Neural processing of itch

Tasuku Akiyama, Ph.D. and **E. Carstens, Ph.D.**

University of California, Davis, Department of Neurobiology, Physiology & Behavior, 1 Shields Avenue, Davis, California 95616

Abstract

While considerable effort has been made to investigate the neural mechanisms of pain, much less effort has been devoted to itch, at least until recently. However, itch is now gaining increasing recognition as a widespread and costly medical and socioeconomic issue. This is accompanied by increasing interest in the underlying neural mechanisms of itch, which has become a vibrant and rapidly-advancing field of research. The goal of the present forefront review is to describe the recent progress that has been made in our understanding of itch mechanisms.

1. Introduction

Itch and pain are defined as "an unpleasant cutaneous sensation which provokes the desire to scratch" (Rothman, 1941) and "unpleasant sensory and emotional experience associated with actual or potential tissue damage" (McCracken et al., 2004), respectively. Itch and pain are similar in that they signal the organism of potentially dangerous stimuli, and are associated with protective motor responses. Itch and pain might share a common pathway, based on following observations. 1) Both sensory qualities are transmitted via spinothalamic tract. 2) Itch is absent in patients congenitally insensitive to pain. 3) Light touch surrounding a region of itch or pain elicits a sensation of itch (alloknesis) or pain (allodynia), respectively. 4) Many spinal neurons respond to both pruritic and algesic stimuli. 5) Brain imaging studies have revealed considerable overlap in areas that are active during itch or pain, such as prefrontal areas, supplementary motor areas (SMA), premotor cortex, anterior insular cortex, anterior midcingulate cortex, primary (S1) and secondary (S2) somatosensory cortices, thalamus, basal ganglia, and cerebellum (Pfab et al., 2012). However, itch and pain differ on a number of points. Firstly, itch-inducing stimuli typically elicit scratching to remove an irritant from the skin surface or to dig out parasites invading the skin, whereas algogenic stimuli typically elicit withdrawal of the stimulated body area away from the stimulus, and/or other integrated escape or aggressive motor responses. Secondly, pain is attenuated by μ-opioids which can elicit or exacerbate itch (Staender and Schmelz, 2006). Conversely, μ-opioid antagonists suppress itch (Heyer et al., 1997) while sometimes inducing hyperalgesia (Levine et al., 1978; Gracely et al., 1983). Thirdly, painful counterstimuli (scratch, cold, heat) inhibit itch. These differences have been used to differentiate between itch and pain in animal models (Shimada & LaMotte, 2008; Akiyama et al., 2010a; LaMotte et al., 2011) (see section 3). Fourthly, while pain occurs on the body

CORRESPONDING AUTHOR: Prof. E. Carstens, University of California, Davis, Department of Neurobiology, Physiology & Behavior, 1 Shields Avenue, Davis, California 95616, Tel.: 1-530-752-7767 (lab), Fax: 1-530-752-5582, eecarstens@ucdavis.edu.

surface as well as in deep tissues (e.g. muscle, joints, or inner organs), itch only occurs at the body surface of the body including skin, cornea, and other mucosal surfaces.

Itch (pruritus) is distinguished as acute or chronic, with the latter defined as pruritus lasting more than 6 weeks (Staender et al., 2007). Chronic pruritus is associated with inflammatory skin diseases as well as systemic diseases and has been classified by several groups. An early classification scheme was based on the origin of itch (Twycross et al., 2003). Later, in 2007, the International Forum for the Study of Itch (IFSI) proposed a clinically-oriented classification scheme (Ständer et al., 2007) consisting of 6 categories: 1) Dermatological (atopic dermatitis, psoriasis, etc.), 2) Systemic (kidney dialysis, liver cholestasis, etc.), 3) Neurological (postherpetic neuralgia, etc.), 4) Psychogenic (e.g., delusional parasitosis), 5) Mixed (overlapping and coexistence of several diseases) and 6) others (undetermined origin). Epidemiological data for each classification of chronic pruritus have been reported by various groups. Among patients with atopic dermatitis, 83–87% reported daily itch (Yosipovitch et al., 2002, Chrostowska-Plak et al., 2009). The incidence of patients with psoriasis reporting itch was 64–85% (Yosipovitch et al., 2000, Sampogna et al., 2004, Prignano et al., 2009). Between 22–90% of haemodialysis patients suffered from uremic itch (Feramisco et al., 2010). In a large epidemiological study of 18,801 hemodialysis patients, moderate to extreme itch was experienced by 42% (Pisoni et al., 2006). The prevalence of itch in primary biliary cirrhosis was variable, ranging from 25–70% (Rishe et al., 2008). Of patients with hepatitis C, 24% reported having itch (Bonacini, 2000). The prevalence of pruritus at 2 years following burn injury was 73% (Carrougher et al., 2013) while another study reported that 87% of burn survivors experience itch on a daily basis (Laura et al., 2012). The prevalence of shingles-associated itch is 17–58% (Oaklander et al., 2003). Among psychiatric inpatients, 36–42% reported idiopathic itch (Kretzmer et al., 2008, Mazeh et al., 2008). Overall, the incidence of chronic itch is high under a variety of different conditions. A population-based cohort study revealed that one out of four people experience chronic itch during their lifetime (Matterne et al., 2013). While the economic costs of chronic pain have been estimated as $560–635 billion per year in the US (Institute of Medicine of the National Academies, 2011), the exact economic costs of chronic itch have not been estimated. NIAMS reported that direct costs of chronic itch (atopic dermatitis) may exceed $3 billion per year (NIAMS, 2009). Considering the high incidence of chronic itch under many different conditions, the economic costs of chronic itch are likely to be much higher. Treatment is challenging, with no current universally accepted therapy for itch (Patel and Yosipovitch, 2010). Although some topical and systemic antipruritic drugs are available, the optimal therapy is not easy to classify due to a lack of knowledge about the mechanisms underlying the various sub-types of itch (Steinhoff et al., 2011).

Pain pathways have been investigated extensively. The spinal cord plays a central role, receiving ascending sensory input from peripheral afferents as well as descending input from supraspinal modulatory curcuits that include the periaqueductal gray (PAG) and rostral ventromedial medulla (RVM) (Basbaum et al., 2009, Heinricher et al., 2009, Dubin and Patapoutian, 2010). In contrast to pain, there have been until recently few studies of the spinal processing and modulation of itch, despite the fact that chronic itch is difficult to treat and can significantly reduce the quality of life as much as chronic pain. Recent studies indicate that itch appears to be transmitted by subsets of spinal nociceptive neurons (see below). Thus, a better understanding of basic mechanisms of itch will not only lead to novel mechanisms-based strategies to treat itch, but will also move forward our understanding of pain signaling.

2 Pruritogens

A list of pruritogens is shown in table 1.

2.1. Amines

Histamine is one of the best-evaluated itch mediators. Histamine produces itch accompanied by skin reactions (wheal and flare) in humans (Lewis, 1927; Weisshaar et al., 1997, Hosogi et al., 2006). Intradermal injection of histamine elicits scratching in most strains of mice (Inagaki et al., 1999, Han et al., 2006b). Histamine in the same dose range elicited little or no scratching in Sprague-Dawley rats (Thomsen et al., 2001; Jinks & Carstens, 2002), except at a high dose that elicited both hindlimb scratching and forelimb wiping following cheek injection (Klein et al., 2011) that may reflect both itch and pain (see section 3). Histamine type 1 and type 4 receptors are involved in histamine-evoked itch (Inagaki et al., 1999, Rossbach et al., 2009). Phospholipase C 3, which is downstream of Gq/G_{11} coupled with the histamine type1 receptor, is activated by histamine and contributes to itch (Han et al., 2006b). 5-HT is another amine showing pruritogenic activity. When applied iontophorecally in humans, 5-HT provokes itch accompanied by flare (Weisshaar et al., 1997, Hosogi et al., 2006). Intradermal injection of 5-HT evokes scratching in rodents via the 5-HT2 receptor (Yamaguchi et al., 1999, Thomsen et al., 2001; Jinks & Carstens, 2002; Nojima and Carstens, 2003a).

2.2. Proteases and tethered ligands

Protease-activated receptors (PARs) are activated by protease-induced cleavage of part of the extracellular domain that acts as a tethered ligand. PARs have been identified in afferent nerves (Steinhoff et al., 2000, Steinhoff et al., 2003) and their role in hyperalgesia and itch has received considerable attention. Cowhage spicules from the bean plant, Mucuna pruriens, have long been known to induce itch (Arthur & Shelley, 1955). The active component is mucunain, a cysteine protease, which acts at PAR-2 and PAR-4 subtypes to produce itch (Reddy et al., 2008). Cowhage spicules elicit histamine-independent itch with little or no accompanying flare (Johanek et al., 2007, Sikand et al., 2009). Tryptase, a serine protease, is stored in mast cell granules and activates PAR-2. Intradermal injection of tryptase elicits scratching in mice (Ui et al., 2006). Tethered ligands, such as SLIGRL (agonist of PAR-2) and AYPGKF (PAR-4 agonist) are also known to elicit scratching in mice, but not rats (Klein et al., 2011). Interestingly, a recent study reported that SLIGRL elicited scratching via Mas-related G-protein-coupled receptors C11 (MrgprC11) rather than PAR-2 (Liu et al., 2011a). Moreover, cathepsin S cysteine protease cleaves PAR-2 and PAR-4 as well as MrgprC11 to produce itch (Reddy et al., 2010 Reddy et al., 2013).

