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Corresponding Author: Hyosang Lee

Authors: Jae Seung Lee[1], Jasmin Sanghyun Han[1], Kyeongho Lee[1], Juwon Bang[1], Hyosang Lee[1,*]

Institution: [1]Department of Brain and Cognitive Sciences, DGIST, Daegu 42988, Republic of Korea,

The peripheral and central mechanisms underlying itch

Jae Seung Lee[1], Jasmin Sanghyun Han[1], Kyeongho Lee, Juwon Bang, Hyosang Lee[2]

Department of Brain and Cognitive Sciences

DGIST

Daegu 42988, Republic of Korea

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[1]J.S.L. and J.S.H. contributed equally to this work.

[2]Author for correspondence

Telephone: 82-53-785-6147

FAX: 82-53-785-6109

e-mail: hyosang22@dgist.ac.kr

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Abstract

Itch is one of the most distressing sensations that substantially impair quality of life. It is a cardinal symptom of many skin diseases and is also caused by a variety of systemic disorders. Unfortunately, currently available itch medications are ineffective in many chronic itch conditions, and they often cause undesirable side effects. To develop novel therapeutic strategies, it is essential to identify primary afferent neurons that selectively respond to itch mediators as well as the central nervous system components that process the sensation of itch and initiate behavioral responses. This review summarizes recent progress in the study of itch, focusing on itch-selective receptors, signaling molecules, neuronal pathways from the primary sensory neurons to the brain, and potential decoding mechanisms by which itch is distinguished from pain.

Introduction

Pruritus, or itch, is a sensation that provokes scratching or the desire to scratch (1). Chronic itch is a major distressing symptom associated with many diseases of dermatological, systemic, neurological, or psychogenic origin (1). For example, extreme itch is a cardinal symptom of atopic dermatitis, which affects an estimated 17% of the world's population, primarily infants and children in urban areas and developed countries (2). Itch is also a common symptom associated with dry skin in the elderly population, experienced by 30-60% of the elderly within any 1-week period (3). Pruritus often arises as a side effect of medications and therapies, leading many patients to discontinue treatment. For example, ~30% of African malarial patients refuse to take the anti-malarial drug chloroquine because of unbearable itch (4). Chronic itch sufferers frequently cause self-harm through uncontrollable itch-scratch cycles.

Despite its evident clinical significance, our knowledge of the etiology and neurobiology of itch is far from complete. In order to develop new and highly selective treatments for a wide variety of persistent itch conditions, we must understand the peripheral and central mechanisms for acute and persistent itch. This review summarizes current knowledge of the molecular and cellular mechanisms underlying itch in the primary afferents and spinal cord and highlights the anatomical structures in the brain that are involved in the sensation and modulation of itch.

Pruriceptive primary afferents

Pruritogens (itch-causing compounds) are produced endogenously, introduced from the environment, or delivered as medications (5-7). They bind to specific receptors expressed in free nerve endings of primary sensory neurons innervating the skin, cornea, and mucous membranes. Activation of itch-specific receptors leads to an induction of receptor potentials, which, in turn, are transformed into action potentials in nerve endings. The electrical signals travel alongside the

primary sensory neurons to reach the central terminals innervating the dorsal horn of the spinal cord or the trigeminal subnucleus caudalis (Vc) (Fig. 1).

During the past decade, significant progress has been made in understanding the peripheral mechanisms of itch. Molecular and mouse genetic approaches have identified itch-sensitive receptors and specific molecular markers that label pruriceptive neurons and also provided potential drug targets to relieve itching. In addition, *in vivo* extracellular recordings have found sensory nerve fibers and spinal cord neurons that can be activated by cutaneous pruritogens. Together with human psychophysical studies, these techniques have underscored the strong correlation between neuronal activity and itch sensation.

Itch can be classified into two categories, histaminergic and nonhistaminergic, according to the degree of responsiveness to histamine (5). Histamine, the best-studied itch substance, is a bioactive amine released by mast cells and epithelial cells. Intradermal application of histamine produces intense itch, with a flare around the application site (1). Histamine binds to specific receptors in the plasma membrane, such as the H1 and H4 receptors, leading to the activation of downstream target molecules within sensory neurons, including G protein, phospholipase C, phospholipase A_2, 12-lipoxygenase, and the capsaicin receptor TRPV1 (8, 9). It has been shown that phospholipase β3 (PLCβ3) mediates an intracellular calcium increase in primary sensory neurons elicited by activation of the histamine H1 receptor (8). Mice deficient in PLCβ3 show a significant defect in scratching behavior elicited by histaminergic pruritogens, including histamine, a selective H1 agonist histamine-trifluoromethyl toluidine, and the mast cell activator compound 48/80 (8). Histamine-evoked electrophysiological and behavioral responses are substantially reduced by genetic deletion of TRPV1 in mice or by specific inhibitors targeting molecules in the histamine signaling pathway (9-11). Mice lacking TRPV1, however, exhibited

normal scratching behavior in response to other pruritogens, such as endothelin-1 (ET-1) or 5-HT, whereas chemical ablation of the central branch of $TRPV1^+$ neurons leads to a remarkable deficit in scratching in response to those pruritogens as well as histamine, suggesting that $TRPV1^+$ neurons are able to detect both histaminergic and nonhistaminergic pruritogens (8, 11).

In recent years, nonhistaminergic itch has been a main focus of itch research because many chronic itch conditions are not readily alleviated by antihistamine. Cowhage is a tropical legume, *Mucuna pruriens*, that evokes intense itch as well as pricking, stinging, and burning sensations in humans and scratching in monkeys and mice (12-17). Because cowhage-evoked itch is not diminished by antihistamine, it has been the preferred tool for exploring neuronal mechanisms of nonhistaminergic itch. When cowhage spicules are inserted into the skin, the cysteine protease mucunain is released and diffuses to reach nearby nerve endings of primary sensory neurons in the epidermis, activating protease-activated receptor (PAR) 2 and 4 (12, 18). PARs, members of the G protein-coupled receptor superfamily, are activated by synthetic peptides that match the sequence of the tethered ligands at the receptor's N-terminus, including Ser-Leu-Ile-Gly-Arg-Leu-NH_2 (SLIGRL) (19). Cutaneous application of SLIGRL elicits scratching behavior in animals. PAR2 and tryptase, the endogenous PAR2 agonist, are markedly increased in the skin of atopic dermatitis patients (20).

