Interleukin-17 Regulates Neuron-Glial Communications, Synaptic Transmission, and Neuropathic Pain after Chemotherapy

Highlights
- IL-17 mediates paclitaxel-induced neuropathic pain by T-cell-independent mechanism
- IL-17 regulates pain via neuron-glial interactions both in the spinal cord and DRG
- Overexpression of IL-17 in spinal astrocytes induces mechanical allodynia
- Blockade of IL-17R suppresses neuronal hyperexcitability induced by paclitaxel

In Brief
Luo et al. find that glia-produced IL-17 regulates synaptic transmission and excitability of spinal SOM* neurons and DRG small neurons by IL-17R in the DRG and spinal pain circuit. These results suggest that IL-17/IL-17R mediate neuron-glial interactions and drive chemotherapy-induced neuropathic pain.
Interleukin-17 Regulates Neuron-Glial Communications, Synaptic Transmission, and Neuropathic Pain after Chemotherapy

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SUMMARY

The proinflammatory cytokine interleukin-17 (IL-17) is implicated in pain regulation. However, the synaptic mechanisms by which IL-17 regulates pain transmission are unknown. Here, we report that glia-produced IL-17 suppresses inhibitory synaptic transmission in the spinal cord pain circuit and drives chemotherapy-induced neuropathic pain. We find that IL-17 not only enhances excitatory postsynaptic currents (EPSCs) but also suppresses inhibitory postsynaptic synaptic currents (IPSCs) and GABA-currents (EPSCs). Selective knockdown of IL-17R in spinal somatostatin-expressing interneurons reduces paclitaxel-induced hypersensitivity. Overexpression of IL-17 in spinal astrocytes is sufficient to induce mechanical allodynia in naive animals. In dorsal root ganglia, IL-17R expression in nociceptive sensory neurons is sufficient and required for inducing neuronal hyperexcitability after paclitaxel. Together, our data show that IL-17/IL-17R mediate neuron-glial interactions and neuronal hyperexcitability in chemotherapy-induced peripheral neuropathy.

INTRODUCTION

Proinflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), interleukin-1β (IL-1β), and IL-18 play important roles in the pathogenesis of chronic pain (Yang et al., 2015; Sweitzer et al., 1999; Guo et al., 2007; Sommer, 1999; Zelenka et al., 2005; Milligan et al., 2001; Miyoshi et al., 2008). Increasing evidence suggests that glial cells, such as microglia and astrocytes, are activated in pathological pain conditions to produce these proinflammatory cytokines. Notably, these cytokines act as neuromodulators and regulate pain via neuron-glial interactions (Ji et al., 2013; Grace et al., 2014). Compared to the inflammatory cytokines TNF, IL-1β, and IL-6, much less is known about the role of IL-17 in pain regulation. IL-17, referred to as IL-17A in literature, is a proinflammatory cytokine produced by Th17 cells (Miossec and Kolls, 2012; Korn et al., 2009). The IL-17 family consists of six ligands (IL-17A–IL-17F) and five receptors (IL-17RA–IL-17RE) in mammals (Gaffen, 2009). IL-17 was shown to regulate rheumatoid arthritis and immune response by increasing the production of IL-6 and IL-8 (Hwang et al., 2004). Binding of IL-17 to its receptor (IL-17RA or IL-17R) induces the activation of nuclear factor-κB (NF-κB) via ACT1 and TNF-receptor-associated factor 6 (TRAF6) in rheumatoid arthritis (Hot and Miossec, 2011). However, little is known about non-transcriptional regulation of IL-17.

Recently, IL-17 was found to regulate inflammatory responses associated with neuropathic pain induced by nerve injury. IL-17 levels are upregulated in injured nerves in neuropathic pain models (Noma et al., 2011; Kleinschitz et al., 2006). IL-17R-deficient mice showed decreased mechanical hyperalgesia compared to normal mice in response to zymosan injection (Segond von Banchet et al., 2013; Richter et al., 2012). IL-17A-deficient mice showed decreased mechanical hyperalgesia compared to normal mice in response to zymosan injection (Segond von Banchet et al., 2013) or partial ligation of the sciatic nerve (Kim and Moalem-Taylor, 2011). Furthermore, intraplantar (Kim and Moalem-Taylor, 2011; McNamee et al., 2011) or intra-articular (Pinto et al., 2010) injection of recombinant IL-17 is sufficient to induce hyperalgesia. Notably, IL-17 can also be produced by spinal cord astrocytes and astrocytic IL-17 may play a role in inflammatory pain (Meng et al., 2013). A recent study found that...
Figure 1. Potentiation of Excitatory Synaptic Transmission and Suppression of Inhibitory Synaptic Transmission by IL-17 in Spinal Lamina Ilo SOM⁺ Neurons in Spinal Cord Slices

(A) Mouse spinal cord slice image showing a recording electrode in a SOM⁺ neuron (red arrow).

(B) Patch-clamp recording sEPSCs traces in lamina Ilo SOM⁺ neurons after perfusion of vehicle or IL-17 (10 ng/mL) in spinal cord slices. Below are enlargements of the recordings in vehicle and IL-17 treatment, respectively.

(C and D) Quantification of frequency and amplitude of sEPSCs showing an increase in frequency (C) but not amplitude (D) of sEPSCs after the perfusion of IL-17 (n = 19; cells). ****p < 0.0001; paired t test. ns, not significant.

(E and F) Corresponding cumulative distributions of inter-event interval (E) and amplitude (F) from one neuron.

(G) Traces of NMDA-eEPSC before (black) and after (red) IL-17 treatment.

(H) Potentiation of the amplitude of NMDA-eEPSC by IL-17 (n = 6; cells). **p < 0.01; ***p < 0.001; paired t test. All the data were mean ± SEM.

(I) Patch-clamp recording sIPSC traces in lamina Ilo SOM⁺ neurons after perfusion of vehicle or IL-17 (10 ng/mL) in spinal cord slices. Below are enlargements of the recordings in vehicle and IL-17 treatment, respectively.

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physiological levels of IL-17 can act directly on interneurons to increase their responsiveness to presynaptic input (Chen et al., 2017a). Despite these previous studies, it remains elusive how IL-17 modulates spinal synaptic transmission in the pain circuit.

Chemotherapy-induced peripheral neuropathy (CIPN) is a common dose-limiting adverse side effect and results in high incidence of neuropathic pain (Sisignano et al., 2014). There is evidence that spinal astrocytes, but not microglia, play an important role in the pathogenesis of paclitaxel-induced neuropathy (Zhang et al., 2012; Luo et al., 2018). CIPN enhances excitability of primary sensory neurons associated with altered gene expression of neuronal kines, such as IL-17, regulate synaptic plasticity in CIPN. It remains elusive as to how cytokines, such as IL-17, regulate synaptic plasticity in CIPN.

