Parallel ascending spinal pathways for affective touch and pain

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The anterolateral pathway consists of ascending spinal tracts that convey pain, temperature and touch information from the spinal cord to the brain1–4. Projection neurons of the anterolateral pathway are attractive therapeutic targets for pain treatment because nociceptive signals emanating from the periphery are channelled through these spinal projection neurons en route to the brain. However, the organizational logic of the anterolateral pathway remains poorly understood. Here we show that two populations of projection neurons that express the structurally related G-protein-coupled receptors (GPCRs) TACR1 and GPR83 form parallel ascending circuit modules that cooperate to convey thermal, tactile and noxious cutaneous signals from the spinal cord to the lateral parabrachial nucleus of the pons. Within this nucleus, axons of spinoparabrachial (SPB) neurons that express Tac1 or Gpr83 innervate distinct sets of subnuclei, and strong optogenetic stimulation of the axon terminals induces distinct escape behaviours and autonomic responses. Moreover, SPB neurons that express Gpr83 are highly sensitive to cutaneous mechanical stimuli and receive strong synaptic inputs from both high- and low-threshold primary mechanosensory neurons. Notably, the valence associated with activation of SPB neurons that express Gpr83 can be either positive or negative, depending on stimulus intensity. These findings reveal anatomically, physiologically and functionally distinct subdivisions of the SPB tract that underlie affective aspects of touch and pain.

Although primary sensory neurons that respond to a range of innocuous or noxious stimuli acting on the skin have been identified and characterized1,2,3, less is known about how their signals are integrated and processed within the spinal cord and conveyed via spinal projection neurons (PNs) to the brain to underlie somatosensory perception and behaviour. Here, we sought to generate mouse genetic tools for spinal cord anterolateral pathway PNs and use them for anatomical, electrophysiological and behavioural analyses to define ascending pathways that underlie affective touch and pain.

Genetically defined spinal PN subsets

We generated a Tac1CreERT2 knock-in mouse line in which CreERT2 is introduced into the Tac1 locus for tamoxifen-dependent reporter expression in cells that express Tac1 (which encodes tachykinin receptor 1; Fig. 1a, Extended Data Fig. 1b, d, e, f) and found that Tac1+ SPB neurons did not express Gad2 (encoding glutamate decarboxylase 2) and represented a large subset (57.3 ± 4.7%) of the total SPB population (Extended Data Fig. 2a, b, e, f), as previously reported4–9. In addition, to identify other, previously unidentified subsets of SPB neurons, we conducted an in silico screen of BAC-GFP transgenic mouse lines from the GENSAT project7. One line, Gpr83–GFP, in which green fluorescent protein (GFP) is expressed in cells that express Gpr83 (encoding G-protein-coupled receptor 83), showed GFP labelling of a major subset of SPB neurons (52.7 ± 4.4% of the total SPB population; Fig. 1b, Extended Data Fig. 1g). Furthermore, a newly generated Gpr83CreERT2 knock-in mouse line showed labelling of PNs of the anterolateral pathway that were Gad2+ and accounted for 45.2 ± 2.4% of SPB neurons (Extended Data Figs. 1c, 2a, b, e, f). Further analysis revealed that the Gpr83+ and Tac1+ SPB populations are numerically comparable and largely non-overlapping, and together account for most (88.4 ± 2.7%) SPB neurons in spinal cord laminae I and IIo (Fig. 1f–h, Extended Data Fig. 2c–g). Tac1+ SPB neurons, a previously reported SPB population6, accounted for 22.1 ± 3.4% of SPB neurons in laminae I and IIo, and Tac1+ is expressed in subsets of both Tac1+ and Gpr83+ SPB neurons (38.2 ± 4.0% and 47.8 ± 9.7%, respectively), suggesting that Tac1+ SPB neurons may correspond to the small subset of SPB neurons that express both Tac1 and Gpr83 (Extended Data Fig. 2j–n). We also generated a Robo3CreERT2 knock-in mouse line (Extended Data Fig. 1a) because Robo3 (which encodes roundabout guidance receptor 3) is transiently expressed in developing commissural neurons8, including PNs of the anterolateral pathway.

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Zonal segregation of SPB axon terminals

The PBN consists of several cytoarchitecturally distinct subnuclei that have distinct input–output connections with other brain regions. We found that the axons of spinal PNs, labelled in their entirety using Cdx2-Cre; Rosa26LSL-synaptophysin-tdTomato mice, terminate within all PBN subnuclei except the ventrolateral subnucleus (PBNL) and the centre region of the external lateral subnucleus (PBNel), whereas Tac1+ SPB neurons form synapses mainly within the central lateral (PBNcl) and internal lateral (PBNi) subnuclei, Gpr83+ SPB neurons form synapses uniquely within the dorsal lateral (PBNdl) and PBNi subnuclei, in addition to the PBNcl (Fig. 2b, c, d). Accordingly, presynaptic terminals of Gpr83+ SPB neurons are associated with Cgrp+ neurons (expressing calcitonin gene-related peptide, encoded by Calca within) the PBNcl, whereas Tac1+ axonal terminals are not (Extended Data Fig. 4a, b). Moreover, Tac1+ SPB neurons form synapses only within the PBNl (Extended Data Fig. 2e–q), which is the sole PBN subnucleus receiving both Tac1+ and Gpr83+ SPB synaptic terminals (Fig. 2c, d). Thus, distinct PBN subnuclei form synaptic terminals within the PBNcl in a zonally segregated manner.

The finding that Tac1+ and Gpr83+ SPB neurons innervate distinct sets of PBNi subnuclei led us to hypothesize that these two SPB populations underlie distinct behavioural responses to somatosensory stimuli. Selective, bilateral optogenetic activation of either Tac1+ or Gpr83+ SPB neuron axon terminals within the PBN cl (Fig. 2e) strongly induced the expression of the immediate-early gene Fos in the respective PBNi subnuclei (Fig. 2f, g) predicted by the synaptic terminal analysis (Fig. 2b, c, d). Strong stimulation of Tac1+ axon terminals also resulted in expression of Fos in the PBNcl, in addition to the PBNl and PBNi (Fig. 2f, g), consistent with the presence of local microcircuits that interconnect these PBNi subnuclei. Behaviourally, high-power (6.5 mW) optogenetic stimulation of either Tac1+ or Gpr83+ SPB neuron terminals led to a robust increase in locomotion in a time-locked manner (Fig. 2f, i), reminiscent of escape behaviours observed in response to noxious stimuli. Notably, whereas stimulation of Gpr83+ terminals promoted forward locomotion, stimulation of Tac1+ terminals induced a pronounced backward 'retreat' behaviour as well as jumping (Fig. 2f). Extended Data Fig. 4d–f, Supplementary Videos 1–3. Both light-activated hyperlocomotion and jumping were abolished following infusion of the glutamatergic synaptic blocker NBQX into the PBN cl (Fig. 2k, l), and induction of Fos was not observed in the spinal cord after photostimulation of SPB axon terminals (Extended Data Fig. 4g, h), suggesting that escape behaviours evoked by stimulation of SPB axon terminals are mediated by glutamatergic synaptic transmission within the PBN and not by back-propagating action potentials and grey (PAG) and the midbrain reticular nucleus (MRN), whereas Tac1+ spinal PNs more densely innervate the superior colliculus, with a compartmentalized array of terminal patches in the intermediate grey layer (SCig; Fig. 1c, d). In the brainstem, both Tac1+ and Gpr83+ PNs innervate the medial accessory olive (MAO) and lateral reticular nucleus (LRN), while Gpr83+ PNs uniquely innervate the dorsal fold of the dorsal accessory olive (DAOd); Extended Data Fig. 2h, i). Analysing anatomical uses using a dual-virus labelling approach (Extended Data Fig. 3) revealed that axons of both Tac1+ and Gpr83+ neurons that project to the posterior thalamus travel through the ventral lateral funiculus of the spinal cord white matter and form collateral branches that terminate in the MAO and LRN of the ventral brainstem. By contrast, axons of Tac1+ neurons projecting to the SCig travel through the dorsal lateral funiculus and form collateral branches terminating in the PAG. Notably, none of these thalamic- and midbrain-projecting Tac1+ or Gpr83+ PNs formed collateral branches terminating within the PBN cl, the most densely innervated brain target of anterolateral pathway PNs. These findings indicate that Tac1+ and Gpr83+ spinal PNs that innervate the posterior thalamus, midbrain and PBN, are distinct populations.

To define brain targets of anterolateral pathway PNs, we visualized axonal projections of Tac1+, Gpr83+ or Robo3+ spinal PNs to the brain using intersectional genetic strategies that combine our newly generated PN CreERT2 mouse lines (Tac1+CreERT2, Gpr83CreERT2 or Robo3+CreERT2), spinal cord–selective Flp mouse lines (Lbx1CreFlpFRT (ref. 16) or Cdx2–Nse–FlpFRT) and the dual-recombinase-dependent reporter Rosa26LSL-tdTomato mouse line. We observed that the lateral parabrachial nucleus of the pons (PBN cl) is the brain region most densely innervated by both Tac1+ and Gpr83+ spinal PNs (Fig. 1c, d, e). In the thalamus, the medial and posterior thalamic nuclei are innervated by both Tac1+ and Gpr83+ spinal PNs in a partially overlapping manner, whereas the ventral posterolateral nucleus (VPL) is innervated by Robo3+ spinal PNs (Fig. 1c, d, Extended Data Fig. 1h). In the midbrain, both Tac1+ and Gpr83+ spinal PNs innervate the lateral region of the periaqueductal grey (PAG) and the midbrain reticular nucleus (MRN), whereas Tac1+ spinal PNs more densely innervate the superior colliculus, with a compartmentalized array of terminal patches in the intermediate grey layer (SCig; Fig. 1c, d). In the brainstem, both Tac1+ and Gpr83+ PNs innervate the medial accessory olive (MAO) and lateral reticular nucleus (LRN), while Gpr83+ PNs uniquely innervate the dorsal fold of the dorsal accessory olive (DAOd); Extended Data Fig. 2h, i). Analysing anatomical uses using a dual-virus labelling approach (Extended Data Fig. 3) revealed that axons of both Tac1+ and Gpr83+ neurons that project to the posterior thalamus travel through the ventral lateral funiculus of the spinal cord white matter and form collateral branches that terminate in the MAO and LRN of the ventral brainstem. By contrast, axons of Tac1+ neurons projecting to the SCig travel through the dorsal lateral funiculus and form collateral branches terminating in the PAG. Notably, none of these thalamic- and midbrain-projecting Tac1+ or Gpr83+ PNs formed collateral branches terminating within the PBN cl, the most densely innervated brain target of anterolateral pathway PNs. These findings indicate that Tac1+ and Gpr83+ spinal PNs that innervate the posterior thalamus, midbrain and PBN, are distinct populations.
activation of other brain regions or the spinal cord. It is noteworthy that light-evoked increases in locomotion were followed by a robust ‘freeze- ing’ behaviour that lasted for the entire light-off period (Extended Data Fig. 4c, Supplementary Video 4). Moreover, activation of either Tacr1+ or Gpr83+ SPB neurons led to an increase in pupil diameter (Fig. 2m, n), which is likely to reflect increased sympathetic tone. Whereas pupillary dilation evoked by Gpr83+ SPB terminal stimulation was transient (showing fast decay and small area under curve), dilation evoked by Tacr1+ SPB terminal stimulation was sustained (Fig. 2m, o). Tacr1+ SPB terminal stimulation additionally induced squinting and blinking, indicative of severe pain (Fig. 2m, p). These findings suggest that high-intensity stimulation of Tacr1+ and Gpr83+ SPB neurons induces nociceptive behaviours, but these two SPB populations differentially influence the mode of escape locomotor behaviour and the temporal dynamics of autonomic responses.

