A spinal neural circuitry for converting touch to itch sensation

Sihan Chen¹,²,³,⁹, Xiao-Fei Gao¹,²,⁴,⁹, Yuxi Zhou¹,²,³, Ben-Long Liu¹,², Xian-Yu Liu¹,², Yufen Zhang¹,⁵, Devin M. Barry¹,², Kun Liu¹,², Yingfu Jiao³, Rita Bardoni⁶, Weifeng Yu³ & Zhou-Feng Chen¹,²,⁷,⁸

Touch and itch sensations are crucial for evoking defensive and emotional responses, and light tactile touch may induce unpleasant itch sensations (mechanical itch or allokinesia). The neural substrate for touch-to-itch conversion in the spinal cord remains elusive. We report that spinal interneurons expressing Tachykinin 2-Cre (Tac2Cre) receive direct Aβ low threshold mechanoreceptor (LTMR) input and form monosynaptic connections with GRPR neurons. Ablation or inhibition markedly reduces mechanical but not acute chemical itch nor noxious touch information. Chemogenetic inhibition of Tac2Cre neurons also displays pronounced deficit in chronic dry skin itch, a type of chemical itch in mice. Consistently, ablation of gastrin-releasing peptide receptor (GRPR) neurons, which are essential for transmitting chemical itch, also abolishes mechanical itch. Together, these results suggest that innocuous touch and chemical itch information converge on GRPR neurons and thus map an exquisite spinal circuitry hard-wired for converting innocuous touch to irritating itch.
Neuropeptide Y (NPY) and its receptor NPY receptor 1 (NPY1R) have been shown to be important for gating mechanical itch\(^\text{21,27}\). On the other hand, recent studies have also implicated the NPY pathway responsible for both histaminergic and nonhistaminergic itch transmission\(^\text{28,29}\), raising the possibility that this neuronal pathway is the excitatory pathway for both sensory inputs\(^\text{28,29}\). Physical contact with textile fibers such as wool or cotton swabs from the seemingly normal skin area neighboring the itchy skin often evokes itching sensations\(^\text{30}\). Because alloknesis can be intolerable under pathological itch conditions, it could be a presenting problem for patients with chronic itch, including atopic dermatitis, dry skin itch, and urticaria\(^\text{31}\). One plausible mechanism is the excitation of the spinal interneurons, which receive input from low threshold mechanoreceptors (LTMRs)\(^\text{11,13,21}\).

Gastrin releasing peptide (GRP) is an itch-specific peptide in sensory neurons and can activate its receptor GRPR in the spinal cord to relay nonhistaminergic itch information to the brain\(^\text{3,22}\), whereas neuropeptide B (NMB) and its receptor (NMBR) mediate histamine-evoked itch via GRPR neurons\(^\text{32,24}\). Loss-of-function and gain-of-function studies demonstrate that the GRP–GRPR neuronal pathway is an itch-specific pathway responsible for both histaminergic and nonhistaminergic itch transmission\(^\text{5,6}\). Recent studies have shown that Ucn3\(^\text{26}\) is expressed in laminae IIi and IIIo, while Tac2\(^\text{34}\) is expressed in laminae IIi and IIIo, suggesting a role for Tac2 neurons in mechanical itch. The spinal cord interneurons expressing Tac2 neurons in laminae IIi and IIIo are activated to convert innocuous touch to irritating itch.

**Results**

**Tac2 neurons are activated by mechanical itch stimulation.** Spinal cord dorsal horn can be divided into distinct laminae according to molecular expression profile, afferent projection and functional allotment\(^\text{34}\). Tac2 neurons are a subset of interneurons that forms a distinct band encompassing the inner layer of lamina II (IIi) and the outer layer of lamina III (IIIo) in the spinal cord (Supplementary Fig. 1a, b)\(^\text{33,35}\). Consistent with the previous study\(^\text{33}\), immunohistochemistry (IHC) shows the overwhelming majority (283/349, 81.1%) of the spinal Tac2 neurons of mice derived from the mating of Tac2\(^\text{Cre}\) mice with A9 reporter mice\(^\text{36}\) (hereafter referred to as Tac2\(^\text{2DTom}\) neurons) express Lmx1b, a transcription factor expressed in glutamatergic interneurons in the spinal cord and brainstem (Supplementary Fig. 1a)\(^\text{37-39}\), while very few (5/386, 1.3%) express Pax2, an inhibitory neuronal marker (Supplementary Fig. 1b)\(^\text{36}\). RNA scope in situ hybridization (ISH) showed that almost all Tac2\(^\text{2DTom}\) neurons (288/295, 97.6%) in the superficial dorsal spinal horn express Vglut2, a marker for excitatory neurons (Supplementary Fig. 1c). This is consistent with RNAseq result\(^\text{39}\), 60.7% (269/443) of which also express Vgr1 (Supplementary Fig. 1d).

To test which sensory modality transmission may require Tac2 neurons, we used c-Fos, a neuronal activity marker, as a surrogate to determine whether Tac2\(^\text{2DTom}\) neurons are activated in response to different types of stimuli. As a control, a free ambulating mouse without evoked stimuli showed little c-Fos activity, as detected by IHC, in the superficial dorsal horn (Fig. 1a, b and Supplementary Fig. 2a). Intradermal injection (i.d.) of chloroquine (CQ), an archetypal pruritogen for chemical itch, evoked robust c-Fos activity, mostly restricted to laminae I and IIo (Fig. 1c, d and Supplementary Fig. 2b). However, c-Fos was barely detected in Tac2\(^\text{2DTom}\) neurons (lamina IIi: 6.7 ± 2.9%; lamina IIIo: 6.5 ± 3.0%) (Fig. 1m, l), consistent with an earlier report that Tac2 is not required for chemical itch\(^\text{33}\). Next, we examined c-Fos induced by mechanical dynamic stimulus using brushing at 2 cm s\(^{-1}\) (Fig. 1e)\(^\text{41}\). While most c-Fos+ neurons were found in laminae III–IIo (Fig. 1f and Supplementary Fig. 2c), few were located in Tac2\(^\text{2DTom}\) neurons (lamina IIi: 9.4 ± 4.7%; lamina IIIo: 3.2 ± 3.0%) (Fig. 1m). To determine whether Tac2\(^\text{2DTom}\) neurons are involved in detecting and transmitting noxious mechanical information, the hindpaw of the mouse was poked with a von Frey filament (1.4 g) (Fig. 1g). Although a von Frey filament evoked c-Fos expression across laminae I–III (Fig. 1h and Supplementary Fig. 2d), there was little co-expression with Tac2\(^\text{2DTom}\) neurons (lamina IIi: 6.7 ± 3.7%; lamina IIIo: 5.0 ± 2.3%) (Fig. 1m). Lastly, we tested whether Tac2\(^\text{2DTom}\) neurons are required for mechanical itch evoked by applying a von Frey filament (0.07 g) to the hairy skin of the nape (Fig. 1i)\(^\text{42}\). Mechanical itch induced c-Fos expression across laminae I–III (Fig. 1j) and Supplementary Fig. 2e). Notably, compared to other stimuli tested, significant amounts of c-Fos were found in Tac2\(^\text{2DTom}\) neurons (laminae IIi: 14.6 ± 6.4%; IIo: 21.8 ± 3.7%) (Fig. 1m). Interestingly, comparison of c-Fos activity in response to chemical itch and innocuous or noxious touch stimuli suggests that overall Tac2\(^\text{2DTom}\) neurons in lamina IIIo are more active than IIi in response to touch stimuli (Fig. 1k), and Tac2\(^\text{2DTom}\) neurons are prone to be activated by mechanical itch-related touch stimulation (Fig. 1l, m). Taken together, these results indicate that Tac2\(^\text{2DTom}\) neurons are more likely to be involved in mechanical itch transmission.
neurons band are considered as lamina IIIo neurons. This enabled us to identify and record Tac2tdTom neurons in these two regions discretely (Fig. 2a, d). Tac2tdTom neurons in lamina IIi displayed four distinct firing patterns: delayed firing (25.7%, 19 of 74), initial firing (10.8%, 8 of 74), tonic firing (31.1%, 23 of 74) and phasic-bursting pattern (24.0%, 19 of 74) (Fig. 2b, c). More strikingly, the dominating firing pattern of Tac2tdTom neurons in lamina IIIo is phasic-bursting (80.6%, 29 of 36) (Fig. 2e, f), whereas the rest comprising single spiking (2.8%, 1 of 36), delayed firing (5.6%, 2 of 36), and initial bursting (11.1%, 4 of 36) (Fig. 2f). The observation that a vast majority of lamina IIIo Tac2 neurons show the same firing pattern is highly unusual, given the heterogeneity of the firing patterns for lamina III interneurons as shown previously7,21.