Somatostatin-positive (SOM+) neurons are a subset of interneurons in the dorsal horn. These neurons are predominantly excitatory and express the vesicular glutamate transporter VGLUT2, a marker for glutamatergic excitatory neurons (Duan et al., 2018; Xie et al., 2018). Recently, Duan et al. (2014) demonstrated that SOM+ neurons are required to sense mechanical pain. These neurons form a pain circuit by receiving input from capsaicin-sensitive C-fibers and sending output to lamina I projection neurons (Todd, 2010; Braz et al., 2014). SOM+ neurons also receive input from inhibitory neurons (Duan et al., 2014). Furthermore, these neurons exhibit remarkable plastic changes after inflammation and nerve injury as well as respond to inflammatory mediators (Park et al., 2011; Xie et al., 2018; Xu et al., 2010). Here, we investigated how IL-17 and IL-17R modulate excitatory and inhibitory synaptic transmission of SOM+ excitatory neurons in normal and pathological pain conditions, and we further tested the involvement of IL-17/IL-17R signaling in the paclitaxel-induced neuropathic pain model. Our findings demonstrate that IL-17 signaling contributes to paclitaxel-induced mechanical allodynia and the dysregulation of excitatory and inhibitory synaptic transmission in spinal SOM+ neurons. Moreover, we reveal an insight into neuron-glial interactions in the spinal cord and DRG by which IL-17 produced by astroglia or satellite glia enhance neuronal activities and excitability to promote neuropathic pain.

RESULTS

IL-17 Enhances Excitatory Synaptic Transmission and Potentiates NMDA-Mediated eEPSCs in Spinal Cord Slices

SOM+ neurons in the spinal dorsal horn are excitatory interneurons (Duan et al., 2014, 2018). These neurons exhibit marked synaptic plasticity in pathological pain conditions (Xie et al., 2018; Xu et al., 2013). We first recorded spontaneous excitatory postsynaptic currents (sEPSCs) in outer lamina II (III) SOM+ neurons in spinal cord slices from SOM-tdTomato mice (Figure 1A). Acute perfusion of IL-17 at a low concentration (10 ng/mL; = 6.7 × 10^−1 nM; 2–5 min) induced a rapid and significant increase in the frequency but did not amplitude of sEPSCs in 19 out of 22 neurons (Figures 1B–1F), suggesting a possible presynaptic mechanism of IL-17 to enhance glutamate release (Figure S6). Notably, IL-17 produced a 66% increase in sEPSC frequency (paired t test; f_{0.01,18} = 9.34; p < 0.0001). Because excitatory synaptic transmission is mainly mediated by AMPA and NMDA receptors (AMPA R and NMDA R) and NMDA R is critical for spinal cord synaptic plasticity and the pathogenesis of pain (Woolf and Salter, 2000; Liu et al., 2008), we further examined the effects of IL-17 on NMDAR-EPSCs evoked by dorsal root entry zone (Lissauer’s tract) stimulation. The amplitude of NMDAR-EPSC was also significantly increased by IL-17 (Figures 1G and 1H; 37%; paired t test; f_{0.01,5} = 4.296; p = 0.0077), suggesting the positive regulation of excitatory synaptic transmission by IL-17.

IL-17 Decreases the Inhibitory Control of SOM+ Neurons and Suppresses GABA-Induced Currents

SOM+ excitatory neurons receive inhibitory input from inhibitory neurons (Duan et al., 2014). We next recorded spontaneous inhibitory postsynaptic synaptic currents (sIPSCs) in lamina II SOM+ neurons by using a pipette solution containing Cs+. After exposure of spinal cord slices to IL-17 (10 ng/mL) for 2–5 min, most of the recorded neurons (7 out of 10) responded to IL-17. IL-17 produced significant decreases in sIPSC frequency (Figures 1I–1K; paired t test; f_{0.01,6} = 4.541; p = 0.0039) and amplitude (Figures 1L, 1M, and 1N; paired t test; f_{0.01,7} = 4.833; p = 0.0019). Because inhibitory synaptic transmission in the spinal cord is mediated by GABA and glycine, two major inhibitory neurotransmitters (Todd, 2010), we further assessed whether IL-17 would also alter GABA- and glycine-evoked currents in lamina II SOM+ neurons. Bath application of GABA (100 µM) and glycine (1 mM) induced marked inward currents. Neither GABA nor glycine current was affected by vehicle perfusion (Figures 1N–1Q). Interestingly, acute application of IL-17 (10 ng/mL) only inhibited GABA-induced currents (Figures 1N and 1O; Student’s t test; f_{0.01,10} = 2.456; p = 0.039) but had no effect on glycine-induced currents in spinal SOM+ neurons (Figures 1P and 1Q; Student’s t test; f_{0.01,8} = 0.1032; p = 0.9204), suggesting specific regulation by IL-17 on GABAB-mediated inhibitory synaptic transmission (Figure S6).

Uregulation of Endogenous IL-17 and IL-17R Regulates Synaptic Plasticity and Excitability of SOM+ Neurons in Paclitaxel-Treated Mice

CIPN results in high incidence of neuropathic pain (Sisignano et al., 2014). A single injection of paclitaxel (6 mg/kg, intraperitoneally [i.p.]) can evoke a remarkable mechanical allodynia on day
7. To address whether paclitaxel alters IL-17 levels in artificial cerebrospinal fluid (CSF) and spinal cord, CSF and spinal cord dorsal horn samples were collected from mice with confirmed mechanical allodynia on day 7 after paclitaxel treatment. ELISA analysis revealed significant increases in IL-17 levels in the CSF and spinal cord dorsal horn samples of paclitaxel-treated mice as compared to vehicle-treated mice (Figures 2A and 2B; Student’s t test; CSF: \( t_{0.05,10} = 2.706, p = 0.022 \); spinal dorsal horn: \( t_{0.01,9} = 3.305, p = 0.009 \)). To examine whether upregulated IL-17 by chemotherapy contributes to spinal cord synaptic plasticity (i.e., central sensitization), a driving force of pathological pain (Woolf and Salter, 2000; Ji et al., 2018), we measured the
frequency and amplitude of sEPSCs or sIPSCs of spinal SOM neurons in paclitaxel-treated mice. Blocking IL-17R with a neutralizing antibody (0.5 μg/mL) resulted in opposite changes in excitatory and inhibitory synaptic transmission in lamina II SOM+ neurons of paclitaxel-treated animals, namely a decrease in the frequency of sEPSCs (Figures 2C–2E; Student’s t test; \( t_{0.05,12} = 2.516; p = 0.027 \)) but increases in sIPSC frequency (Figures 2F and 2G; Student’s t test; \( t_{0.05,14} = 2.552; p = 0.0213 \)) and sIPSC amplitude (Figures 2F and 2H; Student’s t test; \( t_{0.05,14} = 2.226; p = 0.043 \)), suggesting the involvement of endogenous IL-17 in synaptic plasticity after CIPN. Immunohistochemistry in SOM-reporter mice revealed IL-17R immunoreactivity (IR) and IL-17R mRNA expressed on SOM-positive neurons in the spinal dorsal horn (Figures 2I, 2J, and S1A). Additionally, about half of IL-17R expressed on PAX2 (an inhibitory neuronal marker)-positive neurons (Figures 2J and 2K). These data provided a cellular basis for IL-17 directly or indirectly by inhibitory interneurons to regulate the activities of SOM+ neurons (Figure S6).