2.3. Neuropeptides

Substance P (SP) produces itch in humans as well as scratching in mice (Hagermark et al., 1978, Andoh and Kuraishi, 1998a, Andoh et al., 2001, Andoh and Kuraishi, 2003). In human skin, substance P liberates histamine through mast cell degranulation (Hagermark et al., 1978). In mice, SP elicits scratching through a direct action on primary sensory neurons, as well as by release of NO and leukotriene B4 (LTB4) from keratinocytes, rather than mast cell degranulation (Andoh and Kuraishi, 1998a, Andoh et al., 2001, Andoh and Kuraishi, 2003). Intradermal injection of endothelin-1 (ET-1) elicits itch accompanied by a flare response in humans (Ferreira et al., 1989, Katugampola et. al., 2000). ET-1 is produced by mast cells, endothelial cells and keratinocytes in the skin and is a potent pruritogen which can elicit scratching at low concentration (10–100 pmol/site), implying that ET-1 might act as an endogenous pruritogen (McQueen et al., 2007, Gomes et al., 2012, Tsugunobu et al., 2012). ET-1-evoked scratching is mediated by the ET_A receptor, but not via TRPV1 or TRPA1 (McQueen et al., 2007, Jiexian et al., 2011).

2.4. Lipid mediators

Intradermal injection of platelet activating factor (PAF) induced histamine release through degranulation of mast cells, contributing to itch accompanied by flare and wheal reactions (Fjellner and Hagermark, 1985, Petersen et al., 1997, Thomsen et al., 2002). Intradermal injection of PAF elicits scratching in rats (Thomsen et al., 2001). Lysophosphatidic acid (LPA) elicits scratching through mast cell degranulation and/or a Rho/Rho-associated protein kinase (ROCK)-mediated pathway (Hashimoto et al., 2004). LPA produced by autotaxin may contribute to pruritus of cholestasis (Kremer et al., 2012).

Leukotriene B4 (LTB4), a 5-lipoxygenase metabolite, is increased in the skin in an atopic dermatitis mouse model (Andoh et al., 2011a). Intradermal injection of LTB4 elicits scratching in mice through the LTB4 receptor-1 (BLT1) receptor (Andoh and Kuraishi, 1998b, Andoh and Kuraishi, 2005), while 12-lipoxigenase metabolites elicit scratching via the LTB4 receptor-2 (BLT2) receptor (Dae-Kwon et al., 2007, Kim et al., 2008a). LTB4 is a downstream mediator of scratching evoked by SP as well as sphingosylphosphorylcholine (SPC) (Andoh et al., 2009). SPC is increased in the stratum corneum of patients with atopic dermatitis (Okamoto, 2002) and elicits scratching through a Rho/ROCK-mediated pathway (Kim et al., 2008b). Thromboxane A_2, a cyclooxygenase metabolite, is synthesized by keratinocytes (Andoh et al., 2007). Intradermal injection of a stable analogue of Thromboxane A_2 elicits scratching through the thromboxane (TP) receptor expressed in nerve fibers as well as keratinocytes (Andoh et al., 2007).

2.5. Cytokines

Intradermal injection of interleukin-2 (IL-2) elicits transient weak pruritus in healthy humans as well as atopic dermatitis patients (Wahlgren et al., 1995, Darsow et al., 1997). IL-31 is produced by T helper type 2 cells and is overexpressed in pruritic skin compared with non-pruritic skin (Sonkoly et al., 2006). Thus, IL-31 is a promising endogenous pruritogen in inflammatory skin diseases, in particular atopic dermatitis. Injection of IL-31 elicits scratching through a heterodimeric receptor composed of IL-31 receptor A (IL-31RA) and oncostatin M receptor (OSMR) (Dillon et al., 2004).

2.6. Mrgpr agonists

Mrgprs consist of over 50 members, in which MrgprAs, MrgprB4-5, MrgprC11 and MrgprD are restricted to small diameter dorsal root ganglion (DRG) neurons in mice (Dong et al., 2001) and are involved in histamine-independent itch. Chloroquine, the bovine adrenal medulla peptide 8-22 (BAM8-22), and -alanine elicited itch-related scratching through MrgprA3, MrgprC11, and MrgprD, respectively, in mice (Liu et al., 2009, Liu et al., 2012a), and all compounds elicit itch in humans (Abila et al., 1994, Sikand et al., 2011, Liu et al., 2012a). The precursor of BAM8-22, proenkephalin A, is expressed in fibroblasts and keratinocytes (Slominski et al., 2011). This expression is increased under pathological conditions, such as psoriasis, and may contribute to chronic itch.

2.7. Uncategorized

Compound 48/80 is known as a mast cell degranulating agent and elicits itch in humans as well as rodents (Inagaki et al., 2002, Roman et al., 2002). Compound 48/80-evoked itch is mediated through mast cell degranulation as well as a mast cell-independent pathway, such as direct activation of capsaicin-sensitive primary sensory neurons (Eglezos et al., 1992, Inagaki et al., 2002).

The pruritogenic activity of bile acids has been debated. A recent study reported that bile acids selectively act at the G-protein-coupled bile acid receptor-1, also known as TGR5,

expressed in small size sensory neurons, to elicit itch (Kirby et al., 1974, Alemi et al., 2013a). This mechanism could contribute to itch in patients with cholestatic liver diseases.

Oxidative stress, which occurs during many pathophysiological conditions such as inflammation, elicits pain as well as itch through TRPA1 (Andersson et al., 2008, Tong and Ru-Rong, 2012).

Toll-like receptors (TLRs) are important for innate immunity. One member, TLR7, is expressed in small size sensory neurons and its agonist, imiquimod, elicits scratching through the activation of TLR7 as well as the inhibition of background (K_{2P}) and voltage-gated ($K_v1.1$ and $K_v1.2$) potassium channels (Liu et al., 2010a, Kim et al., 2011, Lee et al., 2012).

3. Animal models of itch

The close association between itch and scratching has led to the use of scratching behavior as a readout of itch in most animal models. However, itch may also be associated with other behaviors such as biting or licking the itchy area. These models are discussed, below.

3.1. Rostral back model

Intradermal injection of itch mediators into skin in the rostral back (nape of the neck) in mice and rats elicits bouts of hindlimb scratches directed to the injection site (Kuraishi et al., 1995). Scratch bouts consist of one or more rapid back-and-forth hindpaw motions with the toe claws contacting the site of itch, at a rate of ~12 Hz in mice and ~8 Hz in rats, and lasting approximately 0.5–2 sec (Cuellar et al., 2003, Nojima and Carstens, 2003b; Klein et al., 2011). Scratching is typically quantified as the number or cumulative duration of scratch bouts over time. Because of the constant within-bout scratch frequency, there have been various attempts to automate counts of scratch bouts, based on cage vibration (Brash et al., 2005), magnetic induction (Elliott et al., 2000; Inagaki et al., 2003; Marino et al., 2012), high speed vision (Nie et al., 2009) or scratch sounds (Umeda et al., 2006) with fairly good concordance between automated and direct visual assessments. Attempts have also been made to automate the assessment of scratching in humans as an additional outcome measure of chronic itch (e.g., (Talbot et al., 1991; Benjamin et al., 2004, Murray and Rees, 2011).

3.2. Cheek model

In the rodent rostral back model, hindlimb scratches are the only biomechanically available motor response that can be directed toward the site of itch since the forepaws cannot access this skin location. Thus, hindlimb scratching might reflect other sensations in addition to itch. In contrast, animals can readily access the face with both hindpaws and forepaws. Intradermal microinjection of histamine in the cheek of mice elicited hindlimb scratch bouts directed toward injection site, whereas microinjection of capsaicin elicited singular forelimb wiping motions directed caudo-rostrally across the cheek injection site (Shimada and LaMotte, 2008; Akiyama et al., 2010c, a). Thus, this "cheek" model appears to distinguish between chemical stimuli that elicit itch vs. pain in humans (LaMotte et al., 2011). The parameters of hindlimb scratching were similar between the rostral back and cheek models in mice and rats (Akiyama et al., 2010a; Klein et al., 2011). Forepaw wipes were singular and of much shorter duration (Akiyama et al., 2010a, Klein et al., 2011). The μ-opioid ligands are useful tools to distinguish between itch and pain. The μ-opioid agonist morphine inhibited wiping but not scratching, while the μ-antagonists naltrexone or naloxone inhibited scratching but not wiping, respectively (Akiyama et al., 2010a, Spradley et al., 2012a). This is further evidence that the cheek model can discriminate between itch and pain. The cheek model has the added advantage that behavior can be correlated with responses of trigeminal subnucleus caudalis (Vc) neurons elicited by identical cheek stimuli (Akiyama et al., 2010c).