Fig. 1 Tac2Cre neurons in the spinal cord are activated by mechanical itch stimulation. a, c, e, g, i Schematic of mice in free ambulating state (a), i.d. CQ injection (c), soft brushing (e), von Frey hair applied to the hindpaw (g), von Frey hair applied to the hairy skin (i). b, d, f, h, j Representative images of c-Fos expression (green) in the spinal cord of Tac2tdTom (red) mice corresponding to a, c, e, g, i, respectively. Right: higher magnification of the boxed area. Scale bars, 100 μm (left); 50 μm (right). n = 3 mice per group. k Comparison of the total number of c-Fos positive neurons in laminae IIi and IIIo under different conditions. n = 15 sections from three mice per group. l, m Comparison of the percentage of Tac2tdTom and c-Fos double positive neurons in c-Fos positive neurons in lamina IIi (l) and IIIo (m) under different conditions. n = 15 sections from three mice per group. one-way ANOVA with Tukey post-hoc, ***p = 0.0001. All data are presented as mean ± s.e.m. and error bars represent s.e.m. Source data are provided as a Source Data file.
The location of Tac2tdTom neurons suggests that they may receive mono-LTMR Aβ input. To evaluate the nature of LTMR Aβ primary afferent inputs for Tac2tdTom neurons, we adopted a root stimulation protocol that allowed the best preservation of Aβ fibers\(^4\) and recorded Tac2tdTom neurons from a thick parasagittal spinal cord slice (550 µm) with the root attached. Interestingly, Tac2tdTom in lamina IIi predominantly receive monosynaptic C fiber input (68.0%, 34 of 50), followed by polysynaptic Aδ inputs (44.0%, 22 of 50) and polysynaptic Aβ inputs (28.0%, 14 of 50) (Fig. 2g, h). However, the majority of Tac2tdTom neurons in lamina IIIo, received either monosynaptic (50.0%, 23 of 46) or polysynaptic Aβ inputs (23.9%, 11 of 46) (Fig. 2i, j), while the rest received negligible Aδ and C fiber inputs (Fig. 2j). These results revealed different properties of Tac2tdTom in lamina IIIo and IIi and suggest that Tac2tdTom in lamina IIIo represent a population of excitatory interneurons that are ideally suitable for conveying...
Fig. 3 Optostimulation of Tac2 neurons evoked itch-related scratching behavior. a Schematic of blue light stimulation of Tac2ChR2 neurons. b, c A snapshot (b) and quantification (c) of blue light-induced scratching behaviors in Tac2ChR2 mice. One-way ANOVA with Tukey post-hoc, *p = 0.012, n = 6 mice per group. d The effect of i.t. morphine on blue light-induced scratches. Two-way ANOVA with Bonferroni post-hoc, ***p = 0.00001, n = 9 for Tac2WT/saline, n = 7 for Tac2WT/morphine, n = 11 for Tac2ChR2/saline, n = 8 for Tac2ChR2/morphine. e Blue light-induced scratches decreased after BB-sap treatment. Two-tailed Student’s unpaired t-test, *p = 0.029, n = 7 mice per group. f Double IHC of c-Fos (red) and GFP (green) in the spinal cord of Tac2ChR2 mice following blue light stimulation. g Higher magnification of the boxed area in f. Arrowheads indicate double-stained c-Fos+/Tac2ChR2+ neurons in f, g. Scale bars, 50 μm in f and 10 μm in g. h Quantification of percentages of Tac2ChR2+/c-Fos double positive neurons in c-Fos positive neurons in laminae IIi and IIIo, respectively. n = 3 mice. All data are presented as means ± s.e.m. and error bars represent s.e.m. Source data are provided as a Source Data file.

Opto-activation of Tac2 neurons evokes scratching behaviors.

To determine the role of Tac2 neurons in itch, we examined behavioral response of mice derived from mating between Tac2Cre mice and Ai32 reporter mice expressing channel rhodopsin-eYFP (ChR2-eYFP, referred to as Tac2ChR2) using optogenetic approach (Fig. 3a, b). Optoactivation of Tac2ChR2 neurons provoked scratching bouts, starting at 5 Hz and increasing until 10 Hz (Fig. 3c), revealing that the capacity of Tac2ChR2 neurons to induce scratching bouts reaches a limit at 10 Hz. To determine whether evoked scratching behavior reflects pain or itch, intrathecal (i.t.) injection of morphine was performed to inhibit the spinal nociceptive transmission. i.t. morphine failed to attenuate evoked scratching behaviors at 5 Hz (Fig. 3d), indicating that scratching behavior is likely to be related to itch rather than pain. We also examined whether evoked scratching behavior is itch-related by ablating spinal GRPR neurons with i.t. bombesin-saporin (BB-sap)45. Indeed, the scratching behavior induced by optostimulation of Tac2ChR2 neurons was significantly attenuated (Fig. 3e), suggesting that the evoked scratching behavior was at least partially dependent on GRPR neurons. To confirm whether Tac2ChR2 neurons were indeed activated by optostimulation, we examined the expression of c-Fos using IHC following blue light stimulation. Notably, c-Fos was observed across the dorsal horn laminae, reminiscent of c-Fos pattern induced by mechanical itch (Fig. 1j, 3f, g). Remarkably, most c-Fos positive neurons in laminae II–III were Tac2ChR2 neurons (Fig. 3f–h). Given only a few scratches evoked per ten stimulation in contrast to robust scratching bouts induced by chemical itch, these findings suggest that activation of Tac2 neurons could mimic von Frey-evoked scratching.

Inhibition of Tac2Cre neurons attenuates mechanical itch. Next we used Cre-dependent Gα-coupled designer receptors exclusively activated by designer drugs (DREADDs) to inhibit Tac2Cre neurons followed by intraspinal injection of adeno-associated viruses (AAV2/8-Syn-DIO-h4MDi (Gi)-mCherry) into the cervical cord of mice (Fig. 4a–c). While the baseline of mechanical itch and CQ itch remained the same after virus injection, clozapine injection significantly reduced mechanical itch elicited by von Frey hair stimulation (from 0.07 to 0.4 g) (Fig. 4d and Supplementary movie 1). In contrast, CQ-induced itch was not affected (Fig. 4e).

