Circuit dissection of the role of somatostatin in itch and pain

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Stimuli that elicit itch are detected by sensory neurons that innervate the skin. This information is processed by the spinal cord; however, the way in which this occurs is still poorly understood. Here we investigated the neuronal pathways for itch neurotransmission, particularly the contribution of the neuropeptide somatostatin. We find that in the periphery, somatostatin is exclusively expressed in Nppb+ neurons, and we demonstrate that Nppb+ somatostatin+ cells function as pruricceptors. Employing chemogenetics, pharmacology and cell-specific ablation methods, we demonstrate that somatostatin potentiates itch by inhibiting inhibitory dynorphin neurons, which results in disinhibition of GRPR+ neurons. Furthermore, elimination of somatostatin from primary afferents and/or from spinal interneurons demonstrates differential involvement of the peptide released from these sources in itch and pain. Our results define the neural circuit underlying somatostatin-induced itch and characterize a contrasting antinociceptive role for the peptide.

The somatosensory system helps us evaluate our environment, for instance, alerting us to harmful or potentially damaging conditions. Through this system, noxious stimuli generate itch and pain percepts. The sensation of itch, which warns us to the presence of organisms or substances on or in the skin, triggers removal of these agents. In contrast, painful stimuli produce immediate escape to prevent tissue damage. The presence of painful and itch-inducing stimuli is detected by sensory neurons with cell bodies in the dorsal root ganglion (DRG) or trigeminal ganglion. These nociceptive and pruricceptive neurons transmit signals to the dorsal horn of the spinal cord or spinal trigeminal nucleus.

Many different agents elicit itch, and it is thought that these are detected by specific populations of pruricceptive primary afferent neurons1. One class, those that express the MrgA3 receptor, are likely dedicated for pruritogen detection2. Another population expresses the neuropeptide natriuretic polypeptide B (Nnpb), and since Nnpb is necessary for itch behavior, it has been suggested that these neurons also function as pruricceptors3. Nnpb is thought to transmit signals from peripheral afferents to cells in the dorsal horn that express Npr1 (the receptor for Nnpb)4. Upon activation, these neurons are believed to release gastrin-releasing peptide (GRP), which in turn activates interneurons that express GRPR receptor (GRPR)1. Npr1- and GRPR-expressing interneurons are both selectively required for itch sensation, suggesting a specific neuronal circuit for itch5,6.

Recently, molecular approaches have started to uncover mechanisms for somatosensory information processing in the spinal cord6, and these reveal that the dorsal horn is a site of considerable integration of sensory signals7–11. Itch can be suppressed by other sensory inputs (counterstimuli)—for example, biting or scratching—and this seems to involve modulation within the dorsal horn. The neurons that mediate the suppression of itch by counterstimulation are thought to include a group of inhibitory cells known as B5-I neurons, because they depend on expression of the transcription factor Bhlhb5. Mice lacking B5-I neurons show exaggerated itch responses, suggesting that itch is inhibited by tonic or feedforward input from these cells12. The B5-1 neurons, which account for around one-third of inhibitory interneurons in the superficial dorsal horn, can be subdivided into two populations: those that express the neuropeptides dynorphin and galanin; and those that contain neuronal nitric oxide synthase (nNOS)13–15. Dynorphin inhibits itch, suggesting that B5-I neurons may suppress itch at least partly through dynorphin–kappa opioid receptor (KOR) signaling. However, Duan et al. recently concluded that dynorphin-expressing spinal cord neurons were not involved in suppressing itch16. There is therefore doubt about which cells are responsible for B5-1-mediated itch suppression.

The inhibitory neuropeptide somatostatin is expressed in a small population of DRG neurons17. Transcriptomic studies indicate that these correspond to cells that express Nnpb, together with several itch-related genes18,19, and it has recently been reported that ablation of somatostatin-expressing primary afferents caused itch deficits19. Notably, intrathecal administration of somatostatin elicits scratching behavior20, hinting that it may be involved in enhancing itch. There are conflicting reports suggesting that somatostatin can either promote or attenuate pain20–24. As well as being present in primary afferents, somatostatin is expressed by many dorsal horn excitatory interneurons25, and these are important elements for transmission.
of mechanical pain. Therefore, there is considerable uncertainty about the roles of somatostatin in itch and pain.

Here we have used multiple approaches to examine how itch sensation can be modulated by somatostatin. Using optogenetics, we demonstrate that sensory neurons expressing somatostatin and Nppb function as pruriceptors, and we show that somatostatin potentiates scratching evoked by GRP, Nppb and histamine. By using chemogenetics to interrogate subsets of B5-I neurons, we established that somatostatin-2a receptor (Sst2a)-expressing dynorphin cells are the route through which somatostatin enhances itch. Employing specific lesioning techniques, we show that the disinhibition involving dynorphin cells operates at the level of the GRPR neurons, and we thus define the complete microcircuit through which somatostatin modulates itch. Lastly, we generated and characterized cell-type-specific conditional somatostatin-knockout neurons, and we thus define the complete microcircuit through which somatostatin modulates itch.

To determine the extent of overlap in expression of these neuropeptides, we used these to reveal that somatostatin released from both primary afferents and spinal cord interneurons is required for normal itch behavior. These experiments also established that somatostatin released from peripheral, but not spinal neurons, played a critical role in suppressing heat pain.

**Results**

**Somatostatin and Nppb are co-expressed in a subset of DRG neurons.** Neuropeptides are known to serve various somatosensory signaling roles. We recently studied the neuropeptides neuromedin B (NMB) and Nppb and demonstrated that they are involved in pain and itch mechanisms, respectively. In addition to NMB and Nppb, there are many other neuropeptides expressed in DRG. To characterize these in greater detail, we compared the expression profiles of several neuropeptides (Fig. 1a and Supplementary Fig. 1). Notably, the expression pattern of somatostatin is very similar to that of Nppb (Fig. 1a), and single cell transcriptomic analyses suggest that they are co-expressed. To determine the extent of overlap in expression of these neuropeptides, we performed double-label in situ hybridization (ISH). Figure 1b shows that there was virtually complete co-expression (99% overlap: 161 of 163 Sst+ or Nppb+ neurons). This raised questions of how these neuropeptides are used by the same neuron and what the function is of these neurons.

**Optogenetic activation of somatostatin+ primary afferent neurons elicits itch behavior.** Previously, we demonstrated that Nppb is both necessary and sufficient to produce itch behavior, suggesting that Nppb+ somatostatin+ neurons function as pruriceptors. The tight correspondence in gene expression of somatostatin and Nppb allowed us to test this by genetically manipulating these neurons in mice in which Cre is knocked into the somatostatin locus (SstCre), as was performed recently. We first investigated whether Cre mediates appropriate reporter expression in SstCre mice. Double-label ISH analysis of tissue from reporter-crossed mice (SstCre;Ai9) demonstrated that the majority of reporter-labeled neurons co-expressed Nppb (Supplementary Fig. 2a; 107 of 139 tdT-labeled neurons are Nppb+), and fibers from these neurons innervate the skin (Supplementary Fig. 2b). This indicates that SstCre marks the majority of somatostatin+ primary afferents. The rodent cheek-itch model is widely used to distinguish itch and pain behaviors, and we adapted this by replacing pruritogen injection with optogenetic stimulation. We expressed channelrhodopsin (ChR2) in somatostatin neurons (SstCre;Ai32 mice). To ensure that ChR2 was not expressed in MrgA3 prurceptors, we analyzed co-expression of ChR2 with both Nppb and MrgA3 (Fig. 2a). We found that the majority of Cre-mediated expression (of ChR2) was restricted to Nppb+ neurons (130 of 176 neurons). In addition, almost no MrgA3+ neurons co-expressed ChR2 (1 of 176). This confirmed that the Nppb+ and MrgA3+ neurons were separate populations. To establish that somatostatin neurons can be optogenetically activated and to determine the frequency they can follow, we initially measured responses to light in isolated sensory neurons (Supplementary Fig. 2c). Somatostatin neurons could follow optogenetic activation up to 20 Hz, and we used this frequency for behavioral assays. We activated ChR2 somatostatin+ neurons through a light cannula surgically implanted within 1 mm of the dorsal surface of the trigeminal ganglion (Fig. 2b). Notably, optogenetic activation produced robust scratching responses localized to the ipsilateral cheek (Fig. 2c). Responses to optogenetic stimulation were similar to those induced by injection of histamine into the cheek, except that optogenetic stimulation only elicited scratching, while histamine also evoked cheek wipes. We observed almost no scratching of the contralateral cheek and no behavioral responses to illumination with a nonactivating wavelength of light. These results demonstrated that activating somatostatin afferents was sufficient to generate selective itch behavior.

