in epileptogenesis and epilepsy: anticonvulsant net effect via kappa opioid receptors. Brain 130, 1017–1028.

Ma, Q. (2010). Labeled lines meet and talk: population coding of somatic sensations. J. Clin. Invest. 120, 3773–3778.

Matthes, H.W., Maldonado, R., Simonin, F., Valverde, O., Slowé, S., Kitchen, I., Befort, K., Dierich, A., Le Meur, M., Dollé, P., et al. (1996). Loss of morphine-induced analgesia, reward effect and withdrawal symptoms in mice lacking the mu-opioid-receptor gene. Nature 383, 819–823.

Maxwell, D.J., Belle, M.D., Cheunsuangsang, O., Stewart, A., and Morris, R. (2007). Morphology of inhibitory and excitatory interneurons in superficial laminae of the rat dorsal horn. J. Physiol. 584, 521–533.

Mishra, S.K., and Hoon, M.A. (2013). The cells and circuitry for itch responses in mice. Science 340, 968–971.

Miyamoto, T., Nojima, H., Shinkado, T., Nakahashi, T., and Kuraishi, Y. (2002). Itch-associated response induced by experimental dry skin in mice. Jpn. J. Pharmacol. 88, 285–292.

Morgan, M.M., and Christie, M.J. (2011). Analysis of opioid efficacy, tolerance, addiction and dependence from cell culture to human. Br. J. Pharmacol. 164, 1322–1334.

Nilsson, H.J., Levinsson, A., and Schouenborg, J. (1997). Cutaneous field stimulation (CFS): a new powerful method to combat itch. Pain 71, 49–55.

Pan, Z.Z., Tershner, S.A., and Fields, H.L. (1997). Cellular mechanism for anti-noiception after intrathecal morphine for the prevention of epidural morphine-induced pruritus after cesarean delivery. Chang Gung Med. J. 34, 172–178.

Polgar, E., Gray, S., Riddell, J.S., and Todd, A.J. (2013a). A quantitative systematic review of randomized trials. Eur. J. Anaesthesiol. 18, 346–357.

Polgar, E., Hughes, D.I., Riddell, J.S., Maxwell, D.J., Puškar, Z., and Todd, A.J. (2003). Selective loss of spinal GABAergic or glycinergic neurons is not necessary for development of thermal hyperalgesia in the chronic constriction injury model of neuropathic pain. Pain 104, 229–239.

Polgar, E., Gray, S., Riddell, J.S., and Todd, A.J. (2004). Lack of evidence for significant neuronal loss in laminae I-III of the spinal dorsal horn of the rat in the chronic constriction injury model. Pain 111, 144–150.

Ross, S.E. (2011). Pain and itch: insights into the neural circuits of aversive somatosensation in health and disease. Curr. Opin. Neurobiol. 21, 880–887.

Ross, S.E., Mardinly, A.R., McCord, A.E., Zurawski, J., Cohen, S., Jung, C., F., Duan, B., Oh, S.B., Bean, B.P., Ma, Q., et al. (2013). Activity-dependent silencing reveals functionally distinct itch-generating sensory neurons. Nat. Neurosci. 16, 910–918.

ROSS, S.E. (2011). Pain and itch: insights into the neural circuits of aversive somatosensation in health and disease. Curr. Opin. Neurobiol. 21, 880–887.

Ross, S.E., Mardinly, A.R., McCord, A.E., Zurawski, J., Cohen, S., Jung, C., Hu, L., Mok, S.I., Shah, A., Savner, E.M., et al. (2010). Loss of inhibitory interneurons in the dorsal spinal cord and elevated itch in Bhlhb5 mutant mice. Neuron 65, 886–898.
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Ross, S.E., McCord, A.E., Jung, C., Atan, D., Mok, S.I., Hemberg, M., Kim, T.K., Salogianissi, J., Hu, L., Cohen, S., et al. (2012). Bihinb and Prdm8 form a repressor complex involved in neuronal circuit assembly. Neuron 73, 292–303.

Ruscheweyh, R., and Sandkühler, J. (2002). Lamina-specific membrane and discharge properties of rat spinal dorsal horn neurones in vitro. J. Physiol. 541, 231–244.

Sandkühler, J. (2009). Models and mechanisms of hyperalgesia and allodynia. Physiol. Rev. 89, 707–758.

Sardella, T.C., Polgár, E., Garzillo, F., Furuta, T., Kaneko, T., Watanabe, M., and Todd, A.J. (2011). Dynorphin is expressed primarily by GABAergic neurons that contain galanin in the rat dorsal horn. Mol. Pain 7, 76.

Schlaepfer, T.E., Strain, E.C., Greenberg, B.D., Preston, K.L., Lancaster, E., Bigelow, G.E., Barta, P.E., and Pearson, G.D. (1998). Site of opioid action in the human brain: mu and kappa agonists’ subjective and cerebral blood flow effects. Am. J. Psychiatry 155, 470–473.

Seybold, V.S., Hylden, J.L., and Wilcox, G.L. (1982). Intrathecal substance P and somatostatin in rats: behaviors indicative of sensation. Peptides 3, 49–54.