To further determine the effects of IL-17R in SOM-positive neurons of spinal dorsal horn, Cre-recombinase-dependent adeno-associated virus (AAV) expressing IL-17R-short hairpin RNA (shRNA) (AAV2/9-DIO-EGFP-IL-17R-shRNA) was intra-dorsal horn injected in SOM-Cre mice to selectively knock down IL-17R in SOM+ neurons (Figure S1B). Consistent with IL-17R antibody, selective knockdown of IL-17R in spinal SOM+ neurons significantly suppressed the frequency of sEPSCs, but not sEPSCs amplitude of SOM+ neurons, in paclitaxel-treated mice (Figures 3A–3E; Student’s t test; \( t_{0.05,27} = 4.309; p = 0.0002 \)).

We subsequently determined whether paclitaxel-induced CIPN would alter the excitability of spinal SOM+ neurons. Action potentials (APs) were evoked by superimposed positive current steps. A leftward shift in the input-output curve appeared in paclitaxel-treated mice, and significantly, differences were observed after current injections ranging from 15 to 65 pA (Figures 3F and 3G; two-way ANOVA; treatment: \( F_{1,168} = 94.2; p < 0.0001 \)).
p < 0.0001). Additionally, a more positive resting membrane potential and a lower rheobase were observed in SOM+ neurons taken from paclitaxel-treated mice as compared with that from controls (Figures 3H and 3I). For selective knockdown of IL-17R in SOM-expressing neurons, we expressed IL-17R-targeting shRNA in the spinal cord via intra-spinal cord microinjection. Treatment of AAV2/9-DIO-EGFP-IL-17R-shRNA in SOM-Cre mice prevented paclitaxel-induced hyperexcitability in SOM+ neurons (Figures 3F and 3H–3J). Collectively, these results suggest that endogenous IL-17 is involved in modulating excitatory and inhibitory synaptic transmission and regulating excitability in spinal SOM+ neurons via IL-17R after paclitaxel treatment, suggesting a role of the IL-17/IL-17R pathway in CIPN.

**Cellular Localization of IL-17 and IL-17R in the Spinal Dorsal Horn**

Spinal astrocytes play an important role in the pathogenesis of paclitaxel-induced neuropathy (Zhang et al., 2012; Luo et al., 2018). Paclitaxel-induced IL-17 upregulation in spinal CSF and spinal cord dorsal horn tissues suggests a possible spinal source of IL-17. To determine an astrocytic location of IL-17, we conducted double immunofluorescence of IL-17 with two astrocyte markers, GFAP and ALDH1L1 in spinal cord sections. The result showed that IL-17-IR was primarily colocalized with the GFAP and ALDH1L1, but not with the neuronal marker NeuN and the microglia marker IBA1 in spinal dorsal horn (Figures 4A–4D). Notably, IL-17-IR was found in fine and end processes of astrocytes (Figure 4B), indicating a possibility of IL-17 transport from cell body to end processes for secretion. The selectivity of IL-17-IR was validated by colocalization with td-Tomato signal in astrocytic processes in IL-17-reported mice (Figure S2). To further examine whether these IL-17-labeled dot-like structures are nerve terminals of nociceptors, we performed double staining of IL-17 with CGRP, a marker for peptidergic nociceptors, and IB4, a marker for non-peptidergic nociceptors. Notably, no colocalized immunoreactivities of IL-17 with IB4+ and CGRP+ primary afferent terminals were detected (Figure S3). Thus, IL-17 may not be present on nociceptor terminals in the spinal cord dorsal horn. Fluorescence *in situ* hybridization (FISH) further demonstrated the colocalization of IL-17 mRNA and GFAP (Figures 4E and 4F). Interestingly, IL-17 mRNA puncta were found in processes of astrocytes, suggesting a local translation (Figures 4E and 4F). Differently, IL-17R was primarily expressed on spinal cord neurons (Figure 4G), including SOM+ and PAX2+ neurons (Figures 3I–3K). Some IL-17R immunoreactivities colocalized with GFAP-positive astrocytes but did not with IBA1-positive microglia (Figures 4H and 4I). This localization of IL-17 and IL-17R in spinal astrocytes and neurons, respectively, offers a cellular basis for neuron-astroglial interaction during pain regulation.

**IL-17 Increases the Excitability of Small-Sized Mouse DRG Neurons**

Next, we examined whether IL-17 and IL-17R also display distinct cellular locations in DRG. Immunostaining revealed that IL-17R expressed in small-sized mouse DRG IB4+ and CGRP+ with a predominant in IB4+ neurons but did not in large-sized NF-200+ neurons (Figure 5A). This is consistent with a previous report that IL-17RA is localized to rat DRG neurons (Richter et al., 2012). In contrast, IL-17 mainly expressed in DRG satellite glial cells expressing glutamine synthetase (GS), in IL-17 reporter mice expressing Td-Tomato, or GFP (Figures S4A and S4B). Thus, IL-17 and IL-17R in peripheral nervous systems may also mediate glial-neuron interactions (Figure S7).

To determine a role of IL-17 in regulating the excitability of DRG neurons, we tested the effects of IL-17 on neuronal activities in dissociated small-sized mouse DRG neurons (<25 μm in diameter) using whole-cell patch-clamp recordings. Acute application of IL-17 (10 ng/mL) to mouse DRG neurons *in vitro* induced spontaneous discharge and bursts of APs in some DRG neurons (Figure 5B). Also, IL-17 significantly depolarized the resting membrane potential (Figure 5C; paired t test; t0.01,10 = 4.219; p = 0.0018) and significantly decreased rheobase (Figure 5D; paired t test; t0.01,10 = 4.033; p = 0.0024). Additionally, IL-17 bath application increased the number of AP discharges in response to suprathreshold current injection (Figures 5E and 5F; two-way repeated measures [RMs] ANOVA; treatment: F1,120 = 138.9; p < 0.0001). Therefore, IL-17 increases the excitability of nociceptive neurons by altering rheobase and resting membrane potential in nociceptive neurons, leading to enhanced discharges of APs.

**IL-17R Is Required for Generating Hyperexcitability of Mouse DRG Neurons after Paclitaxel Chemotherapy**

Paclitaxel has been shown to increase the responsiveness and excitability of mouse and human DRG neurons (Chang et al., 2018; Li et al., 2015). We compared the number of APs evoked by a 600-ms current injection through an intracellular electrode in mouse DRG neurons by testing the effects of paclitaxel after bath application (1 μM; 2 h). Only neurons that showed more than one AP in response to stimulation were included in the analysis. Paclitaxel increased the AP firing number in small-sized neurons compared with the vehicle-treated neurons (Figures 6A and 6B; two-way RM ANOVA; treatment: F1,144 = 80.88; p < 0.0001). Strikingly, this excitability increase was suppressed by IL-17RA antibody treatment when compared to control immunoglobulin G (IgG) treatment (Figures 6C and 6D; two-way RM ANOVA; treatment: F1,144 = 114; p < 0.0001). We also measured the number of AP discharges in response to suprathreshold current injection in paclitaxel-induced CIPN mice. The number of AP firings in small-sized DRG neurons from paclitaxel-treated mice was greater than that from control mice (Figures 6E and 6F; two-way RM ANOVA; treatment: F1,130 = 72.5; p < 0.0001). To further confirm the effect of IL-17R in DRG neurons on paclitaxel-induced hyperexcitability, AAV2/9-SYN-EGFP-IL-17Ra-shRNA was intra-DRG injected to selectively knock down IL-17RA on DRG neurons in paclitaxel-treated CIPN mice (Figure 6G). As shown in Figures 6H and 6I, knockdown of IL-17RA in DRG neurons markedly attenuated the frequency of AP firings evoked by a 500-ms current injection (two-way RM ANOVA; treatment: F1,115 = 92.14; p < 0.0001). This result implies direct regulation of neuronal hyperexcitability by IL-17R after chemotherapy (Figure S7).