**Somatostatin interacts with both Nppb and GRP in itch signaling.** Our optogenetic experiments showed that itch can be induced by activation of somatostatin+ primary afferents, suggesting that somatostatin released from these cells acts as an itch transmitter. However, it is unclear how somatostatin interacts with the itch transmitters Nppb and GRP. A simple model would posit that the three neuropeptides interact in such a way as to have additive effects on itch. To investigate this, we measured scratching elicited by Nppb and GRP alone, and compared those responses with scratching elicited by Nppb and GRP co-administered with the somatostatin-receptor antagonist octreotide. Furthermore, we compared Nppb- and GRP-induced scratching with that evoked by co-administration of Nppb or GRP together with the specific somatostatin Sst2-receptor antagonist CYN 154806. We predicted that if somatostatin is involved in GRP- and Nppb-induced itch, then octreotide would increase scratching induced by these neuropeptides, while CYN 154806 would have the opposite effect. Indeed, we found that octreotide potentiated Nppb- and GRP-induced itch behavior, whereas CYN 154806 attenuated these responses (Fig. 3a,b). CYN 154806 also attenuated histamine-induced itch behavior, as would be expected if somatostatin has a physiological role in itch (Fig. 3c). This indicates that somatostatin, GRP and Nppb are transmitters in a connected itch circuit.

**Spinal inhibition of itch involves the dynorphin subset of B5-I neurons.** Previously, somatostatin was shown to hyperpolarize B5-I interneurons, and the itching caused by intrathecal octreotide was absent in mice lacking these neurons. This led to the suggestion that somatostatin-induced itch was mediated by disinhibition involving these cells. However, it was subsequently reported that B5-I neurons are not involved in itch behavior. Furthermore, B5-I neurons can be subdivided into two populations, which show only...
limited (~20%) overlap, based on expression of dynorphin and nNOS\textsuperscript{1,2,16}, and it was not clear which of these was involved in modulating itch. We therefore investigated which type of B5-I neuron is required for pruritogen- and somatostatin-induced itch by manipulating the activity of either dynorphin\textsuperscript{+} or nNOS\textsuperscript{+} neurons. We engineered mice in which we could individually interrogate these populations, based on expression of designer receptors exclusively activated by designer drugs (DREADDs)\textsuperscript{30} (Fig. 4a). Specifically, we injected adenovirus-associated virus (AAV) coding for the Cre-dependent Gq-coupled DREADD hM3Dq (DREADDq) fused to mCherry into one side of the lumbar dorsal horn\textsuperscript{+} of Pdyn\textsuperscript{+} and nNOS\textsuperscript{CreERT2} mice (Fig. 4b). Notably, we found that AAV infection resulted in expression of mCherry in the appropriately targeted populations and that chemogenetic activation of neurons with clozapine-N-oxide (CNO) caused c-Fos expression in the majority (92–95%) of mCherry\textsuperscript{+} cells, confirming that both populations were activated (Fig. 4b–d and Supplementary Fig. 3). In both genotypes, mCherry\textsuperscript{+} cells were most numerous in laminae I–II and scattered through deeper laminae, consistent with the distribution of cells that express nNOS and preprodynorphin (PPD). nNOS\textsuperscript{+} cells can be readily detected with immunocytochemistry, and we confirmed that in nNOS\textsuperscript{CreERT2} mice, 429 of 431 (99.5%) of mCherry\textsuperscript{+} cells in laminae I–II were nNOS-immunoreactive. Since both nNOS and dynorphin are also expressed in some excitatory interneurons, we confirmed expression of mCherry and c-Fos in inhibitory interneurons by immunostaining for the transcription factor Pax2 and/or the Sst\textsubscript{2a} receptor, both of which are restricted to inhibitory interneurons in this region\textsuperscript{15,31,32}. Close to the injection sites in the nNOS\textsuperscript{CreERT2} mice, mCherry was present in 71.8% (range: 58.8–91.7%) of Sst\textsubscript{2a}\textsuperscript{+} (inhibitory) nNOS cells. In the Pdyn\textsuperscript{+} mice, 44% (range: 33.8–50.8%) of cells that contained both Pax2 and Sst\textsubscript{2a} were mCherry\textsuperscript{+}, and since PPD is present in 54% of Sst\textsubscript{2a}\textsuperscript{+} neurons in laminae I–II\textsuperscript{1}, we estimate that 82% of inhibitory dynorphin cells expressed DREADDq in this region. We also confirmed that the DREADDq was not expressed in primary afferents by examining ipsilateral L4 DRGs (4 mice of each genotype) and observing that there were no mCherry-immunoreactive neurons in any of these ganglia (Supplementary Fig. 4).

If somatostatin induces itch by a disinhibitory mechanism involving B5-I neurons, we would expect that chemogenetic activation of one or both of these populations would attenuate pruritogen-evoked itch behavior by increasing inhibitory tone. Consistent with this prediction, we found that activation of dynorphin neurons with CNO markedly attenuated itch responses to the itch-inducing agent chloroquine injected intradermally into the ipsilateral calf (Fig. 4e and Supplementary Fig. 4). In contrast, activation of nNOS neurons had no effect on pruritogen-induced itch (Fig. 4e). Furthermore, in a separate series of experiments involving Pdyn\textsuperscript{+} mice, we tested the effect of intrathecal administration of AAV coding for DREADDq on itch behavior in response to pruritogens injected into the nape of the neck. Administration of CNO to activate the dynorphin neurons attenuated both histamine- and chloroquine-evoked itch. Notably, scratching evoked by intrathecal administration of octreotide was also attenuated when the dynorphin neurons were activated by CNO (Fig. 4f), consistent with the suggestion that somatostatin induces itch by a disinhibitory mechanism involving the dynorphin neurons.

Since activation of nNOS neurons did not alter itch behavior, we investigated their function by testing other somatosensory modalities. Additionally, we wondered whether, in addition to their antipruritic role, dynorphin neurons could modulate responses to other stimuli. We therefore examined behavioral responses to noxious thermal and mechanical stimulation following chemogenetic activation of dynorphin and nNOS neurons in lumbar dorsal horn. Notably, activation of nNOS neurons decreased sensitivity to both noxious heat and mechanical stimuli (Fig. 5a,b), suggesting that they have an antinoceptive, but not an antipruritic, role. Unexpectedly, we found that, as well as inhibiting itch, chemogenetic activation of dynorphin neurons markedly increased sensitivity to von Frey hairs, although it had no effect on responses to heat stimulation (Fig. 5a,b). This pronociceptive effect is likely mediated through activation of dynorphin\textsuperscript{+} excitatory interneurons.

