Genetic and functional evidence for gp130/IL6ST-induced transient receptor potential ankyrin 1 upregulation in uninjured but not injured neurons in a mouse model of neuropathic pain

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Abstract
Peripheral nerve injuries result in pronounced alterations in dorsal root ganglia, which can lead to the development of neuropathic pain. Although the polymodal mechanosensitive transient receptor potential ankyrin 1 (TRPA1) ion channel is emerging as a relevant target for potential analgesic therapies, preclinical studies do not provide unequivocal mechanistic insight into its relevance for neuropathic pain pathogenesis. By using a transgenic mouse model with a conditional depletion of the interleukin-6 (IL-6) signal transducer gp130 in Na\textsubscript{v}1.8 expressing neurons (SNS-gp130\textsuperscript{−/−}), we provide a mechanistic regulatory link between IL-6/gp130 and TRPA1 in the spared nerve injury (SNI) model. Spared nerve injury mice developed profound mechanical hypersensitivity as indicated by decreased withdrawal thresholds in the von Frey behavioral test in vivo, as well as a significant increase in mechanosensitivity of unmyelinated nociceptive primary afferents in ex vivo skin-nerve recordings. In contrast to wild type and control gp130\textsuperscript{+/+} animals, SNS-gp130\textsuperscript{−/−} mice did not develop mechanical hypersensitivity after SNI and exhibited low levels of Trpa1 mRNA in sensory neurons, which were partially restored by adenoviral gp130 re-expression in vitro. Importantly, uninjured but not injured neurons developed increased responsiveness to the TRPA1 agonist cinnamaldehyde, and neurons derived from SNS-gp130\textsuperscript{−/−} mice after SNI were significantly less responsive to cinnamaldehyde. Our study shows for the first time that TRPA1 upregulation is attributed specifically to uninjured neurons in the SNI model, and this depended on the IL-6 signal transducer gp130. We provide a solution to the enigma of TRPA1 regulation after nerve injury and stress its significance as an important target for neuropathic pain disorders.

Keywords: TRPA1, gp130, SNI, Neuropathic pain, DRG, Nerve injury

1. Introduction
Nerve lesions can lead to the development of neuropathic pain, and pronounced alterations of peripheral and central neurons within the pain pathway have been associated with mechanical hypersensitivity developing after nerve injury in patients and several preclinical models of neuropathic pain.\textsuperscript{1,4,57} Major neuroinflammatory processes, such as invasion of monocytes and macrophages into the lesioned nerve but also the dorsal root ganglion (DRG), where cell bodies of nociceptive afferents reside—, and the release of inflammatory mediators by immune and local glia cells contribute to the transition from acute towards chronic neuropathic pain.\textsuperscript{53,78} The glycoprotein gp130, which is encoded by IL6ST, acts as the interleukin-6 (IL-6) signal transducing receptor subunit for the entire IL-6 family of cytokines and can be activated by membrane-bound as well as soluble ligand-bound alpha receptor subunits.\textsuperscript{31,92} gp130 homodimerizes only if activated by IL-6/IL-6R complexes and initiates downstream Jak/Stat signaling, which is essential for innate immunity as well as neuronal functions.\textsuperscript{31,77} As a critical regulator of nociceptor sensitivity to heat and punctate mechanical stimuli, IL-6 controls protein synthesis and nociceptive plasticity through convergent signaling to the eIF4F complex.\textsuperscript{2,58,65,66,73} Interleukin-6 signaling acts as a molecular hub that determines neuronal excitability by suppressing the expression of voltage-gated potassium channels.\textsuperscript{36} Ablation of gp130 strongly affects peripheral nerve regeneration but also mechanonociception.\textsuperscript{54,72} The IL-6/IL-6R/gp130 axis is critically involved in neuropathic pain induced by nerve injury, chronic inflammation, cancer, chemotherapy, diabetes, as well as HIV and herpes infections.\textsuperscript{39,51,80,99} Classical as well as trans-signalling cascades have been extensively studied, and therapeutic interventions targeting the IL-6 pathway have been approved mostly for chronic inflammatory disorders.\textsuperscript{19,65} Several treatment strategies have been used targeting IL-6, IL-6R, or gp130 as well as their soluble forms, eg, sgp130Fc that offers promising benefit for diabetic neuropathy and
inflammatory disease by specifically blocking IL-6 trans-signaling. 

