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The Kv4.3 channel on c-low threshold mechanoreceptors in DRG is a key mediator transforming tactile stimuli into nociceptive signals in chemotherapy-induced neuropathy



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Abstract Oxaliplatin, a chemotherapeutic agent commonly used in colorectal cancer treatment, frequently induces chemotherapy-induced peripheral neuropathy (CIPN), with mechanical allodynia as a dose-limiting neurological complication. However, the precise pathophysiological mechanism underlying this sensory dysfunction remains inadequately elucidated. This study identifies Kv4.3 channel dysfunction in C-low threshold mechanoreceptors (C-LTMRs), a subset of tyrosine hydroxylase –positive (TH⁺) sensory neurons in the dorsal root ganglia (DRG), as the critical driver of oxaliplatin-induced mechanical allodynia. Using electrophysiological, pharmacological, and genetic approaches in mouse models, we have demonstrated that oxaliplatin selectively alters the firing pattern of C-LTMRs and enhances their excitability, particularly in response to low-intensity stimuli. This effect is mediated by Kv4.3 channel dysfunction within C-LTMRs, which underlies the pathological conversion of

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innocuous touch to pain. Critically, pharmacological inhibition or neuron-specific knockdown of Kv4.3 channels exacerbated mechanical allodynia, while Kv4.3 channel activation reversed neuronal hyperexcitability and alleviated oxaliplatin-induced mechanical allodynia. Thus, Kv4.3 dysfunction constitutes a core pathogenic mechanism of oxaliplatin-induced mechanical allodynia. Targeted enhancement of the Kv4.3 channel activity in C-LTMRs represents a promising precision analgesic strategy for this condition.

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1. Introduction

Chemotherapy-induced peripheral neuropathy (CIPN) is a prevalent and severe adverse effect associated with various commonly used chemotherapeutic agents, and is characterized mainly by sensory disturbances, including paresthesia, hypoesthesia, and neuropathic pain. The persistence of these symptoms may negatively impact the quality of life of patients both during and after treatment¹⁻⁴. Oxaliplatin, a third-generation platinum-based chemotherapeutic agent extensively used for advanced colorectal cancer (CRC), is notably associated with a high incidence of CIPN⁵. This adverse effect manifests acutely in approximately 90% of treated patients⁶ and chronic manifestations persist in nearly 70% of patients^{2,7}. Recent studies have primarily focused on enhancing the efficacy of oxaliplatin and reducing its potential side effects through structural modification of Pt(IV) prodrugs⁸ and the use of nanoparticle- or liposome-based delivery systems^{9,10}. Within this neuropathic spectrum, mechanical allodynia and cold hypersensitivity emerge as hallmark clinical manifestations of oxaliplatin-induced peripheral neuropathy (OIPN), simultaneously representing critical dose-limiting factors in chemotherapeutic regimens. Cold hypersensitivity can be alleviated or avoided through patient-initiated warming measures, whereas mechanical allodynia—defined as a pathological pain response to innocuous mechanical stimuli, such as cutaneous contact with clothing—lacks effective patient-modifiable interventions for prevention or mitigation. Currently, there is a paucity of targeted and efficacious analgesic agents for the treatment or prevention of mechanical allodynia¹¹. Therefore, elucidating the molecular and cellular mechanisms underlying oxaliplatin-induced mechanical allodynia, along with the identification of potential analgesic targets, holds significant scientific and translational value. Such research endeavors are crucial as they may directly contribute to the development of mechanism-based analgesic therapies.

The dorsal root ganglia (DRG), which contains the primary sensory neurons of somatosensory afferent pathways, plays a critical role in the initiation and modulation of neuropathic pain. Located in the peripheral nervous system and lacking a protective blood–brain barrier, DRG neurons are particularly vulnerable to chemotherapeutic agents. Preclinical studies have shown that oxaliplatin accumulates in these neurons¹², suggesting that molecular alterations in pain-signaling pathways within the DRG may significantly contribute to the development of CIPN. Among the diverse neuronal subtypes in the DRG, we focus on a specific subpopulation of unmyelinated C-low-threshold mechanoreceptors (C-LTMRs), which are molecularly marked by tyrosine hydroxylase (TH) and exhibit selective expression of vesicular glutamate transporter 3 (VGLUT3) and TAF4^{13,14}. These neurons account for approximately 37% of L4–L6 DRG neurons in

mice¹⁵. Under normal physiological conditions, C-LTMRs mediate pleasant light-touch sensations, contributing to affective comfort associated with innocuous mechanical stimuli^{16,17}. Anatomically, these neurons project peripheral sensory signals to the inner layer of lamina II (L-IIi) in the spinal dorsal horn^{13,14}, where innocuous mechanosensory pathways intersect with nociceptive circuits^{18,19}, indicating a potential site for tactile-affective integration and pain modulation. Previous research has demonstrated that C-LTMRs are involved in tactile hypersensitivity following nerve injury and inflammation in mice^{13,20-22}. These neurons specifically express TAF4, a chemokine-like secreted protein²³ that mitigates postinjury and postinflammatory mechanical allodynia in mice and reduces nociceptive transmission in the spinal cord by restoring inhibitory inputs to spinal neurons^{21,22,24}; *i.e.*, C-LTMRs exhibit the functional capacity to convert innocuous tactile inputs under physiological conditions into pathological mechanical allodynia during disease states. Draxler et al.²⁵ demonstrated that VGLUT3⁺ primary afferents contribute to the development of neuropathic pain in a mouse model of OIPN. However, these VGLUT3⁺ primary afferents encompass three distinct fiber types: A-fibers, TH⁺ C-LTMRs, and TH[−] C-fibers²⁵. As such, whether C-LTMRs exert a unique role in OIPN remains poorly defined.