There may be differences in sensory responses elicited by stimulation of facial skin vs. skin of the lower body (Hunt and Mantyh, 2001, Dussor et al., 2008). The cheek appears to be less sensitive to certain itch mediators compared to the rostral back (Bay et al., 2009), and rats exhibited fewer scratch bouts when 5-HT was delivered to the cheek compared to rostral back (Spradley et al., 2012c). Thus, caution is warranted in estimating the potency of chemically-evoked behavioral responses between the cheek and rostral back models.

3.3. Hindpaw/calf models

Intradermal injection of 5-HT in the mouse hindpaw elicited biting behaviors directed to the injection site, while formalin elicited almost exclusively licking behavior (Hagiwara et al., 1999). Moreover, the 5-HT-evoked biting was suppressed by naloxone, suggesting that it reflects itch. Mice exhibited enhanced biting of the hindpaw in a model of chronic dry skin itch, with no change in pain sensitivity (Nojima et al., 2004; Akiyama et al., 2010d). Spontaneous biting of dry skin-treated hindpaw skin was attenuated by naltrexone but not morphine (Akiyama et al., 2010d). This suggests that biting is a surrogate for scratching to relieve itch in the distal extremities of rodents. In this model, licking of the hindpaw was observed in control mice suggesting that licking is a subtype of grooming behavior (Akiyama et al., 2010d). It is cautioned that use of the plantar surface may not be ideal to assess itch or pain due to weight-bearing and locomotion.

Intradermal injection of pruritogens or algogens into hairy skin on the calf of the lower leg elicits respective biting or licking behaviors that appear to discriminate between itch and pain (LaMotte et al., 2011). An additional advantage of the hindlimb models is that second-order spinal cord neurons activated from the distal hindlimb are located in the lumbar spinal cord and thus readily accessible. However, discrimination between biting and licking requires high-definition video recording and careful visual analysis at slow playback speed. Biting involves high-frequency low-amplitude jaw movements compared to the lower-frequency high-amplitude licking motion of the tongue; these movement parameters may prove useful in developing automated means to distinguish between these two behaviors. Thus, we believe that the "calf" model holds great promise for investigating behavioral-neural correlates of itch.

4. Primary sensory afferents

Itch is mediated by unmyelinated C-fiber afferents as well as thinly myelinated A -fiber afferents. In microneurographic recordings in humans, mechano-insensitive C-fibers preferentially respond to histamine but not cowhage (Schmelz et al., 1997, Namer et al., 2008). In contrast, mechano-sensitive, polymodal C-fibers readily respond to cowhage with lesser or no responses to histamine in humans and primates (Johanek et al., 2008, Namer et al., 2008). Thus, cowhage and histamine appear to activate largely separate populations of C-fibers. While pruritogen-responsive polymodal C-fibers can respond to noxious mechanical stimuli and thus are not pruritogen-specific, histamine-responsive mechano-insensitive C-fibers may be pruritogen-specific although most of them additionally respond to algogens such as capsaicin or bradykinin in humans (Schmelz et al., 2003b). Mechano-sensitive A-fibers responded more vigorously to cowhage than to histamine, but some exclusively responded to histamine in monkeys (Ringkamp et al., 2011). C-fibers and A -fibers may convey two distinct qualities of itch, a slow burning component and a faster pricking component, respectively (Graham et al., 1951). Local anesthesia by procaine abolished the slow component without affecting the fast component. Either local pressure-evoked ischemia or anesthesia by cold produced an area where slow, but not fast, itch was elicited. However, other studies are inconsistent with these observations. The local anesthetic, chloroprocaine, enhanced itch following an intradermal injection of histamine

(Atanassoff et al., 1999). Conduction block of myelinated fibers by nerve compression reduced pricking as well as burning sensations (Ringkamp et al., 2011).

Murine C fibers have been divided into peptidergic and nonpeptidergic subsets mainly on the basis of neurochemical criteria, although there is some overlap (Han et al., 2012, McCoy et al., 2013). The peptidergic neurons typically contain SP and calcotonin gene-related peptide (CGRP), while nonpeptidergic neurons commonly express the purinergic P2X3 receptor and the plant lectin isolectin B4 (IB4) (Hunt and Mantyh, 2001, Dussor et al., 2008). Peptidergic and nonpeptidergic neurons exhibit anatomically distinct distribution patterns in the dorsal horn of the spinal cord as well as in skin. A large subset of nonpeptidergic neurons expresses MrgprD whose central projections terminate mainly in inner lamina II (IIi), while peptidergic neurons mainly terminate in lamina I and outer lamina II (IIo) (Zylka et al., 2005). Within the skin, MrgprD-expressing neurons innervate the most superficial layer of epidermis, the stratum granulosum, while peptidergic neurons innervate the underlying stratum spinosum (Zylka et al., 2005). The sensory function of these two subsets of neurons has been examined and is apparently different. Ablation of MrgprD-expressing neurons selectively reduced mechanical but not thermal nociception, even though they respond to noxious thermal and mechanical stimuli and innervate diverse types of spinal neurons (Cavanaugh et al., 2009, Rau et al., 2009, Wang and Zylka, 2009). Conversely, a large subset of peptidergic neurons expresses TRPV1 and removal of TRPV1-expressing neurons results in a reduction in thermal nociception but not mechanical nociception (Cavanaugh et al., 2009, Rau et al., 2009). Consistent with these observations, genetic ablation of CGRP -expressing neurons impaired thermal but not mechanical nociception (McCoy et al., 2013). Itch is likely to be transmitted by both a unique population of peptidergic neurons expressing MrgprA3, as well as a small subset of nonpeptidergic neurons expressing MrgprD. Genetic ablation of CGRP -expressing neurons reduced MrgprA3 expression by 70%, and impaired scratching evoked by chloroquine (MrgprA3 agonist) and histamine (McCoy et al., 2013). Impaired scratching in these animals is likely attributed to the reduction in MrgprA3-expressing neurons. Genetic ablation of MrgprA3-expressing neurons attenuated scratch responses to chloroquine, BAM8-22 (MrgprC11 agonist) and histamine without affecting pain behaviors (Han et al., 2012). MrgprA3-expressing neurons represent a unique population of DRG neurons, with 63% co-expressing CGRP and IB4 (Han et al., 2012). MrgprA3-expressing sensory neurons are polymodal nociceptors responding to noxious mechanical and chemical stimuli (Han et al., 2012). The involvement of nonpeptidergic neurons in itch was reported by Dong et al (Liu et al., 2012a). The MrgprD agonist, -alanine, elicited itch in humans and scratching in mice; the latter was abolished in MrgprD knockout mice. All MrgprD-expressing neurons exhibited responses to noxious mechanical stimuli. Within this population, about 40% exhibited responses to -alanine as well as noxious heat stimuli, while the remainder responded to neither stimulus. Genetic ablation of neither MrgprA3- nor CGRP -expressing neurons affected -alanine-evoked scratching, implicating the involvement of an additional supopulation of sensory neurons in -alanine-elicited itch (Han et al., 2012, McCoy et al., 2013).

MrgprA3- and MrgprD-expressing neurons that respond to pruritogens are also sensitive to noxious stimuli. This indicates that most primary afferent pruriceptors are not specifically responsive only to pruritogens, but additionally respond to noxious stimuli. Consistent with this, pruriceptors apparently share some of the same signal transduction molecules with nociceptors. TRPV1 and TRPA1 are responsible for mediating burning pain elicited by capsaicin and AITC, respectively. TRPV1 knockout mice show deficits in histamine-evoked scratching (Han et al., 2006a, Imamachi et al., 2009). In contrast, TRPA1 is expressed by a subset of TRPV1-expressing neurons, and TRPA1 knockout mice exhibited a reduced scratching responses to chloroquine and BAM8-22 in a histamine-independent manner (Liu

et al., 2009, Wilson et al., 2011). Thus, histaminergic and non-histaminergic itch pathway utilize distinct channels, namely TRPV1 and TRPA1, respectively. However, cellular and molecular mechanisms underlying chloroquine-evoked itch might be more complicated than previously reported (Than et al., 2013). Phospholipase C (PLC) plays a key role in intracellular signaling by G-protein-coupled receptors (GPCRs). PLC 3 contributes to certain types of itch as well as inflammatory and neuropathic, but not thermal, pain (Xie et al., 1999, Han et al., 2006a, Joseph et al., 2007, Shi et al., 2008, Imamachi et al., 2009). A membrane protein expressed in nociceptors, phosphoinositide interacting regulator of TRP (Pirt)(Kim et al., 2008), binds to phosphatidylinositol (4,5)-bisphosphate (PIP_2), TRPV1, and other ion channels and plays a role in thermal nociception through regulation of TRPV1. Pirt knockout mice exhibited significant reductions in scratch responses elicited by a variety of pruritogens (Patel et al., 2011).