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**Fig. 2 | Somatostatin\textsuperscript{+} primary afferent neurons are sufficient to trigger itch-behavior.** **a.** Triple-label ISH reveals that in Sst\textsuperscript{+};Ai32 mice, expression of ChR2-YFP (green) occurs largely in Nppb neurons (red). MrgA3 neurons (blue) are separate from both Nppb\textsuperscript{+} and ChR2-YFP\textsuperscript{+} neurons. Similar results were obtained from 3 mice. **b.** Schematic diagram illustrating the strategy employed to optogenetically activate trigeminal ganglion (TG) somatostatin\textsuperscript{+} neurons that innervate the face. The implanted fiber optic cannula (blue outline) was passed through the brain to a position approximately 1 mm dorsal to the TG and fixed in place with dental cement. **c.** Unilateral optogenetic activation of the trigeminal ganglion of Sst\textsuperscript{+};Ai32 mice (4 mice of each genotype) and observing that there were no mCherry-immunoreactive neurons in any of these ganglia (Supplementary Fig. 4).
Somatostatin and dynorphin interact with Nppb signaling at the level of GRPR neurons. Previously it was reported that dynorphin can attenuate itch through the KOR and that KOR antagonists can induce itch\(^1\). However, it is unclear how dynorphin acting on KORs modulates the itch evoked by somatostatin and Nppb. To examine this, we again took a pharmacological approach to determine the sequence in the itch pathway involving these neurotransmitters. First, our results placed dynorphin downstream of somatostatin (Fig. 4f), and therefore we reasoned that the KOR agonist ICI119941 should block scratching responses induced by octreotide\(^1\). Second, it follows that if the somatostatin receptor is upstream of the action of KOR, we would predict that administration of a somatostatin-receptor antagonist would not affect itch induced by the KOR antagonist norbinaltorphimine. Lastly, since peripherally induced itch is inhibited by the KOR agonist, then scratching evoked by histamine and Nppb should also be attenuated by the KOR agonist\(^1\).

As expected, we found that the KOR agonist attenuated somatostatin-induced itch, that KOR antagonist-induced itch was unaffected by somatostatin receptor antagonist and that both histamine- and Nppb-induced itch were also attenuated by the KOR agonist (Fig. 6a,b). Together, these results further substantiate the pathway for somatostatin-induced itch, through disinhibition involving the dynorphin subset of B5-I neurons, and provide additional evidence that this pathway interacts with Nppb signaling.

Our results indicate that somatostatin-induced itch is mediated through disinhibition involving dynorphin\(^*\) interneurons and that somatostatin can potentiate Nppb signaling. However, the site of interaction between these pathways is unknown. This could be at the level of either Npr1 neurons or GRPR neurons. To investigate this, we used conjugated toxins to generate selective lesions in the nNOS-containing interneurons and that somatostatin-mediated itch that involves the dynorphin\(^*\) subset of B5-I neurons, which suppress transmission at the level of the GRPR cells (Fig. 6i).

**Somatostatin is required for normal itch and pain responses.** Our findings suggest that the itch-inducing effect of somatostatin is mediated at least in part by dynorphin neurons. However, the origin of the somatostatin that acts on these neurons to cause itch is unknown. Somatostatin is expressed by both primary afferents and excitatory spinal cord interneurons\(^1,3\). Either or both of these populations might be the source of the somatostatin that is involved in regulating itch. To address this issue and provide further evidence of the foot. The calf-itch model activates cells in the middle third of the superficial dorsal horn within the L3 segment\(^1\). Excitatory PPD cells were rarely present in this region (Fig. 5c–g) and are therefore unlikely to be involved in the antipruritic effect seen in the CNO-treated mice. Together, these results establish that dynorphin- and nNOS-containing interneurons modulate responses to several sensory modalities and produce distinct behavioral effects.

as these consistently showed c-Fos expression after treatment with CNO, and included vertical cells (Supplementary Figs. 3a and 5a), which are thought to innervate nociceptive projection neurons in lamina I\(^1\). We also noticed that mCherry\(^*\) excitatory cells were particularly numerous in the medial third of the dorsal horn, where they accounted for ~50% of the mCherry population, whereas they only constituted 12% of mCherry cells elsewhere in the superficial laminae. We recently reported that PPD\(^*\) excitatory neurons are concentrated in the medial part of laminae I–II in the L4 segment, suggesting an association with regions innervated by glabrous skin\(^2\). To confirm this, we immunostained sections through lumbar and cervical enlargements of wild-type mice and compared the distribution of inhibitory (Pax2\(^*\)) and excitatory (Pax2\(^*\)) PPD-immunoreactive neurons. In both enlargements, the excitatory cells were largely restricted to glabrous skin territory, identified by lack of input from VGLUT3\(^+\) C-low threshold mechanoreceptors, which are restricted to hairy skin\(^14\). In contrast, elimination of Npr1 neurons attenuated histamine-induced itch, but had no effect on octreotide-evoked and KOR-antagonist-evoked itch, suggesting that somatostatin and dynorphin act downstream of Npr1 cells (Fig. 6f–h). Together, these data suggest a model for somatostatin-mediated itch that involves the dynorphin\(^*\) subset of B5-I neurons, which suppress transmission at the level of the GRPR cells (Fig. 6i).
that somatostatin acts as a mediator of itch in vivo, we generated mice in which somatostatin could be eliminated in specific cell types (Fig. 7a). The resulting Sstf/f mice (hereafter Sstf) were crossed with Trpv1loxP, Lbx1loxP and Wnt1loxP lines to eliminate somatostatin from DRG neurons, dorsal horn neurons and both classes of neurons, respectively. The resulting mice were born at expected Mendelian ratios, appeared healthy and showed none of the phenotypic abnormalities present in global somatostatin-knockout mice\(^*\). ISH confirmed selective loss of somatostatin mRNA in the expected tissues (Fig. 7b). Our results from blocking the somatostatin receptor (Fig. 3) led to the prediction that mice lacking somatostatin in the dorsal horn should display reduced responses to itch-inducing agents. To test this, we assessed behavioral responses of the mutant mice to several pruritogens. As
Fig. 5 | Chemogenetic activation of PdynCre and nNOSCreERT2 neurons modulates responses to heat and mechanical stimuli, and the pronociceptive effect of dynorphin neuron activation likely involves excitatory interneurons. **a**, Hargreaves assays revealed that, while responses to heat stimulation were unaffected in mice in which dynorphin neurons were chemogenetically activated (CNO), sensitivity was significantly reduced in animals in which nNOS neurons were activated. **b**, von Frey tests showed that chemogenetic activation (CNO) of dynorphin neurons elicited mechanical hyperalgesia, while activation of nNOS neurons caused significantly reduced sensitivity to mechanical stimulation. In all cases, behavioral results of testing the paw ipsilateral to spinal AAV injection in vehicle- and CNO-treated mice (postsurgery) were compared with results from the same animals obtained before intraspinal injection of the AAV (presurgery). Significant differences were assessed using two-way ANOVA with post hoc Sidak’s tests (F, P<0.017; nNOSCreERT2 CNO pre- vs. postsurgery von Frey test; F, P<0.0039 nNOSCreERT2 CNO pre- vs. postsurgery Hargreaves test; F, P<0.0012 PdynCre CNO pre- vs. postsurgery von Frey test). Data represent means ± s.e.m. (n=13 PdynCre CNO, 11 PdynCre veh, 14 nNOSCreERT2 CNO and 13 nNOSCreERT2 veh animals). **c-g**, Plots of the distribution of excitatory and inhibitory dynorphin+ cells show that the excitatory cells are highly concentrated in the region innervated from glabrous skin. (**c**) Immunostaining for VGLUT3 was used to reveal the extent of innervation from hairy skin, since C-low threshold mechanoreceptors (which express VGLUT3) are largely absent from glabrous skin. The VGLUT3 band occupies the whole mediolateral extent of the superficial dorsal horn at L3, but is absent from the medial part of the L4 and L5 segments, which are innervated by afferents from glabrous skin. The junction between these regions is marked (arrowheads). Similar results were obtained from 3 mice. **(d)** The distribution of PPD-immunoreactive cells that are inhibitory (Pax2+, blue circles) and excitatory (Pax2–, red circles) plotted onto outlines of the L3–L5 segments (data pooled from 3 mice). The junction between hairy and glabrous skin territories is marked by a dashed line. Note that excitatory PPD cells are concentrated in the glabrous skin territory and are much less numerous in regions innervated from hairy skin, including the L3 segment. **(e-g)** Examples of immunostaining for PPD (magenta), NeuN (blue) and Pax2 (green) in the medial (med) and lateral (lat) parts of the L3, L4 and L5 segments, respectively. Examples of Pax2+ PPD-immunoreactive neurons are indicated with arrows, and some Pax2– PPD-immunoreactive cells are shown with arrowheads. Similar results were obtained from 3 mice. Scale bars, 100 μm (**c**), 20 μm (**e-g**).
Somatostatin acts upstream of dynorphin\(^*\) inhibitory neurons, which interact with the Nppb itch pathway at the level of GRPR neurons.