The nonselective cationic channel, transient receptor potential ankyrin subtype 1 (TRPA1 or ANKTM1), has been proposed as a multimodal transducer of noxious cold and mechanical stimuli. Increasing pharmacological and genetic evidence moves TRPA1 into focus as a central modifier of pain perception and a very promising drug target of neuropathic pain and its association with the mechanical allodynia caused by nerve injury or even diabetes. The mechanism of TRPA1 regulation after inflammation and nerve injury is debated, and relevant roles for TRPA1 and its splice variants have been reported for nociceptors as well as nonneuronal cells, such as glia or immune cells in the peripheral nociceptive system. Despite the overwhelming evidence supporting the importance of TRPA1 for neuropathic pain and its association with the mechanical allodynia resulting from nerve damage, the mechanisms and signaling cascades regulating TRPA1 expression, in particular in nociceptive primary afferents, are not sufficiently understood. Recent reports link TRPA1 to the proinflammatory cytokine IL-6 in models of chemotherapy-induced and bone cancer pain, yet the signaling cascades regulating TRPA1 expression, in particular in nociceptive primary afferents, are not sufficiently understood. Recent reports link TRPA1 to the proinflammatory cytokine IL-6 in models of chemotherapy-induced and bone cancer pain, yet the signaling cascades regulating TRPA1 expression, in particular in nociceptive primary afferents, are not sufficiently understood. 

2. Methods

2.1. Transgenic model

SNS-gp130Cre mice were bred and genotyped as previously described. Unless otherwise stated, adult male mice were used for behaviour phenotyping, whereas mice of either sex and older than 8 weeks were used in all in vitro experiments. Mice were housed under standard pathogen free conditions, at 24°C on a 12:12 light:dark cycle and had ad libitum access to food and water. For tissue dissection, animals were deeply anesthetized with carbon dioxide and euthanized by cervical dislocation. Behavioral measurements and analyses were performed in awake, unrestrained, age-matched, male mice with an age of 8 to 16 weeks by examiners who were blind to the genotype of the mice. Animals were treated in accordance with ethical guidelines and animal welfare regulations (Medical University of Innsbruck). All experimental procedures were approved by the Austrian National Animal Experiment Ethics Committee of the Austrian Bundesministerium für Wissenschaft und Forschung (BMWF-66.011/0113-I/3b/2010; BMWF-66.011/0051-II/10b/2008).

2.2. Spared nerve injury model

The surgery procedure was adopted from Decosterd and Woolf [Fig.1A]. 

In brief, under xylazine (0.2 mg/Kg, AniMedica, Senden-Bössensell, Germany) and ketamine (2 mg/Kg, Graeub, Bern, Switzerland) anesthesia, the skin on the lateral surface of the thigh was incised and the sciatic nerve was exposed by separating the biceps femoris through incision of the connective tissue without wounding the muscle. For the SNI procedure, the common peroneal and the tibial nerve were ligated with 4-0 Vicryl (Sh-1 plus; Ethicon, Vienna, Austria) and a portion of 3 mm length was excised around the ligation site. Care was taken to avoid any mechanical damage to the sural nerve. After dissection, muscle and skin were sutured using 4-0 Vicryl. Sham treatment involved exposure of the sciatic nerve without ligature and dissection of the nerves. Mice were left to recover at 37°C until they regained consciousness.