A mechanosensory limb of the SPB pathway

PNs of the anterolateral pathway are heterogeneous in their physiological response properties, and the majority are polymodal17–20. To determine the response properties of Gpr83+ and Tacr1+ SPB subsets, we conducted whole-cell patch-clamp recordings from these two neuronal populations using an ex vivo skin–spinal cord preparation (Fig. 3a). Despite heterogeneity in tuning properties among individual Gpr83+ and Tacr1+ SPB neurons, Gpr83+ and Tacr1+ SPB subsets at the population level exhibited distinct responses to mechanical and thermal stimuli (Fig. 3b–g, Extended Data Fig. 5a, b): Gpr83+ SPB neurons were highly sensitive to mechanical stimuli (Fig. 3e), whereas Tacr1+ SPB neurons were more responsive to innocuous thermal stimuli, in particular cool temperature (15 °C), and capsaicin (Fig. 3f, Extended Data Fig. 5c, d). Notably, Gpr83+ and Tacr1+ SPB neurons both responded to noxious cold (0 °C) and noxious heat (54 °C; Fig. 3g), suggesting that both SPB subdivisions convey noxious thermal signals. Consistent with these ex vivo recordings, extensive paw-licking, a nociceptive behaviour elicited by noxious heat (55 °C) or cold (5 °C), and reactivity to noxious mechanical stimuli, as well as aversion to rough floor, were diminished when neurotransmission was suppressed in both Gpr83+ and Tacr1+ spinal neurons (including SPB neurons) simultaneously, but not when it was suppressed in either Gpr83+ or Tacr1+ spinal neurons alone (Extended Data Fig. 6). Together, these physiological and behavioural findings suggest that Gpr83+ and Tacr1+ SPB populations differentially transmit innocuous cutaneous signals to the brain, whereas both populations convey noxious tactile and thermal signals.

To define spinal cord circuit mechanisms that account for the differences in stimulus–response properties of Tacr1+ and Gpr83+ SPB neurons, we next examined the inputs of sensory neurons onto these SPB neurons using channelrhodopsin-assisted circuit mapping in acute spinal cord slices (Fig. 3h–i, Extended Data Fig. 7a). Photostimulation (473 nm) of CGRP+ peptidergic nociceptor terminals, labelled using a newly generated BAC transgenic mouse line, Calca–FlpE (Extended Data Fig. 1n–q), evoked large excitatory postsynaptic currents (EPSCs) and action potential firing in most Tacr1+ SPB neurons, but not in Gpr83+ SPB neurons (Fig. 3j, k) except for a small fraction that exhibited small polysynaptic EPSCs evoked with much longer light pulses (Extended Data Fig. 7b). By contrast, activation of either Mrgprb4+ mechanosensory neuron21 terminals or Ntrk2+ (also known as TrkB) Aδ- or low-threshold mechanoreceptor (LTMR)22 terminals evoked large EPSCs and action potential firing in the majority of Gpr83+ SPB neurons, but not in Tacr1+ SPB neurons (Fig. 3l–o), again except for a small fraction that exhibited small polysynaptic EPSCs evoked with much longer light pulses (Extended Data Fig. 7c, d). Activation of Mrgprd+ polymodal non-peptidergic sensory neuron23 terminals evoked large EPSCs and
action potential firing in both Gpr83+ and Tacr1+ SPB neurons (Extended Data Fig. 7e, f). A morphological correlate of the differential sensory neuron inputs is that dendrites of Tacr1+ SPB neurons are restricted to the most superficial spinal cord lamina, whereas most CGRP+ peptidergic nociceptors terminate, whereas dendrites of Gpr83+ SPB neurons often extend into lamina I/II, the site of non-peptidergic sensory neuron synapses—including those of Mrgprb4+ mechanosensory neurons—and even into laminae III and IV, the site of Aδ-LTMR and Aβ-LTMR synapses (Extended Data Fig. 7g–k). Thus, the distinct physiological responses of Gpr83+ and Tacr1+ SPB neurons to tactile and thermal stimuli can be explained by differences in their dendritic morphologies and synaptic inputs from distinct classes of mechanosensory neurons and nociceptors.

**SPB neuronal subsets and hedonic value**

Several lines of evidence indicate that the organizational properties of the Gpr83+ and Tacr1+ subdivisions of SPB neurons are distinct from those of other ascending pathways of anterolateral spinal tracts. First, we found that in contrast to other brain targets, including the PAG and SCg, which are innervated by axons originating exclusively from the contralateral side of the spinal cord, the PBN, receives bilateral input from both Gpr83+ and Tacr1+ SPB neurons (Fig. 4a, b). Simultaneous retrograde tracing of SPB neurons innervating either side of the PBN (Extended Data Fig. 8a–c) and anterograde tracing of sparsely labelled SPB axons (Fig. 4c–f) revealed that individual SPB neurons project either contralaterally, ipsilaterally or bilaterally. Second, consistent with our observations from dual-virus retrograde labelling experiments (Extended Data Fig. 3), single-axon tracing analyses of sparsely labelled Gpr83+ and Tacr1+ SPB neurons support the idea that most SPB neurons are dedicated anterolateral pathway PNs that innervate the PBN, without forming collateral branches that innervate other brain regions (Fig. 4e). By contrast, anterolateral pathway PNs with axons innervating the inferior olivary complex have collateral branches extending to other brain regions (Extended Data Figs. 3f, i and 8d, e). Third, unlike SPB neurons expressing Tacr1+ and Gpr83+ using a slice preparation of spinal cord. The genetic labelling strategies are described in the Methods. Representative images of dTomato+ Tacr1+ and Gpr83+ SPB neurons in acute spinal cord slices. n = 39, 35 neurons for Tacr1+, Gpr83+, respectively. j, l, n. Representative traces of light-activated currents (left) and action potential firing (right) upon photostimulation of CGRP+ (j), Mrgprb4+ (l) and Ntrk2+ (n) primary afferent terminals. Light-activated EPSCs were abolished in the presence of tetrodotoxin (TTX) and reinstated in the presence of 4-aminopyridine (4-AP) in addition to TTX, implying that the synaptic connections were monosynaptic. Turquoise bars, 0.1-ms (EPSC) and 1-ms (action potential) LED (473 nm) stimuli. k, m, o. Quantifications of peak current density. Mann–Whitney test (two-tailed); n = number of neurons. Error bars, s.e.m.

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**Fig. 3** | SPB neurons that express Tacr1 and Gpr83 exhibit different responses to cutaneous stimuli, which is explained by their distinct synaptic inputs from different subtypes of primary sensory neurons.

a. Schematic of whole-cell patch-clamp recordings from Tacr1+ and Gpr83+ SPB neurons using an ex vivo skin–spinal cord preparation. b, c. Representative traces of action potential firing evoked by indentation with von Frey filaments (b) and application of saline at different temperatures (c). Underbars, times when stimuli were applied to the skin. d. Summary radar plots. e–g. Quantifications of peak instantaneous firing rates after application of mechanical (e) and temperature (innocuous (f) and noxious (g)) stimuli. Mann–Whitney test (two-tailed; comparison for individual stimuli): two-way ANOVA (comparison for different groups of stimuli), F2,30 = 9.77 (e), F2,30 = 4.41 (f); n = 16, 15 neurons for Tacr1+, Gpr83+, respectively. h. Schematic of whole-cell patch-clamp recordings from Tacr1+ and Gpr83+ SPB neurons using a slice preparation of spinal cord.

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**Fig. 4** | SPB neurons that express Tacr1 and Gpr83 form dedicated, bilateral, non-somatotopically organized synaptic inputs to the PBN, a, c.

Schematics of unilateral lumbar injections of AAV viruses for whole-mount alkaline phosphatase staining. b. Top view of whole-mount alkaline phosphatase-stained axonal projections of densely labelled Tacr1+ and Gpr83+ spinal PNs. d. Single axon traces of sparsely labelled Tacr1+ and Gpr83+ SPB neurons. e, r, rostral; c, caudal; d, dorsal; v, ventral. f. Quantifications of the numbers of SPB neurons exhibiting dedicated vs. collateral-forming axons (e) and SPB neurons that innervate the PBN, contralaterally, ipsilaterally or bilaterally (f). g. Schematic of virus injections. h, i. Synaptic terminals of Tacr1+ (h) or Gpr83+ (i) SPB neurons representing hindlimb (GFP), thoracic body (BFP) and forelimb (tdTomato) regions are intermingled within their respective PBN, target subnuclei. n = 3 mice each for Tacr1+ and Gpr83+ SPB neurons.
of SPB neurons, particularly Tac1+ SPB neurons, evokes behaviours associated with negative emotional valence.