To test whether inhibition of Tac2Cre neurons would alter mechanical thresholds and thermal pain, AAV2/8-Syn-DIO-h4MDi (Gi)-mCherry virus was injected into the lumbar spinal cord of mice (Fig. 4a)41. Inhibition of Tac2Cre neurons followed by intraperitoneal (i.p.) injection of diphtheria toxin (DTX) in the Lbx1-Flpo/Tau-DTR/Tac2-Cre mice (Fig. 4f)41. Lastly, intersectional genetic approach was employed to ablate Tac2Cre neurons in the spinal cord of Tac2Cre mice46. Strikingly, mechanical itch evoked by von Frey hair force was almost abolished after intraperitoneal (i.p.) injection of diphtheria toxin (DTX) in the Lbx1-Flpo/Tau-DTR/Tac2-Cre mice (Fig. 4f)41. In contrast, ablation of Tac2Cre neurons did not change CQ-induced itch (Fig. 4g), nor pain or motor behaviors (Supplementary Fig. 3a–I), consistent with previous studies33. The complete ablation of Tac2Cre neurons was confirmed by the absence of NKB immunostaining (Fig. 4h).

Mechanical itch is dependent on GRPR neurons. While mechanical itch and chemical itch have been considered to
function through distinct neuronal pathways in the spinal cord. Recent studies showed that NPY-NPY1R signaling can inhibit both mechanical and chemical itch, indicating that they may share the same pathway. The finding that ablation of GRPR neurons significantly reduced the scratching behavior evoked by optoactivation of Tac2ChR2 neurons prompted us to examine whether mechanical itch transmission is dependent on GRPR neurons. We first examined whether c-Fos is activated in GRPR neurons using Grpr<sup>tdTom</sup> mice. Von Frey hair stimulation applied to the hairy skin of the nape induced c-Fos expression in laminae I–II, including Grpr<sup>tdTom</sup> neurons (Fig. 5a). Throughout laminae I–II, approximately one third of c-Fos were colocalized with Grpr<sup>tdTom</sup> neurons (Fig. 5a, b), suggesting the involvement of GRPR neurons in mechanical itch transmission. To functionally test the role of GRPR neurons, we examined the effect of the spinal ablation of GRPR neurons on mechanical itch using BB-sap (500 ng) approach. The ablation of GRPR neurons was confirmed by the lack of scratching response to CQ (Fig. 5d). BB-sap treatment almost abolished mechanical itch (Fig. 5c and Supplementary movie 2). A comparison of different approaches suggests that the dose of BB-sap (400 ng) used by previous studies is likely to be too low, as mice still showed substantial scratching bouts to CQ injection, suggesting a partial ablation of GRPR neurons (Supplementary Table 1). To test this possibility, we repeated the ablation test using BB-sap (400 ng). Interestingly, we found that mechanical itch is normal even though mice treated with BB-sap showed only approximately 20 scratching bouts to CQ (Supplementary Fig. 4a, b and Table 1). Only when mice treated with BB-sap scratched <5 times did mice fail to show mechanical itch behavior (Supplementary Table 1). We also counted the number of Grpr neurons in spinal cord slices after blank-sap, BB-sap (400 ng), and BB-sap (500 ng) treatment, respectively. The results showed that the number of Grpr neurons was lowest after BB-sap 500 ng treatment (Supplementary Fig. 4c–f), suggesting a complete ablation of GRPR neurons by BB-sap 500 ng. Therefore, a key prerequisite for evaluating whether mechanical itch depends on GRPR neurons is to ablate GRPR neurons completely, manifesting in the absence of CQ-evoked scratching behavior. Recent studies have shown that pharmacological activation of spinal NPY1R can inhibit both mechanical and chemical itch. This promoted us to examine to what extent Npy1r and Grpr or Tac2 are co-expressed in the spinal cord using RNAscope. We found that approximately 35% of Grpr neurons express Npy1r, whereas only 11% express Tac2 (Supplementary Fig. 4g–j). These findings raise the possibility that NPY1R agonists or NPY may act on GRPR neurons via NPY1R to inhibit chemical itch.

Next, we sought to determine the type of inputs that GRPR neurons may receive by recording the response of Grpr<sup>tdTom</sup> neurons located in laminae I–II to the root stimulation. Using the same protocol for recording Tac2<sup>tdTom</sup> neurons, we found that Grpr<sup>tdTom</sup> neurons predominantly received polysynaptic β input (71.4%, 30/42), whereas no monosynaptic Aβ input was detected (Fig. 5e–g). These data suggest that GRPR neurons are endowed with the capacity to receive light touch information indirectly from Tac2 neurons. To examine whether Tac2 neurons may form synaptic contacts with GRPR neurons, we first performed NKB IHC in the cervical spinal cord of Tac2<sup>tdTom</sup> mice (Fig. 6a, b) and Grpr<sup>tdTom</sup> mice (Fig. 6c, d), respectively. Interestingly, the NKB staining was concentrated in laminae I–II where GRPR neurons are located (Fig. 6a–d). This unique distribution pattern of NKB implies that Tac2 neurons project dorsally and their targets are located in laminae I–II, while some arborizing locally within the domain of laminae II–III. Consistent with this view, numerous NKB punctate staining signals were detected surrounding and overlapping with Grpr<sup>tdTom</sup> neurons (Fig. 6c, d). To examine whether Tac2 neurons form monosynaptic contacts with GRPR neurons, we next employed rabies virus circuit tracing method using Grpr<sup>Cre</sup> mice with glycoprotein-deleted rabies virus (RV<sub>dG</sub>...
Fig. 5 Mechanical itch depends on GRPR neurons. a Representative image of c-Fos IHC (green) in the dorsal horn of Grpr<sup>tdTom</sup> (red) mice after von Frey hair stimulation (0.07 g) on the nape skin. Arrowhead indicates c-Fos<sup>+</sup>/ Grpr<sup>tdTom</sup> + neurons. n = 3 mice. Scale bar, 50 μm. b Quantification of the percentage of Grpr<sup>tdTom</sup> +/c-Fos<sup>+</sup> neurons in c-Fos<sup>+</sup> neurons in laminae I-IIo. n = 3 mice. c, d Mechanical itch test (c) and CQ itch test (d) after 500 ng of BB-sap treatment. (c) two-way ANOVA with Bonferroni post-hoc, *p = 0.018,**p = 0.005,**p = 0.00001, n = 10 mice per group; (d) unpaired two-tailed Student’s t-test,**p = 0.00001, n = 10 mice per group. e, f Representative traces showing one Grpr<sup>tdTom</sup> neuron cannot follow 20 μA/20 Hz stimulation. g Percentage of each types of inputs onto Grpr<sup>tdTom</sup> neurons. Data from five mice, n = 42 neurons. All data are presented as means ± s.e.m. and error bars represent s.e.m. Source data are provided as a Source Data file.