5. Spinal Neurotransmitters

5.1. Gastrin releasing peptide (GRP), substance P (SP) and glutamate

The neurotransmitters involved in spinal or trigeminal transmission of itch have recently come under investigation, with particular emphasis on GRP, SP, and glutamate. Neurotoxic ablation of neurokinin-1 (NK-1) receptor-expressing neurons in the superficial dorsal horn of rats attenuated 5-HT-evoked scratching (Carstens et al., 2010), and selective NK-1 antagonists reduced scratching elicited by chloroquine, but not histamine, in mice, implying a role for SP in non-histaminergic itch (Akiyama et al., 2012c). Neurotoxic ablation of GRP receptor-expressing neurons in mice attenuated scratching elicited by a variety of pruritogens without affecting pain-related behaviors (Sun et al., 2009). The GRP receptor is extensively colocalized with MOR1D in the superficial spinal cord, and antagonism of the GRP receptor abolished morphine-induced scratching (Liu et al., 2011b), implicating the GRP receptor in opioid-induced itch. Selective GRP receptor antagonists and knock out of the GRP receptor partially reduced scratching evoked by chloroquine, but not histamine, implying a partial role for GRP in non-histaminergic itch (Sun and Chen, 2007, Akiyama et al., 2012c). Although GRP is expressed in DRG neurons (Sun and Chen, 2007, Liu et al., 2009, Lagerstrom et al., 2010, Liu et al., 2010b, Akiyama et al., 2012c, Alemi et al., 2013b), it is still debatable whether GRP is released from central terminals of primary sensory neurons. MrgprA3-expressing sensory neurons expressed GRP, and their central terminals made synaptic contact with GRPR-expressing spinal neurons (Liu et al., 2009, Han et al., 2012). However, recent *in vitro* studies have shown that GRP-sensitive spinal neurons utilize glutamate rather than GRP as a neurotransmitter (Koga et al., 2011). GRP staining of the rhizotomized spinal cord revealed that the majority of GRP is synthesized locally within the spinal cord (Fleming et al., 2012). Additionally, recent *in vitro* studies using segments of spinal cord with attached dorsal roots have shown that pruritogen-evoked release of GRP was not blocked by pretreatment of capsaicin, which causes depletion of neuropeptides such as CGRP in the central terminals (Alemi et al., 2013b). Thus, the source of GRP acting to promote itch transmission within the spinal cord may be from local spinal neuronal circuits, rather than (or in addition to) release from the intraspinal terminals of non-histaminergic primary afferent pruriceptors. GRP was reported to be expressed in peripheral nerve terminals of primary sensory neurons (Tominaga et al., 2009, Kagami et al., 2013, Nattkemper et al., 2013). Peripheral intradermal injection of GRP elicited scratching through mast cell degranulation (Andoh et al., 2011b). Serum GRP levels in atopic dermatitis patients positively correlated with the itch score (Kagami et al., 2013). Thus, expression of GRP in DRG neurons may reflect a peripheral role of GRP in itch. Moreover, the GRP receptor (GRPR) is expressed in immune cells, such as macrophages, T-cells and neutrophils, and contributes to development of inflammatory diseases (Zhou et al., 2011, Czepielewski et al., 2012). GRP might play a key role in transmission of itch signaling as well as modulating neuroimmune interactions. Glutamate acting at the AMPA/kainate

receptor is also a likely candidate for spinal itch transmission (Koga et al., 2011). Synaptic input to neurons that responded to application of GRP could be blocked with AMPA/kainate receptor antagonists, suggesting that the prominent primary afferent input to these GRP-responsive neurons is glutamatergic. Thus, SP, GRP, and glutamate are good targets for developing novel treatments for itch.

5.2. Nautriuretic polypeptide b (Nppb)

A very recent, striking finding was reported by Mishra and Hoon (2013). Nppb, previously known to be released upon stretching of cardiomyocytes in the heart, was found to be expressed in a subset of small diameter sensory neurons that co-express TRPV1, PLC 3, and MrgprA3 (Mishra and Hoon, 2013). Nppb knockout mice exhibited lack of scratching responses to histamine, chloroquine, ET-1, 5-HT, SLIGRL-NH2, and compound48/80, but exhibited normal pain behaviors. Moreover, intrathecal injection of Nppb elicited scratching behaviors. Mice in which the natriuretic peptide receptor A (Npra) was ablated, exhibited impaired scratching responses to histamine as well as Nppb. Npra was expressed primarily in lamina I. Collectively, these data suggest that Nppb plays a major role in spinal itch transmission. The authors proposed that Nppb is released from primary afferent pruriceptors to excite second-order Npra-expressing spinal neurons and ultimately excite downstream GRPR-expressing spinal neurons that are required for the transmission of itch signals to higher centers. The exact neurocircuitry and relative roles of glutamate, Nppb, SP and GRPR-expressing neurons in itch are important questions that remain to be answered in the exciting and rapidly-advancing field of itch mechanisms research.

5.3. Neurokinins and neuromedins

Neurokinin B encoded by the tachykinin 2 gene is a member of the tachykinin peptides along with SP, and is a possible candidate as a neuropeptide transmitter in spinal itch transmission. However, a recent study revealed that tachykinin 2 null mice exhibited normal scratch responses to compound 48/80, chloroquine, SLIGRL-NH2 and -methyl-5-HT as well as normal responses to noxious heat and mechanical stimuli, implying that neurokinin B does not appear to be essential for the spinal transmission of itch or pain signaling (Mar et al., 2012). Neuromedin B is a member of the mammalian bombesin family of peptides along with GRP, and is another candidate as a neuropeptide transmitter in spinal itch transmission. Intrathecal or intracerebroventricular administration of neuromedin B elicited marked scratching (Van Wimersma Greidanus and Maigret, 1991, Cridland and Henry, 1992, Su and Ko, 2011), while intrathecal administration of neuromedin B produced a transient decrease followed by a delayed increase to above baseline in tail flick latency (Cridland and Henry, 1992). Neurotoxic ablation of neuromedin B receptor-expressing neurons in the superficial dorsal horn did not affect histamine H1 receptor agonist-evoked scratching, but reduced noxious heat-evoked behavioral responses (Mishra et al., 2012), implying a role for neuromedin B in thermal pain. It would be interesting to test whether neuromedin B is involved in the spinal transmission of non-histaminergic itch. CGRP may contribute to the spinal transmission of pain as well as itch. CGRP was expressed by 61–73% of pruritogen-responsive DRG neurons, and 27–83% of algogen-responsive DRG neurons (McCoy et al., 2012). Overall, pathways for itch and pain signaling appear to use the same neurotransmitters. Moreover, it may be expected that nociceptive primary afferents activate pruritogen-responsive spinal neurons as discussed in the next section.

6. Pruritogen-responsive spinal neurons

The dorsal horn is the major site processing information from primary sensory afferents. Superficial dorsal horn neurons (laminae I–II) receive direct input from most nociceptive A - and C-fibers, while deep dorsal horn neurons (laminae III–V) receive direct input from

A δ-fibers (Todd, 2002). Recent molecular studies have further categorized the central projections of nociceptive C-fibers and low-threshold mechanoreceptors (LTMRs) in the spinal cord (Basbaum et al., 2009, Li et al., 2011). Spinal cord neurons in lamina I-IIo, IIm, IIi, III, or III-V receive projections from peptidergic C-fibers, nonpeptidergic C-fibers, C-low-threshold mechanorecptors (LTMRs), Aδ-LTMRs, or Aβ-LTMRs, respectively.

A schematic diagram of excitatory spinal circuits mediating itch is shown in Fig. 1. Approximately 80% of ascending projection neurons express the NK-1 receptor in rat spinal cord, with those located in lamina I receiving input mostly both SP- and CGRP-containing afferents and a few SP-containing afferents lacking CGRP (Todd et al., 2002). Since 5-HT-evoked scratching was reduced in rats following ablation of NK-1-expressing superficial dorsal horn neurons, the latter plausibly play the role in ascending transmission of itch as well as pain signals (Carstens et al., 2010). A later study reported that lamina I projection neurons are innervated by CGRP- as well as IB4-labeled afferents (with a small population expressing both), consistent with the unique characteristics of MgrprA3-expressing neurons (Han et al., 2012, Saeed and Ribeiro-da-Silva, 2012). Since MrgprA3-expressing neurons synapse with GRPR-expressing spinal neurons, some of the latter may be ascending projection neurons. Alternatively, GRPR-expressing spinal neurons might be excitatory interneurons which receive GRP released from Npra-expressing spinal neurons (Mishra and Hoon, 2013). Genetic ablation of testicular orphan nuclear receptor 4 (TR4) resulted in the loss of neurons expressing SP, the vesicular glutamate transporter-2 (VGLUT2), and GRPR, without loss of ascending projection neurons, suggesting that neurons expressing SP, VGLUT2 and GRPR are mainly excitatory interneurons (Todd et al., 2003, Maxwell et al., 2007, Wang et al., 2013). TR4 knockout mice exhibited impaired scratch responses, implying that those excitatory interneurons play a role in spinal excitatory circuits for itch (Wang et al., 2013).

Dorsal horn neurons can be classified into four general categories according to their responses to mechanical stimuli: mechano-insensitive (MI) neurons that respond to neither noxious nor innocuous mechanical stimuli, low-threshold (LT) neurons that do not respond to noxious mechanical stimuli, wide dynamic range (WDR) neurons that respond at higher firing rate to noxious than to innocuous mechanical stimuli, and nociceptive specific (NS) or high-threshold (HT) neurons that respond to noxious but not innocuous mechanical stimuli. WDR and NS neurons are located in both superficial and deep dorsal horn (Price et al., 1978, Chudler et al., 1991, Dado et al., 1994). LT neurons are located mainly in the deep dorsal horn (laminae III-IV) (Price et al., 1978, Chudler et al., 1991).