2.3. Retrograde neuronal labeling

Sensory neurons were labeled with either dextran or Dil/DIO. Two μL of Texas Red-labeled 3000 Da dextran tracer (5% in saline, Invitrogen, ThermoFisher Scientific, Waltham, MA) were injected 7 days before SNI in the center of the hind paw plantar to retrograde label DRG from the injured tibial or common peroneal nerve or in the lateral hind paw plantar area immediately after the SNI surgery to retrograde label DRG from the noninjured sural nerve. For Dil/DIO, sensory neurons projecting their afferent fibers to the hind paw were retrogradely traced by intracutaneous injection of 10 μL 1 mg/mL Dil (D282/DIO1373, Molecular Probes, Vienna, Austria) in 4% DMSO (Sigma, Vienna, Austria) in PBS (PAA, Vienna, Austria) into the lateral, plantar side of the hind paw under brief isoflurane anesthesia. Alternatively, Dil or in some cases DIO crystals were deposited near the cut nerve stumps of the saphenous and anterior tibial nerves right after nerve transection 7-28 days before DRG neurons were used in experiments.

2.4. von Frey and Hargreaves sensory testing

Sensitivity to heat and mechanical stimuli were assessed twice before surgery (baseline measurements days –1 and day 0) as well as on the indicated days after SNI or sham operation on both paws in a blinded manner, as previously described. In brief, for mechanical sensitivity, calibrated von Frey filaments (2.8, 4, 5.7, 8, 11.4, 16, 22.6, 32, and 45.3 mN) were applied to the lateral side of the plantar surface of the paw (sural nerve innervation territory) and the withdrawal threshold was calculated according to the up-and-down method. For assessment of heat hypersensitivity, animals were placed into a Perspex box with a transparent glass floor (Hugo Basile). Heat stimuli were applied to the plantar surface of the hind paws of the mice by the Hargreaves apparatus, which uses focused infrared light onto the hind paw. The light automatically cut off when the paw was removed or 20 seconds after it was switched on, and the latency at shut off was recorded as paw withdrawal latency.

2.5. Skin-nerve preparation and single fiber recordings

Sham- or SNI-treated wild type (wt) mice were used to obtain skin-nerve preparations, and standard single-fiber recordings were performed as we described previously. In brief, the sural nerve and innervated skin of the hind paw were dissected; the preparation was placed corium side up in an organ bath chamber and superfused (appr. 12 mL/minute) with an oxygen-saturated modified synthetic interstitial fluid containing (in mM) 108 NaCl, 3.48 KCl, 3.5 MgSO4, 26 NaHCO3, 1.7 NaH2PO4, 2.0 CaCl2, 9.6 sodium gluconate, 5.5 glucose, and 7.6 sucrose at a temperature of 31.5 ± 0.8°C and a pH of 7.4 ± 0.05. The distal end of the sural nerve was pulled into a separate chamber and electrically isolated from the bath solution using paraffin oil. Fine nerve strands dissected from the nerve bundle were placed on a gold wire recording electrode. Action potentials were recorded, amplified (up to 5000-fold), filtered (low pass 1 KHz, high pass 100 Hz), and stored or analyzed on a PC-type computer with the Spike/Spidi software package. The receptive field was first identified, and activation threshold and conduction velocities of nerve fibers were determined. The fibers were characterized as unmyelinated (C) according to their conduction velocity (<1.0 m/s). The mechanical threshold of each unit was determined.
with calibrated von Frey filaments with a uniform tip diameter of 0.8 mm by applying increasing forces from 1 mN to up to 256 mN, starting with a filament of 22.6 mN. A feedback controlled radiant heat source was used for standard heat stimulation linearly increased the intracutaneous temperature at the receptive field from 31 to 50˚C within 20 seconds. For cold stimulation, ice cold synthetic interstitial fluid was applied to a metal ring isolating the receptive field from the bath which decreased the temperature from 31 to 3˚C within 4 seconds and held this temperature for 20 seconds. Fibers were considered sensitive if 5 or more action potentials were evoked during the stimulus. The threshold was defined as the force or temperature that elicited the second spike of the response.