Tac2 neurons are required for alloknesis and dry skin itch. Next we asked why alloknesis is exacerbated under chronic itch condition. To ascertain whether Tac2 neurons have a role in alloknesis associated with chronic itch, we employed a dry skin model using mice treated with acetone–ether–water (AEW) treatment (<sup>42</sup>), in which the loss of Piezo2-Merkel cell signaling contributed to alloknesis<sup>31</sup>. Mice treated with AEW displayed pronounced alloknesis compared to control mice without AEW treatment (Fig. 7a). Importantly, chemogenetic inhibition of Tac2<sup>Cre</sup> neurons not only reversed enhanced alloknesis associated with dry skin itch (Fig. 7b), but also spontaneous scratching behavior, which reflects chemical itch induced by AEW treatment (Fig. 7c).

In accordance with these findings, we found that c-Fos was induced in Tac2<sup>tdTom</sup> neurons of mice treated with AEW in the absence of von Frey hair stimulation (Fig. 7d). Quantification of c-Fos<sup>+</sup> and Tomato<sup>+</sup>/c-Fos<sup>+</sup> double positive neurons indicates that while c-Fos appears to be evenly distributed across the dorsal horn of the spinal cord, more Tac2<sup>tdTom</sup> neurons were activated by AEW treatment in lamina III than lamina IIIo (Fig. 7e–h). This contrasts with c-Fos pattern in naïve mice in response to mechanical itch stimulus (Fig. 11, m).

Finally, we tested whether Tac2 neurons in lamina IIi and IIIo are differentially activated in mice with dry skin itch by examining the excitability and Aβ-evoked action potentials (Aβ-APs) of Tac2<sup>tdTom</sup> neurons of mice treated with AEW. While resting membrane potentials (RMP) remained unchanged (Fig. 8a, b), the rheobase of action potentials of Tac2<sup>tdTom</sup> neurons in lamina II decreased significantly compared to the control mice (Fig. 8c, 25.4 pA vs. 14.7 pA). In contrast, no such changes were observed for Tac2<sup>tdTom</sup> neurons in lamina III (Fig. 8d), indicating that Tac2<sup>tdTom</sup> neurons in lamina IIi are sensitized under dry skin itch condition. However, the firing patterns of Tac2<sup>tdTom</sup> neurons in lamina III were unchanged in dry skin mice (Fig. 8h). Consistently, increased Aβ-APs were found exclusively in lamina III rather than lamina IIIo Tac2<sup>tdTom</sup> neurons under dry skin condition (Fig. 8e–g). Furthermore, the Aβ-induced synaptic inhibition (eIPSC) was significantly reduced in dry skin itch mice (Fig. 8i, j). To further examine the synaptic inhibition on Tac2<sup>tdTom</sup> neurons in lamina II, we tested the effect of GABA<sub>Rs</sub> antagonist and GlyRs antagonist on Aβ-APs<sup>47</sup>. Of 15 Tac2<sup>tdTom</sup> neurons in lamina IIi without Aβ-APs, 11 showed Aβ-APs after bath application of bicuculline and strychnine (Fig. 8k). These results suggest that Tac2<sup>tdTom</sup> neurons in lamina IIi with Aβ inputs are modulated by GABA/glycine-dependent feedforward inhibition (Fig. 8k, l).

Discussion
How innocuous tactile information is converted into irritating itch sensation is a fascinating question. In this study, we have used a combination of molecular, pharmacological, electrophysiological, chemogenetic, intersectional genetic ablation, and monosynaptic neural circuit tracing approaches to identify Tac2 neurons, which are exclusively located in laminae III–IIIo of the spinal cord as a principal neural circuit for mechanical itch. Importantly, we demonstrate that GRPR neurons are an integral component of the circuitry for touch-to-itch conversion. Notably,
we found that the behavior of Tac2\textsuperscript{dTom} neurons is similar to Ucn3\textsuperscript{tdTom} neurons located in LTM-RZ, which have recently been implicated in mechanical itch transmission\textsuperscript{26}, under both naive and dry skin conditions (Supplementary Table 2). This suggests that Tac2 neurons are a subpopulation of Ucn3\textsuperscript{tdTom} neurons in laminae II–III. Given that Ucn3\textsuperscript{tdTom} neurons are distributed throughout laminae I–III, resulting from tracking transient expression of Ucn3 during postnatal development\textsuperscript{26}, we argue that Tac2 neurons, which are readily identifiable anatomically in the adult spinal cord, represent a bona fide neural circuit for mechanical itch.

The loss of mechanical itch in mice treated with BB-sap suggests a crucial role of GRPR neurons in mechanical itch transmission. Anatomically, rabies virus circuit tracing that revealed monosynaptic connections between Tac2 neurons and GRPR neurons supports this conclusion. The discrepancies between the present and previous studies\textsuperscript{21,26,27} can be ascribed to several methodological differences (see Supplementary Table 1). First, poking the nape of naive mice ten times instead of five is advantageous (Supplementary Table 1), because it permits comparable evaluation of mechanical itch between naive mice and mice treated with BB-sap using the same approach (nape). Second, given that application of von Frey to the nape evokes only a few scratching bouts (<10) per ten stimulations in naive mice, a partial ablation of GRPR neurons, as manifested in approximately 50 scratches induced by pruritogens\textsuperscript{21,27}, is insufficient for blocking mechanical itch. A key difference between our study and the others is the dose of BB-sap used. In our study, we found that 400 ng BB-sap is not sufficient to abolish mechanical itch. However, one caveat is that these different doses may not be absolute and have to be determined empirically by the users. This is because the activity and potency of BB-sap differ from lot to lot and additionally depend on the storage conditions by individual laboratories. We have found a gradual reduced activity of BB-sap over years and accordingly the dose of BB-sap must be adjusted accordingly to achieve complete ablation of GRPR neurons. Because it is difficult to evaluate subtle molecular differences between 400 and 500 ng in the spinal cord, perhaps the most convenient and simple way to verify the completeness of ablation of GRPR neurons is to check if CQ-evoked scratches are abolished after 2 or 3 weeks of injection. If not, additional dose of BB-sap should be injected to abate remaining GRPR neurons, which would ensure the loss of mechanical itch evoked from the nape. Fortunately, a relatively higher dose of BB-sap does not produce observable detrimental effect on mice. Could BB-sap at 500 ng or higher produce nonspecific effect that can be ascribed to the loss of mechanical itch? While this possibility cannot be excluded with certainty, it seems less likely. Most importantly, our finding is consistent with the observation that Grpr neurons express Npy\textsuperscript{1r} (Supplementary Fig. 4g, h), as well as recent pharmacological

![Image of GRPR neurons forming monosynaptic connections with Tac2 neurons](image-url)

**Fig. 6** GRPR neurons form monosynaptic connections with Tac2 neurons. a, c Representative IHC images of NKB staining (green) in the cervical spinal cord of Tac2\textsuperscript{dTom} (red) mice (a) and Grpr\textsuperscript{tdTom} (red) mice (c). b, d High power images of the boxed areas in a, c, respectively. Scale bars, 20 μm in a, c; 5 μm in b, d. e, f Schematic of intraspinal injection of TVA-EGFP/RVG virus and RV-dG-dsRed virus. g Representative ISH image of Tac2 (blue) in the cervical spinal cord after RVdG injections. Arrowheads indicate Grpr\textsuperscript{Cre} starter neurons (yellow) expressing GFP (green) and dsRed (red). Arrows indicate Tac2\textsuperscript{+} input neurons (dsRed\textsuperscript{+} and blue) targeting starter neurons. n = 3 mice. Scale bar, 100 μm.
studies showing that exogenous NPY or NPY1R agonist inhibit chemical itch.\textsuperscript{28,29}