A large family of sensory neuron-specific G protein-coupled receptors known as Mas-related G protein-coupled receptors (Mrgprs) plays a key role in detecting nonhistaminergic pruritogens. MrgprA3 is expressed in a tiny subset of sensory neurons (4-5%) classified as peptidergic C-fibers (21). It has been identified as a receptor for a pruritic antimalarial medication, chloroquine, which evokes intense itch in humans, particularly those of African descent (4, 22). *In vivo* extracellular recordings reveal that $MrgprA3^+$ neurons are not only sensitive to chloroquine but also to histamine, bovine adrenal medulla 8-22 (BAM8-22), cowhage spicules, and even the

pungent vanilloid compound capsaicin. BAM8-22, a proteolytic cleavage product of proenkephalin A, provokes itch in humans, usually accompanied by pricking, stinging, and burning sensations (5). Mice ablated of MrgprA3$^+$ neurons exhibited a substantial deficit in scratching behavior evoked by diverse pruritogens, such as histamine, BAM8-22, SLIGRL, α-methyl-5HT, ET-1, as well as chloroquine (22). The ablated mice, however, respond normally to β-alanine, a supplement for muscle building that causes itch sensations in humans and scratching behavior in mice (23). These findings suggest that MrgrpA3$^+$ sensory neurons are able to detect diverse pruritogens, and a different population of neurons mediates β-alanine-evoked itch.

In fact, MrgprA3 is highly co-expressed with another Mrgpr, MrgprC11, which has been identified as a receptor for pruritic peptides such as BAM8-22, SLIGRL, and the cysteine protease cathepsin S (22, 24, 25). Mice lacking a cluster of Mrgprs, including MrgprC11, exhibit a substantial deficit in scratching behavior to these pruritogens, but their response to other pruritogens, such as histamine and compound 48/80, remains normal (22, 24, 25). Since mice lacking PAR2 exhibit a normal response to subcutaneously injected SLIGRL, MrgprC11 is thought to be a major contributor to SLIGRL-evoked itch (24).

MrgprD has been identified as a receptor that is specifically activated by β-alanine, and mice lacking of MrgprD fail to exhibit scratching after intradermal injection of β-alanine (23). Since MrgprA3 and MrgrpD are expressed in distinct subpopulations of C-fibers in mice, MrgprA3$^+$ neurons are unresponsive to β-alanine in *in vivo* extracellular recordings, and mice ablated of MrgprA3$^+$ neurons are normal in response to β-alanine, these receptors are apparently expressed in two distinct populations of pruriceptive neurons.

It has shown that TRPA1 is necessary for Mrgpr-mediated itch (26). For example, chloroquine and BAM8-22 activate a subset of TRPA1-expressing sensory neurons. Cultured sensory neurons isolated from TRPA1-deficient mice exhibit a remarkable reduction in calcium responses evoked by chloroquine and BAM8-22, but their histamine-evoked responses are unaffected. Conversely, sensory neurons from TRPV1-deficient mice respond normally to chloroquine and BAM8-22, but their histamine-evoked responses are substantially reduced. Thus, TRPV1 and TRPA1 are recruited to serve different types of itch, even though $TRPA1^+$ cells are known to express TRPV1. Mice lacking TRPA1 exhibit almost no scratching behavior upon subcutaneous injection of chloroquine and BAM8-22, but they show normal scratching in response to α-methyl-5HT, indicating that their deficit is specific to certain pruritogens.

ET-1, a potent vasoconstriction peptide, is produced by mast cells, endothelial cells, and keratinocytes (6). ET-1 elicits the sensation of itch accompanied by flare reactions in humans and scratching in mice. A monoamine neurotransmitter, 5-HT, also causes itch by activating the 5-HT-2 receptor after cutaneous application (6).

It is likely that itch is mediated by several different subpopulations of primary sensory neurons. Some itch-causing substances activate mostly overlapping populations of neurons, since their specific receptors are expressed in the same neurons (e.g., chloroquine vs. BAM8-22; MrgprA3 vs. MrgprC11), and other pruritogens activate distinct populations (e.g., chloroquine vs. β-alanine; MrgprA3 vs. MrgprD). The number of itch-responding sensory neurons and extent of overlap between responding populations are variable, depending on the type of pruritogen, method of delivery, and species.

A substantial body of evidence indicates that the vast majority of pruritogen-sensitive sensory

neurons respond not only to pruritogens but also to noxious mechanical, thermal, and/or chemical stimuli (14, 27-30). In human microneurography, for example, intradermal injection of histamine preferentially activates a subset of mechanically insensitive C-fibers (C-MIA) over a time-course matching the psychophysical sensation of itch, whereas topical application of cowhage spicules activates a subset of mechano-heat-sensitive C-fibers (CMH) (13, 14, 31). Most itch-sensitive C-MIAs and CMHs are also activated by intradermal injection of capsaicin. Thus, histamine and cowhage activate two distinct subpopulations of primary sensory neurons that also respond to noxious stimuli in humans. In nonhuman primates, however, both histamine and cowhage activate a subset of CMHs that also responded to noxious stimuli, indicating that those pruritogens activate an overlapping population of neurons that also respond to noxious stimuli (14). In line with this finding, native cowhage spicules and heat-inactivated spicules containing histamine applied to the mouse skin activate a subset of CMH; over 60% of histamine-sensitive CMH also respond to cowhage (17). Conversely, half of the cowhage-responsive fibers are also activated by histamine. Because these histamine- and cowhage-sensitive neurons also respond to noxious stimuli, they represent partially overlapping subpopulations of nociceptors. Other studies have also shown that diverse pruritogens activate partially overlapping subpopulations of nociceptors in rodent trigeminal and dorsal root ganglia (22, 32, 33).