**Fig. 6** | Somatostatin acts upstream of dynorphin\(^*\) inhibitory neurons, which interact with the Nppb itch pathway at the level of GRPR neurons. 

- **a.** Itch responses to intradermally injected histamine (100 µg), and to intrathecally administered Nppb (5 µg) and octreotide (100 ng) were significantly attenuated when the KOR agonist GRP-saporin (100 ng) was co-administered. Significant differences were assessed for indicated treatment groups using two-sided unpaired Student’s t tests (t\(_1\), t\(_2\), t\(_3\) = 10.14, t\(_3\) = 11.78; *P = 0.0001 for all comparisons). Data represent means ± s.e.m. (n = 6 histamine, 5 histamine + KOR agonist, 6 Nppb, 6 Nppb + KOR agonist, 5 octreotide and 6 octreotide + KOR agonist animals). 

- **b.** CYN animals. 

- **c.** Histamine. 

- **d.** Octreotide. 

- **e.** KOR antagonist. 

- **f.** Histamine. 

- **g.** Octreotide. 

- **h.** KOR antagonist. 

- **i.** Schematic diagram of proposed model of the somatostatin-mediated itch microcircuit. Broken red arrows indicate incompletely defined pathways, and blue and green circles are neurons identified by either the receptor or the neuropeptide(s) they express. Npr1, natriuretic polypeptide receptor 1.

Tonic or transient firing patterns (characteristic of inhibitory neurons\(^*\)) and found that all of these cells (8 of 8) were hyperpolarized by bath-applied somatostatin (Supplementary Fig. 7), consistent with expression of Sst\(_2a\) receptor by 91% of inhibitory dynorphin cells\(^*\). In anatomical studies, axonal boutons belonging to somatostatin\(^*\) interneurons can be recognized by their co-expression of somatostatin and VGLUT2, whereas somatostatin primary afferent terminals have very low or undetectable levels of VGLUT2-immunoreactivity\(^*\). We found, as expected, that most eGFP\(^*\) cell bodies in superficial dorsal horn (78%) were Sst\(_2a\)-immunoreactive and that these lay within a dense plexus of boutons that contained both somatostatin and VGLUT2, and therefore presumably originated from local somatostatin interneurons. In addition, all of the cells examined had numerous contacts from axons of local interneurons acts on dynorphin\(^*\) inhibitory interneurons to cause disinhibition.

In addition to a role in itch, somatostatin has been proposed to be either pronociceptive\(^*\) or analgesic\(^*\). To investigate this, we...
noted that mice lacking somatostatin in DRG neurons (Sst\(^{f/f};\)Trpv1\(^{cre}\)) displayed dramatically increased sensitivity to noxious heat (Fig. 8). In contrast, Sst\(^{f/f};\)Lbx1\(^{cre}\) animals exhibited normal responses to noxious heat, while Sst\(^{f/f};\)Wnt1\(^{cre}\) mice displayed responses similar to those of Sst\(^{f/f};\)Trpv1\(^{cre}\) mice. These results indicate that somatostatin released from primary sensory neurons normally suppresses nociceptive responses. This might result from effects in the spinal cord or be due to tonic release of somatostatin from Nppb afferents acting on peripheral endings of nociceptive afferents, as reported previously\(^{22,41}\). Conditional Sst\(^{f/f}\) mice also exhibit phenotypic differences in withdrawal thresholds to von Frey hairs (Supplementary Fig. 6c), suggesting a contribution of somatostatin to this behavior.

Notwithstanding the mechanism involved, the phenotype of somatostatin-deficient mice demonstrates that somatostatin released from primary afferents contributes to the inhibition of pain.