The present finding is further consistent with the observation of augmented allodynia in a mouse model of dry skin itch.\textsuperscript{31,32,37,38} One notable difference between human and mouse chronic itch models is that the latter is a type of chemical itch in nature, for the development and maintenance of scratching behavior are contingent on periodic application of chemicals to the nape as well as enhanced expression of GRP in DRGs and GRPR in the spinal cord.\textsuperscript{6,49,50} Therefore, the fact that histaminergic itch transmitted via NMB and NMBR neurons is also converged on GRPR neurons,\textsuperscript{23} GRPR neurons are the last interneuron station in the spinal cord for integrating and transmitting mechanical itch from the periphery to the brain.\textsuperscript{51}

Our analysis reveals that lamina Iii and llll Tac2 neurons differ in firing patterns and types of input received. While approximately 28.0\% of llll Tac2 neurons can receive polysynaptic A\(\beta\) fiber input, they primarily receive C/A\(\delta\) fiber input with negligible monosynaptic A\(\beta\) fiber input. By contrast, approximately half of llll Tac2 neurons receives monosynaptic A\(\beta\)-LTM R input, making them best suited for transmitting innocuous touch information directly. Consistently, a significantly higher percentage of Tac2 neurons in lamina llll than llll generated A\(\beta\)-evoked action potentials in naive mice. The finding of two discrete subpopulations of Tac2 neurons provides a neuroanatomic basis for explaining why Tac2 neurons transmit mechanical but not chemical itch under naïve conditions. It is conceivable that lamina llll Tac2 neurons may be inactive or “silent” in naïve conditions. The observation of a reduction of feedback inhibition mediated by GABA/glycine for llll Tac2 neurons under a dry skin condition, manifested by increased incidence of A\(\beta\)-

**Fig. 7 Tac2 neurons are required for allodynia and dry skin itch.** a Increased mechanical itch in AEW-treated mice relative to the control. Two-way ANOVA with Bonferroni post-hoc. ***\textit{p} = 0.0001, \(n = 10\) mice per group. b, c Mechanical itch (b) and spontaneous scratching behavior (c) of AEW-treated mice after chemogenetic inhibition of spinal Tac2\textsuperscript{Cre} neurons. (b) two-way ANOVA with Bonferroni post-hoc, *\textit{p} = 0.027, **\textit{p} = 0.005 for group 0.04 g. **\textit{p} = 0.009 for group 0.16 g. ***\textit{p} = 0.0001, \(n = 12\) mice per group; c unpaired two-tailed Student’s t-test, **\textit{p} = 0.001, \(n = 12\) mice per group). d Representative IHC image of c-Fos (green) in the cervical spinal cord of AEW-treated Tac2\textsuperscript{Cre/h4MDi-mCh} (red) mice. Higher magnification of the boxed area (middle), which is shown at higher magnification (right). Arrowheads indicate double c-Fos+/Tac2\textsuperscript{Cre} neurons. Scale bars, 100 \(\mu m\) in left of d; 50 \(\mu m\) in middle of d; 25 \(\mu m\) in right of d. e Quantification of c-Fos+ neurons in laminae I-Ilo, Ili and Iillo, respectively. f The ratio of Tac2\textsuperscript{Cre/h4MDi-mCh} and c-Fos double positive neurons in c-Fos positive neurons in laminae I-Ilo and Ili. g Comparison of the number of c-Fos+ neurons in laminae I-Ilo, Ili and lillo between control mice stimulated by von Frey hair (0.07 g) and AEW mice. h Comparison of the ratio of c-Fos+/Tac2\textsuperscript{Cre/h4MDi-mCh} neurons in lamina I-Ilo versus Ili versus Iillo between control and AEW mice. *\textit{p} = 0.016, unpaired two-tailed Student’s t-test, \(n = 3\) mice per group. All data are presented as means ± s.e.m. and error bars represent s.e.m. Source data are provided as a Source Data file.
evoked APs and higher intrinsic excitability, suggests that they are more active and sensitized relative to normal conditions. Therefore, one plausible explanation may be that lamina II neurons in the spinal cord can activate NPY1R to inhibit the function of GRPR neurons (Supplementary Fig. 4g, h), it is conceivable that Aβ-eIPSCs on lamina II neurons in control mice (left) and dry skin mice (right). Data from five mice per group. g Representative traces for Aβ-eIPSCs in Tac2 neurons in laminae IIi of control mice (left) and dry skin mice (right). Data from three mice per group. h Percentage of Aβ-eIPSCs (%). Data from three mice per group. i The amplitude of Aβ-eIPSCs on lamina II neurons in control mice (left) and after bath application of bicuculline (Bic) and/or strychnine (Stry). Blue arrows indicate stimulation artifacts. Data were from three mice per group. Source data are provided as a Source Data file.

Fig. 8 Increased excitability of Tac2 neurons of dry skin mice. a–d Resting membrane potential (RMP) (a, b) and the rheobase of action potential (c, d) for Tac2 neurons in lamina IIi (a, c) and lamina Ilo (b, d). Control: n = 14 neurons in a, b, 13 neurons in c and 12 neurons in d. Dry skin: n = 17 neurons in a, 14 neurons in b and 13 neurons in d. Two-tailed Mann-Whitney test. ***p < 0.0001, ns not significant. Data were from three mice per group. e Representative traces for Aβ-evoked APs in Tac2 neurons in laminae IIi of control mice (left) and dry skin mice (right). f, g Percentage of Aβ-evoked APs in Tac2 neurons in laminae II (f) and Ilo (g) of control and dry skin mice, respectively. Control: n = 24 neurons in f, n = 11 neurons in g; dry skin: n = 22 neurons in f, n = 11 neurons in g. Chi-square test, ***p < 0.0001, ns not significant. Data from three mice per group. h Firing patterns of Tac2 neurons in control mice (n = 24 neurons) and dry skin mice (n = 22 neurons). Data from five mice per group. i The amplitude of Aβ-eIPSCs on Tac2 neurons in lamina IIi of control mice (left) and dry skin mice (right). j The amplitude of Aβ-eIPSCs on Tac2 neurons in lamina IIi of control mice and dry skin mice. **p = 0.0001, two-tailed unpaired Student’s t-test, n = 18 neurons per group. k Representative traces for Aβ-eIPSCs on Tac2 neurons in lamina IIi of control mice and after bath application of bicuculline or strychnine. ***p < 0.0001, two-tailed paired Student’s t-test, n = 15 neurons per group. Data are presented as means ± s.e.m. Error bars represent s.e.m. 100 nsns