**IL-17 and IL-17R Contribute to Mechanical Hypersensitivity after Chemotherapy**

To test a central role of IL-17 in pain modulation, we compared mechanical pain thresholds of mice following intrathecal injection of IL-17 versus vehicle. Spinal injection of a low dose of...
IL-17 (50 ng, intrathecally [i.t.]) resulted in a transient reduction of paw withdrawal threshold (PWT) at 1 h after injection (Figure 7A). A higher dose of IL-17 (100 ng, i.t.) caused a more persistent reduction in PWT measured at 5 h following injection, with return of mechanical allodynia after 24 h (Figure 7A; two-way RM ANOVA; treatment: $F_{2,165} = 16.59; p < 0.0001$). To address whether astrocyte-derived IL-17 mediates the mechanical allodynia, AAV2/9 expressing IL-17 driven by GFAP was intra-dorsal horn injected to overexpress IL-17 (Figures 7B–7D). These data suggest that IL-17 from astrocytes is sufficient to induce pain hypersensitivity in naive animals. The contribution of endogenous IL-17 to chemotherapy-evoked neuropathic pain was also examined. A single injection of paclitaxel (6 mg/kg, i.p.) evoked a remarkable reduction in PWT measurement on day 7, which was reversed by IL-17R antibody treatment (1 and 10 μg, i.t.) in a dose-dependent manner (Figure 7E; two-way RM ANOVA; treatment: $F_{2,115} = 3.598; p = 0.03$). Intrathecal injection of control IgG (10 μg) produced no changes in PWT. To determine spinal SOM+ neuron expressing IL-17R-mediated effect in paclitaxel-induced CIPN, AAV2/9-DIO-EGFP-IL-17R-shRNA was intra-dorsal horn injected in SOM-Cre mice to selectively knock down IL-17R in spinal dorsal horn SOM+ neurons. Knockdown of

**Figure 4. Photomicrographs Showing IL-17 and IL-17R Expression in Spinal Dorsal Horn**

(A–D) Immunofluorescence double labeling of IL-17 with astrocyte marker GFAP (A) and ALDH1L1 (B), microglia marker IBA1 (C), and neuron marker NeuN (D) in spinal dorsal horn. The top image is enlarged into three separate boxes with single and merged images in each picture. The scale bars represent 50 μm (top) and 20 μm (bottom).

(E and F) Fluorescence in situ hybridization showing the localization of IL-17 mRNA in spinal dorsal horn from naive (E) and paclitaxel-treated (F) mice. The scale bars represent 25 μm (top) and 10 μm (bottom).

(G–I) Immunofluorescence double labeling of IL-17R with neuron marker βIII-tubulin (G), astrocyte marker GFAP (H), and microglia marker IBA1 (I) in spinal dorsal horn. The scale bars represent 50 μm (top) and 20 μm (bottom).

See also Figures S2 and S3.
IL-17R in spinal SOM+ neurons delayed and suppressed paclitaxel-induced mechanical allodynia (Figure 7F; also Figure S1B; two-way RM ANOVA; treatment: $F_{1,57} = 29.53; p < 0.0001$). Moreover, we examined the effect of DRG neuron expressing IL-17R on CIPN. Knockdown of IL-17R in DRG neurons by intra-DRG of AAV2/9-SYN-EGFP-IL-17Ra-shRNA blocked and delayed the development of paclitaxel-induced mechanical allodynia (Figure 7 G; also Figure 6 G; two-way RM ANOVA; treatment: $F_{1,36} = 19.93; p < 0.0001$). These results indicate that endogenous IL-17 and spinal as well as DRG IL-17R are required for the maintenance of chemotherapy-induced neuropathic pain. T cell infiltration into the spinal cord may contribute to nerve-injury-induced mechanical allodynia (Sorge et al., 2015). We therefore measured mechanical response threshold in a T-cell-deficient mouse strain, Rag1 knockout mice, characterized by an absence of functional T cells (Nancy et al., 2012). Intrathecal injection of IL-17R antibody (10 μg) or IL-17 antibody (2 μg) significantly relieved paclitaxel-induced mechanical allodynia (Figure 7H; two-way RM ANOVA; treatment: $F_{2,96} = 12.42; p < 0.0001$), suggesting that (1) T cells may not be required for IL-17 signaling in CIPN and (2) glia-derived IL-17 plays a predominant role in paclitaxel-induced neuropathic pain. As a support, we did not observe colocalization of IL-17 and CD3+ T lymphocytes in the spinal dorsal horn (Figure S5).

**DISCUSSION**

We have provided an insight into how IL-17 promotes chemotherapy-induced neuropathic pain. Our results show that IL-17 and IL-17R regulate neuropathic pain via multiple mechanisms, including neuron-glia interactions, central sensitization, and peripheral sensitization (Figures S6 and S7). In the spinal cord, IL-17 enhances NMDA-receptor-mediated currents, facilitates excitatory synaptic transmission, and increases excitability of dorsal horn SOM+ neurons. In particular, IL-17 suppresses inhibitory synaptic transmission by inhibiting GABA-receptor-mediated currents (Figure S6). In the DRG, IL-17 increases neuronal excitability and IL-17R contributes to paclitaxel-induced nociceptor hyperactivity (Figure S7).

IL-17 and IL-17R Mediate Neuron-Glia Interactions Both in the Central and Peripheral Nervous System

Recent progress has demonstrated the critical role of spinal glial cells in driving chronic pain via production of proinflammatory cytokines and neuron-glia interactions (Ji et al., 2016; McMahon and Malcangio, 2009; Ren and Dubner, 2010; Gosselin et al., 2010; Salter and Stevens, 2017; Tsuda, 2018). Microglia and astrocytes play different roles in CIPN. Paclitaxel was shown to induce astrocyte activation, but not microglia activation, in the spinal cord (Zhang et al., 2012). Activation of p38 mitogen-activated protein (MAP) kinase in spinal microglia contributes to neuropathic pain after nerve trauma and cancer pain (Jin et al., 2003; Yang et al., 2015). However, spinal inhibition of p38 MAP kinase fails to affect chemotherapy-induced mechanical allodynia (Luo et al., 2018). Our results also highlight a role of astrocytes in CIPN. IL-17 is a T-cell-derived cytokine, but we did not see IL-17 expression in T cells (Figure S5). In Rag1 knockout mice with T cell deficiency, paclitaxel-induced CIPN was developed as usual. Furthermore, inhibition of spinal IL-17 signaling was capable of suppressing CIPN in the immune-deficient mice, suggesting that IL-17 signaling in CIPN in both male and female mice may not require T cells. Intriguingly, we found IL-17 immunoreactivity exclusively in GFAP-expressing astrocytes. In contrast, IL-17R was primarily expressed in spinal cord neurons, including SOM+ excitatory...
interneurons and PAX2+ inhibitory interneurons. This localization of IL-17 and IL-17R offers a cellular basis for neuron-astroglial interaction during pain regulation. We also observed respective expression of IL-17 and IL-17R in satellite glial cells and neurons in mouse DRG. Thus, IL-17/IL-17R signaling could promote both central sensitization and peripheral sensitization via neuron-glial interactions in the CNS and peripheral nervous system (PNS).