Itch-signaling spinal neurons should respond to cutaneous application of a pruritogen over a time course matching that of itch sensation. Some studies have taken a non-biased approach to identify ascending projection neurons by antidromic stimulation, and then test if they respond to pruritogens (e.g., Andrew & Craig, 2001; Simone et al., 2004; Davidson et al., 2007, 2012). Other studies have used intradermal injection of a pruritogen as a search stimulus to identify spinal neurons (Carstens, 1997; Jinks & Carstens, 2002; Akiyama et al., 2009a,b). By either approach, pruritogen-responsive neurons were mostly either NS or WDR, with fewer of them being MI (Andrew and Craig, 2001, Jinks and Carstens, 2002, Davidson et al., 2007, Akiyama et al., 2009a, Akiyama et al., 2009b, Akiyama et al., 2010c, Davidson et al., 2012). Andrew and Craig recorded from 190 identified spinothalamic tract (STT) neurons in lamina I of cats; 18 were mechanically- and thermally-insensitive. Interestingly, 10 of the latter responded to iontophoretically applied histamine and 2 of 4 histamine-responsive STT neurons tested did not respond to the algogen, AITC (capsaicin was not tested in this study). Davidson et al. (2012) recorded from 111 STT neurons in adult macaques; 32 responded to either histamine or cowhage and 2 responded to both. All of the histamine- and cowhage-responsive STT neurons were either WDR or NS. Overall,

approximately 30% of nociceptive STT neurons were pruritogen-responsive as well. An important implication of these latter studies (Davidson et al., 2007; 2012) is that itch elicited by histamine vs. cowhage may be mediated via largely separate subpopulations of ascending spinothalamic tract neurons.

Using a pruritogen search stimulus, Jinks & Carstens (2002) identified 21 5-HT-responsive neurons in the superficial dorsal horn of rats, of which 15 were classified as WDR, 5 as NS and 1 as MI. The MI unit responded to noxious chemical and heat stimuli. Using a pruritogen search strategy in mice, we have collectively recorded from 17 histamine-, 7 5-HT-, 58 SLIGRL-NH2-, and 10 chloroquine-responsive lumber spinal neurons; 5 histamine- and 3 SLIGRL-NH2-responsive neurons were MI and the remainder were WDR or NS (Akiyama et al., 2009a, Akiyama et al., 2009b). Overall, the vast majority of pruritogen-responsive spinal neurons, including WDR, NS and MI (but not LT) subtypes, additionally responded to noxious mechanical, thermal, and/or chemical stimuli and thus appears to be a subset of nociceptive spinal neurons.

7. Trigeminal processing of itch

Using calcium imaging of trigeminal ganglion (TG) cells, 15.4% and 5.8% responded to histamine and SLIGRL-NH2, respectively (Akiyama et al., 2010c). Of these, more than 70% additionally responded to capsaicin or AITC. We also recorded from 58 neurons in trigeminal subnucleus caudalis (Vc) with afferent input from the cheek (Akiyama et al., 2010c). Out of 32 pruritogen-responsive Vc neurons, 4 were MI and responded to either capsaicin or AITC. In this study, a subpopulation of nociceptive neurons was isolated using an algogen (AITC) search stimulus and subsequently tested with several pruritogens. Only a minority of these nociceptive neurons (13–41%) additionally responded to the pruritogens histamine, SLIGRL-NH2, or 5-HT. Overall, the vast majority of pruritogen-responsive medullary dorsal horn neurons additionally responds to noxious mechanical, thermal, and/or chemical stimuli and thus appears to be a subset of nociceptive spinal neurons, similar to pruritogen-responsive spinal neurons. There appears to be a larger population of nociceptive neurons, such as those isolated using an AITC search stimulus, the majority or which does not respond to pruritogens. This is consistent with our population-coding model in which itch is proposed to be signaled by pruritogen-sensitive nociceptive neurons, while pain is signaled by the larger population of puritogen-insensitive nociceptive neurons (Fig. 1).

A recent study identified two populations of antidromically identified trigeminothalamic tract neurons in rats (Moser and Giesler, 2013). Pruritogen-responsive neurons were activated by intrathecal application of morphine, while nociceptive neurons which did not respond to pruritogen were inhibited by morphine (Moser and Giesler, 2013). Morphine thus appears to be a convenient tool to identify pruritogen-responsive medullary dorsal horn neurons (see also below).

8. Inhibitory interneurons

Inhibitory interneurons in laminae I–III consist of four distinct neurochemical populations containing neuropeptide Y (NPY), galanin, parvalbumin and neuronal nitric oxide synthase (nNOS) (Tiong et al., 2011). The transcription factor Bhlhb5 is transiently expressed in the dorsal horn of the developing spinal cord to regulate a unique population of inhibitory interneurons that inhibit itch (Ross et al., 2010). Approximately 65% of inhibitory interneurons are innervated by A -fibers and/or C-fibers (Daniele and MacDermott, 2009). This implies that noxious (and possibly pruritic) stimuli activate inhibitory interneurons, which are capable of modulating the spinal transmission of various somatosensory submodalities. Itch can be inhibited by various types of noxious thermal, mechanical, chemical or electrical counterstimuli (Ward et al., 1996; Nillson et al., 1997). Scratching

relieves itch, and recent studies support a spinal site of action by which scratching inhibits pruritogen-responsive neurons. Monkey STT neurons are modulated in a state-dependent manner by cutaneous scratching; scratching inhibited responses elicited by the pruritogen histamine but did not inhibit responses of the same neurons to the algogen capsaicin (Davidson et al., 2009). Consistent with this, pruritogen-responsive mouse spinal neurons are modulated in a state- and also site-dependent manner by cutaneous scratching (Akiyama et al., 2012b). Pruritogen- but not algogen-evoked neuronal responses were inhibited following scratching delivered to the stimulus site (state-dependency), and also during scratching at a distance away (site dependency). Spinal application of glycine and GABA-A and GABA-B receptor antagonists attenuated or abolished scratch-evoked inhibition of spontaneous activity in dorsal horn neurons with input from dry skin (Akiyama et al., 2011b), suggesting that GABA and glycine mediate inhibition of itch-signaling spinal neurons.

Conceivably, spinal inhibitory interneurons are tonically active, based on recent studies showing that decreased activity in, or deletion of, inhibitory spinal interneurons is associated with enhanced itch. Loss of a population of inhibitory interneurons in the superficial dorsal horn of knockout mice lacking the transcription factor Bhlhb5 (Ross et al., 2010), as well as knockout of the glutamate transporter VGLUT2 in certain types of nociceptors (Lagerstrom et al., 2010, Liu et al., 2010c), both resulted in excessive scratching behavior. These findings suggest that a reduction in nociceptive input decreases spinal inhibition, resulting in disinhibition of itch transmission. This is further supported by a recent study (Roberson et al., 2013). When TRPV1- or TRPA1-expressing nociceptors were electrically silenced by entry of a local anesthetic through the open ion channel, cheek application of capsaicin or AITC, which normally elicit forelimb wiping behavior, instead elicited hindlimb scratching. This implies that TRPV1- and TRPA1-expressing nociceptor afferents exert a tonic inhibitory effect on trigeminal itch transmission, and that removal of this tonic inhibition by electrical silencing disinhibited itch-transmitting Vc neurons to result in scratching behavior. A schematic diagram of spinal itch-inhibitory circuits is presented in Fig. 2. In this scenario, decreased input from nociceptors expressing TRPV1 and/or TRPA1 reduced the excitation of Bhlhb5 inhibitory interneurons, thus disinhibiting itch-signaling neurons. Such neurons may be excited via capsaicin or AITC activation of H1R and/or MrgprA3-expressing pruriceptors to elicit scratching (Fig. 1).

9. Opioid modulation of itch

As noted earlier, morphine inhibits pain but can induce or enhance itch, whereas μ-opiate antagonists suppress itch but not pain. One possible explanation for morphine-induced itch is that opioid peptide-expressing inhibitory interneurons in the spinal cord might synapse onto the Bhlhb5 interneurons; activity in the opioid interneurons (or exogenous application of μ-agonists) would inhibit the Bhlhb5 interneurons to disinhbit itch-signaling neurons (Handwerker, 2010). An alternative explanation is that the morphine binds to the μ-opioid receptor isoform MOR1D which heterodimerizes with GRPR co-expressed in itch-signaling spinal neurons (Liu et al., 2011b). In either case, exogenous spinal application of morphine was recently reported to excite pruritogen-responsive trigeminothalamic projection neurons in rats, while inhibiting nociceptive trigeminothalamic neurons (Moser & Giesler, 2013), providing a functional explanation for how systemic morphine induces itch.

The -agonist TRK-820 (Nalfurafine) inhibited pruritogen-evoked scratching in mice (Togashi et al., 2002) and morphine-induced scratching in primates (Ko et al., 2003), indicating a role for the -opioid receptor in the modulation of itch that is worthy of further investigation. This -agonist has proven effective in relieving intractable itch in kidney dialysis patients (Kumagi et al., 2012).