2.6. Dorsal root ganglia neuron culture and microfluorimetric calcium measurements

After SNI, lumbar dorsal root ganglia L3 to L5 with the cell bodies of primary afferents that project into the lesion were harvested, treated enzymatically, and dissociated as we described.
previously. The resulting cell suspension was plated on coverslips coated with poly-L-lysine and laminin and cultivated in serum-free and defined medium (TNB-100 basal medium) (Biochrom) supplemented with nerve growth factor (NGF 25 ng/mL), L-glutamine, penicillin G sodium, and streptomycin sulfate (all from Invitrogen) at 37°C in 5% CO₂. Microfluorimetric Ca²⁺ measurements were performed as previously described. After 2 to 24 hours, the cultures after nondisruptive loading with 3 or 6 μM of the Ca²⁺ sensitive dye Fura-2 AM (Invitrogen) were recorded in extracellular solution containing (in mM): 145 NaCl, 5 KCl, 2 CaCl₂, 1 MgCl₂, 10 D-glucose (all from Sigma), and 10 HEPES (Roth, Karlsruhe, Germany), at pH 7.3 adjusted with NaOH (Merck). Ratioimetric measurements were performed using a Zeiss Axiosvert 200 microscope (Zeiss) with a Fluor 20x/0.75 NA. objective (Zeiss). Fura-2 was excited consecutively at 340 and 380 nm (equal excitation time 55 ms) with a polychrome IV monochromator (TILL Photonics, Gräfelfing, Germany). Fluorescence was filtered by a 510 nm long pass filter with a polychrome IV monochromator (TILL Photonics, Gräfelfing, Germany). Fluorescence was filtered by a 510 nm long pass filter and recorded with a CCD camera (CoolSNAP, Roper Scientific, Munich, Germany) using 8 × 8 binning at 1 second intervals. For data acquisition, MetaFluor 7.1.2.0 (Molecular Devices, Biberach an der Riss, Germany) was used, data traces were filtered by a simple 3-point moving average and off-line analysis was performed with Excel 2007 (Microsoft). All chemicals were diluted in extracellular solution and applied by a gravity driven perfusion system. Only cells with a low and stable baseline Ca²⁺ ratio (<1) were used for analysis. The inclusion criterion for cells responsive to cinnamaldehyde (CA) or capsaicin (Caps) was set to 125% percent increase above baseline ratio 10 seconds before stimulation. Viability of each neuron was tested with a 10 seconds to 125% percent increase above baseline ratio 10 seconds before stimulation. Viability of each neuron was tested with a 10 seconds to 125% percent increase above baseline ratio 10 seconds before stimulation.

2.7. mRNA quantification

RNA was extracted from lumbar L3-5 DRG explants of gp130fl/fl and SNS-gp130−/− mice subjected to the sham or SN1 surgery. PeqGOLD TriFast reagent (Peqlab Biotechnologie, Germany) was used in accordance to manufacturer’s instructions [chloroform (C2432) and absolute ethanol (107017)] were obtained from Merck. The DNA pellet was dissolved in nuclease free water (R0582, ThermoFisher Scientific), and RNA concentration was estimated using NanoDrop 2000 (ThermoFisher Scientific). Reverse transcription of total mRNA was performed as previously described. Genes of interest were quantified by reverse transcription quantitative polymerase chain reaction using TaqMan Gene Expression Assays: 5'-Tctctgtagctgtgaaag-3' (Hprt primers), 5'-CATGGGTTCTGGTCTAACTTGGAC-3' (Sdha primers), 5'-TCCATCGGTCTGCTCCTCCCAT-3' (Piezo1 primers), 5'-GCTTCTAGGTGCAGTCTCCACG-3' (Piezo2 primers), 5'-CTGAGTGCAGTCTCCACG-3' (Pain3 primers), and 5'-GCTTCTAGGTGCAGTCTCCACG-3' (Pain3 primers). Hprt, Sdha, Piezo1, Piezo2, and Tfrc were used as reference genes. Reactions were prepared according to manufacturer’s instructions and loaded on MicroAmp Fast Optical 96-well reaction plates for amplification in duplicates alongside nontemplate controls. The cycling protocol was 10 minutes at 95°C and 40 2-step cycles of 15 seconds at 95°C and 1 minute at 60°C. Threshold was set manually at 0.1, and baselines were automatically calculated. Relative gene expression was calculated using the 2⁻ΔΔCT method and expressed in relation to the respective expression of the geometric mean of the 3 reference genes or the 2⁻ΔΔCT method in which case the expression levels were depicted as fold change normalized to the control condition. No signal was detected in the nontemplate controls.