In this investigation, we employed gene-edited mice, including *TH-Cre* mice and *Ai9-Tomato* reporter mice, to precisely identify C-LTMRs. Our findings revealed that oxaliplatin selectively modulated the electrophysiological properties of TH⁺ neurons, which were identified as C-LTMRs, rather than other TH[−] small-diameter neuronal populations. Furthermore, we observed the neuron-specific pathological downregulation of Kv4.3 potassium channel expression exclusively within C-LTMRs. By employing both pharmacological and genetic manipulation strategies, we established that Kv4.3 channels play a mechanistic role in regulating mechanical nociceptive processing, thereby implicating Kv4.3 dysfunction in C-LTMRs as a molecular basis for oxaliplatin-induced mechanical allodynia. These results suggest that Kv4.3 channels represent potential therapeutic targets for the management or prevention of CIPN associated with oxaliplatin, especially mechanical allodynia.

2. Materials and methods

2.1. Experimental animals

All experimental procedures involving animals were conducted in compliance with the ethical standards approved by the Institutional Animal Care and Welfare Committee of Hebei Medical University (Ethical Approval Reference Number: IACUC-Hebmu-2023065) and adhered to the International Association for the

Study of Pain's guidelines for the ethical use of animals. Adult C57BL/6J mice (7 weeks old, male) were obtained from Changsheng Biotechnology Co., Ltd. (Liaoning, China). The *TH-Cre* and *Ai9-tdTomato* reporter mouse lines were procured from Jinzhihe Biotechnology Co., Ltd. (Jiangsu, China). *TH-Cre-Ai9* mice were generated through the crossbreeding of *TH-Cre* mice with *Ai9-tdTomato* reporter mice.

2.2. Mouse genotyping

Genotyping of the gene-edited mice was conducted through tail DNA analysis. Specifically, 2-mm tail segments were excised and incubated in tissue DNA lysis buffer supplemented with 0.1 mg/mL proteinase K. Samples were digested (55 °C, 2 h) to achieve thorough tissue lysis, with subsequent proteinase K heat inactivation (95 °C, 5 min). The sample was centrifuged at $12,000 \times g$, and the supernatant containing genomic DNA was directly utilized as a template for PCR amplification. The PCR protocol was set to 35 cycles with the following steps: denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s, and extension at 72 °C for 30 s per cycle. The genotyping primers used were as follows: for *TH-Cre* mice, the forward primer was 5'-GAGACA-GAAGTCGGGACCAC-3' and the reverse primer was 5'-AGG-CAAATTTTGGTGTACGG-3'; for *Ai9-tdTomato* reporter mice, the forward primer was 5'-GGCATTAAAGCAGCGTATCC-3' and the reverse primer was 5'-CTGTTCTGTACGGCATGG-3'.

2.3. Animal model and drug administration

The OIPN murine model was generated through a previously validated protocol²⁶. Briefly, oxaliplatin (MedChemExpress, HY-17371) was prepared in a 5% glucose solution and administered *via* intraperitoneal injection at a dose of 6 mg/kg on alternate days, totaling three injections (Days 1, 3, and 5). Animals in the control group were treated with an equal amount of vehicle solution (5% glucose in dH₂O) following the same administration schedule.

For 4-AP (MedChemExpress, HY-B0604) administration, two distinct methods were employed: (1) local delivery of 5 mmol/L 4-AP (4 μ L) to the L4 DRG *via* cannula implantation, and (2) intraplantar injection of 5 mmol/L 4-AP (25 μ L) into the right hind paw.

NS5806 (Cayman, 426834-69-7) was prepared in a solvent mixture comprising DMSO, polyethylene glycol, and 0.9% NaCl at a volume ratio of 1:4:5. The compound was administered intraperitoneally at a concentration of 2.5 mg/kg on Days 14, 15, and 16 following the initial oxaliplatin injection.

2.4. Cell culture

DRG cell isolation was conducted following established protocols²⁷. Specifically, L3–L5 segmental DRGs were rapidly excised from the mice and subjected to enzymatic digestion at 37 °C for 30 min in a mixed enzyme mixture comprising 7.5 mg/mL dispase and 2.5 mg/mL collagenase type II, which was prepared in D-Hanks' balanced salt solution. The enzymatic activity was terminated by the addition of DMEM supplemented with 10% FBS and 1% penicillin–streptomycin, and the tissue lysate was then centrifuged at 1000 rpm for 5 min. After the supernatant was removed, the cell pellet was resuspended in DMEM supplemented with 10% FBS and 1% penicillin–streptomycin and then plated onto poly-D-lysine-coated (0.5 mg/mL) glass coverslips for incubation at 37 °C with 5% CO₂ in a humidified atmosphere. The

current clamp recording and the voltage recording were performed 4 to 8 h after DRG neurons plating.