10. Descending modulation of itch

Scratch-evoked inhibition of spinal itch-signaling neurons involves both segmental and supraspinal circuits. Cold-block or complete transection of the upper cervical spinal cord reduced scratch-evoked inhibition of spontaneous activity in dorsal horn neurons with input from dry skin by 30% and 50%, respectively. This implies that scratch-evoked inhibition is mediated partially via activation of supraspinal neurons that, in turn, engage descending pathways to result in spinal release of glycine and GABA (Fig. 2). The supraspinal circuit is unknown but may involve the same descending pathways that modulate pain. In a human brain imaging study, the midbrain periaqueductal gray (PAG) was activated during the inhibition of histamine-evoked itch by a noxious cold stimulus (Mochizuki et al., 2003), suggesting that the PAG, a well-known center for descending modulation of pain, may also be involved in modulating itch. The locus coeruleus is a major source of descending noradrenergic projections (Ossipov et al., 2010). Neurotoxic destruction of catecholaminergic neurons in the spinal cord enhanced itch-related behaviors, implying that descending noradrenergic neurons inhibit spinal itch signaling (Gotoh et al., 2011). Moreover, swim stress-induced analgesia, which is thought to be mediated by descending antinociceptive pathways, attenuated 5-HT-evoked scratching behavior in rats (Spradley et al., 2012b). The otherwise scant information regarding supraspinal modulation of spinal and trigeminal itch transmission makes this a fruitful area for future research.

11. Sensitization of itch-signaling pathways

Peripheral and central sensitization play important roles in the establishment of chronic pain, and the same processes may contribute to various types of chronic itch. Chronic pain is often associated with ongoing spontaneous pain, hyperalgesia, and allodynia (touch-evoked pain). These conditions can also be experimentally reproduced in human skin by intradermal injection of capsaicin. In primates, capsaicin enhanced the responses of monkey STT neurons to touch and noxious heat, as well as electrical nerve stimulation, suggesting sensitization of the STT neurons (Simone et al., 1991). Chronic itch has parallels with chronic pain (Yosipovitch et al., 2007). Chronic itch can be associated with spontaneous itch, hyperknesis (enhanced itch to a normally itchy stimulus), and alloknesis (itch elicited by an innocuous touch stimulus). Three general mechanisms possibly contributing to chronic itch are shown in Fig. 3, as discussed further below.

11.1. Peripheral Sensitization

Few studies have addressed whether primary afferent pruriceptors are sensitized under chronic itch conditions. Pruritogen-sensitive C-fibers recorded in atopic dermatitis patients exhibited high levels of spontaneous firing (Schmelz et al., 2003a). Using the dry skin model of chronic itch, mice exhibited significantly greater scratching (hyperknesis) following intradermal injections of 5-HT and SLIGRL, but not histamine, within the treatment area (Akiyama et al., 2010b). DRG cells taken from the dry skin-treated mice exhibited significantly greater responses to 5-HT and SLIGRL, but not histamine, consistent with the behavioral results (Akiyama et al., 2010b). A potential mediator of peripheral sensitization is nerve growth factor (NGF), which is elevated in dry skin and might contribute to peripheral sensitization of pruriceptors (Tominaga et al., 2007). Intradermally administered NGF enhanced itch induced by cowhage but not histamine in humans (Rukwied et al., 2013). A possible role for PAR-2 in peripheral sensitization comes from our study showing that SLIGRL, but not BAM8-22, enhanced the response of DRG neurons to subsequently-applied chloroquine and BAM8-22 (Akiyama et al., 2012c).

11.2. Central Sensitization

To investigate if chronic dry skin itch sensitizes spinal neurons, we recorded from superficial dorsal horn neurons receiving afferent input from a dry skin-treated hindpaw (Akiyama et al., 2011a). These neurons exhibited heightened spontaneous activity and enhanced responses to SLIGRL, but not histamine, compared to units recorded in control animals. However, mechanically-evoked responses were not enhanced, suggesting that the enhanced response to SLIGRL was due to peripheral sensitization of pruriceptors in the dry skin area and consistent with our results noted in the previous section.

A possible central mechanism of enhanced itch transmission is long-term potentiation (LTP). Knockout mice lacking toll-like receptor 3 (TLR3) exhibited significantly reduced scratching to histamine and variety of other pruritogens, as well as impaired LTP in spinal neurons (Liu et al., 2012b), supporting a role for TLR3 in central sensitization of spinal itch-transmitting neurons. In addition, TLR3 knockout mice with experimental dry skin exhibited almost no spontaneous scratching behavior compared to the robust scratching observed in wildtype mice (Liu et al., 2012b). Interestingly, the dry skin-treated wild type mice showed a 25-fold increase in TLR3 expression in the skin, suggesting that TLR3 may also be involved in peripheral sensitization of dry skin itch (Liu et al., 2012b).

Alloknesis is a common and often distressing symptom of many chronic itch patients. Alloknesis has been suggested to be mediated by mechanoreceptor afferent input to sensitized itch-signaling spinal neurons, but there is currently no evidence for this. We recently developed a novel animal model of alloknesis involving innocuous mechanical stimulation of rostral back skin in the C57BL/6 mouse (Akiyama et al., 2012a). C57BL/6 mice do not normally respond to this low-threshold mechanical stimulus. However, following intradermal injection of histamine and certain other pruritogens, lightly touching skin surrounding the injection site reliably elicited discrete hindlimb scratch bouts directed to the stimulus. The touch-evoked scratching developed more slowly and lasted longer compared to the scratching that began shortly after the pruritogen injection and usually ceased within 30 min. Touch-evoked scratching was observed following histamine, 5-HT, a PAR-4 agonist, and BAM8-22, but not SLIGRL or chloroquine. We also observed touch-evoked scratching in dry skin-treated animals, suggesting that dry skin itch is associated with alloknesis. In recordings from primate STT neurons, innocuous mechanical stimuli elicited greater responses after vs. before intradermal histamine injection (Davidson et al., 2012). In contrast, cowhage did not affect mechanically-evoked responses. Innocuous mechanical stimuli elicited greater responses in pruritogen-responsive rat trigeminothalamic tract neurons after compared to before the intrathecal application of morphine (Moser and Giesler, 2013). The enhancement of mechanically-evoked responses of ascending sensory neurons may thus represent a mechanism by which certain pruritogens induce alloknesis.

11.3. Dysfunction of Inhibitory Interneurons

As noted above, loss of Bhlhb5 inhibitory interneurons resulted in spontaneous scratching (Ross et al., 2010), consistent with disinhibition of itch-signaling spinal neurons. Future studies are needed to determine if the number of Bhlhb5 inhibitory interneurons is reduced under conditions of chronic itch.

As more animal models of chronic itch conditions become available, it will be important to determine if the animals exhibit ongoing spontaneous scratching, hyperknesis and alloknesis, if peripheral and/or central itch-signaling neurons exhibit sensitization, and if there are functional changes in spinal inhibitory interneruons. The identification of the molecular players involved in these various processes will provide important targets for the future development of treatments for chronic itch.

12. Theories of itch

It has been debated for over a century whether itch and pain are mediated via distinct pathways, a concept known as specificity theory or labeled-line coding, or if itch is a low-level form of pain on the same sensory continuum, a concept known as the intensity (or frequency) theory (von Frey, 1922). Intensity theory holds that a common population of sensory neurons responds to both pruritic and noxious stimuli, with itch being signaled by a low firing rate and pain by a higher firing rate in these neurons. Indeed, many spinal neurons in our studies exhibited relatively lower firing rates to pruritogens and higher-frequency responses to algogens. However, the concept of intensity coding is not supported by observations that electrical stimulation at certain points on the skin surface elicits a distinct sensation of itch that increases in intensity, but does change to pain, with increasing stimulus frequency (Tuckett, 1982). Instead, it is the authors' opinion that current evidence supports the concept of specificity or labeled-line coding for distinct sensations of itch and pain. However, it must be recognized that most pruritogen-sensitive primary afferents and second-order spinal and trigeminal neurons also respond to noxious stimuli, rather than being pruritogen-specific, thereby introducing complications for a simple labeled-line theory of neural coding. The interested reader is directed to additional references describing alternative theories for itch and pain coding (McMahon & Koltzenburg, 1992; Handwerker, 2010; Namer & Reeh, 2013).

The concept of labeled-line coding for itch is supported by studies implicating GRPR-expressing dorsal horn neurons in selectively mediating itch but not pain (Sun et al., 2009), as noted above. Further support comes from recent observations of human mechano-insensitive C-fibers that responded to histamine over a time course matching that of concomitant itch sensation (Schmelz et al., 1996), and mechano-insensitive lamina I STT neurons in cats that similarly responded to histamine (Andrew and Craig, 2001). A recent striking observation supports labeled-line coding. In knockout mice lacking TRPV1 globally, TRPV1 was selectively re-expressed in MrgprA3-expressing sensory neurons. In these mice, cheek injection of capsaicin, which normally evokes pain-related wiping, instead elicited itch-related scratching behavior (Han et al., 2012). This implies that MrgprA3-expressing pruriceptors are linked to a labeled line for itch sensation, even though they can be excited by other types of stimuli including capsaicin and noxious pinch (Han et al., 2012). Consistent with this, application of capsaicin-impregnated cowhage spicules within the superficial epidermis (Sikand et al., 2009), where MrgprA3-expressing primary afferent terminals are located, or topical application of capsaicin (Green, 1990; Green & Shaffer, 1993), elicited a dominant sensation of itch in humans. However, intradermal injection of capsaicin elicits burning pain (LaMotte et al., 1991), and it remains a challenge to explain how the nervous system discriminates between capsaicin-evoked itch and pain sensations, as discussed further, below.