2.8. Adenovirus vector construction and dorsal root ganglion cultures transduction

Plasmids for the gp130 adenoviral construct were produced according to the Gateway system procedure (Invitrogen).

2.8.1. Polymerase chain reaction products

For a first polymerase chain reaction (PCR), the primers _a and _c were used to add a Kozak sequence at the 5’ end and anchors for attB1 and attB2 sites at the 5’ and 3’ ends of the gp130 gene. In a second PCR, the attB6 sites were completed using the primers 1107_20 and 1106_20.

Primer_a:

CAAAAAAGCAGGCTCCATGTCAGCACCAAGGATTTGGC.

Primer_c:

CAAGAAAGCTGGGTCCTGCGGCATGTAGCCAC.

2.8.2. BP-reaction

The BP Clonase enzyme mix was used to recombine the gp130 gene flanked by attB sites with the donor vector pDONOR207. The donor vector confers gentamycin resistance and contains the ccdB gene, ie, lethal for E. coli flanked by attP sites. In case of successful recombination, the resulting vector was an entry clone in which the gp130 gene is flanked by attL sites. Cells were transformed with the plasmids and grown on selective media containing gentamycin. After plasmid isolation, the resulting pENTR vector (pDONOR207-mgp130) was verified by DNA sequencing.

2.8.3. LR reaction

In this step, the gp130 gene flanked by attL sites (entry clone) was transferred into a destination vector with attR sites using an LR Clonase enzyme mix (Invitrogen). The destination vector contains a dext cassette, which is exchanged by the recombination, the V5 epitope, and an ampicillin resistance gene. The final vector was an expression clone containing the gp130 gene tagged with the V5 epitope: pAd/CMV-mgp130-V5.

2.8.4. Virus production

HEK 293T cells were transfected with the expression clone and grown until confluence. The cells were lysed, and the supernatant was collected for further amplifications. The collected supernatant was purified using Vivapure AdenoPACK 20 kit (Sartorius Stedim Biotech GmbH, Gottingen, Germany; Cat. # VS-AVP020).

2.8.5. Dorsal root ganglion neuron cultures transduction

Dorsal root ganglion neurons were transduced with 0.1 to 10 μL/mL pAd/CMV-mgp130-V5 adenovirus and cultured for 48 hours. The concentration of 1 μL/mL of virus was nontoxic and used for this study. An empty pAd/CMV-V5 was used as control.

2.9. Statistical analysis

Statistical analysis was performed per animal (N) and per nerve fiber or number of cells (n). For statistical analysis, Sigma Stat 3, Origin Pro 8, and GraphPad Prism 9 software were used.
Depending on sample size, distribution, and number of variables, appropriate statistical tests were used and are indicated in the figure legends. Violin plots indicate median (thick dashed line) and first and third quartile (thin dashed lines). Differences were considered statistically significant at $P < 0.05$.