Pruriceptive neurons in the spinal cord and spinal trigeminal nucleus

Itch-sensitive primary sensory neurons release specific neurotransmitters onto postsynaptic neurons in the spinal cord and Vc, where the itch signal is further processed by local excitatory and inhibitory neurons as well as descending synaptic inputs from the brain, before the itch information is transmitted to supraspinal regions, such as the thalamus and parabrachial nucleus (PB), via ascending neuronal pathways (Fig. 1). Projection neurons comprise only a small fraction of the neurons in the dorsal horn (~5% of lamina I neurons in the L4 segment of the rat

spinal cord), and many of them send collateral projections to synapse with multiple brain regions (34)

In conjunction with glutamate and substance P, gastrin-releasing peptide (GRP) has been considered as a key neuropeptide transmitter that is released from either the central terminals of itch-sensitive primary sensory neurons or local excitatory neurons, activating dorsal horn neurons that express the GRP receptor (GRPR) (7, 35, 36). GRP is a homolog of bombesin, a 14-amino acid peptide originally isolated from frog skin; intrathecal or intradermal injection of bombesin provokes scratching in animals (36, 37). Mice lacking GRPR or ablated of $GRPR^+$ neurons display a substantial reduction in scratching behavior in response to a variety of pruritogens, such as compound 48/80, SLIGRL, and chloroquine, but normal responses to painful stimuli, indicating that $GRPR^+$ neurons are selectively involved in itch signaling (35, 37). Consistent with this finding, mice deficient in the testicular orphan nuclear receptor exhibit a near-complete absence of scratching behavior in response to histamine, α-Me-5-HT, and chloroquine (38). In these mice, ~80% of GRP^+ or $GRPR^+$ neurons are ablated, suggesting the importance of GRP signaling in itch responses (38)

B-type natriuretic peptide (BNP, also known as natriuretic polypeptide B) is another itch-specific neurotransmitter expressed in a subset of primary sensory neurons that co-express MrgprA3, TRPV1, and PLCβ3 (36). It is released from the central terminals of these neurons to activate the postsynaptic second-order neurons in the spinal cord. Mice lacking in BNP exhibit greatly attenuated responses to a range of pruritic agents but retain normal reactions to thermal, touch, and proprioceptive stimuli. Conversely, intrathecal injection of BNP induces robust scratching in mice. Consistent with these findings, ablation of spinal interneurons expressing NPRA, a receptor for BNP, leads to a major attenuation in itch responses to intradermal injection of histamine or

intrathecal administration of BNP, but normal reactions to other somatosensory stimuli. Thus, BNP signaling plays a key role in itch transmission. It has been proposed that $NPRA^+$ neurons are the elements upstream of $GRPR^+$ neurons in itch-transmitting circuits (36).

In order to determine the neural basis of itch processing in the spinal cord, *in vivo* single-unit recordings have been performed from the spinal cord and Vc, following application of itch stimuli to the skin. The dorsal horn neurons can be classified according to their responsiveness to innocuous and noxious mechanical stimuli, into: 1) mechano-insenstive (MI), 2) low-threshold, 3) wide dynamic range (WDR), and 4) nociceptive-specific (NS) neurons (7, 39). WDR neurons respond to both innocuous and noxious mechanical stimuli, with higher-frequency discharges in response to noxious stimuli.

In vivo recordings have shown that the vast majority of itch-sensitive neurons in the spinal cord and Vc are WDR and NS neurons, and only a few are MI neurons. Most of these neurons are nociceptors that also respond to noxious stimuli. For example, in nonhuman primates, histamine and cowhage activate two largely separate subpopulations of neurons in the spinal cord, all of which are either WDR or NS neurons that also respond to noxious mechanical or thermal stimuli, indicating that histaminergic and nonhistaminergic itch are carried by two separate subpopulations responding to nociceptive stimuli (28, 30, 40). Antidromic mapping has revealed that the neurons involved are STT neurons projecting to the ventrobasal and posterior nucleus of the thalamus. Similarly, in rodents, the dorsal horn of the spinal cord and Vc contain a number of pruritogen-sensitive neurons, mostly WDR and NS neurons that also respond to noxious stimuli (7, 29, 32, 33, 41, 42). These findings also support the concept that itch information is mediated in the spinal cord and Vc by a subset of nociceptive neurons responding to noxious stimuli. Only a small fraction of itch-sensitive neurons are MI. Previous work in cats has identified a small number of histamine-sensitive MI neurons from the spinothalamic tract (STT) (43). Half of all

histamine-sensitive neurons are unresponsive to the noxious chemical mustard oil, indicating the existence of itch-specific neurons that are insensitive to noxious mechanical or chemical stimuli. The response of CMHs was not examined in this study.

It is likely that the vast majority of pruritogen-responsive neurons are local interneurons, since only a small subset of pruriceptive neurons in the spinal cord and Vc are projection neurons that innervate either the thalamus or PB in mice (44).

Neural coding mechanisms of itch and pain

Itch is closely linked to pain, and electrophysiological studies have shown that the majority of itch-sensitive neurons are nociceptors, raising an important question as to how itch is differentiated from pain. Both itch and pain are complex sensory and emotional experiences created by neuronal activities in the peripheral and central nervous systems. They are detected by a subset of primary afferents in the somatosensory system, mainly by slowly conducting unmyelinated C-fibers and thinly myelinated Aδ-fibers (5, 16, 45). A number of receptors and signaling molecules share in transducing itch and pain within sensory neurons. As such, pruriceptive and noxious stimuli often activate the shared populations of neurons. Moreover, alleviation of pain can produce a sensation of itch; morphine, for example, inhibits pain but causes a sensation of itch in humans and scratching behavior in animals (46). Conversely, itch is temporarily relieved by scratching. Finally, both itch and pain elicit unpleasant sensations. However, they clearly evoke qualitatively different sensations and behavioral responses, suggesting that there should be a mechanism by which itch and pain are differentiated.