In addition to negative valence associated with noxious stimuli, observations in human patients with anterolateral cordotomy have implicated the anterolateral pathway in conveying signals associated with positive valence and pleasurable properties of gentle touch. Our finding that Gpr83+ SPB neurons are much more responsive than Tac1+ SPB neurons to light mechanical forces acting on the skin and receive strong synaptic inputs from mechanosensory neurons, including LTMRs, prompted us to ask whether Gpr83+ SPB neurons convey signals that underlie positive valence associated with light touch as well as negative valence associated with noxious stimuli. To address this, we developed an optogenetic stimulation–coupled instrumental conditioning assay in which mice receive selective optogenetic stimulation of either Tac1+ or Gpr83+ SPB axon terminals in the PBN, upon pressing an active lever, but not an inactive lever (Fig. 5d). Remarkably, low-intensity, self-administered photostimulation (0.4 mW) of Gpr83+ SPB neurons, but not Tac1+ SPB neurons, promoted positive reinforcement (increased lever-pressing) over time, whereas moderate-intensity photostimulation (1 mW) of either Tac1+ or Gpr83+ SPB neurons served as a punishment signal (decreased lever-pressing) (Fig. 5e, f, Extended Data Fig. 9e, f). Notably, elevated lever-pressing and positive reinforcement associated with weak optogenetic stimulation of Gpr83+ SPB neurons were observed for several days after photostimulation was uncoupled from pressing of the active lever. Similarly, in a real-time place preference paradigm, only Gpr83+ (Extended Data Fig. 10b, c), Rosa26h–Tac1+/–;Reeler mice exhibited preference for the stimulated side of the chamber following the period of moderate-intensity photostimulation (1 mW), but not strong photostimulation (6.5 mW) (Fig. 5b, c).

To begin to define cellular- and circuit-level correlates of intensity-dependent changes in behaviours associated with different valences (positive or negative), we next examined Fos induction in the PBN, after photostimulation of SPB axon terminals with different optical strengths. High-intensity photostimulation (6.5 mW) of Gpr83+ SPB axon terminals resulted in strong Fos induction in all three PBN subnuclei with which Gpr83+ SPB axon terminals are associated, whereas low-intensity photostimulation (0.4 mW) induced Fos expression only in the PBNent (Fig. 5g, h). Moreover, the number of Fos+ neurons within the PBNent, including a Fos+ CGRP+ double-positive population, correlated with stimulation intensity (Fig. 5h–j), suggesting that neurons within the PBNent may control behaviours associated with different valences in a scalable manner. Together, these findings indicate that the Gpr83+ mechanosensory limb of the SPB tract can be associated with either positive or negative emotional valence depending on stimulus intensity.

Discussion

Studies of the anterolateral pathway have mainly focused on its role in pain and temperature sensation, and subdivisions of the anterolateral pathway that may mediate affective touch have been poorly understood. We propose that the Gpr83+ SPB pathway is a unique subdivision of the anterolateral pathway that conveys tactile information to higher brain centres via the PBN, to underlie affective touch (Extended Data Fig. 10a). Support for this model includes the observations that Gpr83+ SPB neurons are highly sensitive to mechanical stimuli, receive strong synaptic inputs from primary mechanosensory neurons and convey tactile information bilaterally to the PBN, in a manner that is non-topographically organized. In addition, low-intensity stimulation of Gpr83+ SPB neurons is appetitive, whereas high-intensity stimulation of these neurons is aversive. It is noteworthy that Gpr83+ SPB neurons receive synaptic inputs from both LTMRs and HTMRs, suggesting that the Gpr83+ SPB pathway underlies either positive or negative valence associated with cutaneous mechanosensation depending on the properties or intensity of a tactile stimulus.
The current view of ascending pain pathways emphasizes the involvement of Tac1+ PNs in transmitting nociceptive signals from the spinal cord to the brain. However, therapeutic strategies in humans that target TACR1-expressing neurons, and TACR1 itself, to treat pain have been minimally successful, consistent with the existence of additional, Tac1+ SPB neurons seen in rat. Our physiological and behavioural findings suggest that Tac1+ and Grpr83+ SPB neurons form parallel ascending circuit motifs that cooperate to convey nociceptive signals to the brain (Extended Data Fig. 10a). These two SPB modules receive synaptic inputs from distinct but overlapping sets of nociceptors and project to distinct but overlapping PBN, subnuclei, which presumably engage different downstream brain regions associated with processing nociceptive signals. Indeed, strong activation of Tac1+ and Grpr83+ SPB neurons generates spatiotemporally distinct patterns of escape locomotion, autonomic (pupillary) reactions and place aversion, supporting the idea that these two SPB circuit motifs mediate different aspects of pain perception and behavioural responses to noxious stimuli. Intriguingly, TACR1 and GRPR3 are structurally highly related members of the GPCR family (Extended Data Fig. 10b), both coupled to Gi, signalling pathways, suggesting that they may modulate the activities of Tac1+ and Grpr83+ SPB neurons, respectively. Future studies of the TACR1 and GRPR3 GPCRs, and the spinal PNs that express them, may reveal new therapeutic approaches for treating disorders associated with pain and affective touch.

Online content
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Mice
Mice were handled and housed in accordance with Harvard Medical School and IACUC guidelines. Mice were kept in a temperature- and humidity-controlled room with a 12-h light/dark cycle. Mice (2–24 weeks of age) from both genders were used in experiments. Knock-in mouse lines generated in this study include the Rosa26LSL-EYFP, Tacr1CreERT2, Gpr83CreERT2, AvilCre and Tau2STOP (this line will be described elsewhere) mouse lines. These knock-in mouse lines were generated in the Janelia Research Campus Gene Targeting and Transgenic Facility using conventional ES cell targeting strategies. Briefly, a 3X-STOP-ires-CreERT2 cassette was introduced via homologous recombination into the first common coding exon that is shared by different splice variants of the Rosa26 gene for the Rosa26CreERT2 knock-in mouse line. CreERT2 or FlpO cassettes were introduced via homologous recombination into the Tacr1, Gpr83 and Avil (advinillin) genes, replacing the first coding ATGs to generate the Tacr1CreERT2, Gpr83CreERT2 and AvilCre mouse lines, respectively. Detailed sequence elements of the targeting vectors are described in Extended Data Fig. 1a–c. Rosa26LSL-EYFP, Tacr1CreERT2, Gpr83CreERT2 and AvilCre heterozygous mice were generated by mating chimaeric mice to germline FlpE (Actb-FlpE) (JAX#003800) or germline Cre (Elisa-Cre) (JAX#003724) mice to remove the neomycin selection cassette. Other published knock-in mouse lines used in this study include Lbx1Cre (ref. 19), Rosa26LSL-ESF-Fbxw7Cre ( Ai65) (JAX#021875), Rosa26LSL-ESF-FlpO-EIIa-Cre (Ai14) (JAX#007908), Rosa26LSL-ESF-TfCre (Ai3) (JAX#007903), Rosa26LSL-ESF-synaptophysin-FlpO (Ai2) (JAX#007904), Rosa26LSL-ESF-synaptophysin-GFP (Ai34) (JAX#012570), Rosa26LSL-ESF-tdTomato (generated from the cross between Ai65 and Elisa-Cre mouse lines; germline excision of LSL), Rosa26LSL-ESF-Rex1Cre (Ai34) (JAX#024846), Rosa26LSL-ESF-FlpO (ref. 13), Rosa26LSL-ESF-synaptophysin-GFP (Ai65) (JAX#007905), Tacr1CreERT2 (JAX#021877), Gad2CreERT2 (JAX#023140), AvilCre (ref. 14), Ntrk2CreERT2 (ref. 15), Mrgrpbc4Cre (ref. 16) and MrgrpdCre (ref. 17). The Calca–FlpF BAC transgenic mouse line was generated by introducing a FlpF cassette downstream of the first coding ATG of the Calca gene in a bacterial artificial chromosome (RP23-181A2). The Gpr83–GPFBAC transgenic mouse line was imported from the MMRRC (stock no. 010442-UCD). Other published transgenic mouse lines used in this study include Cdx2–Cre (JAX#009352), Calca–GFP (MMRRC, stock no. 011187-UCD) and Calcd–NSE–FlpF18.

Tamoxifen treatment
Tamoxifen (T5648, Sigma) was dissolved in 100% ethanol (20 mg ml⁻¹), mixed with a 2× volume of sunflower seed oil (SS007, Sigma), vortexed for 20 min and vacuum centrifuged for 30 min for ethanol evaporation. Tamoxifen in sunflower seed oil (10 mg ml⁻¹) was delivered via oral gavage to pregnant female mice for embryonic treatment (3–4 mg at E11.5 for Robo3CreERT2) or to mice at weaning ages (P19–P24) for postnatal treatments (1–1.5 mg for Tacr1CreERT2 and 2–2.5 mg Gpr83CreERT2). The number of tamoxifen treatments for Tacr1CreERT2 and Gpr83CreERT2 varied depending on reporter mouse lines and Cre-dependent viruses used for experiments; Rosa26LSL-ESF-FlpO-EIIa-Cre (Ai165), Rosa26LSL-ESF-FlpO-EIIa-Cre (Ai14) and Rosa26LSL-ESF-FlpO-EIIa-Cre (Ai13), one dose; Rosa26LSL-ESF-Rex1Cre, two doses 2 d apart; Rosa26LSL-ESF-FlpO-EIIa-Cre, three doses on three consecutive days; AAV1–FLEX–PLAP, one dose 5–7 d after virus injection; all other Cre-dependent viruses, two or three doses administered 5–14 d after virus injections.

Surgical procedures
Spinal cord injections. Mice (P12–P20) were anesthetized via continuous inhalation of isoflurane (1.5–2.5%) using an isoflurane vaporizer (VetEquip) during the surgery. Laminectomies were performed to expose either cervical, thoracic or lumbar spinal cords, and a total of 300–450 nl of AAV viruses were directly injected into two or three adjacent spots in the spinal cord using pulled glass pipettes (Wiretrol II, Drummond) and a microsyringe pump injector (UMP3, World Precision Instruments). For sparse labelling experiments described in Fig. 4, 150 nl of 1/10-diluted AAV1–hSyn–FlpO–WPRE (1.37E+12 gc ml⁻¹) was injected into one location in the lumbar spinal cord.

Brain injections and fibroptic/dual opto-fluid cannula implants.
Mice (6–10 weeks of age) were placed on a stereotaxic frame (Kopf Instruments) and anaesthetized via continuous inhalation of isoflurane (1.5–2.5%) using an isoflurane vaporizer during the surgery. Burr holes were made on the skull using a dental drill, and 150–250 nl of Alexa Fluor–conjugated CTB (ThermoFisher) or 100–300 nl of AAV viruses were injected into the target brain regions using pulled glass pipettes (Wiretrol II, Drummond) and a microsyringe pump injector (UMP3, World Precision Instruments). For fibroptic or dual opto-fluid cannula implants, fibroptic cannulas (400 μm in diameter, 0.53NA, Doric Lenses) or dual opto-fluid cannulas (DIOFC, Doric Lenses) were bilaterally implanted into the PBN, and secured using a gel-type Super Glue (Loctite) with an accelerator application followed by application of dental cement (Metabond, Parkell). The coordinates used for stereotaxic injections and implantation were as follows: PBNl, −5.2 to −5.0 mm posterior to bregma, ±1.4 to ±1.6 mm from midline and −2.8 to −3.0 ventral to dura; MGl–SFPp, −3.05 to −3.15 mm posterior to bregma, −1.70 to −1.75 mm from midline and −3.0 to −3.2 mm ventral to dura; SCig, −3.35 to −3.50 mm posterior to bregma, −0.75 to −1.25 mm from midline and −1.35 to −1.45 mm ventral to dura.