2.5. Behavioral tests

Prior to the behavioral assessments, all the mice were acclimatized to the testing environment for a minimum of 60 min to ensure environmental adaptation. To mitigate observer bias, all experimental procedures were conducted under double-blind conditions.

2.5.1. Von Frey test

Mechanical thresholds were assessed *via* calibrated von Frey filaments (Stoelting, USA). The mice were acclimated for 30 min in individual plexiglass chambers with mesh platforms before plantar stimulation. Paw withdrawal thresholds were determined *via* the up-and-down method, with the threshold defined as the lowest filament force eliciting at least three positive responses during the assessment.

2.5.2. Cotton swab test

Light touch sensitivity was evaluated using the cotton swab test, as previously described²⁸. Briefly, the cotton swab tip was fluffed to three times its original volume, and five gentle strokes were applied from the heel to the toes of the hind paw, with a 3-min interval between each stroke. Positive responses, including paw withdrawal, shaking, or sustained licking, were recorded, whereas the absence of such behaviors was considered a negative response. The response frequencies and average scores were documented. The scoring criteria were as follows: 0 = no response; 1 = transient rapid paw movement or brief paw lifting; 2 = prolonged lifting of the paw toward the body for more than 2 s or forceful lateral lifting above the body level; and 3 = flinching responses or licking of the stimulated paw.

2.5.3. Light brush test

Tactile sensitivity was evaluated using a brush applied in five consecutive heel-to-toe strokes across the hind paw plantar surface. Positive responses (paw withdrawal/shaking/sustained licking) were quantified as both response frequency and mean scores. The scoring criteria were identical to those used in the cotton swab test.

2.5.4. Sticky tape test

The adhesive tape removal assay was performed by affixing a standardized 10 mm $\times$ 5 mm surgical-grade adhesive patch to the central plantar region of the hind paws. The latency to initiate removal attempts (*e.g.*, biting, licking, or paw shaking) and the frequency of such behaviors were recorded.

2.5.5. Cold plate test

To assess nociceptive responses to cold stimulation, the mice were subjected to a 4 °C cold plate (IITC Life Science, Harvard Apparatus). The latency to exhibit nocifensive behaviors, including hind paw withdrawal, licking, or jumping, was recorded as the primary outcome measure. The tests employed a triple-trial paradigm with 15-min intertrial intervals and a cutoff time of 45 s implemented as a safety measure to minimize potential tissue trauma.

2.5.6. Hot plate test

Thermal nociception was evaluated using a precision-controlled hot plate apparatus (IITC Life Science, Harvard Apparatus)

maintained at three distinct temperatures: 50, 52, and 54 °C. The latency to display nocifensive behaviors (hind paw withdrawal, licking, or jumping) was recorded for each temperature condition. The testing protocol included three trials per temperature with 15-min intertrial intervals, and a 45-s cutoff time was established to prevent thermal tissue injury.

2.5.7. Hargreaves test

Thermal nociceptive latency was quantified using a radiant heat source (Taimeng, PL-200, China) targeted to the central plantar region of rodent hindpaws. Testing employed a triplicate trial design with 15-min intertrial intervals, incorporating a 30-s ceiling latency to preclude thermal injury.

2.6. Electrophysiology

Whole-cell patch-clamp electrophysiological recordings were acquired using an Axon 700B amplifier with pClamp 10.3 (Molecular Devices) at 4 h post-plating under thermoregulated conditions (25 ± 2 °C). Current-clamp and voltage-clamp configurations were employed to characterize APs and I_A currents, respectively. The signals were subjected to low-pass filtering at 2 kHz and were sampled at 10 kHz. The patch pipettes exhibited a resistance of 2–3 M Ω . The intracellular solution used for current-clamp recordings comprised the following (in mmol/L): 140 KCl, 2 MgCl₂, 11 EGTA, 1 CaCl₂, and 10 HEPES (the pH was adjusted to 7.4 with KOH, osmolarity 290 ± 5 mOsm). The extracellular mixture contained (in mmol/L) 150 NaCl, 5 KCl, 2.5 CaCl₂, 1 MgCl₂, 10 glucose, and 10 HEPES (the pH was adjusted to 7.4 with NaOH, osmolarity 310 ± 5 mOsm). Key electrophysiological parameters were quantified from APs elicited at the rheobase. For I_A current recordings, the intracellular solution comprised the following (in mmol/L): 1 MgCl₂, 140 KCl, 0.5 CaCl₂, 10 HEPES, 0.3 Na-GTP, 5 EGTA, and 3 Mg-ATP (the pH was adjusted to 7.4 with KOH). The extracellular mixture consisted (in mmol/L) 150 choline chloride, 5 KCl, 0.03 CaCl₂, 1 MgCl₂, 10 glucose, and 10 HEPES (pH 7.4 adjusted with KOH). For I_A current recording, we used a two-step voltage protocol in accordance with the method reported by Hu et al.²⁹.