Discussion

Here using optogenetics, chemogenetics, pharmacology and conditional genetic knockouts, we delineated roles for somatostatin in itch and pain sensation. First, using optogenetic activation, we showed that sensory neurons expressing somatostatin are sufficient to evoke itch behavior (Fig. 2). Second, we demonstrated that somatostatin directly potentiated itch elicited by Nppb and GRP and that a somatostatin-receptor antagonist attenuated histaminergic itch (Fig. 3). Third, genetic knockout of somatostatin established
that it is required for normal itch behavior (Fig. 7), and our studies defined a disinhibitory spinal cord microcircuit through which somatostatin modulates itch (Figs. 3, 4 and 6). Lastly, we showed that somatostatin released from primary afferents was involved in inhibiting pain behavior (Fig. 8). Therefore, our studies revealed, at both molecular and cellular levels, the mechanisms by which somatostatin modulates itch, and we showed that somatostatin also played an important role in heat nociception. The co-localization of somatostatin and Nppb in a subclass of sensory neurons raised the question of how these neuropeptides interact in itch processing. Our results revealed that they act on distinct neural substrates and that the pathways engaged by these transmitters, although initially separate, converged and interacted (Figs. 3, 4 and 6). Although somatostatin presumably acts at least partly through dynorphin or KOR signaling to regulate itch, inhibition involving GABA and glycine has also been shown to play a critical role in suppressing pruritogen-evoked activity1,42,43. GABA, the principal fast transmitter used by the dynorphin and galanin interneurons44, is therefore also likely to have contributed to the antipruritic effect of stimulating the dynorphin neurons and to be involved in somatostatin-evoked itch. Since neuropeptides have longer-lasting actions than amino-acid transmitters, we suggest that peptidergic mechanisms involving somatostatin and dynorphin probably modify the excitability of neurons in the spinal cord to control longer-term behavioral responses, whereas fast transmitters underlie the rapid suppression of itch by counterstimuli. Recently, another study examined the effects of chemogenetic and optogenetic activation of spinal cord somatostatin neurons and found that this potentiates mechanical sensitivity26, in line with the proposed role of these neurons in gating mechanical pain. In addition, the authors reported that low-frequency optogenetic stimulation of these neurons increases histamine-evoked scratching behavior and that this effect is reduced by intrathecal administration of the somatostatin receptor antagonist CYN 154806 (250 ng)36, suggesting that it is mediated at least in part by somatostatin released from these cells. However, this finding is difficult to interpret, because we show here that intrathecal treatment with a somewhat higher dose (1 μg) of CYN 154806 strongly suppressed histamine-evoked itch (Fig. 3c). Nonetheless, these findings are consistent with our conclusion that somatostatin release from the spinal cord contributes to itch neurotransmission. Previously, we showed that somatostatin is expressed in the majority of GRP neurons35, and so co-release of GRP and somatostatin from these cells could independently contribute to itch. Molecularly defined classes of primary afferent neurons that detect and transmit signals for thermal, tactile and itch stimuli have been identified, and it has been suggested that sensation is primarily encoded by these specifically tuned receptor cells. However, since somatostatin afferents express TRPV1, they could be activated by noxious stimuli. Nonetheless, our optogenetic findings show that selective stimulation of these cells results in itch, but not pain behaviors. This suggests that a coding mechanism allows these two types of stimulus to be distinguished. One potential mechanism would be the 'leaky gate' model46. This proposes that although pruritic and nociceptive inputs converge on GRP neurons, frequency coding by these cells determines whether pain or itch behavior is evoked, through a feedforward inhibition involving enkephalinergic neurons. As predicted by this model, Sun et al.46 found that ablation of GRP cells resulted in a dramatic reduction of itch and an increase in certain types of pain. However, their ablation appears to have extended beyond the GRP neurons, since they reported a marked loss of cells that express PKCy, which shows minimal overlap with GRP15,47. Loss of additional populations of excitatory interneurons therefore complicates interpretation of their behavioral findings. Consistent with the idea that somatostatin-Nppb neurons are dedicated itch chemoreceptors, the IL31Ra itch receptor is exclusively expressed by these cells12,16,25,48. The somatostatin primary sensory neurons are molecularly distinct from MrgA3 neurons12,44 (Fig. 2) and therefore represent an additional population of pruritoid afferents.

There has been great interest in determining the mechanisms by which circuits in the spinal cord integrate and modify incoming sensory signals. We studied the effects of activating dynorphin neurons, most of which represent a subset of B5-I cells. Consistent with the suggestion that B5-I cells include neurons responsible for suppressing itch15, and that KOR agonists act locally within the spinal cord to reduce itch (Fig. 6 and ref. 15), we found that chemogenetic activation of these neurons suppressed pruritogen-evoked itch behavior (Fig. 4). Furthermore, in line with the view that somatostatin-induced itch is mediated through a disinhibitory mechanism, activating the dynorphin neurons also attenuated scratching evoked by intrathecally administered octreotide (Fig. 4f). This antipruritic role was specific for the dynorphin neurons, since activating the other main class of B5-I cells, those that express nNOS, reduced responses to noxious stimuli but had no effect on itch behavior.

Previously, Duan et al. reported that dynorphin spinal cord neurons have a role in gating mechanical pain, since mice lacking dynorphin-lineage neurons were hypersensitive to mechanical stimulation but showed normal itch behavior49. Notably, our experimental approach differed substantially from that of Duan et al. We engineered mice in which mature dynorphin neurons express DREADDq, whereas Duan et al. used an intersectional ablation strategy. This would have captured inhibitory interneurons that transiently express dynorphin21, but apparently excluded excitatory dynorphin cells, as well as ~40% of the galanin-inhibitory neurons (see Fig. 5 and Supplementary Fig. 7 of Duan et al.). Because of these differences, the neurons we activated only partially overlap with those that they ablated. This presumably accounts for the difference in our findings with mechanical pain tests and for the discrepancy between the antipruritic effect that we observed and the lack of a significant effect on itch reported in their study.

Our findings reveal that somatostatin is also important in controlling pain (Fig. 8). In particular, they suggest that somatostatin released from primary afferents tonically suppressed responses to noxious heat. This resolves previous conflicting reports, which suggested that somatostatin could either promote or attenuate pain20,21. It has long been known that activity in pain pathways can suppress itch49. Notably, our findings suggest that somatostatin released by pruritoid primary afferents suppresses pain, meaning that itch may also inhibit pain.

The activity in pruriceptors in the skin is conveyed via the spinal cord to the brain, where the perceptual quality of itch is produced. By investigating the function of somatostatin in spinal processing, we show that it plays important roles in transmitting and integrating sensory information. In particular, we demonstrate a mechanism whereby two neuropeptides, somatostatin and Nppb, that are released from the same primary afferent, cooperate in a modality-specific dorsal horn circuit that underpins the evolutionarily important sensation of itch.

Methods

Methods, including statements of data availability and any associated accession codes and references, are available at https://doi.org/10.1038/s41593-018-0119-z.

Received: 9 June 2017; Accepted: 25 January 2018; Published online: 19 March 2018

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Acknowledgements
We thank the NIDCR Gene targeting facility for help generating chimeric Sstf/f mice. We also thank C. Birchmeier, Max Delbruck Center, Berlin; M. Krashes, NIH, Bethesda; and Q. Ma, Dana-Faber Cancer Institute, Boston, for generously providing mice. We are very grateful to T. Furuta, Kyoto University, Kyoto, for the gift of PPD antibody, to A. Bell and D. Hughes for comments on the manuscript, to X. Gu for help in some of the experiments and to R. Kentrath and C. Watt for expert technical help. This work was supported by the intramural research program of the National Institute of Dental and Craniofacial Research (NIDCR)-National Institutes of Health (MAH) and grants from the Medical Research Council (MR/L003430/1), the Biotechnology and Biological Sciences Research Council (grant N006119), the Wellcome Trust (102645) (A.J.T.), the Swiss National Science Foundation (156393) (H.U.Z.) and the Natural Science Foundation of China (6172417). The Sstf/f mice used in this study were generated from ES cells obtained from the National Center for Research Resources (NCRR)-NIH-supported KOMP repository and engineered by the Welcome Trust Sanger Institute and the Mouse Biology Program.

Author contributions
J.H., E.P.S.R., A.J.T. and M.A.H. designed the experiments. J.H., E.P.S.R., S.K.M., H.J.S., P.-Y.T., M.K.N., J.K.A.B. and A.C.D. performed experiments. H.W. and H.U.Z. provided assistance and M.W. provided reagents. A.J.T. and M.A.H. wrote and edited the paper, with comments from all other authors.

Competing interests
The authors declare no competing financial interests.

Additional information
Supplementary information is available for this paper at https://doi.org/10.1038/natureneuroscience. Reprints and permissions information is available at www.nature.com/reprints. Correspondence and requests for materials should be addressed to A.J.T. or M.A.H.

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Methods

Animals. Mice were 20–30 g and 2–4 months old, unless otherwise stated. The following lines: Ssttm2.1(cre)1, Aii2, Aii9, PdynCre, nNOS7;7, Egr1mitt, TrpV1cre2, Wnt1cre3 and Sstf/f(Ssttm1 (KOMP)) were bred and intercrossed to generate experimental animals as described in the Materials and Methods section. All experiments using mice followed NIH guidelines and were approved by either the National Institute of Dental and Craniofacial ACUC or the Ethical Review Process Applications Panel of the University of Glasgow and performed in accordance with the UK Animals (Scientific Procedures) Act 1986.