Viruses
The following AAV viruses were used in this study: AAV1–CAG–FLEX–synaptophysin–GFP–WPRE (1.088E+14 gc ml⁻¹), AAV1–CAG–FLEX–synaptophysin–BFP–WPRE (1.5059E+14 gc ml⁻¹), AAV1–CAG–FLEX–synaptophysin–tdTomato–WPRE (1.1482E+14 gc ml⁻¹), AAV2–Retro–CAG–FLEX–tdTomato–WPRE (2.2248E+13 gc ml⁻¹), AAV2–Retro–EF1α–FlpO–WPRE (2.683E+13 gc ml⁻¹), AAV1–Hsyn–Con/Fon–EYFP–WPRE (1.04E+13 gc ml⁻¹) and AAV1–Hsyn–FlpO–WPRE (1.37E+13 gc ml⁻¹). The pAAV–FLEX–synaptophysin–GFP expression vector was a gift from S. Arber. pAAV–FLEX–synaptophysin–BFP and pAAV–FLEX–synaptophysin–tdTomato expression vectors were generated by swapping the GFP sequence with BFP and tdTomato sequences, respectively. pAAV–CAG–FLEX–tdTomato–WPRE (Addgene#51503) and pAAV–Hsyn–Con/Fon–EYFP–WPRE (Addgene#55650) expression vectors were purchased from Addgene. pAAV–EF1α–FlpO–WPRE and pAAV–Hsyn–FlpO–WPRE expression vectors were generated using standard approaches. All the AAV viruses were produced and packaged at the Boston Children’s Hospital Viral Core Facility except for the AAV1–FLEX–PLAP virus, which was a gift from C. Čepko.

Dual-virus retrograde labelling experiments. The traditional view of the anterolateral pathway is that most spinal PN axons form collateral branches that innervate multiple regions of the brain, including the PBN,3; this is based on double labelling of neurons by retrograde tracers injected into different brain regions. However, one potential caveat of this approach is that some brain targets of the anterolateral pathway, particularly in the hindbrain, including the PBN, the pons and brainstem, are located near main bundles of ascending anterolateral pathway axons, potentially complicating interpretation of retrograde labelling experiments because PNs may be labelled by uptake of tracers into fibres of passage as well as axon terminals. To circumvent this concern, we selectively labelled Tacr1 or Gpr833 spinal PNs that innervated rostral brain targets, including the MGl, SFPp and the SCig of the midbrain, by combining lumbar spinal cord injections of AAV1–CreFon–EYFP viruses3 and brain injections of AAV2–retro–FlpO viruses39 into Tacr1CreERT2 or Gpr83CreERT2 mice (Extended Data Fig. 3).

AAV1–FLEX–synaptophysin virus injections into three spinal axial levels. AAV1–FLEX–synaptophysin viruses3 expressing one of three different fluorescent proteins (GFP, BFP and tdTomato) were injected into three spinal axial levels (lumbar enlargement, mid-thoracic and cervical enlargement) of either Tacr1CreERT2 or Gpr83CreERT2 mice.
visualize synaptic terminals of SPB neurons representing hindlimbs, thoracic body regions and forelimbs, respectively (Fig. 4g–i, Extended Data Fig. 8f).

**RNAscope in situ hybridization**
Adult mice were euthanized with carbon dioxide. Lumbar spinal cords were dissected and immediately embedded in OCT (1437365, Fisher) and frozen with dry ice–cooled methylbutane. The spinal cord tissues were cryosectioned (25 μm) using a cryostat (Leica), and transverse sections were collected on glass slides (12-550-15, Fisher). mRNA transcripts were detected using the RNAcope Fluorescent Multiplex Assay (Advanced Cell Diagnostics) and RNAcope Fluorescent Multiplex Reagent Kit (cat. no. 320850). The RNAcope catalogue probes were used to detect Gpr83 (cat. no. 317431), Tac1 (cat. no. 410331) and tdTomato (cat. no. 317041-C2) mRNA molecules.

**Immunohistochemistry**
Mice (6–8 weeks of age) were anaesthetized with CO₂ and transcardially perfused with 5–10 mL of modified Ames Medium (A1420, Sigma) in 1× PBS, followed by 20–30 mL of 4% paraformaldehyde (PFA) (P6148, Sigma, or 15714-S, EMS) in 1× PBS at room temperature (RT). Brains and vertebral columns, including spinal cords and dorsal root ganglia, were roughly dissected from perfused mice and post-fixed in 4% PFA at 4 °C overnight. Tissues were washed in 1× PBS for over 3 h, and brains and spinal cord tissues were finely dissected out from the rest of the tissue. Brain and spinal cord tissues were cryoprotected in 30% sucrose in 1× PBS at 4 °C for 2 d, embedded in OCT compound (1437365, Fisher), frozen using dry ice and kept at −80 °C. Brain (coronal sections) and spinal cord (transverse or horizontal sections) tissues were cryosectioned (30–40 μm) using a cryostat (Leica). Spinal cord sections were collected on glass slides (12-550-15, Fisher), and brain sections were collected on glass slides or in 1× PBS. Sections (on slides for spinal cord sections, on slides or as floating sections for brain sections) were washed three times for 5 min each with 1× PBS containing 0.1% Triton X-100 (0.1% PBST), incubated with blocking solutions (0.1% PBST containing 5% normal goat serum (S-1000, Vector Labs) or normal donkey serum (005-000-121, Jackson ImmunoResearch)) for 1 h at RT, incubated with primary antibodies diluted in blocking solutions at 4 °C overnight, washed three times for 10 min each with 0.1% PBST, incubated with secondary antibodies diluted in blocking solutions at 4 °C overnight, washed again three times for 10 min each with 0.1% PBST, incubated with secondary antibodies diluted in blocking solutions at 4 °C overnight, washed again three times for 10 min each with 0.1% PBST, incubated with secondary antibodies diluted in blocking solutions at 4 °C overnight, washed again three times for 10 min each with 0.1% PBST, incubated with secondary antibodies diluted in blocking solutions at 4 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times for 5 s each (with 10-s light-off periods between photostimulation periods). Littermate controls were photostimulated with either of the protocols above. For thermal stimulation (Extended Data Fig. 6e, f), mice were placed either on a 55 °C hot plate for 30 s or on a 5 °C cold plate for 10 min. Littermate controls were placed on a plate at room temperature for 10 min. L5 h after the delivery of each stimulation, mice were perfused and processed for Fos immuno histochemical analysis.

**Ex vivo whole-cell patch-clamp recordings using a skin-spinal cord preparation**

A semi-intact skin–spinal cord preparation was used as previously described with a few modifications. Briefly, mice (6–9 weeks of age) were deeply anesthetized with ketamine/xylazine (90 and 10 mg kg⁻¹, respectively) and transcardially perfused through the left ventricle with oxygenated (95% O₂, and 5% CO₂) sucrose-based artificial cerebrospinal fluid (ACSF) (in mM: 234 sucrose, 2.5 KCl, 0.5 CaCl₂, 10 MgSO₄, 1.25 NaH₂PO₄, 26 NaHCO₃, 11 glucose) at RT. Immediately after perfusion, the skin was incised along the dorsal midline and the spinal cord was quickly exposed via dorsal laminectomy. The right hindlimb and the spinal cord (C2–S6) were dissected, transferred onto a Sylgard-coated dissection/recording dish and submerged in the same sucrose-based ACSF, which was circulated at 50 ml min⁻¹ for superfusion of the spinal cord. Next, the skin piece innervated by the saphenous nerve and the femoral cutaneous nerve was dissected free of surrounding tissues. The L2 and L3 DRGs were left attached on the spine. Dural and pial membranes were carefully removed, and the spinal cord was pinned onto the Sylgard chamber with the right dorsal horn facing upward. After the dissection, the chamber was transferred to the electrophysiology rig, and the skin–spinal cord preparation was perfused with normal ACSF solution (in mM: 117 NaCl, 3.6 KCl, 1.25 CaCl₂, 1.2 MgCl₂, 1.2 NaHPO₄, 25 NaHCO₃, 11 glucose) saturated with 95% O₂ and 5% CO₂ at 32 °C. The tissues were rinsed with ACSF for at least 30 min to wash out sucrose. Thereafter, recordings were performed for up to 6 h post-dissection. Neurons were visualized using a fixed stage upright microscope (BX51WI, Olympus) equipped with a 40× water-immersion objective and a CCD camera (ORCA-ER, Hamamatsu Photonics). An narrow-beam infrared LED (L850D-06, Murubeni) was positioned outside the solution meniscus. Either TacICre or GPR83 λIama ISA PBN neurons were identified by CTB (injected into the PBN) and GFP (or tdTomato) double fluorescence. Whole-cell patch-clamp recordings were then performed using a thin-walled single-filamented borosilicate glass pipette pulled with a microelectrode puller (PC-10, Narishige International). The pipette resistance ranged from 3 to 12 MΩ. Electrodes were filled with an intracellular solution (in mM: 135 K-gluconate, 5 KCl, 0.5 CaCl₂, 5 EGTA, 5 HEPES, 5 MgATP, pH 7.2). Signals were acquired using a Multiclamp 700B amplifier (Molecular Devices). The data were low-pass filtered at 2 kHz, digitized at 10 kHz with an A/D converter (Digidata 1322A, Molecular Devices) and stored using a data-acquisition program (Clampex version 10, Molecular Devices). The liquid junction potential was not corrected. Action potential (AP) amplitudes were recorded in current-clamp mode. For ChR2-assisted circuit mapping, primary afferent synaptic terminals were stimulated with wide-field blue LED illumination through the 40× objective (pE-300, CooLED, 0.1 ms, 1 ms and 10 ms pulse width, light intensity = 27 mW). Cell capacitance, current amplitude, latency and jitter were analysed using Clampfit (version 10, Molecular Devices). For pharmacology experiments, cells were recorded in a solution containing 1 μM tetrodotoxin (TTX) (1069, Tocris) followed by the addition of 4-aminopyridine (4-AP; 500 μM (940, Tocris) to the bath.