2.7. Tissue processing and immunostaining

DRGs were fixed in cold 4% PFA overnight, and then dehydrated sequentially in 10% and 30% sucrose solutions at 4 °C for 12 h each. Tissues were embedded in Tissue-Tek OCT compound (Sakura, 4583) and frozen at -80 °C overnight. The samples were sectioned into 10 μ m slices. At room temperature, sections on adhesive-coated slides were washed with PBS three times (10 min each). The sections were permeabilized with 0.3% Triton X-100 and 3% BSA in PBS at 37 °C for 1 h. After non-specific binding was blocked with 10% goat serum for 1 h, all the sections were incubated overnight at 4 °C with primary antibodies against Kv4.3 (1:250, 75-017, NeuroMab), Piezo2 (1:200, #NBP1-78624, Novus Biologicals), Kv7.2 (1:100, PA1-929, Invitrogen), HCN1 (1:50, 55222-1-AP, Proteintech), TH (1:80, ARG11149, Arigo), TH (1:200, 25859-1-AP, Proteintech), CGRP (1:200, ab283568, Abcam), NFH (1:200, ab207176, Abcam), and parvalbumin (1:200, 29312-1-AP, Proteintech). Following three washes, the tissue sections were subjected to 2 h of ambient incubation with either fluorescent-tagged secondary antibodies or iB4-AF647 (1:400, I32450, Invitrogen), followed by sequential washes and

coverslipping with DAPI-containing antifade mounting medium (SouthernBiotech, 0100-20).

Skin tissue samples were collected from the dorsal region of the mice, fixed in 4% PFA, embedded in paraffin, and then sectioned into 10- μ m slices. The tissue sections were deparaffinized by sequential immersion in xylene two times (20 min each), absolute ethanol two times (10 min each), and then graded ethanol solutions (95%, 85%, and 75%, 5 min each), followed by a final rinse with distilled water. Antigen retrieval was performed using $1 \times$ citrate buffer (pH 6.0) under high-temperature and high-pressure conditions. Endogenous peroxidase activity was quenched by incubating the sections with 3% H₂O₂ at room temperature, and then, 10% goat serum was used to block nonspecific staining at 37 °C for 30 min. The sections were incubated with the anti-Kv4.3 primary antibody (1:250, 75-017, NeuroMab) at 4 °C overnight, followed by three times PBS washes (10 min each). An HRP-conjugated goat anti-mouse secondary antibody (Pnuofei, PN0080) was used to incubate the samples at 37 °C for 1 h. After three 5-min washes with PBS, the sections were incubated with TYR-488 (Pnuofei, PN0100) at 37 °C for 30 min, followed by additional washes with PBS. Microwave antigen retrieval was repeated using $1 \times$ citrate buffer (5 min at high heat), followed by cooling the sections to room temperature. After reblocking with 10% goat serum at 37 °C for 30 min, the sections were incubated at 4 °C overnight with an anti-TH primary antibody (1:200, 25859-1-AP, Proteintech). Following PBS washes, the sections were incubated with an HRP-conjugated goat anti-rabbit secondary antibody (Pnuofei, PN0046) for 1 h at 37 °C and then with TYR-555 (Pnuofei, PN0101) for 30 min at 37 °C. After three 5-min rinses with PBS, the sections were mounted with DAPI-containing antifade mounting medium.

2.8. DRG microinjection

Mice were anesthetized with 2% isoflurane. After shaving and disinfecting the surgical area, the animals were positioned in a prone orientation. The surgical site was located at the space between the fourth and fifth lumbar vertebrae and was identified along the line connecting the bilateral anterior superior iliac spines. A skin incision was made at the site, followed by blunt dissection of the muscular layer to fully expose the transverse and spinous processes of the third and fourth lumbar vertebrae. Using microscissors, the transverse and spinous processes of L3 and L4, along with the surrounding soft tissues, were meticulously removed to achieve complete visualization of the L3 and L4 DRGs. A microsyringe equipped with a glass electrode was vertically inserted into the DRG to a depth of 0.2–0.3 mm, and 2 μ L of viral solution was administered at a rate of 200 nL/min. The glass electrode remained *in situ* for 10 min to facilitate viral absorption before being withdrawn. The muscular layers and skin were subsequently sutured in sequence.

The AAV constructs employed in this study included AAV9-EF1 α -DIO-hM4D(Gi)-mCherry and AAV9-EF1 α -DIO-mCherry, obtained from Vigene Biotechnology Co., Ltd. (Shandong, China), as well as AAV2/9-TH-mir30-sh-*kcnk3* and AAV2/9-TH-mir30-sh-*Scr*, sourced from Hanbio Technology Co., Ltd. (Shanghai, China). All viral preparations presented titers exceeding 1×10^{12} vg/mL.