The targeted M8W4A ES cell clone F09 with knock-in insertion into the Sst gene was obtained from MBP UC Davis and used to generate chimeric mice. Chimeras were crossed with C57BL/6 and then with Gt(ROSA)26Sor1(cre) mice to produce animals with a Cre-dependent conditional Sst allele consisting of loxp sites surrounding exon 2. These mice were next crossed with TrpV1cre, Wnt1cre and Lox1 mice to produce conditional-knockout mice; controls were homozygous Sstf/f lines isolated without Cre. Age- and sex-matched Sstf/f KO mice and littermates were used, and there were no substantial phenotypic differences between sexes.

Genotyping was performed with TGGTGAGATTATGAAGAGCAAGCG, GCCGACGTGTTCCCAATAGCCATC for wild-type alleles and TGGTGAGATTATGAAGAGCAAGCG, GCCGACGTGTTCCCAATAGCCATC for mutant alleles.

Optogenetic stimulation. For light-mediated activation of trigeminal somatostatin neurons, Sstf/f(Aii) mice were implanted with a 200-μm-diameter optical fiber (Thorlabs) positioned within 1 mm of the ganglion. Briefly, mice were anesthetized and mounted in a stereotaxic frame (Stoelting, USA). The skull was exposed and a hole drilled and fiber implanted with the following coordinates: Z, 6.1 mm; X, 1.2 mm; Y, 2.0 mm from bregma. The cannula was secured using acrylic dental cement, and after the cement dried, the skin was trimmed and glued. Mice were allowed to recover and experiments were initiated approximately 3 weeks after surgery.

To measure optogenetically elicited behavior, mice were placed in clear plastic enclosures with an optical cannula that could rotate to allow free movement of the mouse. Behavioral responses were recorded during the experiment. Mice were habituated for 30 min with the tethered optical cannula. Light was delivered from a Thorlabs LED driver (1,000 mA, 20 Hz). For all animals, scratching bouts were counted for 30 min without illumination, followed by 30 min with continuous 20 Hz, 590-nm light and finally, bouts were counted over 30 min with continuous 20-Hz, 470-nm illumination. Counts of scratching bouts for individual animals were averaged over two sessions performed on consecutive days. Separate Sstf/f(Aii) mice were assessed for histamine-activated scratching (10 μL injected into the cervical). For in vitro testing of the optogenetic excitation of somatostatin primary afferent neurons, DRGs from Sstf/f(Aii) mice were incubated with 5 mg/mL colchicine/dispaize for 30 min and were mechanically dissociated. Dissociated primary cultures were seeded onto poly-d-lysine-treated cover slips. DRG neurons were cultured with Dulbecco’s Modified Eagle Medium/F-12 supplemented with 10% FBS, 100 U/mL Penicillin and 100 μg/mL Streptomycin, nerve growth factor (100 ng/mL) and glial-derived neurotrophic factor (50 ng/mL) for 2–4 d. Whole-cell recordings were performed on DRG neurons expressing ChR2-YFP with an Axon 700B amplifier, 1440 Digitizer and pCLAMP 10 software (Molecular Devices). Bath solution contained 140 mM NaCl, 4 mM KCl, 2 mM CaCl2, 1 mM MgCl2, 10 mM HEPES. Pipette solution contained 140 mM KCl, 10 mM EGTA, 10 mM HEPES, 3 mM MgATP, 3 mM MgCl2, 0.5 mM NaGTP. Light pulses were generated by Prizmatix blue LED fiber-coupled LED light source and Prizmatix pulser in the following setting: 1 Hz, 25 ms/975 ms (on/off); 5 Hz, 25 ms/175 ms; 20 Hz, 25 ms/25 ms; 40 Hz, 10 ms/15 ms.

Chemogenetic activation. Intraperitoneal injections were performed by using a modification of the method described by Foster et al.15. Mice were anesthetized with isoflurane and placed in a stereotaxic frame with 2 vertebral clamps attached to the T12 and L1 vertebrae. The spaces between the laminae of T12–T13 and T13–L1 vertebrae were exposed, and a small incision was made in the dura on the right side of the midline in each space. A hole was drilled through the lamina of the T13 vertebra on the right hand side, and an incision was also made through the dura beneath this hole. Drilling a hole through the lamina of the neck (histamine or chloroquine), or delivered intrathecally (octreotide), and scratching bouts counted over 30 min.

Conjugated peptide–mediated cell ablation. Ablation of Nprl- and GRPR–expressing spinal cord interneurons was accomplished by intrathecal (segment L3/4) injection of Npbr-saporin (4 μg in 10 μL; Advanced Targeting Systems) and GRPR-saporin (2.5 μg) respectively. We have previously shown that these treatments are highly selective for the corresponding neuronal populations1. Behavioral assays were initiated 2 weeks after toxin injection.

I itch behavioral test. All other itch tests were performed as previously described3. Briefly, mice were habituated for 1 h at room temperature (18–22 °C) in separate, clear, plastic containers (10 × 10 × 12 cm). The experimenter was blinded to genotype. I itch-inducing substances histamine (100 μg), chloroquine (100 μg), SLIGRL-NH₂ (100 μg), 2-methyl serotonin (30 μg), endothelin (25 ng) and compound 48/80 (80 μg) were injected intraperitoneally into the neck (10 μL), and numbers of scratching bouts directed to the nape of the neck assessed over 30 min. We cannot completely eliminate the possibility that we were observing nociceptive rather than pruritic behaviors, but since we used established pruritogens in these assays we interpret the scratching responses we measured as...
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Mice were reacted for mCherry (chicken antibody), Sst2a, Pax2 (rabbit antibody, anaesthetized with pentobarbital (30 mg i.p.) and perfused with 4% freshly for immunocytochemical staining as described previously61. The sections were of the same species as the Sst2a antibody; and in addition, PPD may be below the presence of the other markers. In addition, to determine the proportion of c-Fos immunoreactive were identified. The stained neurons were then examined for the up–down method57.

In situ hybridization. Single- and double-labelISH was performed at high stringency as described previously. The probe used to test Sst6 mice corresponded to the entire exon 2 of Sst. ISH experiments quantifying overlap of somatostatin with Nppb and of somatostatin with tdTomato were performed on 2–3 sections prepared from 3 wild-type and 3 Sst6 mice, and representative images are displayed. RNAscope, a multiplexed fluorescent in situ hybridization technique (Advanced Cell Diagnostics), was performed according to the manufacturer’s instructions on fresh frozen tissue sections.

Behavioral testing of Sst6 mice. Thermal sensitivity was tested with a Hargreaves apparatus (Ugo-Basile). Animals were acclimated for 1 h in a plastic cage on a glass plate. A radiant heat source was targeted to the plantar surface of the hindpaw and with the latency of 20 s to prevent tissue damage. For von Frey measurements, mice were acclimatized in a plastic cage with a wire mesh floor for 1 h and then tested with von Frey filaments with logarithmically incremental stiffness (starting at 0.4 g). Each filament was applied for 5 s to the hindpaw, and the presence or absence of a withdrawal response was noted. The filament with the next stiffness was then applied, depending on the response to the previous filament, and this was continued until six positive responses were recorded. The 50% withdrawal was determined by the up–down method12.