**Tactile and thermal behavioural experiments**

Both male and female mice (2–6 months old) of mixed genetic backgrounds were used for behavioural analyses (except for the real-time texture aversion assay, in which only male mice were used). All mice (experimental mice and littermate controls) were group housed. Littermates from the same genetic crosses were used as controls for each group to control for variability in mouse strains and genetic

**Whole-cell patch-clamp recordings using an acute spinal cord slice preparation**

ReaChR, a red-shifted variant of channelrhodopsin, was selectively expressed in distinct primary sensory neuron subtypes using intersec- tional genetic strategies that combine a sensory neuron subtype specific Flp (or Cre) recombinase mouse line, a pan-sensory-neuron-specific AiatCre line (Extended Data Figs. 1i–m, 7a), and the dual recombinase dependent ReaChR mouse line Rosa26LSL-FSeReaChR (Fig. 3h). Whole-cell patch-clamp recording was then performed on Gpr83CreERT2 and Tac1Cre SPB neurons labelled with tdTomato by viral delivery of AAV2–ReaChR–FLEx–tdTomato into the PBN of quadruple transgenic mice harbouring either the Tac1Cre or Gpr83CreERT2 allele. Acute transverse spinal cord slices were used for whole-cell patch-clamp recordings of retrogradely labelled Tac1Cre or Gpr83CreERT2 SPB neurons. Specifically, mice (5–7 weeks of age) were anesthetized via continuous inhalation of isoflurane (1.5%–2.5%) while vertebral columns were dissected. Lumbar enlargements were dissected out from vertebral columns in an ice-cold choline solution (in mM: 92 choline chloride, 2.5 KCl, 1.2 NaHPO₄, 30 NaHCO₃, 20 HEPES, 25 glucose, 5 sodium ascorbate, 2 thiourea, 3 sodium pyruvate, 10 MgSO₄, 37Na₂H₂O, 0.5 CaCl₂·2H₂O) and mounted in 0.3% LMP agarose (16520-100, Life Technology). The lumbar spinal cord slices were sliced in a transverse plane (350 μm) (VT1200S, Leica), and the spinal cord slices were allowed to recover at 34 °C for 30 min in oxygenated (95% O₂ and 5% CO₂) HEPES holding solution (in mM: 86 NaCl, 2.5 KCl, 1.2 NaHPO₄, 35 NaHCO₃, 20 HEPES, 25 glucose, 5 sodium ascorbate, 2 thiourea, 3 sodium pyruvate, 1 MgSO₄·3H₂O, 2 CaCl₂·2H₂O). After recovery, spinal cord slices were placed at RT for 30 min before recordings. Spinal cord slices were then superfused with oxygenated (95% O₂ and 5% CO₂) ACSF recording ACSF (in mM: 2.5 CaCl₂, 1.0 NaHPO₄·H₂O, 119 NaCl, 2.5 KCl, 1.3 MgSO₄·7H₂O, 26 NaHCO₃, 25 glucose, 1.3 sodium L-ascorbate) at RT in a recording chamber mounted on a SlicеScope Pro 6000 electrophysiology rig (Scientifica). Cells were visualized by fluorescence to identify tdTomato-positive cells, followed by infrared differential interference contrast microscopy for patching (ORCA-Flash 4.0, Hamamatsu Photonics; SlicеScope Pro 6000, Scientifica). Whole-cell voltage-clamp recordings of retrogradely labelled SPB neurons within superficial lamina were obtained under visual guidance using a 40× objective. The pipette resistance ranged from 3 to 4 MΩ, and the electrodes were filled with an intracellular solution (in mM: 135 K-gluc, 3.5 KCl, 0.1 CaCl₂, 3.5 EGTA, 6 HEPES, 5 MgATP, pH 7.2). Signals were acquired using a Multiclamp 700B amplifier (Molecular Devices). The data were low-pass filtered at 2 kHz, digitized at 10 kHz with an A/D converter (Digidata 1440A, Molecular Devices) and stored using a data-acquisition program (Clampex version 10, Molecular Devices). The liquid junction potential was not corrected. Action potential (AP) discharges were recorded in current-clamp mode. For ChR2-assisted circuit mapping, primary afferent synaptic terminals were stimulated with wide-field blue LED illumination through the 40× objective (pE-300, CooLED, 0.1 ms, 1 ms and 10 ms pulse width light intensity = 27 mW). Cell capacitance, current amplitude, latency and jitter were analysed using Clampfit (version 10, Molecular Devices). For pharmacology experiments, cells were recorded in a solution containing 1 μM tetrodotoxin (TTX) (1069, Tocris) followed by the addition of 4-aminopyridine (4-AP; 500 μM (940, Tocris) to the bath.
backgrounds. Experiments were performed and analysed by investigators who were blinded to genotype.

**Von Frey test.** Mice were placed in clear plastic chambers on an elevated wire mesh, and the plantar surface of the hindpaw was stimulated with a set of calibrated von Frey filaments (North Coast Medical) 10 times each (0.008–4 g). The number of paw withdrawal responses was scored for each von Frey filament.

**Hot and cold plates.** Mice were placed on a 55 °C hot plate or a 5 °C cold plate (IITC) and their behaviours were recorded using a camera (Hero 4, GoPro). Cut-off times were 20 s and 3 min for 55 °C hot plate and 5 °C cold plate, respectively. The number of paw-licking episodes (hindpaw licking for hot plate and forepaw licking for cold plate) were manually scored by analysing video recordings.

**Real-time texture aversion.** Male mice were habituated in test chambers (black acrylic, 12 inches × 11.8 inches × 6 inches, 0.25-inch thickness) for 2 d (10–20 min each day) before the test day. On the test day, mice were first placed in test chambers with a fresh sheet of dusty pink construction paper (338293, Office Depot) on the floor, and their baseline preference behaviours were recorded for 10 min using a digital USB 2.0 CMOS video camera (60516, Stoeiling) mounted directly above the test chambers. Mice were then transferred to the test chambers with sandpapers of two different textures (extra fine grit SP400 (smooth) and coarse grit SP150 (rough), McMaster-Carr) on either side of the floor and their behaviours were recorded for 10 min. The mouse centroid was tracked, and the percentage of time spent on each side of the test chamber was analysed using a custom program written in Bonsai software (2.3.1)41.

**Optogenetic behavioural experiments**

The same criteria were used for mouse handling as described above. Prior to experiments, fibreoptic cannulas implanted into the PBN, were attached through zirconium sleeves (Doric Lenses) to branching fibreoptic patchcords (Doric Lenses) connected to a blue LED (Doric Lenses) and a programmable LED driver (Doric Lenses). Optogenetic stimulation was controlled by the combination of custom programs written in Bonsai software and Doric Neuroscience Studio (4.1.5.2) through an Arduino circuit board (Uno, Arduino) and custom sketches written in Arduino software (1.8.7). Approximately the initial half of the experiments were performed and analysed by investigators who were not blinded to genotype, and the remainder of the experiments were repeated by investigators who were blinded to genotype.

**Locomotion and grooming analyses.** Mice were habituated in test chambers (Clear acrylic, 8.5 inches × 4.5 inches × 5.5 inches, 0.25 inch thickness) for 2 d (20 min each day) before the test day. On the test day, mice were placed in test chambers and photostimulation was delivered as described in the figure legends. Locomotor and grooming behaviours were recorded (30 frames s−1) with two separate digital USB 2.0 CMOS video cameras mounted directly above and in front of the test chambers. The mouse centroid was tracked, and speed and velocity of mouse locomotion were analysed at 2 Hz with video files filmed by the camera mounted above of the test chambers using a custom program written in Bonsai software. Mouse grooming and jumping behaviours were analysed manually using video files filmed by the camera mounted in front of the test chambers by investigators who were blinded to genotype.

**NBQX infusion.** 300 nl of either saline or the selective AMPA receptor antagonist NBQX (10 mM dissolved in saline, 1044, Tocris) was bilaterally infused into the PBN, through fluid injectors (Doric Lenses) inserted into the dual opto-fluid cannulas implanted into the PBN, (infusion rate was 100 nl min−1). After the infusion, fluid injectors were swapped with optical injectors (Doric Lenses) for photostimulation. Locomotor behaviour was monitored 0.5–1.5 h after the infusion.

**Pupilometry.** Mice were implanted with custom-cut headplates, which were then secured by application of dental cement (Metabond, Parkell). After 3–5 d of recovery, mice were head-fixed and acclimated on the custom-built behavioural apparatus for 2 d (20–30 min each day) before the test day. On the test day, mice were head-fixed and acclimated on the behavioural apparatus for 10–20 min, and photostimulation was delivered as described in the figure legends. Pupillary reactions were recorded (30 frames s−1) using a digital USB 2.0 CMOS video cameras mounted close to one eye. An infrared illuminator was used to obtain high-contrast images of the pupil. The pupil diameter was tracked at 10 Hz using a custom program written in Bonsai software, and peak amplitude and area under the curve (AUC) were analysed using GraphPad Prism (Version 8, GraphPad Software). Relative change in pupil diameter (∆D/D) was calculated as follows.

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\frac{\Delta D}{D} = \frac{\text{pupil diameter} - \text{average baseline pupil diameter}}{\text{average baseline pupil diameter}}
\]

The average baseline pupil diameter was the average of pupil diameter during the 1-min baseline period before the beginning of the first optogenetic stimulation. The long, abrupt downward lines in the raw traces of pupil diameter were used as a measure of the number of blinks/squints.

**Real-time place preference.** Mice were habituated in test chambers (black acrylic, 12 inches × 11.8 inches × 6 inches, 0.25 inch thickness) for 2 d (20 min each day) before the test day. On the test day, mice were placed in test chambers and their preference behaviours during pre-stimulation, stimulation and post-stimulation sessions (10 min each session) were recorded using a digital USB 2.0 CMOS video camera mounted directly above the test chambers. The mouse centroid was tracked in real time using a custom program written in Bonsai software, and photostimulation (10 Hz, 10-ms pulse width) was constantly delivered through Doric Neuroscience Studio and Arduino coupled to the Bonsai program while mice stayed on one side—the ‘stimulated’ side—of the chambers (counterbalanced between the two sides).

**Lever-pressing assay.** Two levers (ENV-110M, Med Associates) were placed side by side on one side of the test chambers (black acrylic, 10 inches × 8 inches × 8 inches, 0.25-inch thickness). The original snap action switches inside of the levers were swapped with ones that have a lower operating force threshold (480-3021-ND, Digi-Key). The levers were calibrated using von Frey filaments to operate following 4–6 g force application. Mice were habituated in test chambers for 2 d (1 h each day) before the test day, and the number of lever presses during the 1-h session on the third day was used as baseline. During the subsequent 8 d (‘light on’ sessions, 1-h session each day), mice received an optogenetic stimulation (5 s, 10 Hz) when they pressed the active lever, but not the inactive lever (counterbalanced between left and right sides). The active lever was coupled to optogenetic stimulation through Doric Neuroscience Studio and Arduino under control of a custom program written in Bonsai software. On the following 5 d (‘light off’ sessions, 1-h session each day), optogenetic stimulation was uncoupled from lever-pressing. The number of lever presses was recorded in real time using custom programs written in Arduino and Bonsai software.