3. Results

3.1. Spared nerve injury induced mechanical but not heat hypersensitivity in vivo and ex vivo

As expected, and in line with previous reports,19,42,93 SNI-treated mice (Fig. 1A) showed unaltered thermal withdrawal behavior throughout the entire observation period. Heat nociception, as indicated by heat-induced paw withdrawal latency, was similar in both paws in the Hargreaves test in vivo (Fig. 1B). Correspondingly, heat responses and threshold temperatures of unmyelinated heat-responsive C fibers in vitro were comparable with controls (Fig. 1C and D). Likewise, conduction velocities were similar in control and SNI-treated primary nociceptors (Fig. 1E). Analysis of the different C-fiber subpopulations did not indicate loss or gain of specific neuron populations, which suggests that the overall composition of uninjured nociceptive primary afferents within the sural nerve was not affected by SNI (Fig. 1F). In contrast and importantly, SNI induced a severe and persistent decrease in mechanical von Frey thresholds in vivo (Fig. 1G), which was accompanied by a significant increase of the number of mechanosensitive C fibers responding to low mechanical stimuli in the sural nerve: More than 80% of nociceptors responded to mechanical forces below 16 mN after SNI (Fig. 1H). Overall, mechanical thresholds of unmyelinated primary afferents were significantly decreased in the ex vivo skin nerve preparation 7 days after injury (Fig. 1).

3.2. gp130 depletion ameliorated mechanical hypersensitivity

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injured neurons of control mice, responded to CA 7 days after SNI. In addition, this approach revealed a striking difference between the 2 genotypes with dramatically and specifically increased numbers of CA-responsive uninjured neurons between the 2 genotypes with dramatically and specifically.

**4. Discussion**

It is generally accepted that the mechanosensitive ion channel TRPA1 contributes to the development of neuropathic pain and hypersensitivity. Its importance for neuropathic changes emerges not only in nociceptive primary afferents but also in Schwann cells and macrophages. Therefore, it is not surprising that TRPA1 has been proposed as one of the most promising targets for analgesic drug development. Despite seminal preclinical studies stressing the importance of TRPA1 for neuropathic pain and the transition towards pain chronification, mechanistic insight into the regulation of the channel and how it contributes to the pathogenesis of neuropathic pain is still largely missing. The murine SNI model offers the unique advantage to separately explore injured and uninjured neurons lying next to each other.
within the DRG, which is not the case in other traumatic neuropathy models, such as spinal nerve ligation or chronic constriction injury. We discovered that injured and uninjured neurons regulate TRPA1 expression in an inverse manner in this model: While injured neurons lost responsiveness to TRPA1 agonists within 7 to 14 days after injury, their uninjured neighbors became more responsive, neurons lost responsiveness to TRPA1 agonists within 7 to 14 days after injury, their uninjured neighbors became more responsive, and this was associated with decreased mechanical activation thresholds in vivo and nociceptor sensitization to mechanical stimuli in vitro. We, for the first time, provide evidence that TRPA1 activity is regulated in an inverse manner in two genotypes (Fisher exact test, gp130\(^{fl/fl}\); n = 157, P < 0.0001) and 28 days after SNI (Fisher exact test, gp130\(^{fl/fl}\); n = 172, P = 0.0001). (C–D) The magnitude of CA evoked Ca\(^{2+}\) transients was unaltered 7 days after SNI (gp130\(^{fl/fl}\) mean \pm SEM: 0.7084 \pm 0.081, Mann–Whitney U test) but developed a decrease in SNS-gp130 \(^{-/-}\) DRG neurons 14 days after SNI, indicating a further decay of TPRA1 at later stages of neuropathy (for gp130\(^{fl/fl}\) 0.8789 \pm 0.062 and for SNS-gp130 \(^{-/-}\) 0.5480 \pm 0.081, Mann–Whitney U test P = 0.0054). (E) The percentage of neurons responding to Caps (Caps\(_{IR}\)) was similar in both genotypes (Fisher exact test, gp130\(^{fl/fl}\); n = 215, P = 0.2320). For 7 days N = 10/group, for 14 days N = 7/group, and for 28 days N = 4/group. CA\(_{C}\): cinnamaldehyde responsive neurons; CA\(_{IC}\): cinnamaldehyde irresponsive neurons; Caps\(_{C}\): capsaicin responsive neurons; Caps\(_{IC}\): capsaicin irresponsive neurons. *P < 0.05, **P < 0.01, ***P < 0.001, DRG, dorsal root ganglion; SNI, spared nerve injury; TRPA1, transient receptor potential ankyrin 1.