There have been debates as to the neural basis of itch processing. Itch has been considered for many years a sub-modality of pain in which itch and pain are served by the same population of neurons, and the sensation is determined by the pattern of neuronal activity; itch is elicited when

sensory neurons are activated weakly, whereas pain is evoked when neurons are strongly activated (5). This so-called intensity theory was supported by the experimental finding that most itch-sensitive neurons also respond to painful stimuli. Moreover, high doses of pruritogen produce pain, whereas low doses of algogen evoke itch. For instance, intradermal injection of a high concentration of histamine elicits pain (47, 48). Conversely, capsaicin, which normally causes intense burning pain when injected intradermally, elicits itch when delivered topically or applied as heat-inactivated cowhage spicules coated with capsaicin (15, 48).

Primary afferents and postsynaptic neurons in the spinal cord exhibit relatively higher-frequency discharges in response to noxious stimuli than do pruritogens (17, 28, 30, 32, 33, 40-42, 49). For example, capsaicin provokes higher-frequency discharges in the trigeminothalamic and STT neurons than do histamine or cowhage (17, 28, 40, 49). How information contained in the pattern of activity is used to produce the specific sensation of itch in the higher-order neurons in the brain remains to be determined. There is experimental evidence that challenges the intensity theory. For instance, an itch sensation provoked by neuronal or cutaneous electrical stimulation does not transform into pain at higher-frequency stimulation(50, 51). Also, a specific firing pattern of CMHs does not correlate with itch and pain sensation in humans (52). Since these studies focused on primary afferents, it remains unclear whether intensity coding is a valid mechanism in the spinal cord and brain.

A number of pieces of genetic and behavioral evidence support another theory, the “labeled line” theory, in which specific populations of neurons are dedicated to serving either itch or pain. For example, mice ablated of either MrgprA3^{+} primary sensory neurons, GRPR^{+} neurons in the spinal cord, or NPRA^{+} neurons in the spinal cord, or mice lacking BNP all exhibit a selective deficit in scratching behavior in response to a variety of pruritogens, whereas these manipulations have no effect on nociception (21, 35, 36). In an elegant genetic experiment, TRPV1-knockout mice were

engineered to express TRPV1 only in itch-sensitive MrgprA3^{+} primary sensory neurons (21). When capsaicin was applied, these animals exhibited only itch-related behaviors without pain-related behaviors; even though MrgprA3^{+} neurons were found to respond to noxious stimuli in electrophysiological recordings. To accommodate the discrepancies in the electrophysiological and behavioral findings, a new theory, known as "population coding" was proposed, in which the sensation of itch is elicited by activating "pruriceptive nociceptors" that respond to both pruriceptive and noxious stimuli in electrophysiological recordings; in contrast, the sensation of pain is elicited by noxious stimuli, which activate an additional population responding only to noxious stimuli (5, 7). The activity in the "nociceptive-specific" population is speculated to prevent or mask itch transmission by the pruriceptive nociceptors, possibly via local inhibitory mechanisms.

Several lines of evidence support this model. First, selective deletion of vesicular glutamate transporter 2 in a subset of nociceptive neurons leads to spontaneous scratching and capsaicin-evoked itch in mice, suggesting that baseline glutamatergic signaling inhibits itch transmission (53, 54). Second, activity-dependent silencing of a subset of nociceptors using the lidocaine derivative QX-314, followed by activation of another subpopulation of nociceptors, elicits scratching rather than pain behavior in mice (55). QX-314 permeates through activated ion channels such as TRPV1 and inhibits neuronal firing by blocking voltage-gated channels inside the cell. Finally, selective ablation of inhibitory interneurons expressing the transcription factor basic helix–loop–helix domain-containing, class B5 (BHLHB5) results in a substantial elevation of spontaneous scratching, suggesting a potential role for these neurons in suppressing itch transmission by pruriceptive nociceptors (56). A recent study has shown that transplantation of precursors of cortical inhibitory neurons in the spinal cord can rescue the itch-related phenotypes of these mice, presumably by restoring inhibitory mechanisms in the spinal cord (57). The local circuit involving BHLHB5 neurons remains to be determined.

Itch processing in the brain

One of the major insights that has emerged from pain studies is that chronic pain is a disease not only of the primary sensory neurons and spinal cord but also of the brain. Chronic pain causes functional and structural alterations in the brain and also affects other brain functions such as emotion, motivation, and memory. Developing an effective itch treatment, therefore, must take into consideration a therapeutic strategy targeting the brain.

Itch is the multidimensional experience that involves perception of the sensory and emotional-affective aspects of itch as well as an urge to scratch that uses the motor system (1). The perception of itch occurs in the brain by interpreting the neuronal activities stemming from projection neurons in the spinal cord and Vc that receive itch signals from pruriceptive primary afferents.

Most of our current knowledge on itch processing in the brain is derived from a small number of brain imaging studies performed on human subjects, which have utilized techniques such as positron emission tomography, functional magnetic resonance imaging, and magnetoencephalography. Itch was produced in healthy subjects by histamine, cowhage, or electrical stimulus delivered locally to the skin, leading to increased or decreased cerebral activity as well as the sensation of itch (Table 1). A few studies focused on itch processing in the brain of chronic itch patients suffering from atopic dermatitis or end-stage renal disease (ESRD), and other studies tested cerebral activity during suppression of itch (Table 2).