**Statistical analysis**

Statistical analyses were performed using GraphPad Prism (Version 8, GraphPad Software). The number of mice used and the statistical analyses, including post hoc multiple-comparisons tests, used for individual experiments are indicated in the figure legends. The following symbols are used in the figure legends for P values: ns, not significant;
\*P < 0.05; **P < 0.01; ***P < 0.001. The exact P values can be found in Supplementary Table 1. Statistical methods to predetermine sample size were not used. Sample sizes were based on previous studies from our lab and others. The mice were randomly allocated into different experimental groups whenever possible. For place preference assays and lever-pressing assays, the sides of chambers and the levers associated with photostimulation were randomized and counterbalanced. For histological experiments, images were collected and analysed by investigators who were blinded to genotype whenever possible. Blinding was not used for electrophysiological recordings as we used all mice with the correct genotype, not comparing to littermate controls, by our study design. Electrophysiological data were analysed automatically with the same software (Clampfit 10.4) run for each experimental group. Tactile and thermal behavioural experiments and data analysis were performed by investigators who were blinded to genotype. For optogenetic behavioural experiments, approximately the initial half of the experiments were performed by investigators who were not blinded to genotype to make sure that new experimental setups and optogenetic activations were working, and the remainder of the experiments were repeated by investigators who were blinded to genotype, and the results were successfully reproduced. The data were analysed either by investigators who were blinded to genotype or automatically using Bonsai software (2.3.1) with the same scripts run for each experimental group.

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

**Data availability**
The data generated in this study are available from the corresponding author upon reasonable request. Source data are provided with this paper.

**Code availability**
The custom codes used in the study are available at GitHub (https://github.com/SebastianChoi/Choi-et-al-Nature2020) or upon request.

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**Author contributions**
S.C. and D.D.G. conceived and designed the project. S.C. screened and characterizing the new anterolateral pathway PN mouse lines and designed, executed and analysed the histology, behaviour and spinal cord slice recording experiments. M.A.B., A.R.M. and N.I. helped execute and analyse histology and behavioural experiments. D.Z. helped with RNAscope analyses. M.M.D. and R.L.W. helped execute and analyse behavioural experiments. The ex vivo spinal cord physiological recordings were done by J.H., Y.O. and S.C. and analysed by J.H., Y.O., S.E.R. and H.R.K. N.H. and S.G. generated the Calca-FlpE BAC transgenic mouse line. L.B. and C.S. characterized the Calca-FlpE and AvilFlox/Flox mouse lines, respectively. M.G. provided the Lbx1Flox/Flox mouse line. S.C. and D.D.G. wrote the manuscript with input from all authors.

**Competing interests**
The authors declare no competing interests.

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Extended Data Fig. 1 | Generation of CreERT2 mouse lines for genetic labelling of anterolateral pathway neurons and Flp mouse lines for labelling of primary sensory neurons.

a–c, Gene targeting strategies used to generate the *Robo3*∫STOP-IRESCreERT2 (a), *Tacr1*CreERT2 (b) and *Gpr83*CreERT2 (c) mouse lines. A 3X-STOP-IRESCreERT2 cassette was introduced via homologous recombination into the first common coding exon that is shared by different splice variants of the *Robo3* gene. A CreERT2 cassette was introduced via homologous recombination into the *Tacr1* gene, replacing the first coding ATG. A CreERT2 cassette was introduced via homologous recombination into the *Gpr83* gene, replacing the first coding ATG. IRES, internal ribosome entry site; s.int, synthetic intron; WPRE, Woodchuck hepatitis virus (WHP) posttranscriptional regulatory element; pA, poly(A); f, FRT site; kz, Kozak sequence.

d–f, A horizontal section of the lumbar spinal cord. 93.7 ± 2.6% of tdTomato+ neurons were *Tacr1*+, while 96.6 ± 2.4% of *Tacr1*+ neurons were tdTomato+.

n = 3 mice.

g, A transverse section of a *Gpr83*-GFP mouse. Green and red dots represent GFP and *Gpr83* mRNA molecules, respectively, detected with gene-specific RNAscope probes. 96.0 ± 1.2% of GFP+ cells were *Gpr83*+, while 84.5 ± 5.0% of *Gpr83*+ cells were GFP+.

n = 2 mice.

h, Distribution of tdTomato-expressing *Robo3*+ neurons in the spinal cord dorsal horn (top) and their thalamic projections terminating in the VPL (bottom). n = 2 mice.

i–k, The *AvilFlpO* mouse line labels the majority of DRG neurons (99.0 ± 0.1% of NeuN+ neurons are tdTomato+) (i), nodose ganglia neurons (80.8 ± 5.1% of NeuN+ neurons are tdTomato+) (j) and sympathetic ganglia neurons (98.6 ± 0.3% of TH+ neurons are tdTomato+) (k). A transverse section of the vertebral column. tdTomato+ advillin-expressing neurons and their axons are visualized in the spinal cord (asterisk), DRGs (arrows), and sympathetic ganglia (arrowheads). m, A coronal section of the brainstem. tdTomato+ axons of advillin-expressing neurons innervate the nucleus of the solitary tract (arrowhead), the dorsal column nuclei (arrow), and the trigeminal nucleus (asterisk).

n–q, Characterization of the *Calca-FlpE* mouse line.

n = 2 mice.

A cross section of the lumbar DRG (n–p) and a transverse section of the lumbar spinal cord (q). n–p, 91.9 ± 1.5% of tdTomato+ neurons were CGRP+, while 92.3 ± 1.5% of CGRP+ neurons were tdTomato+. q, tdTomato-expressing axons of CGRP+ DRG neurons are CGRP immunoreactive in the spinal cord dorsal horn.
Extended Data Fig. 2 | See next page for caption.
Extended Data Fig. 2 | Comparative analysis of the *Gpr83*, *Tac1* and *Tac1* SPB populations. 

**a**, Distribution of EYFP-expressing *Tac1* (top) or *Gpr83* (bottom) spinal neurons and mCherry-expressing *Gad2* neurons in the superficial lamina of the spinal cord dorsal horn. 

**b**, Quantification of % of *Gad2*-negative neurons in EYFP+ neurons. 97.5 ± 1.4% of *Tac1* neurons and 99.5 ± 0.5% of *Gpr83* neurons were *Gad2*-negative. 

**c**, Distribution of tdTomato-expressing *Tac1* neurons and GFP-expressing *Gpr83* neurons in the spinal cord dorsal horn. 

**d**, Quantification of co-expression of tdTomato and GFP. 80.2 ± 1.5% and 87.0 ± 2.5% of tdTomato-expressing *Tac1* neurons are not positive for GFP expression in laminae I and IIo, respectively. Conversely, 78.0 ± 1.8% and 92.0 ± 1.4% of GFP-expressing *Gpr83* neurons are not positive for tdTomato expression in laminae I and IIo, respectively. 

**e**, Distribution of EYFP-expressing *Tac1* neurons, *Gpr83* neurons or both in the superficial lamina of the spinal cord dorsal horn. The SPB neurons were retrogradely labelled with CTB injected into the PBN. Arrowheads, CTB and EYFP double-positive neurons. 

**f**, Quantification for % of *Tac1* SPB neurons, *Gpr83* SPB neurons, and either *Tac1* or *Gpr83* SPB neurons. 

**g**, % of *Tac1*, *Gpr83*, *Tac1* *Gpr83* and *Tac1* *Gpr83* SPB neurons calculated from experiments in **e, f, h, i**. Coronal sections of the ventral brain stem of *Tac1* CreERT2 (h) or *Gpr83* CreERT2 (i) mice whose lumbar spinal cords were injected with AAV1–FLEX–synaptophysin–GFP viruses. MAO, medial accessory olivary nucleus; DAOdf, dorsal accessory olivary nucleus dorsal fold; DAOvf, dorsal accessory olivary nucleus ventral fold; PO, primary olivary nucleus. 

**j**, Distribution of tdTomato-expressing *Tac1* neurons in the superficial lamina of the spinal cord dorsal horn. The SPB neurons were retrogradely labelled with CTB injected into the PBN. Arrowhead, CTB and tdTomato double-positive neuron. 

**k**, Quantification of % of *Tac1* SPB neurons. 

**l**, Schematic of injections of AAV2-retro-FlpO viruses into the PBN. 

**m**, Distribution of tdTomato-expressing *Tac1* (left) or *Gpr83* (right) SPB neurons and *Tac1* neurons in the spinal cord dorsal horn. tdTomato (red) and *Tac1* (green) mRNA molecules were detected with gene-specific RNAscope probes. Filled arrowheads, double-positive neurons; empty arrowheads, tdTomato+ SPB neurons that do not express *Tac1*. 