Mechanistically, hypersensitivity evoked by mechanical trauma is associated with intraneuronal and perineuronal monocyte and macrophage invasion and increased levels of oxidative stress by-products. AT2R in macrophages that infiltrate the site of injury trigger an intercellular redox communication and activation of the cell damage or pain-sensing ion channel TRPA1.\(^{61}\) Attenuation of monocyte or macrophage infiltration results in reduced pain-like behaviors, which are ablated by perineural administration of a TRPA1 antagonist, suggesting that pain-like behaviors may be entirely mediated by TRPA1.\(^{68}\) However, invading macrophages and monocytes can release a multitude of different bioactive compounds including immune mediators such as IL-6,\(^{34}\) and this is reflected by increasing levels of IL-6 but not gp130 in injured nerves and ganglia.\(^{3,6,2,4,9}\)

Apart from invading macrophages releasing IL-6 in peripheral ganglia,\(^{41,52}\) neurons themselves are capable to synthesize IL-6 and contribute to increased IL-6 levels within neuropathic DRG.\(^{56}\) More recently, DRG satellite cells are emerging as an important IL-6 source after peripheral nerve injury.\(^{25}\) Another gp130 using cytokine, ciliary neurotrophic factor (CNTF), is highly expressed in Schwann cells and supports the neuroinflammatory response through the signal transducer and activator of transcription 3

**Figure 3.** Reduced TRPA1-responsiveness in SNS-gp130\(^{-/-}\) neurons after SNI. (A) Representative recording of Ca\(^{2+}\) transients responding to 200 \(\mu M\) cinnamaldehyde (CA) and 100 nM capsaicin (Caps). (B) 7 days after SNI, a significantly smaller percentage of small size SNS-gp130\(^{-/-}\) neurons responded to CA (CA\(_{C}\)) in comparison with gp130\(^{fl/fl}\) controls (Fisher exact test, gp130\(^{fl/fl}\); n = 355; SNS-gp130 \(^{-/-}\); n = 248, P = 0.2089). This difference between the 2 genotypes became even more pronounced at later stages of SNI with less total CA-responsive SNS-gp130 \(^{-/-}\) neurons at 14 (Fisher exact test, gp130\(^{fl/fl}\); n = 174; SNS-gp130 \(^{-/-}\); n = 157, P < 0.0001) and 28 days after SNI (Fisher exact test, gp130\(^{fl/fl}\); n = 174; SNS-gp130 \(^{-/-}\); n = 172, P = 0.0001). (C–D) The magnitude of CA evoked Ca\(^{2+}\) transients was unaltered 7 days after SNI (gp130\(^{fl/fl}\) mean \pm SEM: 0.7084 \pm 0.081, Mann–Whitney U test) but developed a decrease in SNS-gp130 \(^{-/-}\) DRG neurons 14 days after SNI, indicating a further decay of TRPA1 at later stages of neuropathy (for gp130\(^{fl/fl}\) 0.8789 \pm 0.062 and for SNS-gp130 \(^{-/-}\) 0.5480 \pm 0.081, Mann–Whitney U test P = 0.0054). (E) The percentage of neurons responding to Caps (Caps\(_{IR}\)) was similar in both genotypes (Fisher exact test, gp130\(^{fl/fl}\); n = 215, P = 0.2320). For 7 days N = 10/group, for 14 days N = 7/group, and for 28 days N = 4/group. CA\(_{C}\): cinnamaldehyde responsive neurons; CA\(_{IC}\): cinnamaldehyde irresponsive neurons; Caps\(_{C}\): capsaicin responsive neurons; Caps\(_{IC}\): capsaicin irresponsive neurons. *P < 0.05, **P < 0.01, ***P < 0.001, DRG, dorsal root ganglion; SNI, spared nerve injury; TRPA1, transient receptor potential ankyrin 1.
(STAT3) and induction of IL-6 in sensory neurons. This Schwann cell–derived CNTF to neuronal STAT3 to neuronal IL-6 axis seems to mediate the onset and progression of the neuroinflammatory cascade resulting from nerve injury. Other central regulators, such as CCL2, affecting IL-6 levels further support the importance of IL-6/gp130 in the pathogenesis of neuropathic pain.