2.9. Implantation of DRG cannula in mice

A cannula targeting the DRG was surgically implanted to allow for focal application of substances, as previously described^{30–32}.

Isoflurane anesthesia was administered to the mice, with 2% for anesthesia induction, and 1%–1.5% for anesthesia maintenance, and the mice were positioned in a prone posture on a thermoregulated surgical platform. The L4–L5 intervertebral space was identified through palpation of the iliac crest and alignment of the spinal processes. Following aseptic preparation, a midline incision was made to expose the L4 lamina *via* layered blunt dissection. A cranial drill (0.8 mm diameter) was used to create an aperture in the right lamina of the fourth lumbar vertebra adjacent to the spinous process, with a downward and slightly medial trajectory. Drilling was terminated promptly upon the detection of loss of resistance and the onset of right hindlimb kicking, which confirmed successful access to the dorsal root ganglion. Tail twitching during drilling indicated inadvertent spinal cord penetration, necessitating immediate cessation of the procedure. The surgical site was cleansed with sterile cotton swabs, and a micropipette cannula (inner diameter: 0.64 mm, length: 4 mm) was implanted. Dental cement was uniformly applied to secure the muscular layer, cannula, and skin, followed by a 10-min curing period.

2.10. Western blot

Lysis buffer was prepared with RIPA lysis buffer supplemented with 1% protease inhibitor cocktail. DRG tissues (L3–L5) were mechanically homogenized in precooled lysis buffer, followed by centrifugation ($12,000 \times g$, 10 min, 4 °C) to isolate the supernatant. SDS–PAGE was used to separate protein samples. Then proteins were subsequently transferred onto PVDF membranes. At room temperature, 5% nonfat dry milk was used to block the membranes for 2 h, followed by incubation with primary antibodies at 4 °C overnight. DyLight 800/700-conjugated secondary antibodies (Rockland, USA) were used to label the primary antibodies in membranes for 2 h at room temperature. Protein signals were detected using the Li-Cor Odyssey infrared imaging system. The following primary antibodies were utilized: anti-Kv4.3 (1:500, ER1802-91, HUAAN Bio), anti-Kv4.1 (1:200, YT2513, Immunoway), anti-Nav1.7 (1:1000, ARG56140, Arigo), anti-Nav1.8 (1:500, ER1706-30, HUAAN Bio), anti-Nav1.9 (1:1000, ARG42591, Arigo), anti-Cav3.2 (1:100, sc-377510, Santa Cruz), and anti- β -actin (1:1000, #4967, CST), anti-Piezo2 (1:1000, #NBPI-78624, Novus Biologicals), anti-Kv7.2 (1:200, PA1-929, Invitrogen), anti-HCN1 (1:500, 55222-1-AP, Proteintech).

2.11. Membrane protein and nuclear protein extraction

Subcellular fractionation of L3–L5 dorsal root ganglia (DRG) collected from four mice was performed using the Minute™ Plasma Membrane/Protein Isolation and Cell Fractionation Kit (Invent, #SM-005) according to the manufacturer's instructions. Briefly, fresh DRG tissues were placed in a filter-equipped cartridge and homogenized on ice with 200 μ L of Buffer A using a pestle. An additional 300 μ L of Buffer A was added and mixed thoroughly. The homogenate was lysed on ice for 5 min with the tube cap open, then centrifuged at $16,000 \times g$ for 30 s at 4 °C. The pellet was resuspended by vortexing and centrifuged at $700 \times g$ for 1 min at 4 °C. The resulting pellet (nuclear fraction) was solubilized in RIPA lysis buffer. The supernatant was transferred to a new 1.5 mL tube and centrifuged at $16,000 \times g$ for 30 min at 4 °C. The pellet was retained, resuspended in 200 μ L of Buffer B, and centrifuged at $7800 \times g$ for 5 min at 4 °C. The supernatant was transferred to a 2.0 mL tube, mixed with 1.6 mL of ice-cold PBS, and centrifuged again at $16,000 \times g$ for 30 min at 4 °C. The

final pellet (membrane fraction) was dissolved in Minute™ Denaturing Protein Solubilization Reagent (Invent, #WA-009). Protein concentrations were determined using a BCA assay kit. $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ (anti- $\text{Na}^+ - \text{K}^+ - \text{ATPase}$, 1:1000, #RT1412, HUABIO) and PCNA (anti-PCNA, 1:1000, #ET1605-38, HUABIO) were used as loading controls for membrane and nuclear fractions, respectively.

2.12. Real-time quantitative PCR

Total RNA was isolated from the DRG tissues using a commercial RNA extraction kit (Promega, USA). cDNA was generated by the Promega Reverse Transcription System following the manufacturer's protocols. Quantitative PCR was carried out with a commercial kit (GoTaq qPCR Master Mix, Promega). Transcript quantification was conducted with β -actin as the endogenous reference, with relative expression values derived through a comparative threshold cycle ($2^{-\Delta\Delta C_t}$). The gene-specific sequences of primers were listed in Supporting Information Table S1.