**n**, Quantification of co-expression of tdTomato and *Tac1* in laminae I and IIo. 

**o**, Schematic of lumbar injections of an AAV1–FLEX–synaptophysin–tdTomato virus. 

**p**, Distribution of tdTomato-positive synaptic terminals of *Tac1* SPB neurons in the PBN. 

**q**, Quantification of distribution of tdTomato-positive synaptic terminals of *Tac1* SPB neurons in the PBN. *n* = number of mice (indicated in the graph). Error bars, s.e.m.
Extended Data Fig. 3 | Tacr1<sup>+</sup> and Gpr83<sup>+</sup> spinal PNs that innervate the posterior thalamus, midbrain or pons are distinct populations. a, d, g, Schematics of lumbar spinal cord injections of AAV1–C on/Fon–EYFP viruses and brain injections of AAV2–retro–FlpO viruses into the SCig of Tacr1<sup>CreERT2</sup> mice (a) (n = 3 mice), the MGm/SPFp of Tacr1<sup>CreERT2</sup> mice (d) (n = 2 mice) or Gpr83<sup>CreERT2</sup> mice (g) (n = 3 mice). b, e, h, Transverse sections of cervical spinal cords of Tacr1<sup>CreERT2</sup> (b, e) or Gpr83<sup>CreERT2</sup> mice (h). White dotted lines, tdTomato-expressing axons travelling through spinal cord white matter. DLF, dorsal lateral funiculus; VLF, ventral lateral funiculus. c, f, i, Coronal sections of target brain regions of Tacr1<sup>+</sup> (c, f) or Gpr83<sup>+</sup> (i) spinal PNs. AQ, cerebral aqueduct.
Extended Data Fig. 4 | Strong axon terminal stimulation of Tacr1+ and Gpr83+ SPB neurons produces distinct locomotor behaviours.

a, Association of synaptic terminals of Tacr1+ and Gpr83+ SPB neurons with Calca-GFP-expressing cell bodies and neurites in the PBN. b, Quantification of the number of synaptophysin–tdTomato puncta associated with GFP+ cell bodies and neurites. The numbers were normalized with the total GFP+ area (to normalize for the variability of total GFP+ area) and the total number of synaptophysin–tdTomato puncta within the entire PBN (to normalize for the variability of virus injections). AU, arbitrary unit. Two-tailed t-test; n = 4 mice each for Tacr1+ and Gpr83+ SPB neurons.

c, Quantification of average speed during light-off periods following light-on periods (473 nm, 6.5 mW, 10 ms pulse width). One-way ANOVA (Dunnett’s multiple-comparisons test); F(3,16) = 10.60 (2 Hz), F(3,16) = 40.12 (5 Hz), F(3,16) = 20.48 (10 Hz). d, Average velocity of mice over time (6.5 mW, 2 Hz, 10 ms pulse width). Positive values indicate forward movement whereas negative values indicate backward movement. Shaded areas, s.e.m. e, f, Quantification of average velocity during light-on periods with 2 Hz (e) and 5 Hz (f) photostimulation. Note that mice receiving Tacr1+ SPB neuron terminal stimulation exhibited net negative velocity during the 2 Hz photostimulation and lack a velocity increase despite the dramatic increase in speed during 5 Hz photostimulation. Two-tailed t-test; n = 6, 5 mice for Tacr1+, Gpr83+, respectively. g, Distribution of Fos+ neurons in the spinal cord dorsal horn following either photostimulation of axon terminals of SPB neurons (Tacr1+ or Gpr83+) or a capsaicin (0.1%) injection into a hindpaw. Photostimulation of axon terminals of SPB neurons did not induce significant Fos expression in the spinal cord, whereas a hindpaw injection of capsaicin induced strong Fos expression in the medial region of the superficial lamina of the spinal cord dorsal horn. d, dorsal; v, ventral; m, medial; l, lateral. n = 4, 3, 5, 2 mice for control, Gpr83+, Tacr1+, capsaicin, respectively. h, Quantification of the number of Fos+ neurons in lamina I and II. The number of Fos+ cells was quantified in the medial 200 μm of the spinal cord dorsal horn. One-way ANOVA (Tukey’s multiple-comparisons test). Error bars, s.e.m.
Extended Data Fig. 5 | Physiological response properties of Tacr1+ and Gpr83+ SPB neurons. a, b, Summary violin plots of peak instantaneous firing rates of Gpr83+ (a) and Tacr1+ (b) SPB neurons in response to von Frey indentations and thermal stimuli. Red lines indicate median, while blue lines indicate quartiles. Friedman test (Dunn's multiple-comparisons test). n = 16, 15 neurons for Tacr1+, Gpr83+, respectively. c, Representative traces of action potential firing evoked by topical capsaicin (0.05%) treatment. Arrows, time when capsaicin was applied to the skin. d, Quantification of peak instantaneous firing rates upon capsaicin application. Mann-Whitney test (two-tailed); P value is indicated; n = 11, 7 neurons for Tacr1+, Gpr83+, respectively; error bars, s.e.m.
Extended Data Fig. 6 | Simultaneous inhibition of the synaptic outputs of both Tacr1+ and Gpr83+ SPB neurons attenuates nocifensive behaviours in response to noxious cutaneous stimuli. 

a, Hindpaw licking was scored while Tacr1CreERT2; Lbx1FlpO; Rosa26 LSL-FSF-TeTx mice, Gpr83 CreERT2; Lbx1FlpO; Rosa26 LSL-FSF-TeTx mice or Tacr1CreERT2; Gpr83 CreERT2; Lbx1FlpO; Rosa26 LSL-FSF-TeTx mice were placed on the 55 °C hot plate (cut-off time, 20 s). These intersectional strategies target the entire Tacr1+ and Gpr83+ spinal populations, of which 34.2% (20.5% PBNL-projecting, 6.6% PAG-projecting and 7.1% MGm/SPFp-projecting PNs are combined) and 30.9% (14.0% PBNL-projecting, 4.6% PAG-projecting and 12.3% MGm/SPFp-projecting PNs are combined) are Tacr1+ and Gpr83+ PNs (laminae I and IIo and the LSN are combined), respectively (a detailed description of the quantification is in the methods). Two-tailed t-test. 

b, Forepaw licking was scored while mice were placed on the 5 °C cold plate (cut-off time, 3 min). Two-tailed t-test. 

c, Paw withdrawal frequency following hindpaw skin indentation using von Frey filaments. Two-way ANOVA; P-value is indicated; $F_{1,43} = 8.65$ for Tacr1/Gpr83-TeTx. 

d, Real-time texture aversion assay (150 grit sand paper vs 400 grit sand paper). % of time spent in rough side of sand paper (150 grit) was measured (normalized to baseline preference). Two-tailed t-test. 

The suppression of neurotransmission in the quadruple transgenic mice was confirmed by reduced Fos induction in the PBNL following exposure of mice to noxious thermal stimuli. 

Distribution of Fos+ neurons in the PBNL following thermal stimulation. 

Quantification of the number of Fos+ neurons in the PBNL. One-way ANOVA (Tukey’s multiple-comparisons test); $F_{1,5} = 8.97$ (5 °C), $F_{2,8} = 27.09$ (55 °C). n = number of mice (indicated in the graphs). Error bars, s.e.m.
Extended Data Fig. 7 | Gpr83+ and Tacr1+ SPB neurons receive strong synaptic inputs from Mrgprd+ polymodal non-peptidergic sensory neurons and weak, sparse, and polysynaptic inputs from distinct primary sensory neurons, and exhibit distinct dendritic morphologies. a, Distribution of CGRP+, Mrgprb4+, Mrgprd+ and Ntrk2+ primary afferent synaptic terminals in the spinal cord dorsal horn. The Rosa26\textsuperscript{cre}\textsuperscript{-}\textsuperscript{FLP-E} reporter mouse line\textsuperscript{33} was used in combination with sensory neuron Cre/FlpE mouse lines and Avil\textsuperscript{flpO}/Avil\textsuperscript{cre} mouse lines. Note that CGRP+, Mrgprb4+, Mrgprd+ and Ntrk2+ primary afferent synaptic terminals mainly innervate laminae I and IIo, IIid, IIid, and IIiv and III, respectively. b–d, Quantifications of peak current density in Tacr1+ (c, d) and Gpr83+ SPB neurons elicited by long light pulse-stimulation (1 ms and 10 ms) of CGRP+ (b), Mrgprb4+ and Ntrk2+ primary afferent terminals. The same neurons, stimulated with different durations of light stimulation, are connected by dotted lines. Note that only a small fraction of Gpr83+ SPB neurons exhibited long-latency (21.68 ± 2.66 ms), high-jitter (2.97 ± 0.85 ms) polysynaptic EPSCs with 10-ms-long photostimulation of CGRP+ afferent terminals and, conversely, only a small fraction of Tacr1+ SPB neurons exhibited long-latency (14.29 ± 3.49 ms), high-jitter (4.31 ± 2.31 ms) polysynaptic EPSCs with 10-ms-long photostimulation of Mrgprb4+ afferent terminals. 2 out of 7 Tacr1+ SPB neurons exhibited long-latency (11.89 ± 4.18 ms) but relatively low-jitter (0.57 ± 0.21 ms) synaptic EPSCs after 10-ms-long photostimulation of Ntrk2+ afferent terminals. e, Representative traces of light-activated currents (left) and AP firing (right) upon photostimulation of Mrgprd+ primary afferent terminals. Turquoise bars, 0.1 ms (EPSCs) and 1 ms (APs) LED (473 nm) stimulations. f, Quantifications of peak current density. Mann–Whitney test (two-tailed); n = number of neurons. g, Schematic of injections of AAV2-retro-FlpO viruses into the PBN. h, Distribution of tdTomato-expressing dendrites of Tacr1+ (top) and Gpr83+ (bottom) SPB neurons. Lamina IId is labelled using IB4 binding. Arrowheads, Gpr83+ dendrites that are extended into deeper laminae of the spinal cord dorsal horn. i, Quantification of distance between the cell bodies and the outer boundary of IB4+ lamina IId (dotted line). #, note that a small number of Gpr83+ SPB neurons have their cell bodies located within lamina IId. n = 65, 60 neurons for Tacr1+, Gpr83+, respectively. j, k, Quantifications of total length of dendrites in a spinal cord section image within (J) or below (K) IB4+ lamina IId (normalized to the total length of the IB4+ lamina IId in the same spinal cord section image). Two-tailed t-test; n = 18, 23 sections (40 μm) for Tacr1+, Gpr83+, respectively. Error bars, s.e.m.
Extended Data Fig. 8 | Anatomical analyses of axonal projections of anterolateral pathway PNs innervating the PBN, and the inferior olivary complex. 

**a**, Schematic of dual-CTB injections into the PBN. 

**b**, Distribution of CTB-labelled neurons in the spinal cord laminae I and IIo and the LSN. 

**c**, Quantification of % of SPB neurons that innervate the PBN, contralaterally, ipsilaterally or bilaterally. n = 3 mice. Error bars, s.e.m. 

**d**, Bottom view of a single axon trace of sparsely labelled Gpr83+ spinal PN that innervate the inferior olivary complex. Arrowhead, an axon branch travelling up to the rostral brain. r, rostral; c, caudal; m, medial; l, lateral. 

**e**, Quantification of the number of inferior olivary complex-projecting spinal PNs that exhibit dedicated vs. collateral-forming axons. 