IL-17 and IL-17R Modulate Excitatory Synaptic Transmission in the Spinal Cord Pain Circuit

Enhanced excitatory synaptic transmission has been shown in spinal cord neurons, including SOM+ neurons, in various pathological pain conditions (Yang et al., 2015; Chen et al., 2015). Our data indicate that IL-17 is both sufficient and required for inducing this synaptic plasticity. Exogenous IL-17 rapidly increased EPSCs in spinal cord slices from naive animals. Spinal cord slices from paclitaxel-treated animals exhibited an increase in sEPSCs, which was suppressed by IL-17R antibody and IL-17R-shRNA, suggesting an endogenous role of IL-17 in CIPN. Mechanistically, IL-17 acutely enhanced the amplitude of NMDA-evoked currents following dorsal root stimulation, suggesting that IL-17 increases NMDAR activity via rapid post-translational regulation. This is consistent with the previous report that IL-17 acts on spinal nociceptive neurons co-expressing IL-17R and NR1 to modulate pain (Meng et al., 2013). NMDAR plays a critical role in the induction and maintenance of central sensitization during persistent pain conditions (Woolf and Thompson, 1991; South et al., 2003; Liu et al., 2008). It remains to be investigated how IL-17 modulates NMDAR activity. It is possible that IL-17 activates protein kinases, such as extracellular-regulated kinase (ERK) and protein kinase C (PKC), to enhance NMDAR activation and neuronal excitability (Hu and Gereau, 2003). For example, TNF-α increases NMDA currents in spinal cord lamina II neurons via ERK phosphorylation (Xu et al., 2010). In addition, Ca2+/calmodulin-dependent protein kinase II (Ca2+/CaMKII) is involved in Ca2+ signaling and hyperalgesia. CaMKII inhibitors decreased many types of hyperalgesia, including neuropathic pain (Katano et al., 2011). CaMKII inhibitor KN-93 suppressed the neuropathic pain and NMDA GluN2B upregulation (Liu et al., 2018). Yao et al. (2016) reported that IL-17 significantly increased phospho-CaMKII (p-CaMKII) levels in the spinal cord. L4 spinal nerve ligation (SNL)-induced p-CaMKII can be blocked by IL-17 antibody or IL-17 genetic deficiency. CaMKII inhibitor...
markedly relieved SNL-induced hyperalgesia and pCREB. CREB is essential for the regulation of pro-nociceptive genes and long-term neuronal plasticity (Ji et al., 2003). In neuropathic pain model, an increased phosphorylation of CREB can be induced, which is associated with spinal central sensitization (Miletic et al., 2002). Spinal rIL-17 significantly increased pCREB levels,

Figure 7. IL-17 and IL-17R Contribute to Paclitaxel-Induced Neuropathic Pain in Wild-Type Mice and Immune-Deficient Rag1 KO Mice

(A) Intrathecal injection of IL-17 induces a transient and dose-dependent mechanical allodynia (i.e., reduction in PWT). *p < 0.05; **p < 0.01 versus vehicle; #p < 0.05 versus 50 ng IL-17 group; two-way ANOVA; n = 12; mice.

(B) Overexpression of IL-17 in spinal astrocytes by intra-dorsal horn injection of AAV2/9-GFAP-IL-17-EGFP induces mechanical allodynia.

(C) Western blot showing an increased IL-17 expression in spinal dorsal horn from AAV2/9-GFAP-IL-17-EGFP treatment mice. *p < 0.05 versus control; Student’s t test; n = 6; mice.

(D) Fluorescent images showing that IL-17-EGFP colocalized with GFAP-IR (left) but not with NeuN-IR (right) in spinal dorsal horn in astrocyte IL-17-overexpressed mice. Scale bar: 30 μm.

(E) Mechanical allodynia, induced by paclitaxel (6 mg/kg, i.p.) is attenuated by intrathecal injection of IL-17R antibody (1 and 10 μg). **p < 0.01 versus control IgG (10 μg, i.t.); two-way ANOVA; n = 7–11; mice.

(F) Specific knockdown of IL-17R in spinal dorsal horn SOM+ neurons by intra-dorsal horn injection of AAV2/9-DIO-EGFP-IL-17R-shRNA blocked paclitaxel-induced mechanical allodynia. *p < 0.01 versus control shRNA; 4p < 0.01 versus baseline (before i.p. PAX); two-way ANOVA; n = 6 and 8; mice.

(G) Knockdown of IL-17R in DRG neurons by intra-DRG injection of AAV2/9-hSYN-EGFP-IL-17R-shRNA prevented and delayed paclitaxel-induced mechanical allodynia. *p < 0.05; **p < 0.01 versus baseline (before i.p. PAX); two-way ANOVA; n = 4 and 5; mice.

(H) Mechanical allodynia is attenuated by intrathecal injection of IL-17R antibody (10 μg) or IL-17 antibody (2 μg) in Rag1 KO mice. *p < 0.05; ***p < 0.001 versus control IgG (10 μg, i.t.); two-way ANOVA; n = 5–6; mice. All data were mean ± SEM. See also Figure S5.
and blockage of IL-17 signaling decreased pCREB (Yao et al., 2016). Thus, IL-17 may play a role in spinal pain modulation via ERK, CaM-KII, and PKC signaling pathways regulating spinal synaptic transmission.

**Modulation of Inhibitory Synaptic Transmission by IL-17 and IL-17R**

One of the most interesting findings of this study is the profound suppression of inhibitory synaptic transmission in lamina II SOM+ neurons. Disinhibition, i.e., loss of inhibitory synaptic transmission, is emerging as a key mechanism of neuropathic pain (Coull et al., 2003; Zeilhofer et al., 2012a; Lu et al., 2013; Coull et al., 2005). Removal of spinal inhibition, especially presynaptic GABAergic inhibition, not only reduces the fidelity of normal sensory processing but also provokes symptoms very much reminiscent of inflammatory and neuropathic pain syndromes (Zeilhofer et al., 2012b; Takazawa et al., 2017; Chen et al., 2014). Increasing evidence suggests that disinhibition is a key mechanism by which glial mediators (e.g., IL-1 and brain-derived neurotrophic factor [BDNF]) regulate central sensitization and chronic pain (Chen et al., 2018; Coull et al., 2005). Our study shows that exogenous IL-17 rapidly (within 1 min) and drastically decreased the frequency and amplitude of iIPSCs. Mechanistically, IL-17 specifically suppressed GABA-induced currents, but not glycine-induced currents. Although TNF-α and IL-1β have also been shown to regulate inhibitory synaptic transmission in spinal cord neurons (Kawasaki et al., 2008; Zhang et al., 2010; Chirila et al., 2014), they act on different pain circuits in the spinal cord. Multiple mechanisms have been implicated in disinhibition in pathological pain (Zeilhofer et al., 2012b). Microglia-produced BDNF was shown to suppress GABA currents in lamina I projection neurons (Coull et al., 2005). Our data suggest that IL-17 can elicit a very rapid loss of inhibition to open the spinal gate, which enables low-threshold synaptic input to spinal cord substantia gelatinosa (SG) neurons (Chang et al., 2018; Li et al., 2015; Zhang and Dougherty, 2014). Peripheral mechanisms of pain modulation by IL-17 have been investigated. For example, IL-17 sensitizes joint nociceptors to mechanical stimuli to facilitate arthritic pain (Richer et al., 2012). It was also reported that neuronal IL-17R regulates mechanical, but not thermal, hyperalgesia by upregulation of TRPV4, but not TRPV1, in DRG neurons (Segond von Banchet et al., 2013). We observed rapid excitability increase in both mouse and human DRG neurons following IL-17 treatment, suggesting possible post-translational modulations to some key ion channels, such as sodium channels. Our work in progress shows that IL-17 also increased sodium currents (data not shown). Interestingly, we found that the enhanced excitability in paclitaxel-pretreated small mouse DRG neurons can be abolished by a neutralizing antibody against IL-17R antibody and IL-17R-shRNA. Because the recordings were conducted in dissociated neurons and the physiological concentration of IL-17 is not present in culture medium, our result suggests a possibility that IL-17R may directly regulate neuronal activity in the absence of IL-17. Future studies will examine how IL-17R interacts with ion channels, such as Nav1.7. We should not rule out the possibility that satellite glial cells may also be attached to neurons in our culture conditions and communicate with neurons by releasing IL-17. Our working hypothesis of peripheral glial regulation of chemotherapy-evoked neuropathic pain via IL-17/IL-17R signaling is illustrated in Figure S7.