2.13. Tissue-specific gene expression analysis

The gene expression data of the DRG neurons were acquired from the XSpeciesDRGAtlas database (<http://research-pub.gene.com/XSpeciesDRGAtlas/>). Bubble plots were generated by RStudio.

2.14. RNAscope combined with immunofluorescence co-detection

According to the manufacturer's protocol of the RNAscope™ Multiplex Fluorescent Reagent Kit v2 (Advanced Cell Diagnostic, ACD, 323100), we performed RNAscope *in situ* hybridization combined with immunofluorescence staining. Briefly, fresh mouse DRG tissues were embedded in Tissue-Tek® O.C.T. compound (Sakura, 4583), rapidly frozen in liquid nitrogen, and sectioned at 16 μ m thickness using a Leica cryostat. The sections were mounted on SuperFrost™ Plus slides (Thermo Fisher, 4951PLUS-001E), fixed in pre-chilled 10% neutral buffered formalin at 4 °C for 1 h, and then washed twice in $1 \times$ PBS for 5 min each. Subsequently, the sections were dehydrated in a graded ethanol series (50%, 70%, and 100%) for 5 min each at room temperature. The hydrogen peroxide reagent was applied for 10 min at room temperature, followed by Pretreat Pro reagent 10 min at 40 °C. Target probes were hybridized for 2.5 h at 40 °C in a HybEZ oven. Amplification steps were then performed sequentially using AMP 1 (30 min), AMP 2 (30 min), AMP 3 (15 min), and HRP-C1 (15 min), followed by incubation with Opal 520 ($1 \times$, 30 min) and HRP blocker (15 min), all carried out at 40 °C in the HybEZ oven. After RNAscope processing, the sections were blocked with 10% normal goat serum for 1 h and then incubated overnight at 4 °C with the following primary antibodies: anti-TH (1:200, 25859-1-AP, Proteintech), anti-CGRP (1:200, ab283568, Abcam), and anti-NFH (1:200, ab207176, Abcam). Corresponding secondary antibodies were applied for 2 h at room temperature. Finally, nuclei were counterstained with DAPI, and slides were coverslipped with anti-fade mounting medium (Invitrogen, P10144). Custom target and standard control probes were provided by ACD including RNAscope™ Probe-Mm-Kcnd3-O1 (ACD, 490451), RNAscope™ Positive Control Probe-Mm-Ppib (ACD, 313911), and RNAscope™ Negative Control Probe-DapB (ACD, 310043).

2.15. Quantification and statistical analysis

The data are shown as mean $\pm$ standard error of mean (SEM). Continuous variables underwent normality assessment *via* the Shapiro–Wilk and Kolmogorov–Smirnov tests. Two-group comparisons utilized unpaired Student's *t* tests (normally distributed data) or Mann–Whitney U tests (nonnormal distributed data). Paired *t* tests were used to evaluate the differences before and after the intervention. One-way ANOVA with Tukey's *post hoc* test was employed for parametric data, following confirmation of normality and homogeneity of variance for multiple group comparisons. The AP frequency under varying currents was analyzed by two-way ANOVA. The behavioral data collected over days were analyzed using two-way repeated-measures ANOVA. Statistical analyses were conducted using SPSS version 25.0, with significance set at $P < 0.05$.

3. Results

3.1. TH^+ and TH^- small neurons show markedly distinct electrophysiological properties

Among C-type unmyelinated primary sensory neurons, TH -positive C-LTMRs exhibit distinct functional properties and are uniquely capable of transducing two antagonistic modalities of tactile perception: pleasurable tactile sensation and injury-evoked mechanical nociception³³. To investigate the electrophysiological characteristics of C-LTMRs, we generated a *TH-Cre-Ai9* conditional reporter mouse line in which the majority of C-LTMRs in the DRG were fluorescently labeled with tdTomato, enabling the precise identification of C-LTMRs (TH^+) from other small-diameter neuronal populations (TH^-)¹⁴. Acutely dissociated DRG neurons from these mice were subjected to whole-cell patch-clamp recordings (Fig. 1B), with a focus on small-diameter neurons (somatic diameter $<30\ \mu\text{m}$), which constitute the principal cellular substrates mediating peripheral nociceptive signal transduction³⁴. Electrophysiological analysis revealed significant differences between TH^+ and TH^- neurons. TH^+ neurons displayed a delayed firing pattern, characterized by prolonged latency to the first action potential (AP) at the rheobase current ($115.55 \pm 21.85\ \text{ms}$ in TH^+ neurons vs. $16.24 \pm 2.41\ \text{ms}$ in TH^- neurons), followed by sustained repetitive firing (Fig. 1C and F), which is consistent with previous findings³⁵. Additionally, TH^+ neurons presented a lower rheobase ($56.67 \pm 9.07\ \text{pA}$) than did TH^- neurons ($116.47 \pm 24.22\ \text{pA}$, Fig. 1G). However, other AP properties, including the resting membrane potential (RMP), AP threshold, AP half-width, AP amplitude, and after-hyperpolarization (AHP) amplitude, were comparable between TH^+ and TH^- neurons (Fig. 1D, E and H–K). These findings demonstrated that TH^+ and TH^- small-diameter neurons exhibited intrinsic electrophysiological divergence under physiological conditions.