**f**, Synaptic terminals of Tac1+ (left) or Gpr83+ (right) PNs, representing hindlimb regions (GFP) and forelimb regions (tdTomato), are segregated in the inferior olivary complex. n = 3 mice each for Tac1+ and Gpr83+ PNs.
Extended Data Fig. 9 | Photostimulation of either Tacr1 or Gpr83 SPB neuron axon terminals promotes rostral grooming, and produces distinct behaviours in instrumental conditioning assays. a, Duration of rostral grooming of control (black line), Gpr83CreERT2;Lbx1flpO;Rosa26LSL-FLS-Fsf-reAChR (green line) or Tacr1CreERT2;Lbx1flpO;Rosa26LSL-FLS-Fsf-reAChR (red line) mice over time. Bin size, 30s. Axon terminals in the PBN were stimulated with blue LED (473 nm, 1 mW, 10 Hz, 10 ms pulse width) for 30 s 4 times (with 1 min light-off periods between photostimulation periods). Turquoise bars, 30-s-long light-on periods. 
b, Quantification of average duration of rostral grooming during light-on periods for 0.4 mW, 1 mW, and 6.5 mW photostimulation. One-way ANOVA (Dunnett’s multiple-comparisons test); \( F_{2,18} = 7.60 \) (1 mW), \( F_{2,16} = 7.49 \) (6.5 mW); \( n = 6,6,9 \) mice (0.4 mW), \( 6,7,9 \) mice (1 mW), \( 8,5,6 \) mice (6.5 mW) for control, Gpr83, Tacr1, respectively. c, Schematic of lumbar injections of AAV1–hSyn–FlpO viruses. d, Quantification of total duration of grooming of different body parts during light-on periods. Axon terminals in the PBN, were stimulated with blue LED (473 nm, 10 mW, 5 or 10 Hz, 10 ms pulse width) 4 times for 1 min each (with 1 min light-off periods between photostimulation periods). \( n = 4 \) trials (2 mice; 2 trials per mouse, 5 Hz and 10 Hz stimulation) for Tacr1 SPB neuron terminal stimulation, \( n = 6 \) trials (3 mice; 2 trials per mouse, 5 Hz and 10 Hz stimulation) for Gpr83 SPB neuron terminal stimulation. Paired \( t \)-test (two-tailed). e, Weak self-administered photostimulation (0.4 mW) of Gpr83 SPB neuron terminals led to an increase in the number of presses for the active lever, but not the inactive lever over time. f, Self-administered photostimulation (1 mW) of Tacr1 SPB neurons led to a decrease in the number of presses for the active lever, but not inactive lever over time. Turquoise boxes indicate 8 d of light-on sessions. \( n = 7 \) mice (Gpr83, 0.4 mW; Tacr1, 1 mW). Two-way repeated measures ANOVA; \( F_{1,6} = 8.23 \) (Gpr83, 0.4 mW), \( F_{1,6} = 9.43 \) (Tacr1, 1 mW). Error bars, s.e.m.
Extended Data Fig. 10 | Summary of two parallel ascending SPB pathways and a phylogenetic tree of structurally-related GPCR family proteins.

**a**, Summary cartoon of two parallel ascending SPB pathways for affective touch and pain. **b**, A phylogenetic tree generated using a multiple sequence alignment algorithm, ClustalW2 (EMBL-EBI). The top 14 mouse proteins that have the highest amino acid sequence similarity to mouse GPR83 were used for this analysis.
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Policy information about availability of computer code

**Data collection**

- Electrophysiology data were acquired using Clampex (version 10, Molecular Devices). Histological data were acquired using Zeiss Zen software (2.3 SP1). Behavioral experiments were conducted using Doric Neuroscience Studio (4.1.5.2) and custom codes written in Bonsai (2.3.1) and Arduino (1.8.7) software, which are available at GitHub (https://github.com/SebastianChoi/Choi-et-al-Nature2020) or upon request.

**Data analysis**

- GraphPad Prism (Version 8, GraphPad Software), Image J (2.0.0-rc-69/1.52p), Zeiss Zen software (2.3 SP1), Clampfit (10.4), and custom codes written in Bonsai (2.3.1) and CellProfiler (3.1.9) software. Custom codes are available at GitHub (https://github.com/SebastianChoi/Choi-et-al-Nature2020) or upon request.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

All data are available from the corresponding author upon reasonable request.
Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- Life sciences
- Behavioural & social sciences
- Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

**Sample size**

Statistical methods to predetermine sample size were not used. Sample sizes were based on previous studies from our lab and others (Li et al., Cell, 2011), (Abraira et al., Cell, 2017), (Hachiska et al., eLife, 2016), (Rodriguez et al., Nature Neuroscience, 2017).

**Data exclusions**

For real-time place preference and texture aversion assays, animals that spent less than 20% or more than 80% in one side of chambers during baseline/prestimulation period were excluded from further experiments. For lever-pressing assay, animals that exhibit less than 10 lever-presses during baseline session were excluded from further experiments. Those animals were excluded from further experiments because they exhibited anxiety and did not engage in experimental tasks (e.g. sitting in one corner of the chambers, not exploring chambers).

**Replication**

We performed experiments with multiple animals to confirm reproducibility. All attempts at replication were successful. The number of replications is noted in the figure legends.

**Randomization**

The mice were randomly allocated into different experimental groups whenever possible. For place preference assays and lever pressing assays, the sides of chambers and the levers associated with photostimulation were randomized and counterbalanced.

**Blinding**

For histological experiments, images were collected and analyzed by investigators who were blinded to genotype whenever possible. Blinding was not used for electrophysiological recordings as we used all mice with the correct genotype, not comparing to littermate controls by our study design. Electrophysiological data were analyzed automatically with the same software (Clampfit (10.4)) run for each experimental group. Tactile and thermal behavioral experiments were performed and analyzed by investigators who were blinded to genotype. For optogenetic behavioral experiments, approximately the initial half of the experiments were performed by investigators who were not blinded to genotype to make sure that new experimental setups and optogenetic activations were working, and the remainder of the experiments were repeated by investigators who were blinded to genotype, and the results were successfully reproduced. The data were analyzed either by investigators who were blinded to genotype or automatically using Bonsai software (2.3.1) with the same scripts run for each experimental group.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

| Materials & experimental systems | Methods |
|---------------------------------|---------|
| n/a                             | n/a     |
| ☒ Antibodies                    | ☒ ChIP-seq |
| ☒ Eukaryotic cell lines         | ☒ Flow cytometry |
| ☒ Palaeontology                 |         |
| ☒ Animals and other organisms   | ☒ MRI-based neuroimaging |
| ☒ Human research participants   |         |
| ☒ Clinical data                 |         |

**Antibodies**

**Antibodies used**

IB4 (Alexa 647 conjugated, I32450, ThermoFisher) was diluted at 1:300 and incubated together with secondary antibodies. Primary antibodies used in this study include rabbit anti-DsRed (1:1000, 632496, Clontech), goat anti-mCherry (1:1000, ABO040-200, Acris), chicken anti-GFP (1:1000, GFP-1020, Aves Labs), rabbit anti-GFP (1:1000, A-11122, Life Technologies), mouse anti-NeuN (1:1000, MAB377, Millipore), rabbit anti-tagRFP (for BFP detection, 1:1000, EVN-AB233-C100, Axxora), rabbit anti-TACR1 (1:2000, Sigma), rabbit anti-PKC \(\gamma\) (1:1000, SC-211, Santa Cruz Biotechnology), mouse anti-c-Fos (1:1000, M-1752-100, Biosensis), rabbit anti-phospho-c-Fos (1:500, #5348, Cell Signaling), and rabbit anti-CGRP (1:1000, 24112, Immunostar). Secondary antibodies included Alexa 488 or 546 conjugated goat anti-rabbit antibodies, Alexa 488 or 546 conjugated goat anti-chicken antibodies, Alexa 488 or 647 conjugated goat anti-mouse antibodies (IgG1, IgG2b), Alexa 647 conjugated goat anti-guinea pig antibodies, Alexa 488 conjugated donkey anti-chicken antibodies, Alexa 546 conjugated donkey anti-goat antibodies, and Alexa 488 or 647 conjugated donkey anti-rabbit antibodies. All secondary antibodies were purchased from Life Technologies except for Alexa 488 conjugated donkey anti-chicken antibodies, which was purchased from Jackson ImmunoResearch, and used at 1:500 dilution.
Validation

| Antibody                        | Dilution | Reference (Year) |
|---------------------------------|----------|------------------|
| rabbit anti-DsRed (1:1000)      |          | Abraira et al.   |
| goat anti-mCherry (1:1000)      |          | Abraira et al.   |
| chicken anti-GFP (1:1000)       |          | Abraira et al.   |
| rabbit anti-GFP (1:1000)        |          | Abraira et al.   |
| mouse anti-NeuN (1:1000)        |          | Abraira et al.   |
| rabbit anti-tagRFP (1:1000)     |          | Tang et al.      |
| rabbit anti-TACR1 (1:2000)      |          | Polgar et al.    |
| mouse anti-c-Fos (1:1000)       |          | Bai et al.       |
| rabbit anti-phospho-c-Fos (1:500) |        | Wilson et al.    |
| rabbit anti-CGRP (1:1000)       |          | Bai et al.       |

Animals and other organisms

Policy information about studies involving animals: ARRIVE guidelines recommended for reporting animal research

| Category           | Information                                                                 |
|--------------------|-----------------------------------------------------------------------------|
| Laboratory animals | Mice were handled and housed in accordance with Harvard Medical School and IACUC guidelines. Mice were kept in a temperature- and humidity-controlled room with a 12-hour light/dark cycle. Mice (2-24 weeks of age) from both genders were used in experiments. Robo3CreERT2, Tacr1CreERT2, Gpr83CreERT2, AdvillinFlipO, TauF5FIAP knockin mouse lines were generated and characterized in this study. Other published knockin mouse lines used in this study include Lbx1FipO, Rosa26FSF-LSL-tdTomato (Ai65) (JAX#021875), Rosa26LSL-tfTomato (Ai14) (JAX#007908), Rosa26LSL-EYFP (Ai3) (JAX#007903), Rosa26LSL-synaptophysin-tfTomato (Ai34) (JAX#012570), Rosa26FSF-tfTomato (generated from the cross between Ai65 and Ella-Cre mouse lines; germline excision of LSL), Rosa26LSL-Fsf-creR (JAX#024846), Rosa26LSL-Fsf-TeR, Rosa26FSF-LSL-Synaptophysin-GFP, AdvillinCre, Nrk2CreER, MrgrpbCre, MrgrpdCre, Tac1RES2-Cre (JAX#021877), Gad2NLS-mCherry (JAX#023140). The Calca-FlpE BAC transgenic mouse line was generated and characterized in this study. The Gpr83-EGFP BAC transgenic mouse line was imported from the MMRRC (Stock number: 010442-UCD). Other published transgenic mouse lines used in this study include Cdx2-Cre (JAX#009352), Cdx2-NSE-FipO, and Calca-GFP (MMRRC, Stock number: 011187-UCD). |
| Wild animals       | No wild animals were used.                                                   |
| Field-collected samples | No field-collected samples were used.                                      |
| Ethics oversight   | Experimental protocols were approved by Harvard Medical School IACUC following the NIH Guide for the Care and Use of Laboratory Animals. |

Note that full information on the approval of the study protocol must also be provided in the manuscript.