As mentioned above, itch-mediating projection neurons in the spinal cord and Vc are a small subset of WDR and NS neurons that also respond to noxious stimuli. Thus, it is highly likely that itch and pain share common neuronal pathways to and within the brain. Consistent with this idea, brain regions activated by pruritic and noxious stimuli overlap extensively (58-60). Pruriceptive

STT neurons send their axons across the midline of the spinal cord and ascend within the anterolateral column pathway to reach the contralateral thalamus, particularly within the ventroposterior medial or posterior thalamic nuclei (28, 30, 40, 43, 61). As is true for pain, itch information may be further relayed to the primary (S1) and secondary (S2) somatosensory cortices, insular cortex (IC), and cingulate cortex (61, 62). As such, the thalamus is one of the prominent regions consistently activated by histamine and cowhage in multiple studies (59, 63-66). Interestingly, cowhage elicits more extensive activation in the thalamus than does histamine, as well as in other regions such as the IC, claustrum, globus pallidum, and putamen, consistent with the finding that cowhage-responsive STT neurons in nonhuman primates innervate larger areas within the thalamus than do histamine-responsive STT neurons (40, 66). Overall, both pruritogens activate mostly overlapping regions. The S1 and S2 are specialized for receiving sensory input and are thought to participate in processing the sensory-discriminative aspect of itch. Since activity in the S1 is positively correlated with the intensity of an itch stimulus, this region is speculated to play an important role in interpreting the intensity of stimuli (58). Several studies report the activation of the S2, but its role in itch is unclear (59, 64, 66, 67).

The IC is known to mediate the integration of autonomic, visceral, and limbic functions (68). It is connected to many other regions in the brain, including the cingulate gyrus, frontal, parietal, and temporal lobes, as well as subcortical structures such as the thalamus, amygdala, and brainstem (69, 70). Previous studies have shown that the IC can be roughly divided into several subregions with unique connectivity and functional features (71). The anterior agranular insular cortex (aIC) is connected mainly to other cortical areas and plays a role in awareness of bodily states by integrating autonomic and interoceptive information, whereas the posterior granular part (pIC) receives nociceptive inputs from the primary afferents via the brainstem and thalamic nuclei to mediate pain processing (68, 72, 73). It has been shown that itch activates both the aIC and pIC (60, 65). The activity in the aIC is positively correlated with the unpleasant sensation of itch,

while the activity of the pIC is correlated with the intensity of the itch stimulus (58-60, 64-66). In ESRD patients with chronic itch, bilateral activation of the insular cortex is observed even in the absence of pruritogen stimulation (67).

The cingulate cortex is one of critical brain regions contributing to the processing of the affective component of pain (74). For example, the anterior cingulate cortex (ACC) is activated upon anticipation or response to acute noxious stimulus or during chronic pain (75). Consistently, surgical damage to the cingulate cortex in humans decreases the affective response to noxious stimuli, while leaving intact the ability to localize the unpleasant stimuli (74, 76). The ACC is also activated by itch stimuli, mainly in its dorsal part (dACC) as well as the anterior part of the midcingulate cortex (aMCC) (58-60, 63-67, 77). Since electrical stimulation of the MCC evokes the motivation to act, the dACC/aMCC appears to be associated with recognizing itch stimuli and preparing motor behavior (58, 60, 63-65, 78, 79). Many studies have found activation of other motor-related regions, including the supplementary motor area, premotor cortex, primary motor cortex, and cerebellum (58-60, 63-67, 77, 79, 80). These regions may play a role in recognizing the location of itch stimuli as well as organizing and executing motor responses such as scratching (58-60, 63-67, 77, 79, 80). Like the S1, activity in these regions is correlated with the intensity of the itch stimulus (58, 63). Interestingly, scratching the itchy skin also elicits activation of these brain regions, and the activity is higher when scratching provokes pleasure than when it does not (81-83).

In rodents, the majority (~80%) of projection neurons in the superficial dorsal horn of the spinal cord innervate the PB, which is connected to the amygdala and hypothalamus and also to the IC (84). As described above, *in vivo* single-unit recordings in mice have identified pruriceptive neurons in the spinal cord and Vc that project to PB, implicating the spino-parabrachio-amygdaloid pathway in itch processing (85-87). Brain imaging studies, however, have failed to

detect activity changes in the PB, probably because most of the projection neurons in the spinal cord in humans send their axons to the thalamus rather than the PB.

The amygdala is also one of the key cerebral structures participating in the sensation, expression, and modulation of pain (86, 87). In particular, the central nucleus of the amygdala (CeA) is considered the output nucleus of the amygdala and integrates nociceptive information from the cerebral cortex and thalamus as well as nociceptive inputs from the PB (88). A recent study has shown that inhibition of GABAA receptors by bilateral microinjection of bicuculline into the rat CeA dramatically increases scratching behavior in acute and chronic itch models, suggesting a role for inhibitory mechanisms in the CeA in itch modulation (89). Although the amygdala appears to be involved in itch processing, its role is still unclear, because it was activated in response to a combinatorial application of histamine and cowhage in one study but inactivated by individual treatment in another study (59, 66, 90).

It has been reported that reversible cold-block or complete transection of the upper cervical spinal cord causes a 30-50% reduction in the inhibition of ongoing spontaneous firing of the dorsal horn neurons caused by scratching in a mouse model of chronic dry itchy skin, indicating that supraspinal mechanisms are partially involved in itch inhibition by scratching (91). Indeed, mosquito allergy-elicited biting behavior is inhibited by an intrathecal α(2)-adrenoceptor antagonist but increased by the catecholaminergic neurotoxin 6-hydroxydopamine and the α-adrenoceptor antagonist, indicating that the descending noradrenergic system tonically inhibits itch signaling in the spinal cord (92). Conversely, serotonergic neurons in the rostral ventromedial medulla appear to control itch transmission in the dorsal horn of spinal cord by facilitating GRP-mediated signaling (93). On the other hand, electrical stimulation of PAG inhibits the spiking responses of STT neurons activated by subcutaneous histamine (27). A human PET study has demonstrated that application of a painfully cold stimulus to histamine-evoked itch increases

activity in the PAG, suggesting the possibility of a descending inhibitory mechanism of itch in the PAG (63). PAG was activated during scratching the cowhage-evoked itch in one study but inactivated in another study (82, 94). Thus, the role of PAG in itch suppression is still unsettled, and further investigation is needed.