Whereas cytokines, such as LIF or CNTF that use gp130 as their signal transducer subunit of their heteromeric receptors, are critically important for neuronal regenerative processes, IL-6 acting by gp130 homomeric receptors may have a broader function. Interleukin-6, like its related cytokines, promotes neuronal regeneration through gp130 signaling, but also sensitizes nociceptors to mechanical stimuli, and IL-6 deficiency causes deficits in nociceptive and sensory properties of peripheral neurons. IL-6−/− and SNS-gp130−/− mice are protected from mechanical allodynia and a first hint towards an IL-6/gp130 mechanism involving the primary nociceptive afferent either by targeting an ion channel serving transduction or synaptic transmission mechanisms both involving TRPA1. This was supported by upregulated TRPA1 expression in cultured neurons after rescue or overexpression of gp130 with an adenoviral vector approach. Despite this observation, but similar to results in previous studies, TRPA1 mRNA upregulation was not detectable in DRG explants from SNI-treated mice. Although mRNA levels do not necessarily reflect protein levels, quantitative estimation of protein expression on a single cell level remains challenging.

For the induction and maintenance of mechanical hypersensitivity experimentally induced by inflammation, tumor, or nerve injury, based on these findings, we explored this link in the murine SNI model of neuropathic pain. Like IL6−/− mice, mice with a selective depletion of gp130 in nociceptors were protected from SNI-induced mechanical hypersensitivity, suggesting that IL-6 acting on gp130 expressed by nociceptors is critically involved in a process involving the primary nociceptive afferent either by targeting an ion channel serving transduction or synaptic transmission mechanisms both involving TRPA1. This was supported by upregulated TRPA1 expression in cultured neurons after rescue or overexpression of gp130 with an adenoviral vector approach. Despite this observation, but similar to results in previous studies, TRPA1 mRNA upregulation was not detectable in DRG explants from SNI-treated mice. Although mRNA levels do not necessarily reflect protein levels, quantitative estimation of protein expression on a single cell level remains challenging.
sensory neurons obtained from SNS-gp130−/− mice and signatures of mechanical hypersensitivity were largely absent in these mice.

Our current study provides a solution to the enigmatic and partially inconsistent reports of TRPA1 expression in neuropathic pain models where TRPA1 expression seems to depend on the impact of the injury on the respective neurons. Varying numbers of primary afferent neurons may be injured, for example, in the chronic constriction injury or the ligation models. These cannot easily be determined if the impact of the injury model affects the entire nerve, and this may even more apply to chemotherapymediated neuropathic pain models.

Overall, the current findings support our idea that IL-6/gp130 signaling, likely by STAT3 as previously published,30 not only sets mechanosensitivity in healthy conditions but is also critically regulating TRPA1 in uninjured neurons, indirectly affected by neuropathic conditions. The differential upregulation of TRPA1 exclusively in uninjured but not in injured neurons provides important novel mechanistic insight into the critical role of TRPA1 in neuropathic pain pathogenesis and stresses the importance of this ion channel as a relevant drug target for neuropathic pain disorders.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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