3.2. Oxaliplatin selectively alters the firing patterns of TH^+ neurons rather than those of TH^- neurons

To investigate the impact of oxaliplatin on the electrophysiological properties of two subpopulations of small DRG neurons, we established an OIPN mouse model (Fig. 1A). Beginning on Day 7, the mice in the oxaliplatin-treated group exhibited pronounced neuropathic pain, as evidenced by sustained mechanical allodynia

and cold hypersensitivity (Supporting Information Fig. S1A–S1H), which is consistent with previous studies^{26,36}. Following validation of the OIPN model, whole-cell current-clamp recordings of acutely dissociated DRG neurons revealed subtype-specific hyperexcitability in TH^+ neurons. Specifically, oxaliplatin treatment resulted in a 6.301 mV depolarization of RMP (Fig. 1E), a 65.4% reduction in AP latency (Fig. 1F), and a 26.3% decrease in AP threshold (Fig. 1H) compared with those in TH^+ control neurons. No significant differences in other electrophysiological parameters were detected between oxaliplatin-treated and control TH^+ neurons (Fig. 1G and I–K). In contrast, TH^- neurons in the oxaliplatin-treated group exhibited a significant 44.39% reduction in AP threshold, with no notable changes in the other parameters (Fig. 1E–K). Additionally, under oxaliplatin-induced neuropathic conditions, TH^+ neurons presented significantly lower rheobase currents (Fig. 1G) and lower AHP amplitudes (Fig. 1K) than did TH^- neurons, further indicating heightened intrinsic excitability in the TH^+ subpopulation during pathological sensitization.

Although oxaliplatin induced hyperexcitability in both TH^+ and TH^- small neurons, as evidenced by an overall increase in firing frequency, their responses to varying current intensities revealed distinct patterns. TH^+ neurons exhibited a marked increase in average AP number during lower-intensity stimulation (100–400 pA) (Fig. 1M), whereas TH^- neurons displayed this effect under higher-intensity stimulation (250–500 pA) (Fig. 1L). Notably, oxaliplatin selectively abolished the unique delayed firing pattern characteristic of TH^+ neurons (Fig. 1F). Collectively, these findings demonstrated that oxaliplatin induced hyperexcitability in small DRG neurons and selectively modulated the firing patterns of TH^+ neurons, distinguishing them from TH^- neurons.

3.3. The chemogenetic silencing of DRG TH^+ neurons reverses oxaliplatin-induced mechanical allodynia

Given that oxaliplatin selectively altered the firing patterns of TH^+ neurons in the DRG, a subpopulation corresponding to C-LTMRs, we hypothesized that these neurons may functionally mediate the development of oxaliplatin-induced mechanical hyperalgesia and tactile allodynia through the aberrant amplification of mechanosensory signals. To investigate the functional contribution of TH^+ neurons to pain behaviors, we employed a chemogenetic strategy to achieve acute neuronal inhibition during behavioral assessments^{37,38}. Specifically, for the experimental group, we expressed an inhibitory DREADD, hM4D(Gi), in the unilateral L3/L4 DRG by microinjecting the Cre-recombinase-dependent virus AAV9-EF1 α -DIO-hM4D(Gi)-mCherry into *TH-Cre* mice. The control group, which received AAV9-EF1 α -DIO-mCherry, underwent virus injection simultaneously (Fig. 2A). Following a 4-week viral expression period, an OIPN model was established, and behavioral phenotyping was conducted on day 8 after initial oxaliplatin administration (Fig. 2B). And mCherry fluorescence was detected in the DRG at 4 weeks after AAV9 injection, confirming successful viral infection (Fig. 2C). Prior to behavioral testing, both the control and experimental groups received intraperitoneal (i.p.) injections of the DREADD ligand clozapine-*N*-oxide (CNO, 2 mg/kg) to activate the inhibitory DREADD receptor hM4D(Gi), with nociceptive threshold assessments performed 30 min post-injection. As expected, hM4D(Gi) receptor expression under baseline physiological conditions did not alter baseline responsiveness to mechanical, thermal, or cold stimuli in mice (Fig. 2K