Conclusions

Compared to the extensive research on pain, itch has received relatively less attention until recently. However, our knowledge of the molecular and neuronal circuit mechanisms of itch detection in the skin and itch transmission within the spinal cord and Vc has greatly expanded during the past decade. Identification of itch-specific receptors and neurotransmitters has provided important insights into novel therapeutic strategies that selectively target itch-mediating neurons.

Unfortunately, our current knowledge of the supraspinal processing of itch is limited and relies mainly on a small number of brain imaging studies. Those studies have revealed that cutaneous pruritogen application activates brain regions involved in somatosensory, limbic, and motor-related functions, such as S1, the thalamus, ACC, IC, supplementary motor area, primary motor cortex, and cerebellum. Since the same brain regions are also activated by noxious stimuli, it is puzzling how itch is distinguished from pain in the brain. Given their distinct sensations and different behavioral responses (scratching vs. withdrawal), there should be a mechanism by which these two sensory modalities are differentiated. One possibility is that a single population mediates both itch and pain, using distinct patterns of neuronal activation, spike timing, or other mechanisms. Another possibility is that the brain regions contain two intermingled or closely adjacent subpopulations that are each specialized for either itch or pain but cannot be detected in brain imaging studies because of limited spatial resolution. Thus, it is essential to study itch in the brain using molecular genetic approaches to identify itch-mediating brain regions at the level of neuronal circuits and to decipher the neuronal circuit mechanism underlying the interrelationship between itch and pain. A combinatorial approach of the advanced techniques to reveal neuronal circuits, including *in vivo* imaging such as multi-photon microscopy and microendoscopy, functional manipulation tools such as optogenetics and chemogenetics, and population-specific

neuronal tracing methods based on pseudorabies and herpes virus, will help us to achieve these goals (95-98).

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Table 1. Brain activity upon itch stimulation (58-60, 63-67, 77-80, 90, 99, 100)

Brain imaging studies have shown that cutaneous application of pruritogens (cowhage and histamine) or electrical stimulation lead to activity changes in multiple brain regions of healthy subjects and chronic itch patients (R, right hemisphere; L, left hemisphere; bi, both hemispheres). Unless otherwise indicated with (↓), all regions were activated during pruritic stimulation. *Mixed activity (both activation and deactivation) was found within a region. PFC, prefrontal cortex; dlPFC, dorsolateral prefrontal cortex; OFC, orbitofrontal cortex; mOFC, medial orbitofrontal cortex; middleOFC, middle orbitofrontal cortex; lOFC, lateral orbitofrontal cortex; MFC, medial frontal cortex; SFG, superior frontal gyrus; MFG, middle frontal gyrus; IFG, inferior frontal gyrus; iPFG, inferior prefrontal gyrus; preCG, precentral gyrus; postCG, postcentral gyrus; SMA, supplementary motor area; preSMA, presupplementary motor area; PMC, premotor cortex; M1, primary motor cortex; S1, primary somatosensory cortex; S2, secondary somatosensory cortex; CC, cingulate cortex; ACC, anterior cingulate cortex; dACC, dorsal anterior cingulate cortex; vACC, ventral anterior cingulate cortex; preACC, pregenual anterior cingulate cortex; periACC, perigenual anterior cingulate cortex; subACC, subgenual anterior cingulate cortex; aMCC, anterior midcingulate cortex; MCG, midcingulate gyrus; PCC, posterior cingulate cortex; dPCC, dorsal posterior cingulate cortex; vPCC, ventral posterior cingulate cortex; RSC, restrosplenial cingulate cortex; PC, parietal cortex; aPC, anterior parietal cortex; pPC, posterior parietal cortex; iPC, inferior parietal cortex; SMG, supramarginal gyrus; IPL, inferior parietal lobe; SPL, superior parietal lobe; IC, insular cortex; aIC, anterior insular cortex; pIC, posterior insular cortex; BG, basal ganglia; TH, thalamus; AD, atopic dermatitis; ESRD, end-stage renal disease; LS, lesional skin; NLS, nonlesional skin.

Table 2. Brain activity during suppression of itch(63, 81-83, 101, 102)

Activity changes in brain regions during suppression of itch transmission by cold block, scratching (active scratching by subjects or passive scratching by experimenters), butorphanol (a

mixed action opioid), or acupuncture (R, right hemisphere; L, left hemisphere; bi, both hemispheres). Unless otherwise indicated with (↓), all regions were activated during pruritic stimulation. Brain imaging was performed following a VAC application to the itchy skin during the sensation of itch was either increasing* or at peak**. dlPFC, dorsolateral prefrontal cortex; dmPFC, dorsal part of medial prefrontal cortex; vmPFC, ventral part of medial prefrontal cortex; lPFC, lateral prefrontal cortex; vlPFC, ventral lateral prefrontal cortex; OFC, orbitofrontal cortex; MFG, middle frontal gyrus; MFC, medial frontal cortex; LFC, lateral frontal cortex; SMA, supplementary motor area; PMC, premotor cortex; M1, primary motor cortex; S1, primary somatosensory cortex; S2, secondary somatosensory cortex; ACC, anterior cingulate cortex; preACC, pregenual anterior cingulate cortex; subACC, subgenual anterior cingulate cortex; MCC, midcingulate cortex; PCC, posterior cingulate cortex; dPCC, dorsal posterior cingulate cortex; vPCC, ventral posterior cingulate cortex; IC, insular cortex; aIC, anterior insular cortex; pIC, posterior insular cortex; NAc, nucleus accumbens; PC, parietal cortex; BG, basal ganglia; TH, thalamus; VTA, ventral tegmental area; PAG periaqueductal gray; AD, atopic dermatitis; ESRD, end-stage renal disease; NLS, nonlesional skin; VAC, verum acupuncture.