**Translational Potential**

IL-17 levels in the sciatic nerve are elevated after nerve injury (Noma et al., 2011). Our data show that IL-17 levels are also elevated in the CSF and spinal cord in paclitaxel-treated mice. Importantly, intrathecal injection of IL-17R antibody or selective knockdown of IL-17R in DRG neurons or spinal SOM+ neurons effectively alleviated paclitaxel-induced neuropathic pain. Chemotherapy has been shown to activate cancer-associated fibroblasts, which leads to renewal of cancer-initiating cells and maintenance of colorectal cancer by IL-17 secretion (Lotti et al., 2013). Thus, targeting IL-17 signaling may not only alleviate neuropathic pain but also improve anti-cancer efficacy after chemotherapy. The translational potential of this study is enhanced by demonstrating hyperexcitability of human sensory
neurons in response to IL-17. IL-17 blockers have been developed for treating inflammatory diseases, such as psoriasis and arthritis (Kivelevitch and Menter, 2015). Brodalumab (Kyntheum) is a human anti-interleukin-17 receptor A (IL-17RA) monoclonal antibody available for use in patients with moderate to severe plaque psoriasis (Blair, 2018). Because mouse IL-17RA antibody is effective in suppressing neuronal hyperexcitability after paclitaxel, brodalumab could be used to treat CIPN and neuropathic pain.

STAR METHODS

Detailed methods are provided in the online version of this paper and include the following:

- **KEY RESOURCES TABLE**
- **LEAD CONTACT AND MATERIALS AVAILABILITY**
- **EXPERIMENTAL MODEL AND SUBJECT DETAILS**
- **METHOD DETAILS**
  - Reagents and Drug Injection
  - ELISA
  - Immunohistochemistry
  - Fluorescent In Situ Hybridization (ISH) Using RNAscope and Double Staining with Immunohistochemistry
  - Preparation of Spinal Cord Slices and Whole-Cell Patch-Clamp Recordings
  - Preparation of Mouse DRG Neurons and Whole-Cell Patch Clamp Recordings
  - Western Blot
  - Surgical Procedures and Behavioral Testing
- **QUANTIFICATION AND STATISTICAL ANALYSIS**
- **DATA AND CODE AVAILABILITY**

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.celrep.2019.10.085.

ACKNOWLEDGMENTS

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AUTHOR CONTRIBUTIONS

H.L. developed the project, performed electrophysiology in mouse spinal slices, and prepared final figures. H.-Z.L. conducted intra-dorsal horn injection of AAV virus stereotaxic injection and immunohistochemical and all the behavioral experiments. W.-W.Z. conducted intra-DRG injection of AAV virus stereotaxic injection and DRG electrophysiological recording. M.M., N.L., G.C., and Z.-Z.X. conducted in situ hybridization and ELISA experiments. Y.-Q.Z. supervised the project. Y.-Q.Z. and H.L. wrote the paper.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES


## STAR METHODS

### KEY RESOURCES TABLE

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**Bacterial and Virus Strains**

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LEAD CONTACT AND MATERIALS AVAILABILITY

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Yu-Qiu Zhang (yuqiuzhang@fudan.edu.cn). This study did not generate new unique reagents.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Most experiments were performed on adult C57BL/6J mice (8-10 weeks, male, purchased from Charles River). Some electrophysiology experiments were conducted in transgenic C57BL/6J mice (5-6 weeks). These mice express tdTomato fluorescence in somatostatin (SOM+) neurons, after Som-Cre mice (JAX013044) were crossed with tdTomato Cre-reporter mice (Rosa26-floxed stop tdTomato mice, JAX007914), both from Jackson Laboratory, to generate conditional transgenic mice that express tdTomato in SOM+ neurons. Rag1 knockout mice were purchased from Shanghai Model Organisms (NM-KO-00069). IL-17-Cre (JAX016879) and IL-17-GFP (JAX018472) mice were purchased from Jackson Laboratory. All animal procedures were approved by the Institutional Animal Care & Use Committee (IACUC) of Fudan University (Permit Number: SYXK2009-0082). Animals were randomly assigned to each group. The behavioral testing and immunohistochemical experiments described herein were performed by experiments who were blind to the treatments.

Intraperitoneal (i.p.) injections of paclitaxel (PAX, 6 mg/kg for a single injection or 2 mg/kg for multiple injections on days 0, 2, 4, and 6) were conducted to generate chemotherapy-associated neuropathic pain. Seven days following the injection, spinal dorsal horn and CSF samples were collected.

METHOD DETAILS

Reagents and Drug Injection

We purchased the following reagents from R&D System Inc. (MN, USA): recombinant mouse IL-17A protein (421-ML), mouse IL-17 receptor A (IL-17 RA or IL-17R) antibody (MAB4481) and control IgG. GABA and glycine were obtained from Sigma-Aldrich. IL-17 was prepared as a 1000X stock solution in 4 mM HCl and used in experiments at a concentration of 10 ng/mL. All the compounds were prepared in artificial cerebrospinal fluid (ASCF). Picrotoxin, strychnine, AP-5, and CNQX were purchased from Sigma-Aldrich.

IL-17 or vehicle was delivered into the CSF space between the L5 and L6 vertebrae via a lumbar puncture made by a 30 gauge needle. Prior to puncture, the animal’s heads were covered by a piece of cloth. Ten microliters of solution were injected with a microsyringe, with successful spinal puncture confirmed by a brisk tail-flick.