In view of the present findings that Npy1r is expressed in GRPR neurons (Supplementary Fig. 4g, h), it is conceivable that exogenous administration of NPY or NPY1R agonists in the spinal cord can activate NPY1R to inhibit the function of GRPR neurons and thus inhibit both chemical and mechanical itch. Since not all GRPR neurons express NPY1R, the inhibitory effect of NPY or NPY1R agonists on chemical itch could depend on the
type of pruritogens or subtypes of GRPR neurons expressing NPY1R. Despite these studies, it seems less likely that NPY–NPY1R signaling, or inhibitory neural circuits in general, is involved in mechanical itch inhibition endogenously for several reasons. Firstly, analogously to acute pain stimuli to which animals respond with withdrawal behavior, innocuous light touch does not penetrate the skin. To protect from a potential harm, withdrawal or light scratching/wiping is sufficient to distant from or remove trivial irritants on the skin. By contrast, activating an endogenous inhibitory neural circuit usually requires more intense scratching behavior. Indeed, once the von Frey hair is removed, mice no longer scratch the spot being touched. Consistent with this notion, ablation or inhibition of various populations of spinal inhibitory neurons does not impair acute thermal and mechanical pain behaviors. Secondly, GABA/glycine are adequate for regulating Tac2/Ucn3δTom neurons through feedforward inhibition via GABAergic/glycinergic neurons. Because mechanical itch travels through GRPR neurons, which are directly subject to GABA/glycine-mediated inhibition of galanin neurons, the physiological relevance of inhibitory regulation of Tac2/Ucn3δTom neurons is currently unclear. It is possible that Tac2/Ucn3δTom neurons are kept in a quiescent state under an inhibitory control, which may explain why mechanical itch is rather infrequently experienced. Nonetheless, caution is warranted while extrapolating the endogenous function of a receptor from behaviors resulting from its pharmacological activation or inhibition, which could trigger a novel signaling pathway that may not occur in vivo.

On the other hand, there is substantial evidence that the spinal administration of a NPY2R antagonist exhibited robust pain- but not itch-related scratching behavior, suggesting a role of NPY signaling in gating spontaneous pain. Accordingly, von Frey stimulation applied to the nape or cheek may evoke pain-related scratching behavior due to mechanical hypersensitivity caused by the ablation of NPY neurons. While not tested, one can predict that galanin neurons, which mediate direct inhibition of GRPR neurons, may also be important for gating mechanical itch. Collectively, these data suggest that mechanical itch can be simultaneously gated at the level of Tac2 neurons and GRPR neurons.

In summary, our results show that lamina IIIa Tac2 neurons function as a key entry node for receiving and encoding innocuous touch, which is accessible to GRPR neurons via the Tac2–GRPR monosynaptic neuronal connection. GRPR neurons subserve as convergent and integrating circuit for chemical and mechanical itch and represent the last output station from the spinal cord to the brain. Under pathological itch conditions, lamina IIIa Tac2 and laminae I–IIo GRPR neurons might be sensitized to convey heightened alloknesis, in part due to disinhibition, thereby exacerbating chronic itch conditions. Hence, our study suggests a previously unknown spinal circuitry that is exquisitely hard-wired for touch-to-itch conversion.

**Methods**

**Animals.** Experiments were carried out on C57BL/6J (Stock no.000664, Jax mice), Tac2Cre mice, GrprCre mice, Ai32 mice (Stock no. 024109, Jax mice), Aip mice (Stock no.007909, Jax mice), Lbx1Cre mice, Tac2DTom mice and their wild-type littermates unless indicated otherwise. All mice were housed under a 12 h light/dark cycle. Mice were housed in clear plastic cages with no more than 5 mice per cage in a controlled environment at a constant temperature of ~23 °C and humidity of 50 ± 10% with food and water available ad libitum. Male animals of 2–3 months of age were used in the experiments. All experiments conformed to guidelines set by the National Institutes of Health and the International Association for the Study of Pain and were reviewed and approved by the Institutional Animal Care and Use Committee (IACU C) at Washington University School of Medicine.

**Acute pain behavior test.** Mice should have 3 days of acclimation in all the acute pain behavior tests. Mechanical sensitivity was assessed using a set of calibrated von Frey filaments. The lateral plantar surfaces of the hindpaw were stimulated with defined von Frey filaments for five times with 10-s intervals. The smallest filament that evoked reflexive flinches of the paw on three of the five trials was taken as the paw withdrawal threshold. To measure tail flick threshold to noxious mechanical stimulation, a Randall–Selitto Analgesy-meter was used.
instrument generates a mechanical force that increased linearly with time. Mice were held gently, and the force was applied directly to the dorsal surface of the tail (2.5 cm from its end via a cone-shaped plunger. The tail flick threshold is defined as the average force of five trials with 10-min intervals, in grams, at which the mouse attempts to flick its tail (cut-off force 250 g). Thermal sensitivity was determined by Hargreaves test, hot plate and tail flick tests. For Hargreaves test, the plantar paw surface was exposed to a beam of radiant heat with 10-min intervals. The paw withdrawal latency was tested five times per animal and averaged for analysis. For hot plate test, the latency for the mouse to lick its hindpaw or jump from the hotplate (48, 52, and 56 °C) was recorded. For the tail flick test, the end of tail was exposed to a beam of radiant heat with 10-min intervals. The tail flicking latency was tested five times per animal and averaged for analysis.

**Rotarod test.** Mice were placed on a rotarod apparatus that accelerates 5–20 revolution per minute (r.p.m.) for 5 min and trained to maintain its stability. The rotarod test latencies of mice to fall off were recorded for analysis.

**Bombesin-saporin treatment.** Blank-saporin or bombesin-saporin (Advanced Targeting) was intracranially (i.1) injected at 500 or 400 ng per 10 µl 14 days before behavioral tests.

**Dry skin itch.** The dry skin model was implemented as described49. Briefly, the nape of mice was shaved at least 3 days before experiments. A mixture of acetone (Sigma, Cat. No. 179124) and diethyl ether (Sigma, Cat. No. 309966) (1:1) was painted on the neck skin for 15 s, followed immediately by a 30-s of distilled water application (AEW). This regiment was administered twice daily for 5–7 days. Littermate control mice received water only for 45 s on the same schedule. Spontaneous scratches were examined for 60 min in the early morning on day 5–7.

**Mechanical itch or allodynia test.** The nape of mice was shaved at least 3 days before experiments. Mice were acclimated in a recording chamber (20 × 10 × 12.5 cm) for 30 min to acclimate. Mice were briefly removed from the chamber and intradermally (i.d.) injected at the nape with CQ at the dose of 200 µg in 50 µl saline. Hind limb scratching behavior towards the injected area was counted by observers blinded to the group or genotype of the mice.

**Optogenetic stimulation behavior.** Tac2CHR2 mice and wild-type littermates (Tac2WT) were used for optical stimulation experiments. One day prior to the experiments, each mouse was placed in a plastic home cage (27.16 × 1.25 cm) for 30 min to acclimate. For stimulation, the fiber optic ferrule was connected via a ferrule sleeve to a fiber optic cable with a connector (Thorlabs) that was attached to a fiber-coupled 473 nm blue laser (BL473T8-150FC, Shanghai Laser and Optics Co.) with an ADR-800A adjustable power supply. The animal was allowed to acclimate to be tethered to the cable for 30 min prior to stimulation. Laser power output from the fiber optic was measured using a photometer (Thor Labs) and set to 10 mW from the fiber tip (fibers were implanted and % efficiency of power was recorded prior to implantation to ensure 10 mW final power at the photometer tip). An Arduino UNO Rev 3 circuit board (Arduino) was programmed and attached to the laser via a BNC input to control the frequency and timing of the stimulation. For 30-s stimulation, three trials (30-s on, 270-s off) were performed for each frequency (1, 3, 10, 20 or 30 Hz) with 1-day break between each frequency. For morphine injection, morphine (0.3 nmol in 10 µl saline) was i.t. injected 30 min before stimulation. Control mice were i.t. injected with saline. The mean value of the three trials for behavior responses was used in the results and analysis. Mice were recorded with a video camera from a side angle and played back on computer for assessments of the number of scratches by observers blinded to the animal groups.