Figure legends

Figure 1. Itch signaling pathway

a. Schematic illustrating the transmission of itch from the primary sensory neurons to the brain. Itch stimuli (pruritogens) activate itch-sensing neurons in the dorsal root ganglion (DRG) that innervate the skin, which then stimulate second-order neurons in the spinal cord and multiple brain regions. Indicated in the tables are pruritogens, itch-selective molecules and receptors expressed in the primary sensory neurons and spinal cord, and brain regions activated by cutaneous application of a pruritogen. STT, spinothalamic tract; SPA, spino-parabrachio-amygdaloid pathway; PFC, prefrontal cortex; SMA, supplementary motor area; PMC, premotor cortex; M1, primary motor cortex; S1, primary somatosensory cortex; S2, secondary somatosensory cortex; CC, cingulate cortex; IC, insular cortex; BG, basal ganglia; GRP, gastrin-releasing peptide; GRPR, gastrin-releasing peptide receptor; BNP, B-type natriuretic peptide; NPRA, natriuretic peptide receptor A; NK-1, neurokinin-1; H1, histamine H1 receptor; PLCβ3, phospholipase C β3; PLA2, phospholipase A2; 5-HT, 5-hydroxytryptamine (serotonin); 5-HT-2, 5-HT receptor subtype 2; PAR2, protease-activated receptor 2; Mrgpr, Mas-related G-protein-coupled receptor; ET-1, endothelin-1; ET_A, endothelin-1 receptor A; BAM8-22, bovine adrenal medullary peptide 8-22.

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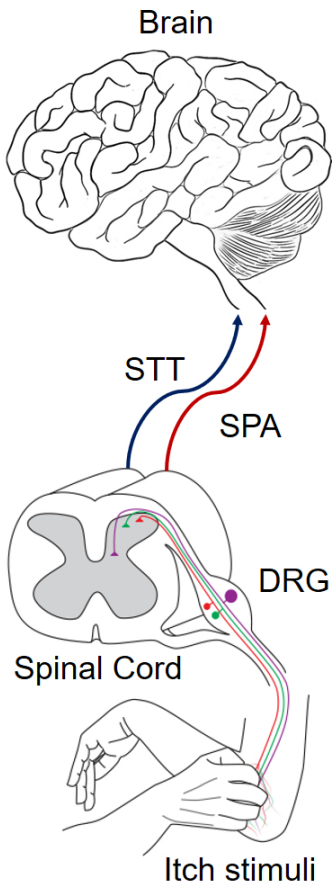
Brain

PFC	S1	Parietal cortex
SMA	S2	Precuneus
PMC	Thalamus	BG
M1	IC	
Cerebellum	CC	

Spinal cord

Neurotransmitter	Receptor
GRP	GRPR
BNP	NPRA
NK-1	NK-1 receptor



Primary sensory neuron

Pruritogen	Signaling molecule
Histamine	H1, H4 PLCβ3 PLA2 12-lipoxygenase
5-HT	5-HT-2
Tryptase	PAR2
SLIGRL	PAR2 MrgprC11
Cathepsin S	PAR2 MrgprC11
ET-1	ET_A
Chloroquine	MrgprA3
BAM8-22	MrgprC11
β-alanine	MrgprD