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**Experimental Models: Organisms/Strains**

| B6.Cg-Gt(Rosa)26Sortm14(CAG-tdTomato)Hze/J | The Jackson Laboratory | JAX007914 |
| Ssttm2.1(cre)Zjh/J | The Jackson Laboratory | JAX013044 |
| IL17a tm1.1(icre)Stck/J | The Jackson Laboratory | JAX016879 |
| IL17A-IRES-GFP-KI | The Jackson Laboratory | JAX018472 |
| Rag1 knockout mice | Shanghai Model Organisms | NM-KO-00069 |

**Software and Algorithms**

| GraphPad Prism 6 | GraphPad Software | https://www.graphpad.com/scientific-software/prism/ |
| Photoshop CS6 | Adobe | https://www.adobe.com/ |
ELISA
ELISA was performed using CSF and spinal cord tissue samples. The spinal cords were homogenized in lysis buffer containing protease and phosphatase inhibitors (Sigma Chemical Co) and centrifuged (12,500 × g for 10 min) to obtain extract proteins. CSF was collected from the cisterna magna as we previously demonstrated (Chen et al., 2015). For each ELISA assay, 50 μg of protein or 5 μL of CSF were used. ELISA was conducted according to the manufacturer’s instructions (R&D Systems Inc., MN, USA., Cat# PM1700) and the standard curve was included in each experiment.

Immunohistochemistry
Mice were deeply anesthetized with urethane and then transecardially perfused with normal saline followed by 4% paraformaldehyde in 0.1 M PB. The L4–L5 segments of the spinal cord were removed and postfixed for 24 h at 4°C and then dehydrated in gradient sucrose at 4°C. Transverse spinal cord sections (30 μm) were cut on a cryostat (Model 1900, Leica). The sections were blocked with PBS containing 10% donkey serum and 0.3% Triton X-100 for 2h at RT and then incubated for 48 h at 4°C with a mixture of rabbit anti-IL-17 antibody (1:50, Santa Cruz, sc-7927) together with mouse anti-NeuN (1:2000, Millipore, MAB377), goat anti-IBA-1 (1:500, Abcam, ab5076), mouse anti-GFAP (1:2000, Sigma, G6171), mouse anti ALDH1L1(1:5, NeuroMab Facility, 73-140) mouse anti-CD3 (1:100, eBioscience, 14-0030-81), mouse anti-NGF (1:1000, Sigma, C7113), mouse anti NF200 (1:1000, Sigma, N0142) antibodies, or rabbit anti-IL-17R antibody (1:200, Abcam, ab180904) together with anti GFAP/IBA-1 antibodies, goat anti Pax2(1:500, R&D, AF336-SP), mouse antiβ3 tubulin (1:1000, Sigma, T8578). The sections were then incubated with a mixture of Alexa Fluor 488- or 546-conjugated secondary antibodies (1:200, Invitrogen, USA), or IB-Alexa Fluor 488 (1: 200, Invitrogen, A21206) for 2h, or DAPI (1:30000, Sigma, 32670) for 2 min at RT. The specificity of immunostaining and primary antibodies was verified by omitting the primary antibodies, by testing IL-17 mutant mice, and also by in situ hybridization (see below). The stained sections were observed and images captured with a confocal laser-scanning microscope (Model FV1000, Olympus).

Fluorescent In Situ Hybridization (ISH) Using RNAscope and Double Staining with Immunohistochemistry
Mice were deeply anesthetized with isoflurane and transcardially perfused with PBS, followed by 4% paraformaldehyde. After the perfusion, the L4–L5 DRGs and L4–L5 spinal cord segments were removed and postfixed in the same fixative for 2 h at 4°C. Then, the tissues were cryopreserved in 30% sucrose/ PBS solution for 2 days. DRG (12 μm) and spinal cord sections (14 μm) were cut using a cryostat. ISH was performed using RNAscope system (Advanced Cell Diagnostics) according to the manufacturer’s protocol. Pretreatment consisted of dehydration, followed by incubation with hydrogen peroxide and protease IV at room temperature. Subsequently, the protocol for the Multiplex Fluorescent Kit v2 was followed using commercial probes for IL-17A (Mm-il17a, #319571), IL-17AR (Mm-il17ra-C2, #403741-C2), and SOM/SST (Mm-Sst-C3, #404631-C3). After ISH, some spinal cord sections were labeled with anti-GFAP antibody (mouse, 1:1000, Sigma, St. Louis, MO, USA, MAB360) and counterstained with DAPI (1:30000, Sigma, 32670). The spinal cord and DRG images were captured using a Nikon fluorescence microscope.

Preparation of Spinal Cord Slices and Whole-Cell Patch-Clamp Recordings
The L4–L5 lumbar spinal cord segment was rapidly removed under urethane anesthesia (1.5 - 2.0 g/kg, i.p.) and transferred to ice-cold cutting ACSF containing (in mM): NaCl 125, KCl 2.5, CaCl2 2, MgCl 2 1, NaHCO 3 26, D-glucose 25. The L4–L5 segments of the spinal cord were removed and postfixed for 24 h at 4°C and then dehydrated in gradient sucrose at 4°C. Transverse spinal cord sections (30 μm) were cut on a cryostat (Model 1900, Leica). The sections were blocked with PBS containing 10% donkey serum and 0.3% Triton X-100 for 2h at RT and then incubated for 48 h at 4°C with a mixture of rabbit anti-IL-17 antibody (1:50, Santa Cruz, sc-7927) together with mouse anti-NeuN (1:2000, Millipore, MAB377), goat anti-IBA-1 (1:500, Abcam, ab5076), mouse anti-GFAP (1:2000, Sigma, G6171), mouse anti ALDH1L1(1:5, NeuroMab Facility, 73-140) mouse anti-CD3 (1:100, eBioscience, 14-0030-81), mouse anti-NGF (1:1000, Sigma, C7113), mouse anti NF200 (1:1000, Sigma, N0142) antibodies, or rabbit anti-IL-17R antibody (1:200, Abcam, ab180904) together with anti GFAP/IBA-1 antibodies, goat anti Pax2(1:500, R&D, AF336-SP), mouse antiβ3 tubulin (1:1000, Sigma, T8578). The sections were then incubated with a mixture of Alexa Fluor 488- or 546-conjugated secondary antibodies (1:200, Invitrogen, USA), or IB-Alexa Fluor 488 (1: 200, Invitrogen, A21206) for 2h, or DAPI (1:30000, Sigma, 32670) for 2 min at RT. The specificity of immunostaining and primary antibodies was verified by omitting the primary antibodies, by testing IL-17 mutant mice, and also by in situ hybridization (see below). The stained sections were observed and images captured with a confocal laser-scanning microscope (Model FV1000, Olympus).