**RNAseq in situ hybridization (ISH).** The spinal cord sections were processed according to the manufacturer’s instructions in the RNAscope Fluorescent Multiplex Assay v2 manual for fixed frozen tissue (Advanced Cell Diagnostics), and conjugated biotin Fluoromount-G 3 reagent board (Arduini) was processed. Behavioral and histochemical experiments were performed 2 weeks after the second DXT injection.
signal was shown up in small dots rather than filling up entire neurons. For co-localization studies, dots associated with single DAPI stained nuclei were assessed as being co-localized. Cell counting was done by a person who was blinded to the experimental design.

Monosynaptic retrograde tracing. Briefly, 400 nl of the mixture of rAAV2/9-Ef1a-DIO-EGFP-TVA and rAAV2/9-Ef1a-DIO-RVG (2.0 × 10²⁵ vg·mL⁻¹, BrainVTA Co., Ltd., Wuhan, China) was injected into the cervical spinal cord of GrprtdTom mice. Two weeks later, the spinal cord of mice was injected with 200 nl of RV-ENVA-dG-dsRed (2.0 × 10⁸ IFU·mL⁻¹, BrainVTA Co., Ltd., Wuhan, China) at the same site. One week later, the mice were perfused, and the spinal cord was sectioned and imaged under a Nikon C2+ confocal microscope (Nikon Instruments, Inc.). The sections were then used for RNAscope ISH using Tac2 probe (Advanced Cell Diagnostics) and imaged under the confocal microscope. Images taken before and after ISH were aligned and merged for analysis.

Electrophysiology. Adult mice (8–10 weeks old) were deeply anesthetized with ketamine cocktail (ketamine, 90 mg·kg⁻¹ and xylazine, 10 mg·kg⁻¹). Then they were perfused with 30 ml cold 4°C NMDG slicing solution (in mM, 93 NMDG, 2.5 KCl, 1.25 NaH₂PO₄, 30 NaHCO₃, 20 HEPES, 25 Dextrose, 12.1 N HCl 5 Ascorbic acid, 2 Thiamore, 3 Na₃ pyruvate, 10 MgSO₄, 0.5 CaCl₂, 12 N-acetylcycteine, pH was adjusted to 7.3–7.4 with NMDG). Spinal cord was isolated under oxygenated (95% O₂, 5% CO₂) sucrose-based dissection solution (in mM, 209 Sucrose, 2 KCl, 1.25 NaH₂PO₄, 5 MgCl₂, 0.5 CaCl₂, 26 NaHCO₃, 10 HEPES, pH was adjusted to 7.3–7.4) and the lumbar region was embedded in agar. Sections of the lumbar were obtained at 400 μm thick using a vibrating slicer (Vibratome 1000plus). The slices were then collected in chamber containing 37°C oxygenated holding solution (in mM, 92 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 30 NaHCO₃, 20 HEPES, 25 Dextrose, 2 MgCl₂, 2 CaCl₂, pH was adjusted to 7.3–7.4) for 1 h.

Neurons were visualized with 593 nm light (TXRRED filter) under an upright microscope (Olympus BX1WD). Slices were mounted in a chamber (Warner RC 26 G) and perfused with oxygenated ACSF at 2 ml·min⁻¹ (in mM, 124 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 24 NaHCO₃, 5 HEPES, 12.5 Dextrose, 1 MgCl₂, 2 CaCl₂, pH was adjusted to 7.3–7.4). Patch pipettes were pulled to a resistance of 6–8 MΩ. Signals were amplified with Multiclamp 700B and Digidata 1500 A and pClamp 10.0 software (Molecular Devices). Signals were filtered at 2 kHz and digitized at 10 kHz. Data were analyzed with Clampfit 10.7, Mini Analysis 6.0.1 (Synaptosoft) and Prism 7 software (GraphPad). Traces were plotted using Origin 2015 software (OriginLab).

For the root stimulation recording, the lumbar spinal cord of mice was removed, embedded using gelatin and glued with the spinal cord midline parallel to the vibratome blade. Parasagittal sections of the lumbar cord were obtained at 500–550 μm, 7–10 mm dorsal roots were kept. L4 or L5 root was sucked and injected currents by a suction electrode. Tacgαδ₇ or GrprtdTom neurons were recorded by another electrode filled with normal pipette solution (in mM, 130 K gluconate, 10 NaCl, 0.2 EGTA, 10 HEPES, 1 MgATP, 5 NaGTP, 1 MgCl₂, pH was adjusted to 7.2). After establishing whole-cell configuration, the resting membrane potential was noted immediately. If the resting membrane potential was positive to the resting membrane potential, the data were discarded. Evoked EPSCs were recorded from a holding potential of −70 mV, evoked IPSCs were recorded by holding membrane potential at 0 mV when eEPSCs were minimized. Stimulus duration was 0.1 ms. Stimulus intensities were determined by performing extracellular recordings of compound action potentials from the dorsal root. The Discrete fibers were classified according to the following criteria: Aβ fibers (5–20 μA), Lf/HT-Aβ (20–50 μA) and C fibers (100–500 μA). Neurons showing no failures at 20 Hz for Aβ, 2 Hz for Aδ, and 1 Hz for C were considered monosynaptic. Onset latencies varied <2 ms for monosynaptic. A fiber mediated EPSCs. To examine feed-forward, bicusculine (10 μM, MilliporeSigma, St. Louis, MO) and/or strychnine (2 μM, MilliporeSigma, St. Louis, MO) were used to disinhibit the dorsal horn neurons. Aβ-evoked IPSP, EPSP, or APs were detected by current clamp recording at the resting membrane potential.

Statistics. Statistical methods are indicated when used. Values are reported as the mean ± standard error of the mean (s.e.m.). Statistical analyses were performed using Prism 7 (v7.0d, GraphPad, San Diego, CA). For parametric comparison between two group, an F-test was conducted to determine the similarity in the variances between the groups, and statistical significance was analyzed using the Student’s t-test. For multiple comparisons, Bartlett’s test for equal variances was used to ensure the variances between multiple groups and then one way or two-way analysis of variance (ANOVA) followed by post hoc test was used to test statistical significance. A p value of less than 0.05 was considered statistically significant.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability. The authors declare that all data supporting the findings of this study are available within the paper and its supplementary information files.
32. Steinhoff, M. S., von Mentzer, B., Geppetti, P., Pothoulakis, C. & Bunnett, N. W. Tachykinins and their receptors: contributions to physiological control and the mechanisms of disease. Physiol. Rev. 94, 265–301 (2014).

33. Mar, L., Yang, F. C. & Ma, Q. Genetic marking and characterization of Tα2-expressing neurons in the central and peripheral nervous system. Mol. Brain 5, 3 (2012).

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

35. Polgar, E., Furuta, T., Kaneko, T. & Todd, A. Characterization of neurons that express preprotachykinin B in the dorsal horn of the rat spinal cord. Neuroscience 139, 687–697 (2006).

36. Madisen, L. et al. A robust and high-throughput Cre reporting and characterization system for the whole mouse brain. Nat. Neurosci. 13, 133–140 (2010).

37. Xiang, C. et al. The transcription factor, Lmx1b, is necessary for the development of the principal trigeminal nucleus-basedlemniscal pathway. Mol. Cell Neurosci. 44, 394–403 (2010).