Nevertheless, many of the available molecular-genetic, behavioral and electrophysiological studies suggest that pruriceptors are a subset of nociceptors that respond to noxious mechanical, chemical, and/or thermal stimuli. This raises the question as to how noxious stimuli elicit pain without simultaneously eliciting itch. One possibility is that noxious stimuli activate inhibitory interneurons that suppress itch transmission. Although noxious stimuli can inhibit responses of spinal neurons to pruritogens, this inhibition is state-dependent (Davidson et al., 2009, Akiyama et al., 2011b). Most pruritogen-sensitive spinal neurons are also excited by capsaicin, so that scratching becomes an excitatory rather than inhibitory stimulus to further excite the neuron. If such neurons are dedicated to only signal itch, then it remains difficult to explain how a noxious stimulus only elicits pain. Another possibility is that noxious stimuli do elicit both itch and pain simultaneously, whereby the sensation of itch is masked or occluded by the larger pain signal in order to discriminate

between the two sensory qualities. An additional related concept is population coding (Akiyama et al., 2009a,b; Ma, 2010). Itch is postulated to be signaled by the activation of a subset of spinal neurons that responds to both pruritogens and noxious stimuli. Noxious stimulation activates a larger population of nociceptive spinal neurons, including those responsive to pruritogens, to signal pain. The CNS decodes activity in the former and latter neuronal populations as itch and pain, respectively. According to this idea, MrgprA3-expressing sensory neurons would be expected to project to a subset of GRPR-expressing spinal neurons that respond to both pruritogens and noxious stimuli. MrgprA3-expressing sensory neurons do respond to noxious mechanical and chemical stimuli, and their central spinal terminals make synaptic contact with GRPR-expressing spinal neurons (Han et al., 2012). Since ablation of neither MrgprA3-expressing neurons nor GRPR-expressing spinal neurons has any effect on nociception (Sun et al., 2009, Han et al., 2012), the CNS may decode activity in a larger population of nociceptive spinal neurons as pain regardless of activity in a smaller subset of spinal neurons responsive to both pruritogens and noxious stimuli.

Acknowledgments

List of Abbreviations

5-HT	5-hydroxytryptamine (serotonin)
5-HT2	5-HT receptor subtype 2
12-HETE	12-Hydroxyeicosatetraenoic acid
AITC	allyl isothiocyanate
BAM8-22	bovine adrenal medullary peptide 8-22
BLT	LTB4 receptor
DRG	dorsal root ganglion
ET-1	endothelin-1
ETA-1	endothelin receptor A-1
GABA	gamma aminobutyric acid
Glu	glutamate
GRP	gastrin releasing peptide
GRPR	gastrin releasing peptide receptor
H1R	histamine receptor-1
IB-4	isolectin B4
IL31	interleukin 31
K2P	two-pore-domain potassium channels
Kv	voltage-gated potassium channel
LPA	lysophosphatidic acid
LTB4	leukotriene B4
LT	low threshold

LTP	long-term potentiation
MI	mechanically insensitive
MOR1D	morphine receptor-1D isoform
Mrgpr	Mas-related G-protein-coupled receptor
NGF	nerve growth factor
NK-1	neurokinin-1 receptor
NO	nitric oxide
Nppb	natriuretic polypeptide B
NS	nociceptive specific
OSMR	oncostatin M receptor
PAF	platelet activating factor
PAG	periaqueductal gray
PAR	protease-activated receptor
Rho-ROCK	Rho-associated protein kinase
RVM	rostral ventromedial medulla
SP	substance P
SPC	sphingosylphosphorylcholine
STT	spinothalamic tract
TG	trigeminal ganglion
TGR5	G protein-coupled bile acid receptor 1 (GPBAR1)
TLR3	toll-like receptor 3
TLR7	toll-like receptor 7
TP	thromboxane receptor
TR4	testicular orphan nuclear receptor-4
TRPA1	transient receptor potential ankyrin 1
TRPV1	transient receptor potential vanilloid 1
TXA2	thromboxane A2
Vc	trigeminal subnucleus caudalis
VGLUT2	vesicular glutamate transporter-2
WDR	wide dynamic range

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Highlights

There are histamine-dependent and –independent types of itch

Molecular detectors of pruritogens include Mrgprs, PARs, and many others

Spinal transmitters include gastrin releasing peptide and natriuretic polypeptide B

Pruritogen-sensitive sensory neurons usually also respond to pain-producing stimuli

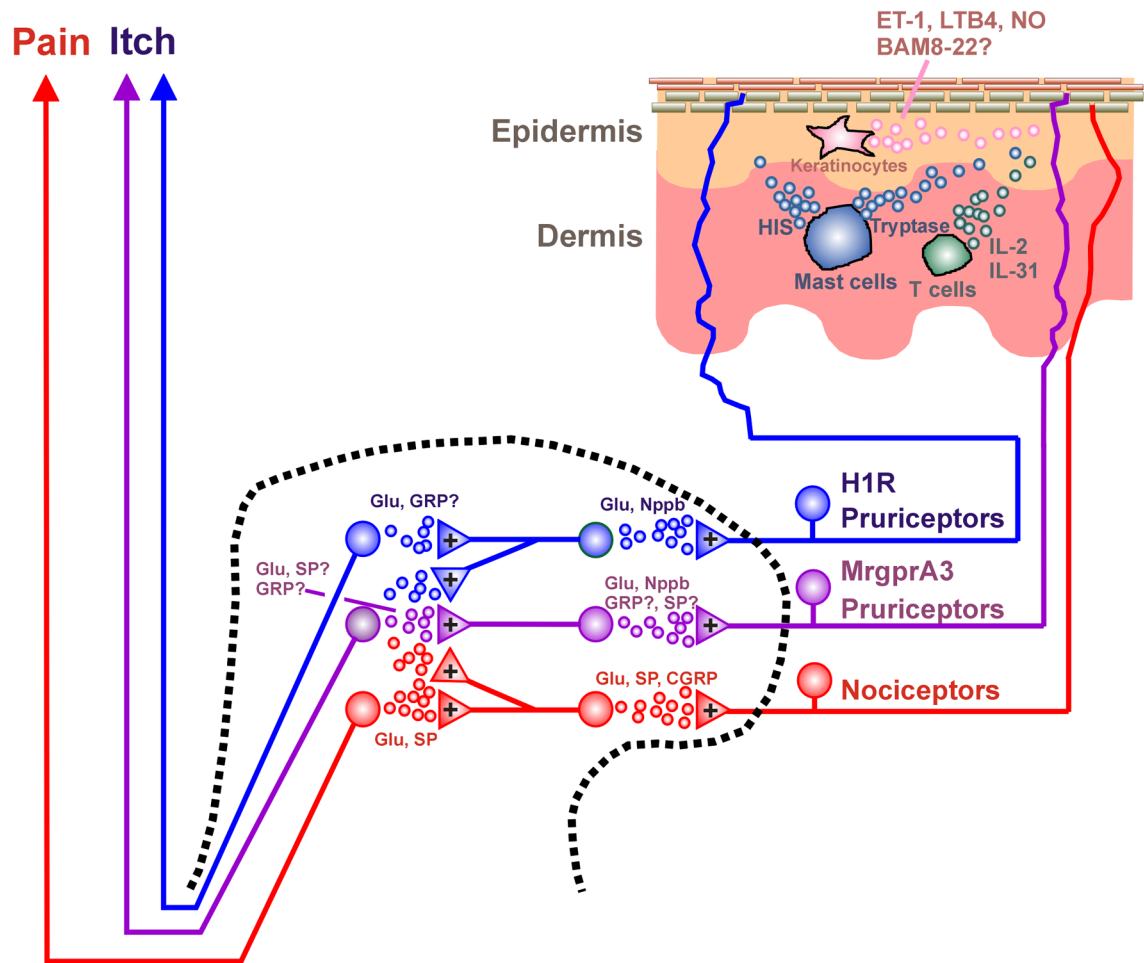Pruritogen-responsive sensory neurons connect to an itch-specific central pathway