Preparation of Mouse DRG Neurons and Whole-Cell Patch Clamp Recordings
Mouse DRGs were aseptically dissected from 5 to 8 week-old mice and digested with collagenase (0.2 mg/ml, Roche)/dispase-II (3 mg/ml, Roche) for 120 min. Cells were placed on glass coverslips coated with poly-D-lysine and grown in a neurobasal defined medium (10% fetal bovine serum and 2% B27 supplement) at 37°C and 5% CO2 for 24 h before experiments.
Whole-cell patch-clamp recordings in small-diameter (<25 μm in mice) DRG neurons were conducted at room temperature as we previously demonstrated (Chen et al., 2017b). We used an Axopatch-200B amplifier with a Digidata 1440A digitizer (Axon Instruments) to measure action potentials and resting membrane potential. The patch pipettes were pulled from borosilicate capillaries (World Precision Instruments, Inc.). The resistance of the pipettes was 3–4 MΩ when filled with the pipette solution. The recording chamber (300 μl) was continuously superfused at a flow rate of 1.2 ml/min. Series resistance was compensated (> 80%) and leak subtraction was performed. Data were low pass-filtered at 2 KHz and sampled at 10 KHz. The software program pClamp10.6 (Axon Instruments) was used during experiments and Clampfit 10.6 was used for analysis. The pipette solution contained (in mM): potassium gluconate 126, NaCl 10, MgCl₂ 1, EGTA 10, Na-ATP 2 and Mg-GTP 0.1, adjusted to pH 7.3 with KOH. The external solution contained: NaCl 140, KCl 5, CaCl₂ 2, MgCl₂ 1, HEPES 10, glucose 10, adjusted to pH 7.4 with NaOH. In current-clamp experiments, the action potentials were evoked by a current injection. The resting membrane potential was measured without a current injection.

Western Blot
Mice were sacrificed by overdoses of urethane, and the L4–L6 spinal cord segment was rapidly removed. The dorsal horn tissues were homogenized in lysis buffer containing a mixture of protease inhibitors and phenylmethylsulfonyl fluoride (Roche Diagnostics). Equal amount of protein was loaded and separated on 10% SDS-PAGE gels (Bio-Rad) and transferred to PVDF membranes (Millipore). The membranes were blocked with 5% nonfat milk in Tris-buffered saline (pH 7.6) with 0.1% Tween-20 for 2 h at room temperature, and incubated overnight at 4°C with primary antibodies, followed by HRP-conjugated secondary antibodies for 2 h at room temperature. GAPDH antibody was probed as a loading control. Signals were visualized using enhanced chemiluminescence (ECL, Pierce), and captured by ChemiDoc XRS system (Bio-Rad). We used the following primary antibodies: rabbit anti-interleukin 17 A (1:250, Sigma, PRS4887) and secondary antibodies: HRP-GAPDH (1:20000, Aksomics, Shanghai, China, KC-5G5), HRP-conjugated goat anti-rabbit (1:5000, Jackson 111-035-003). All western blot analysis was performed three to four times, and consistent results were obtained. A Bio-Rad image analysis system was then used to measure the integrated optic density of the specific bands.

Surgical Procedures and Behavioral Testing
AAV virus and stereotaxic injection
We purchased the following adeno-associated virus: pAAV-hSYN-DIO-IL17RA shRNA-EGFP (AAV2/9, titer: 1.13 × 10¹³ V.G./ml), pAAV-hSYN-DIO-EGFP (AAV2/9, titer: 1.51 × 10¹³ V.G./ml), pAAV-hGFAP-IL17A-T2A-EGFP (AAV2/9, titer: 7.88 × 10¹² V.G./ml), pAAV-hGFAP-T2A-EGFP(AAV2/9, titer: 4.28 × 10¹² V.G./ml) were obtained from Shanghai Sunbio Medical Biotechnology Co.,Ltd. (Shanghai, China), pAAV-SYN-MCS-EGFP-3FLAG-micro30shRNA (IL17Ra-shRNA) (AAV2/9, titer: 3 × 10¹³ V.G./ml) were purchased from Obio Technology Co.,Ltd. (Shanghai, China). The design of IL-17R shRNA oligo (shRNA, 5’-CCTACCGTTTGTCTACTT-3’) referred to works of Chen et al. (2013) or shControl (the scrambled sequence) was ligated into the above designated plasmid vector construct. IL-17A (NM_010552.3) designed to coexpress enhanced green fluorescent protein (EGFP) and driven by the GFAP promoter.

Mice were anesthetized with pentobarbital sodium (50 mg/kg intraperitoneally [i.p.]). Back hair of mice was shaved and the surgical site was sterilized with iodophor and 75% ethanol.

Intra-dorsal horn injection referred previous description (Haenraets et al., 2017) with modifications, the vertebral column was mounted in a stereotaxic frame (Stoelting, USA) and the dura in intervertebral spaces was incised to expose the spinal cord at L2-L4. No laminctomy was performed to maximally avoid trauma. Micropipette was tilted inserted into the dorsal spinal cord at an angle (45 degrees) and at 420 μm from the dura (equivalent to a vertical depth of 300 μm from the dura) to target at the superficial dorsal horn. Adeno-associated viruses (AAV) were injected into the right side of the spinal cord at 2 injection sites (400-600 nL of AAV was injected for each site at a rate of 40 nl/min) using an air pressure system (NANOLITER 2010 injector, WPI, USA) by connecting to glass pipettes (tip diameter of 10-30 μm). The micropipette was withdrawn 5 min after viral injection for diffusion. Incision was closed with stitches. The animals were allowed to recover from anesthesia on a heating blanket before returning to their home cage. Antibiotics (Ceftriaxone sodium, 0.1 g/kg) was injected intraperitoneally for three days after surgery to prevent infection. Relevant experiments were performed 3 weeks after viral inoculation to allow maximal and stable expression.

Intra-DRG injection was performed referring the previous reports (Luo et al., 2012; Unger et al., 2017), a sagittal incision about 1.5 cm length of the skin was made at the level of the lumbar 4 (L4) and lumbar 5 (L5) vertebrae. Remove the muscle and expose the vertebral plate, zygaphophysis, and transverse process of spine column. The spinal column was fixed on the stereotaxic instrument (Stoelting, USA). The left part of the L4 and L5 vertebral plate, zygaphophysis, and transverse process were removed with skull drill under microscope. Clean the tissue and blood with microforceps and cotton swaps to expose the L4 and L5 dorsal root ganglion (DRG). Micro syringe pump with glass electrode was adjusted at 45-degree inserting into DRG with depth about 0.2-0.3 mm. Adeno-associated virus (AAV) was injected into the unilateral L4 and L5 DRG at two sites (150nl of AAV was injected into each site with 30nl/min injection speed). The micro syringe pump was withdrawn 5 min after injection for virus diffusion. The incision was closed with 4-0 suture. The mice were allowed to recover in a warm temperature before sent back to their homecage. The DRGs with virus injection were detected through immunohistochemistry after 3 weeks of maximal expression.
Behavior
Animals were habituated to the testing environment for at least 2 days before testing. Animals were kept in boxes on an elevated metal mesh floor. Mechanical allodynia was assessed by measuring paw withdrawal thresholds in response to stimulation from a series of von Frey hairs (0.16-2.0 g, Stoelting Company). The withdrawal threshold was determined using Dixon’s up-down method.

QUANTIFICATION AND STATISTICAL ANALYSIS
All data were expressed as mean ± SEM and analyzed using Prism 6.0 (GraphPad Software, San Diego, CA). ELISA and behavioral data were analyzed using Student’s t test (two groups) or 2-way RM ANOVA followed by post hoc Bonferroni test. Electrophysiological data were tested using Paired t test, Student’s t test, 2-way or 2-way RM ANOVA followed by post hoc Student-Newmann-Keuls test were used to identify significant differences. The statistical details of experiments can be found in the figure legends and results. The criterion for statistical significance was p < 0.05.

DATA AND CODE AVAILABILITY
The published article includes all data generated or analyzed during this study. This study did not generate code.