Immunocytochemistry for chemogenetic experiments. All of the mice that had received intraspinal injections of AAV2.flex.hM3Dq-mCherry were deeply anesthetized with pentobarbital (30 mg i.p.) and perfused with 4% freshly diluted in phosphate-buffered saline containing 0.3% Triton X-100 and 5% normal antibodies (Jackson ImmunoResearch, West Grove, PA, USA). All antibodies were 10% fetal calf serum, and this was revealed with fluorescence-labeled species-specific secondary antibodies (Jackson ImmunoResearch, West Grove, PA, USA). All antibodies were diluted in phosphate-buffered saline containing 0.3% Triton X-100 and 5% normal donkey serum. Sections were scanned with a confocal microscope to confirm adequate expression of the mH3Dq-mCherry fusion protein in the appropriate spinal segments. In two cases, intraspinal injections were not successful, as judged by lack of mCherry staining in the appropriate spinal segments, and for these mice the corresponding behavioral data were excluded from the study.

To determine the neurochemical phenotype of neurons that expressed the mCherry fusion protein and to examine c-Fos staining following chemogenetic activation, an additional 5 mice of each genotype received intraspinal injections of AAV2.flex.hM3Dq-mCherry into the L3 and L5 segments. These mice were injected with either CNO (n = 3 mice per genotype) or vehicle (n = 2 mice per genotype), and 2 h later they were perfused with fixative, as described above. Transverse spinal sections were cut from the L3 and L5 segments. Sections from the appropriate spinal segments were cut from the L3 and L5 segments. Sections from the appropriate spinal segments were cut from the L3 and L5 segments. Sections from nNOSCreERT2 mice were immunostained for mCherry (chicken antibody Abcam, ab205402, 1:10,000), nNOS (rabbit antibody, Millipore, 07–571, 1:2000), Sst6 (guinea pig, Gramsch Laboratories, SS-870, 1:2000) and c-Fos (goat antibody, Santa Cruz biotech, sc22-G, 1:2000). Sections from PdynCre mice were reacted for mCherry (chicken antibody, Abcam, ab205402, 1:10,000), nNOS (rabbit antibody, Millipore, 07–571, 1:2000), Sst6, Pax2 (rabbit antibody, Life Technologies, 716000, 1:1000) and c-Fos. Two sections from each mouse were analyzed with Neurolucida software (MBF, Bioscience, Williston, VT, USA). All cells in the superficial dorsal horn (laminae I–II) that were mCherry-immunoreactive were identified. The stained neurons were then examined for the presence of the other markers. In addition, to determine the proportion of c-Fos(+/−) cells that were mCherry(+/−), we counted neurons that were c-Fos(+/−) but lacked mCherry. Note that for technical reasons we did not use antibodies against preprodynorphin (PDP), as our PDP antibody is raised in guinea pig, the same species as the Sst6 antibody, and in addition, PDP may be below the detection threshold to allow unambiguous identification of all dynorphin-expressing neurons.

Both mCherry antibodies were raised against recombinant full-length protein corresponding to mCherry. Specificity was demonstrated by finding an identical distribution of staining to that seen with native fluorescence of mCherry protein of staining and by the lack of staining in regions of tissue that do not contain mCherry. The c-Fos antibody was raised against a peptide corresponding to the N-terminus of human Fos, and its specificity has been shown in previous studies by the restriction of staining to neurons in somatotopically appropriate areas after noxious or pruritic stimulation37. The nNOS antibody was directed against a synthetic peptide corresponding to the N-terminus of rat nNOS and labels a single band of 155 kDa in rat brain extracts. The antibody against Pax2 was raised against amino acids 188 to 385 of the mouse protein and recognizes bands of the appropriate size on western blots of mouse embryonic kidney7. The Sst6 antibody was generated against the C-terminal 15 amino acids of the mouse receptor, and staining is abolished by incubation with the immunizing peptide (manufacturer’s specification).

Distribution of excitatory and inhibitory dynorphin cells. Three wild-type C57BL/6 mice (either sex, 19–20 g) were perfused with fixative as described above. Spinal cord segments L3, L4 and L5 were removed from all three mice and C7, T5 and C8 from two of them. Whole segments werecryostat (18–22 °C) for at least 30 min, and then transferred into recording solution that contained the following (in mM): 3.0 KCl, 1.2 NaH2PO4, 0.5 CaCl2, 1.3 MgCl2, 8.7 MgSO4, 26 NaHCO3, 215 sucrose, oxygenated with 95% O2 and 5% CO2. The recording apparatus (Ugo-Basile) was used to record PPD and VGLUT3. Two or three rats with that had been reacted with the first antibody were analyzed using a modification of the dissector method11. All PPD-immunoreactive neurons with the bottom surface between reference and look-up sections were initially plotted onto an outline of the dorsal horn, and then the presence or absence of Pax2 staining was recorded for each selected cell. The sections that had been reacted with VGLUT3 antibody were then examined, and those that were clearly detectable were analyzed by the optical fractionator. The NeuN antibody was raised against cell nuclei extracted from mouse brain and found to react with a protein specific for neurons, which has subsequently been identified as the splicing factor Fox3. The antibody against VGLUT3 was raised against amino acids 522–588 of the mouse protein and detects a single protein band at 60–62 kDa.

Somatostatin action on dynorphin cells. Eight PdynCre mice of either sex (18–23 g, aged 5–9 weeks) received intraspinal injections of AAV.flex.eGFP (4.3 × 1010 to 1.7 × 1010 GC in 300 nL diluent). These were performed as described above, except that injections were made through incisions on either side of the T13 vertebra into the L3 or L5 segments, and the mice survived between 7 and 11 days after surgery. Five of these animals were used for electrophysiological experiments. The other three were decapitated under general anesthesia with isoflurane (1–3%). Spinal cords were isolated in ice-cold dissecting solution that contained the following (in mM): 3.0 KCl, 1.2 NaH2PO4, 0.5 CaCl2, 1.3 MgCl2, 8.7 MgSO4, 26 NaHCO3, 20 HEPEs, 25 glucose, 215 sucrose, oxygenated with 95% O2 and 5% CO2. The dorsal horn was removed, and dorsal segments L7, T10, C6, C7 and C8 from two mice. In each case, the segments were cut into four regions of the spinal cord, showing that this route of administration causes injected spine. Neurons were identified and added to the outline drawing. The PPD antibody12 was raised against a peptide corresponding to amino acids 229–248 at the C-terminus of rat PPD, and has been shown to label PPD but not dynorphin or enkephalin. The NeuN antibody was raised against cell nuclei extracted from mouse brain and found to react with a protein specific for neurons, which has subsequently been identified as the splicing factor Fox3. The antibody against VGLUT3 was raised against amino acids 522–588 of the mouse protein and detects a single protein band at 60–62 kDa.
(2 μM, Tocris Bioscience) was administered via the recording solution, and any change in membrane potential was recorded in current-clamp mode. Around 5 min after the start of somatostatin application, the same voltage and current step protocols were repeated to assess somatostatin-mediated modulatory effects in the recorded cell (n = 8).

Three of the PdynCre mice that had received intraspinal injection of AAV.flex.eGFP were deeply anaesthetized and perfused with fixative. Injected spinal cord segments were removed and processed for immunocytochemistry as described above. Parasagittal sections were immunoreacted to reveal Sst2a-immunoreactive somatostatin (rabbit antibody, Peninsula labs, T-4103, 1:500) and VGLUT2 (chicken antibody, Synaptic Systems, 135416, 1:500). Five eGFP+ cells that were Sst2a-immunoreactive were selected from each mouse before immunostaining for somatostatin was observed, and these were scanned with a confocal microscope to include as much of the dendritic tree as was visible in the section. The cell bodies and dendritic trees were reconstructed with NeuronLucida software based on eGFP fluorescence. The other channels were then viewed, and contacts from somatostatin+VGLUT2+ boutons were marked. The VGLUT2 antibody was raised against a synthetic peptide corresponding to amino acids 566–582 of rat VGLUT2 and detects a single band of appropriate molecular weight on western blots (manufacturer’s specification). The somatostatin antibody is reported to show 100% cross-reactivity with somatostatin-28 and somatostatin-25, but none with substance P or neuropeptide Y, and staining is blocked by preincubation with somatostatin65.