Fig. 1.
Schematic diagram of excitatory circuits for itch. Dashed line is cross-section of spinal cord dorsal horn. Upper right shows cross-section through skin. + denotes excitatory synapse.
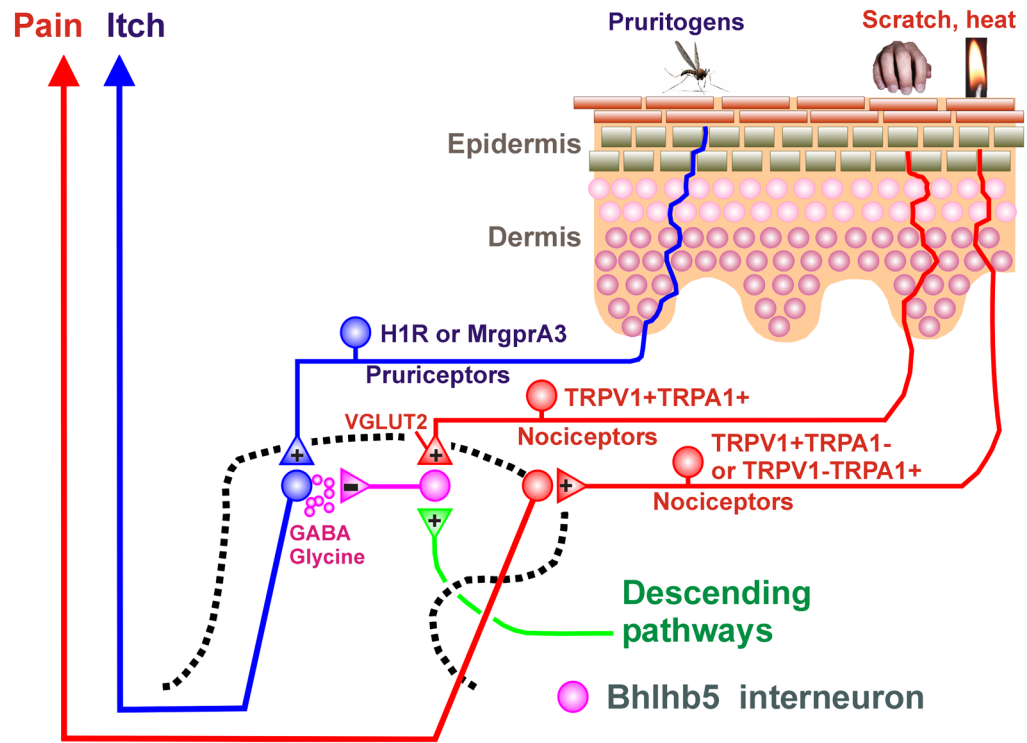
Fig. 2.
Schematic diagram of inhibitory spinal circuits for itch. +, − denote excitatory and inhibitory synapses, respectively.
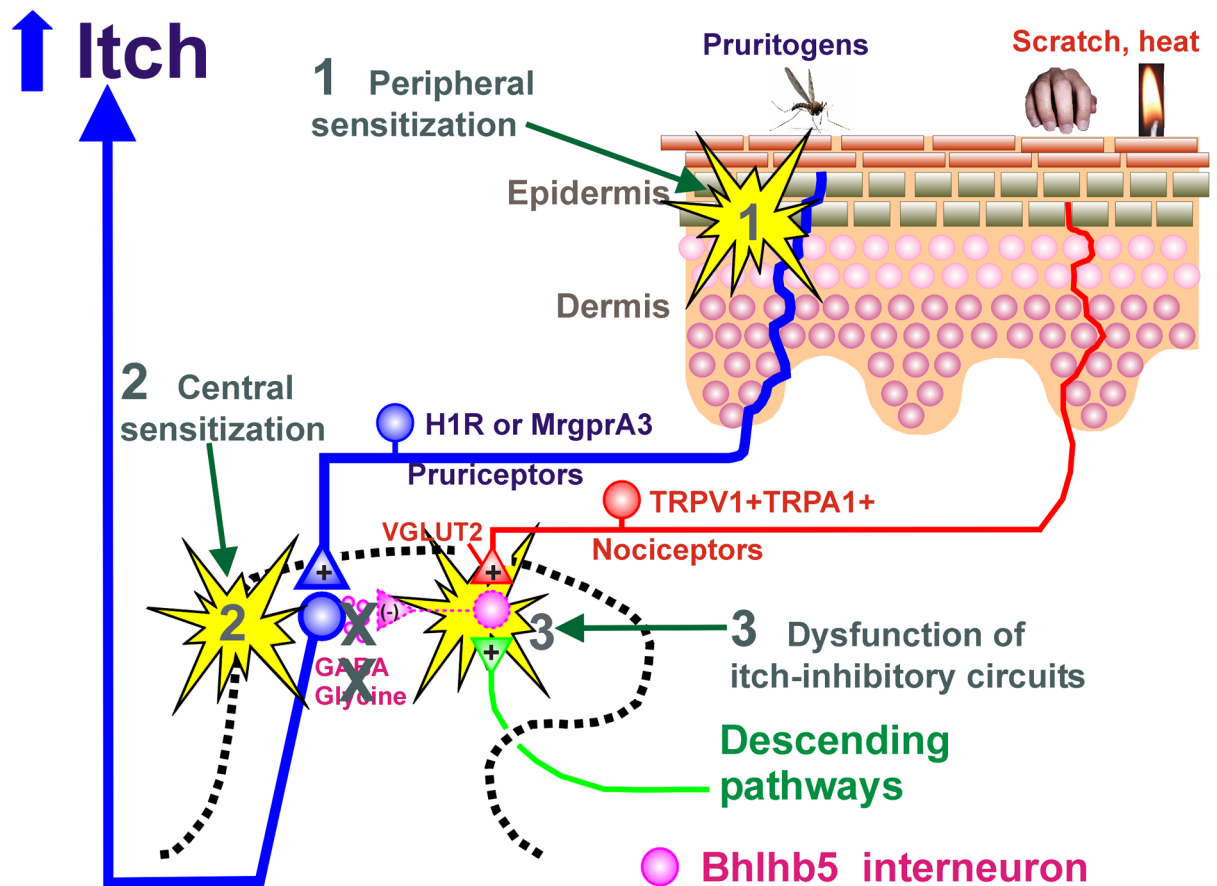
Fig. 3.
Schematic diagram of mechanisms underlying itch sensitization. 1. Peripheral sensitization may occur through PAR-2. 2. Central sensitization may occur through TLR3. 3. Dysfunction of itch inhibitory circuits may contribute to itch sensitization. See text for details.

Table 1

Pruritogens and mechanisms

category	pruritogen	Scratching			itch humans	mechanisms
		CD-1 mice	C57BL/6 mice	SD rats		
amines	histamine	+	+	−	+	H1/H4 receptor, PLC- 3, TRPV1
	5-HT	+	+	+	+	5-HT2 receptor
proteases (tethered ligands)	SLIGRL	+	+	−	+	PAR-2, MrgprC11
	AYPGKF	+	+	−	N.T.	PAR-4
	tryptase	+	N.T.	N.T.	N.T.	PAR-2
	mucunain	+	N.T.	N.T.	+	PAR-2, PAR-4
	cathepsin S	N.T.	N.T.	N.T.	+	PAR-2, PAR-4, MgprC11
neuropeptides	substance P	+	N.T.	−	+	mast cell degranulation, NK1 receptor, LTB4, NO
	endothelin-1	+	+	+	+	ETA receptor
lipid mediators	PAF	N.T.	N.T.	+	+	PAF receptor
	LPA	+	N.T.	N.T.	N.T.	histamine, Rho-ROCK
	LTB4	+	N.T.	N.T.	N.T.	BLT1 receptor
	TXA2	+	N.T.	N.T.	N.T.	TP receptor
	SPC	+	N.T.	N.T.	N.T.	Rho-ROCK, LTB4
	12-HETE	+	N.T.	N.T.	N.T.	BLT2 receptor
cytokines	IL-2	N.T.	N.T.	N.T.	+	mast cell degranulation?
	IL-31	N.T.	+	N.T.	N.T.	IL31RA/OSMR
Mrgpr agonists	chloroquine	+	+	−	+*	MrgprA3, TRPA1, mast cell degranulation
	BAM8-22	N.T.	+	N.T.	+	MrgprC11, TRPA1
	-alanine	N.T.	+	N.T.	+	MrgprD
uncategorized	compound 48/80	+	+	−	+	mast cell degranulation
	Bile acids	N.T.	+	N.T.	+	TGR5
	oxidative stress	N.T.	+	N.T.	N.T.	TRPA1
	TLR7 agonists	N.T.	+	N.T.	N.T.	TLR7, K2P, Kv1.1, Kv1.2

+, induction of itch or scratching; −, no induction of itch or scratching; N.T., not tested;

*
oral administration

Abbreviations: 5-HT, 5-hydroxytryptamine (serotonin); PAF, platelet activating factor; LPA, lysophosphatidic acid, LTB4, leukotriene B4; TXA2, thromboxane A2; SPC, sphingosylphosphorylcholine; 12-HETE, 12-Hydroxyeicosatetraenoic acid; BAM8-22, bovine adrenal medullary peptide 8-22; TLR7, toll-like receptor 7; H, histamine; PAR, protease-activated receptor; NO, nitric oxide; ETA, endothelin-1 A, Rho-ROCK, Rho-associated protein kinase; BLT, LTB4 receptor; IL31, interleukin 31; OSMR, oncostatin M receptor; Mrgpr, Mas-related G-protein-coupled receptor; TP, thromboxane receptor; TGR5, G protein-coupled bile acid receptor 1 (GPBAR1); TRPA1, transient receptor potential ankyrin 1; K2P, two-pore-domain potassium channels; Kv, voltage-gated potassium channel.