38. Ding, Y. Q. et al. Lmx1b controls the differentiation and migration of the superficial dorsal horn neurons of the spinal cord. Development 131, 3693–3703 (2004).

39. Cheng, L. et al. Tlx3 and Tlx1 are post-mitotic selector genes determining glutationamatergic GABAergic cell fates. Nat. Neurosci. 7, 510–517 (2004).

40. Haring, M. et al. Neuronal atlas of the dorsal horn defines its architecture and links sensory input to transcriptional cell types. Nat. Neurosci. 21, 869–880 (2018).

41. Duan, B. et al. Identification of spinal circuits transmitting and gating mechanical pain. Cell 159, 1417–1432 (2014).

42. Akiyama, T. et al. Mouse model of touch-evoked itch (allokinesia). J. Invest. Dermatol. 132, 1886–1891 (2012).

43. Cheng, L. et al. Identification of spinal circuits involved in touch-evoked dynamic mechanical pain. Nat. Neurosci. 20, 804–814 (2017).

44. Madisen, L. et al. A toolbox of Cre-dependent optogenetic transgenic mice for light-induced activation and silencing. Nat. Neurosci. 15, 793–802 (2012).

45. Armbruster, B. N., Li, X., Pausch, M. H., Herlitze, S. & Roth, B. L. Evolving the lock to fit the key to create a family of G protein-coupled receptors potently activated by an inert ligand. Proc. Natl Acad. Sci. USA 104, 5163–5168 (2007).

46. Bourane, S. et al. Identification of a spinal circuit for light touch and fine motor control. Cell 160, 503–515 (2015).

47. Munanairi, A. et al. Non-canonical opioid signaling inhibits itch transmission in the spinal cord of mice. Cell Rep. 23, 866–877 (2018).

48. Callaway, E. M. & Luo, L. Monosynaptic circuit tracing with glycoprotein-conjugated viral vectors. Nature 523, 405–410 (2015).

49. Zhao, Z. Q. et al. Chronic itch development in sensory neurons requires BRAF signaling pathways. J. Clin. Invest. 123, 4769–4780 (2013).

50. Liu, X. et al. Spinal GRPR and NPRA contribute to chronic itch in a murine model of allergic contact dermatitis. J. Invest. Dermatol. 140, 1856–1866 e7 (2020).

51. Aresh, B. et al. Spinal cord interneurons expressing the gastrin-releasing peptide receptor convey itch through VGLUT2-mediated signaling. Pain 158, 945–961 (2017).

52. Lu, Y. et al. A feed-forward spinal cord glycinergic neural circuit gates mechanical allodynia. J. Clin. Invest. 123, 4050–4062 (2013).

53. Baba, H., Doubell, T. P. & Woolf, C. J. Peripheral inflammation facilitates Akt/α β-1 fibronectin-mediated synaptic input to the substantia gelatinosa of the adult rat spinal cord. J. Neurosci. 19, 859–867 (1999).

54. Liu, M. Z. et al. Synaptic control of spinal GRPR(+) neurons by local and long-range inhibitory inputs. Proc. Natl Acad. Sci. USA https://doi.org/10.1073/pnas.1905658116 (2019).

55. Ross, S. E. et al. Loss of inhibitory interneurons in the dorsal spinal cord and elevated itch in Bhlhb5 mutant mice. Neuron 65, 886–898 (2000).

56. Freitag, F. B., Ahemaiti, A., Jakobsson, J. E. T., Weman, H. M. & Lagerström, M. C. Spinal gastrin releasing peptide receptor expressing interneurons are controlled by local phasic and tonic inhibition. Sci. Rep. 9, 16573 (2019).

57. Bardoni, R. et al. Pain inhibits GRPR neurons via GABAergic signaling in the spinal cord. Sci. Rep. 9, 15804 (2019).

58. Smith, P. A., Morán, T. D., Abdulla, F., Tumber, K. K. & Taylor, B. K. Spinal mechanisms of NPY analgesia. Peptides 28, 464–474 (2007).

59. Solway, B., Bose, S. C., Corder, G., Donahue, R. R. & Taylor, B. K. Tonic inhibition of chronic pain by neuropeptide Y. Proc. Natl Acad. Sci. USA 108, 7224–7229 (2011).

60. Nelson, T. S. et al. Facilitation of neuropathic pain by the NPY Y1 receptor-expressing subpopulation of excitatory interneurons in the dorsal horn. Sci. Rep. 9, 7248 (2019).

61. Chen, S., Liu, X. Y., Jiao, Y., Chen, Z. F. & Yu, W. NPY1R signaling gates spontaneous and mechanical, but not thermal, pain transmission. Mol. Pain. 15, 17448091988753 (2019).

62. Malet, M., Leiguarda, C., Gaston, G., McCarthy, C. & Brumovsky, P. Spinal activation of the NPY Y1 receptor reduces mechanical and cold allodynia in rats with chronic constriction injury. Peptides 92, 38–45 (2017).

63. Polgar, E., Shehab, S. A., Watt, C. & Todd, A. J. GAβAergic neurons that contain neuropeptide Y selectively target cells with the neurokinin 1 receptor in laminae III and IV of the rat spinal cord. J. Neurosci. 19, 2637–2646 (1999).

64. Barry, D. M. et al. Critical evaluation of the expression of gastrin-releasing peptide in dorsal root ganglia and spinal cord. Mol. Pain 7, 174480916643724 (2016).

65. Christensen, A. J. et al. In vivo interrogation of spinal mechanosensory circuits. Cell Rep. 17, 1699–1710 (2016).

66. Wang, F. et al. RNAscope: a novel in situ RNA analysis platform for formalin-fixed, paraffin-embedded tissues. J. Mol. Diagn. 14, 22–29 (2012).

67. Cheng, L. Z. et al. Identification of spinal circuits involved in touch-evoked dynamic mechanical pain. Nat. Neurosci. 20, 804–814 (2017).

68. Torsney, C. & MacDermott, A. B. Disinhibition opens the gate to pathological pain signaling in superficial neurokinin 1 receptor-expressing neurons in rat spinal cord. J. Neurosci. 26, 1833–1843 (2006).

Acknowledgements
We thank G.T., J. Yin for technical support and P.A.T. and S.W. for comments. We also thank A. Zhu for helping with RNAscope, B. Duan for sharing unpublished data and discussion, Q. Ma for Tan-Cre mice and M. Goulding for Lhx1-Elpe mice. Virus preparation was performed by M.L. of the Hope Center Viral Vectors Core at Washington University School of Medicine. The project has been supported by the NIH grants RO1AR065318-06, RO1NS094344, and RO1 DA037261-01A1 (Z.F.C.) and National Natural Science Foundation of China (Grant No. 81571048) (Y.F.J. and W.Y.).

Author contributions
Z.F.C. conceived and supervised the project. S.H.C., X.F.G., Y.X.Z., B.L.L., D.M.B., K. L., R.B., and X.Y.L. conducted experiments, Y.F.J. and W.Y. supported the project. Z.F.C. wrote the manuscript with inputs from all authors.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary information is available at https://doi.org/10.1038/s41467-020-18895-7.

Correspondence
and requests for materials should be addressed to W.Y. or Z.-F.C.

Peer review information
Nature Communications thanks the anonymous reviewers for their contribution to the peer review of this work.

Reprints and permission information is available at http://www.nature.com/reprints

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.