Statistical analysis. Data are expressed as mean ± s.e.m. Statistical analysis was performed in Prism (GraphPad). Differences between two groups were examined using a two-sided Student’s t test, with P < 0.05 considered significant and P > 0.05 considered nonsignificant. When comparisons were made between different groups of mice, ANOVA was used and when repeated effects were assessed in a single group of mice (Fig. 2 only) repeated-measures ANOVA was used. No statistical methods were used to predetermine sample sizes, but our sample sizes are similar to those reported in previous publications11,16,23,45. Data distribution was assumed to be normal, but was not formally tested. Data collection was not randomized, and data analysis and collection were not performed blind to the conditions of the experiment except where noted (chemogenetic experiments). Animals and data points, except where noted, were not excluded from analysis. All relevant data are available from authors.

Life Sciences Reporting Summary. Further information on experimental design is available in the Life Sciences Reporting Summary.

Data availability. The data that support the findings of this study are available from the corresponding authors upon reasonable request.

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64. Mullen, R. J., Buck, C. R. & Smith, A. M. NeuN, a neuronal specific nuclear protein in vertebrates. Development 116, 201–211 (1992).
65. Proudflock, F., Spike, R. C. & Todd, A. J. Immunocytochemical study of somatostatin, neuropeptide, GABA, and glycine in rat spinal dorsal horn. J. Comp. Neurol. 327, 289–297 (1993).
### Experimental design

1. **Sample size**
   
   Describe how sample size was determined.
   
   No statistical methods were used to pre-determine sample sizes but our sample sizes are similar to those reported in previous publications Sun, Y.G. & Chen, Z.F. A gastrin-releasing peptide receptor mediates the itch sensation in the spinal cord. Nature 448, 700-3 (2007). Stantcheva, K.K. et al. A subpopulation of itch-sensing neurons marked by Ret and somatostatin expression. EMBO Rep 17, 585-600 (2016). Sun, S. et al. Leaky Gate Model: Intensity-Dependent Coding of Pain and Itch in the Spinal Cord. Neuron 93, 840-853 (2017).

2. **Data exclusions**
   
   Describe any data exclusions.
   
   Mice in which intraspinal injection was not successful, as judged by mCherry staining, were excluded from analysis of behavioral results.

3. **Replication**
   
   Describe whether the experimental findings were reliably reproduced.
   
   The experimental findings were reliably reproduced in cases where experiments were repeated.

4. **Randomization**
   
   Describe how samples/organisms/participants were allocated into experimental groups.
   
   The order for testing animals was randomized. Genotype or treatment groups were recorded post-testing.

5. **Blinding**
   
   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.
   
   For studies with chemogenetic and knockout experiments, the investigator was blinded.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.
6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

| n/a | Confirmed |
|-----|-----------|
| ☑   | The exact sample size \((n)\) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.) |
| ☑   | A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☑   | A statement indicating how many times each experiment was replicated |
| ☑   | The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section) |
| ☑   | A description of any assumptions or corrections, such as an adjustment for multiple comparisons |
| ☑   | The test results (e.g. \(P\) values) given as exact values whenever possible and with confidence intervals noted |
| ☑   | A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range) |
| ☑   | Clearly defined error bars |

*See the web collection on statistics for biologists for further resources and guidance.*

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### Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.  

**Graphpad Prism7 and Neurolucida for Confocal 11.01.**

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

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### Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

**No restrictions.**

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

**For fos, Gutierrez-Mecinas, M. et al. Preprotachykinin A is expressed by a distinct population of excitatory neurons in the mouse superficial spinal dorsal horn including cells that respond to noxious and pruritic stimuli. Pain 158, 440-456 (2017). For Pax2, Dressler, G.R. & Douglass, E.C. Pax-2 is a DNA-binding protein expressed in embryonic kidney and Wilms tumor. Proc Natl Acad Sci U S A 89, 1179-83 (1992). For PPD, Lee, T., Kaneko, T., Taki, K. & Mizuno, N. Preprodynorphin-, preproenkephalin- and preprotachykinin-expressing neurons in the rat neostriatum: an analysis by immunocytochemistry and retrograde tracing. J Comp Neurol 386, 229-44 (1997). For NeuN, Mullen, R.J., Buck, C.R. & Smith, A.M. NeuN, a neuronal specific nuclear protein in vertebrates. Development 116, 201-11 (1992). For SST, Proudluck, F., Spike, R.C. & Todd, A.J. Immunocytochemical study of somatostatin, neotensin, GABA, and glycine in rat spinal dorsal horn. J Comp Neurol 327, 289-97 (1993). For mCherry, nNOS, Sst2a, VGLUT2, and VGLUT3 manufacturer’s specifications validate their specificity.**


10. Eukaryotic cell lines
   a. State the source of each eukaryotic cell line used. No eukaryotic cell lines were used.
   b. Describe the method of cell line authentication used. No eukaryotic cell lines were used.
   c. Report whether the cell lines were tested for mycoplasma contamination. No eukaryotic cell lines were used.
   d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

Animals and human research participants

11. Description of research animals

   From Methods section of our paper. Mice were 20-30g (2-4 months old) unless otherwise stated. The following lines: Ssttm2.1(cre) 42, Ai32 59, Ai9 60, PdynCre 41, nNOSCreERT2 42, Lbx1-cre 61, Tg(Trpv1-cre) 62, Wnt1-cre 63, and Sst fl/fl (Sst tm1a (KOMP)) were bred and intercrossed to generate experimental animals as described in the text. All experiments using mice followed NIH guidelines and were either approved by the National Institute of Dental and Craniofacial ACUC, or were approved by the Ethical Review Process Applications Panel of the University of Glasgow and performed in accordance with the UK Animals (Scientific Procedures) Act 1986.

   The targeted JM8A3 ES-cell clone F04 with knockin insertion into the Sst gene was obtained from MBP UC Davis and was used to generate chimeric mice. Chimeras were crossed with C57BL6 and then with Gt(Rosa)26Sortm1(FLP1) mice to produce animals with a Cre-dependent conditional Sst allele consisting of loxP sites surrounding exon 2. These mice were next crossed with Trpv1-cre, Wnt1-cre, and Lbx1-cre mice, to produce conditional knockout mice; controls were homozygous Sst fl/fl littermates without Cre. Age and sex matched Sst fl/fl cKO mice and littermates were used, and there were no significant phenotypic differences between sexes. Genotyping was performed with TGGTGAGATTATGAAGAGCAAGCG, GGCAGCTGTTCCCAATAGCCATC wild-type, and TGGTGAGATTATGAAGAGCAAGCG, ATCATTAATTGCGTTGCGCCATCTC, mutant alleles.

   Animals were maintained in a temperature-controlled environment with a 12-hour light/12-hour dark cycle and free access to food and water. Mice were group housed 4-5 animal per cage, except following surgical procedures when they were single housed. Unless otherwise noted, male C57BL/6N mice (Charles River) at least 6 weeks old were used for pharmacological and conjugated-peptide ablation studies.

Policy information about studies involving human research participants

12. Description of human research participants

   Study did not involve human research participation.