Shimada, S.G., and LaMotte, R.H. (2008). Behavioral differentiation between itch and pain in mouse. Pain 139, 681–687.

Simmons, D.R., Spike, R.C., and Todd, A.J. (1995). Galanin is contained in GABAergic neurons in the rat spinal dorsal horn. Neurosci. Lett. 187, 119–122.

Spike, R.C., Todd, A.J., and Johnston, H.M. (1993). Coexistence of NADPH diaphorase with GABA, glycine, and acetylcholine in rat spinal cord. J. Comp. Neurol. 335, 320–333.

Stevens, C.W., and Yaksh, T.L. (1986). Dynorphin A and related peptides administered intrathecally in the rat: a search for putative kappa opiate receptor activity. J. Pharmacol. Exp. Ther. 238, 833–838.

Sun, Y.G., Zhao, Z.Q., Meng, X.L., Yin, J., Liu, X.Y., and Chen, Z.F. (2009). Cellular basis of itch sensation. Science 325, 1531–1534.

Szanvasz, S., Harmon, D., and Murphy, D. (2003). Neuraxial opioid-induced pruritus: a review. J. Clin. Anesth. 15, 234–239.

Tiong, S.Y., Polgár, E., van Kralingen, J.C., Watanabe, M., and Todd, A.J. (2011). Galanin-immunoreactivity identifies a distinct population of inhibitory interneurons in laminae I-III of the rat spinal cord. Mol. Pain 7, 36.

Todd, A.J. (2010). Neuronal circuitry for pain processing in the dorsal horn. Nat. Rev. Neurosci. 11, 823–836.

Todd, A.J., Spike, R.C., and Polgár, E. (1998). A quantitative study of neurons which express neurokinin-1 or somatostatin sst2a receptor in rat spinal dorsal horn. Neuroscience 85, 459–473.

Togashi, Y., Umeuchi, H., Okano, K., Ando, N., Yoshizawa, Y., Honda, T., Kawamura, K., Endoh, T., Utsumi, J., Kamei, J., et al. (2002). Antipruritic activity of the kappa-opioid receptor agonist, TRK-820. Eur. J. Pharmacol. 435, 259–264.

Vanderah, T.W. (2010). Delta and kappa opioid receptors as suitable drug targets for pain. Clin. J. Pain 26 (Suppl 10), S10–S15.

Ward, L., Wright, E., and McMahon, S.B. (1996). A comparison of the effects of noxious and innocuous counterstimuli on experimentally induced itch and pain. Pain 64, 129–138.

Weisshaar, E., and Dalgard, F. (2009). Epidemiology of itch: adding to the burden of skin morbidity. Acta Derm. Venereol. 89, 339–350.

Wikström, B., Gellert, R., Ladefoged, S.D., Danda, Y., Akai, M., Ide, K., Ogasawara, M., Kawashima, Y., Ueno, K., Mori, A., and Ueno, Y. (2006). Kappa-opioid system in uremic pruritus: multicenter, randomized, double-blind, placebo-controlled clinical studies. J. Am. Soc. Nephrol. 16, 3742–3747.

Williams, J.T., Ingram, S.L., Henderson, G., Chavkin, C., von Zastrow, M., Schulz, S., Koch, T., Evans, C.J., and Christie, M.J. (2013). Regulation of μ-opioid receptors: desensitization, phosphorylation, internalization, and tolerance. Pharmacol. Rev. 65, 223–254.

Xin, L., Geller, E.B., and Adler, M.W. (1997). Body temperature and analgesic effects of selective mu and kappa opioid receptor agonists microdialyzed into rat brain. J. Pharmacol. Exp. Ther. 281, 499–507.

Yasaka, T., Kato, G., Furue, H., Rashid, M.H., Sonohata, M., Tamae, A., Murata, Y., Masuko, S., and Yoshimura, M. (2007). Cell-type-specific excitatory and inhibitory circuits involving primary afferents in the substantia gelatinosa of the rat spinal dorsal horn in vitro. J. Physiol. 581, 603–618.

Yasaka, T., Tiong, S.Y., Hughes, D.I., Riddell, J.S., and Todd, A.J. (2010). Populations of inhibitory and excitatory interneurons in lamina II of the adult rat spinal dorsal horn revealed by a combined electrophysiological and anatomical approach. Pain 751, 475–488.

Yosipovitch, G. (2008). Epidemiology of itching in skin and systemic diseases. In Itch: Basic Mechanisms and Therapy, G. Yosipovitch, M.W. Greaves, A.B. Fleischer, and F. McGlone, eds. (New York: Marcel Dekker), pp. 183–191.

Yosipovitch, G., Duque, M.I., Fast, K., Dawn, A.G., and Coighill, R.C. (2007). Scratching and noxious heat stimuli inhibit itch in humans: a psychophysical study. Br. J. Dermatol. 156, 629–634.

Zheng, J., Lu, Y., and Perl, E.R. (2010). Inhibitory neurones of the spinal substantia gelatinosa mediate interaction of signals from primary afferents. J. Physiol. 588, 2065–2075.