Fig. 1

Table 1. Brain activity upon itch stimulation

Subject	Imaging	Stimulus (method)	Location	Brain region	Reference
Healthy	PET	Histamine (Intracutaneous injection)	Right forearm	SFG(bi), MFG(bi), IFG(R), SMA(bi), PMC(bi), ACC(L), IPL(R), Cerebellum(bi)	ref. 78
Healthy	PET	Histamine (skin prick)	Right forearm	PFC(L), SMA(L), PMC(L), M1(L), S1(L), PC(SMG(L))	ref. 79
Healthy	PET	Histamine (skin prick)	Right forearm	PFC(L), preCG(L), IFG(L), MFG(L) SMA(L), PMC(L), ACC(L), PC (postcentral(R) & superior(R))	ref. 58
Healthy	fMRI	Histamine (skin prick)	Right foot	periACC(bi), aIC(bi), pIC(L), BG (caudate(bi), ventral caudate(R), ventral putamen(R)), TH(bi)	ref. 65
Healthy	fMRI	Histamine (skin prick)	Left forearm	IFG(R), IC(R), preCG(L)↓, MFG↓, preACC↓, subACC↓, cerebellum↓	ref. 90
Healthy	fMRI	Histamine (skin prick) at 25℃ (skin)	Right forearm	dlPFC(bi), preSMA(R), aIC(L), iPC(bi), TH(bi), OFC(L)↓, MFC(R)↓, M1(L)↓, dACC(R)↓	ref. 99
Healthy	PET	Histamine (iontophoresis)	Right foot	dlPFC(bi), PMC(R), CC(L), aPC(R), pPC(R), TH(L)	ref. 63
Healthy	fMRI	Histamine (iontophoresis)	Left wrist	preSMA(L), ACC(R), PCC(R), aIC(bi), pIC(L), BG(L)	ref. 60
Healthy	PET	Histamine (iontophoresis)	Left hand	SFG(L), preCG(R), postCG(R), MCG(R)	ref. 80
Healthy	fMRI	Histamine (iontophoresis)	Right forearm	M1(L), S1(L), precuneus(L), IPL(L), SPL(L)	ref. 77
Healthy	fMRI	Histamine (microdialysis)	Left forearm	SFG(R), MFG(bi), IFG(L), preCG(bi), SMA(bi), PMC(bi), S1(R), S2(bi), aMCC(bi), precuneus(R), IPL(bi), SPL(R), aIC(bi), BG (caudate body(bi), caudate tail(bi)), TH(bi), cerebellum, subACC(bi)↓, amygdala(bi)↓	ref. 59
Healthy	fMRI	Cowhage	Right forearm	PMC(L), S1(L), S2(bi), PCC(bi), ACC(L), precuneus(L), IC(bi), SMG(bi), angular gyrus(bi), hippocampus(L), putamen(bi), TH(bi), cerebellum(L)	ref. 66
Healthy	fMRI	Histamine (iontophoresis) & cowhage	Right forearm	SFG(R), OFC(L), IFG(R), SMG(L), S1(L), S2(R), ACC(bi), PCC(bi), IC(bi), angular gyrus(L), IPL(L), SPL(L), precuneus(bi), BG (putamen(bi), lateral globus pallidus(L)), Amygdala(bi), TH(L)	ref. 66
Healthy	fMRI	Allergen (skin prick)	Right foot	mOFC(L), middle OFC(L), lOFC(L), SMA(L), PMC(L), M1(L), S1(L), periACC, subACC(bi), pPC(L), BG (caudate(bi)), TH(R)	ref. 65
Healthy	fMRI	Electrical stimulus	Left wrist	PFC(R), SMA(R), PMC(R), S2(bi), ACC(L), PCC(L), precuneus(R), IC(bi), aPC(bi), pPC(R), TH, cerebellum(L)	ref. 64
AD (no active phase)	PET	Histamine (iontophoresis)	Left hand	SFG(bi), MFG(R), IFG(bi), preCG(bi), postCG(R), IC(L), PC (SMG(R)), BG (pallidum(L)), TH(R), cerebellum(bi)	ref. 80
AD	fMRI	Histamine (iontophoresis)	Right forearm (LS)	dlPFC(bi), IPFG(L), SFG(bi), MFG(R), IFG(L), PMC(bi), vACC(bi), dACC(bi), vPCC(bi), dPCC(bi), RSC(bi), precuneus(bi), aIC(R), pIC(R), IPL(R), SPL(R), BG (caudate(L), putamen(L))	ref. 77
AD	fMRI	Histamine (skin prick) at 25℃ (skin)	Right forearm (NLS)	PFC↓, SMA↓, PMC↓, S1↓, S2↓, CC↓, IC↓, BG↓	ref. 100
AD	fMRI	Histamine (skin prick) at 25℃ (skin)	Right forearm (LS)	*PFC↑↓, PMC↓, S1↓, S2↓, CC↓, IC↑, *PC↑↓, BG↑	ref. 100
ESRD	fMRI	Histamine (iontophoresis)	Right forearm	OFC(L), SFG(R), MFG(R), IFG(L), S1(L), ACC(L), PCC(bi), paracingulate gyrus(L), precuneus(bi), SPL(bi), SMG(L), angular gyrus(L)	ref. 67
ESRD	fMRI	Cowhage	Right forearm	PMC(bi), M1(R), S1(L), S2(L), ACC(bi), PCC(R), precuneus(bi), IC(L), angular gyrus(L), SMG(L), BG (putamen(L)), TH(R)	ref. 67

Table 2. Brain activity during itch suppression

Subject	Imaging	Itch (suppression)	Location	Brain region	Reference
Healthy	PET	Histamine - iontophoresis (Cold pain (5℃))	Right foot (itch) Left foot (pain)	S2(bi), TH(R), Midbrain(R) (including PAG)	ref. 63
Healthy	fMRI	Histamine - iontophoresis (Passive scratching)	Right hand	MFC(bi), LFC(bi), PMC(bi), M1(bi), S1(bi), S2(bi), subACC(bi), preACC(bi), dPCC(bi), vPCC(bi), aIC(bi), pIC(bi), PC(bi), BG (putamen(bi)), TH(bi), cerebellum(bi)	ref. 81
Healthy	fMRI	Cowhage i (Passive scratching)	Right forearm	S1, S2, PCC, precuneus, hippocampus, subthalamic nucleus, vlPFC↓, OFC↓, M1↓, ACC↓, IC↓, TH↓, BG (Putamen)↓	ref. 82
Healthy	fMRI	Cowhage (Active scratching)	Right forearm	dlPFC, SMA, PMC, M1, S1, S2, ACC, PCC, precuneus, BG (Caudate), TH, cerebellum, vlPFC↓, OFC↓, frontal medial cortex↓, ACC↓, IC↓, NAc↓, hippocampus↓, amygdala↓, cerebellum (anterior lobe, culmen)↓, midbrain (VTA, PAG, Dorsal nucleus of the raphé)↓	ref. 82
Healthy	fMRI	Cowhage (Active scratching)	Right forearm	dmPFC(R), lPFC(bi), PMC(R), M1(bi), S1(bi), ACC(R), MCC(bi), IC(bi), PC(bi), TH(bi), cerebellum(bi)	ref. 83
Healthy	fMRI	Histamine - iontophoresis (Butorphanol)	Right forearm	subACC(R)	ref. 101
Healthy	fMRI	Cowhage (Butorphanol)	Right forearm	S1(L)↓, PCC(L)↓, IC(R)↓, TH(L)↓, cerebellum (culmen(L))↓	ref. 101
AD, psoriasis, ESRD	fMRI	Cowhage (Active scratching)	Right forearm	dmPFC(L), vmPFC(L), lPFC(bi), SMA, PMC(bi), M1(L), S1(L), ACC(R), MCC(R), precuneus(R), IC(bi), PC(bi), TH(bi)	ref. 83
AD	*fMRI	Allergen - skin prick (VAC)	Left forearm (NLS)	aIC(R)↓, NAc(R)↓, putamen(R)↓, globus pallidus(R)↓, caudate(R)↓	ref. 102
AD	**fMRI	Allergen - skin prick (VAC)	Left forearm (NLS)	MFG(R)↓, PMC(R)↓, M1(bi)↓, S1(bi)↓, S2(R)↓, PCC(L)↓	ref. 102