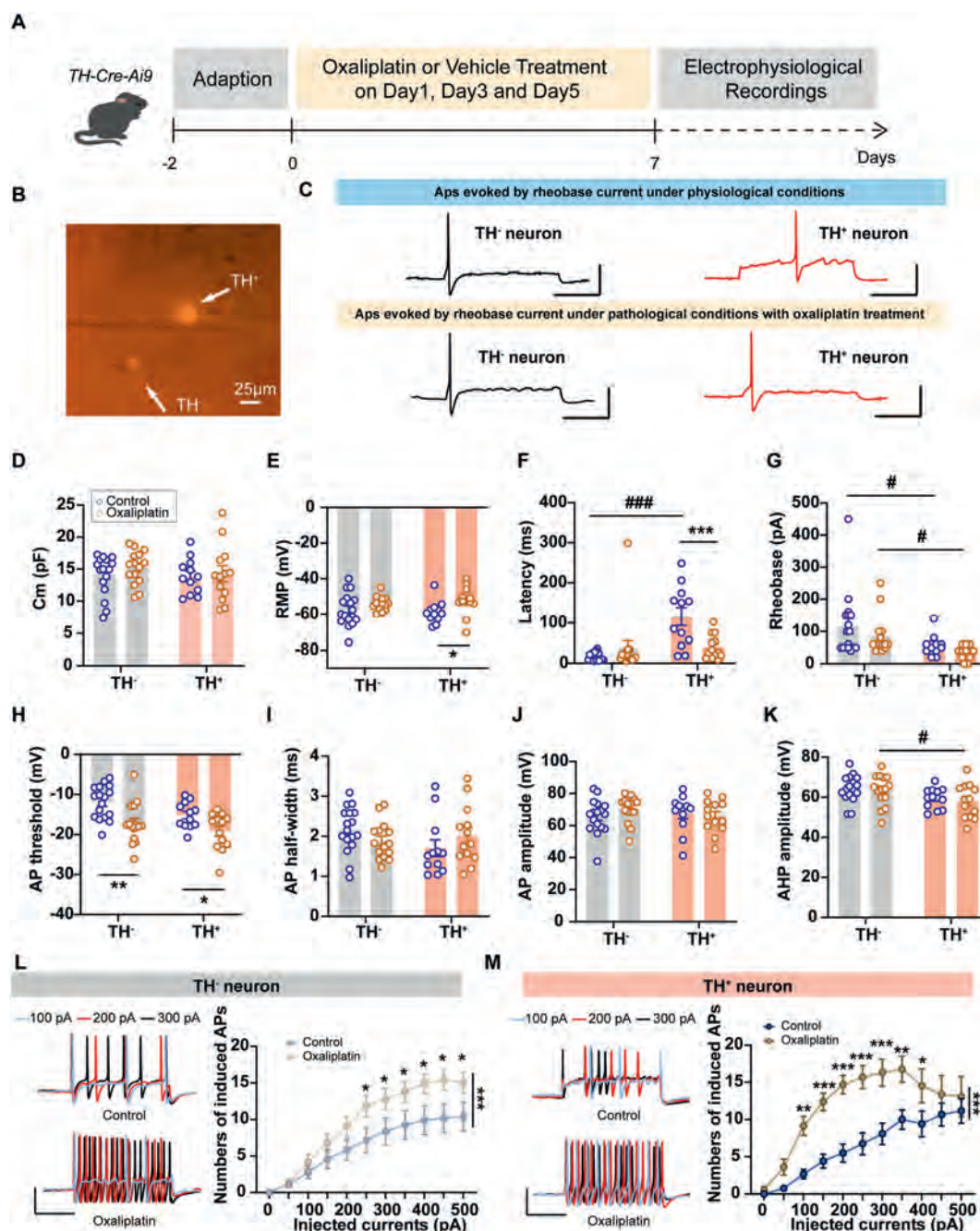


Figure 1 Oxaliplatin chemotherapy selectively modulates the electrophysiological properties of TH^+ DRG neurons. (A) *TH-Cre-Ai9* mice were intraperitoneally injected with oxaliplatin (6 mg/kg) or vehicle control every other day for a total of three doses. Electrophysiological recordings were conducted between Days 8–14 following initial oxaliplatin administration. (B) Schematic representation of whole-cell patch-clamp recordings performed using Tomato fluorescence-positive (TH^+) and Tomato fluorescence-negative (TH^-) neurons. Scale bar: 25 μm . (C) Representative traces of evoked APs in TH^- and TH^+ small-diameter DRG neurons from *TH-Cre-Ai9* mice, comparing naive (upper panel) and oxaliplatin-treated conditions (lower panel). Scale bars: 200 ms and 50 mV. (D–K) Quantitative analysis of electrophysiological parameters, including membrane capacitance (C_m) (D), RMP (E), AP latency (F), current threshold (rheobase) (G), AP threshold (H), AP half-width (I), AP amplitude (J), and AHP amplitude (K) in TH^- ($n = 17$ cells in Control; $n = 15$ cells in Oxaliplatin) and TH^+ ($n = 12$ cells in Control; $n = 13$ cells in Oxaliplatin) DRG neurons from *TH-Cre-Ai9* mice. Statistical comparisons were performed using two-way ANOVA with Sidak's *post hoc* test. (L, M) Representative traces and quantitative analysis of APs evoked by current injection in TH^- (L) ($n = 17$ cells in Control; $n = 15$ cells in Oxaliplatin) and TH^+ (M) ($n = 12$ cells in Control; $n = 13$ cells in Oxaliplatin) small-diameter DRG neurons from *TH-Cre-Ai9* mice under naive and oxaliplatin-treated conditions. Scale bars: 200 ms and 50 mV. Group effects were assessed by two-way ANOVA followed by Bonferroni's *post hoc* test. All data are presented as mean $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. Control; # $P < 0.05$, ### $P < 0.001$ vs. TH^- neurons.

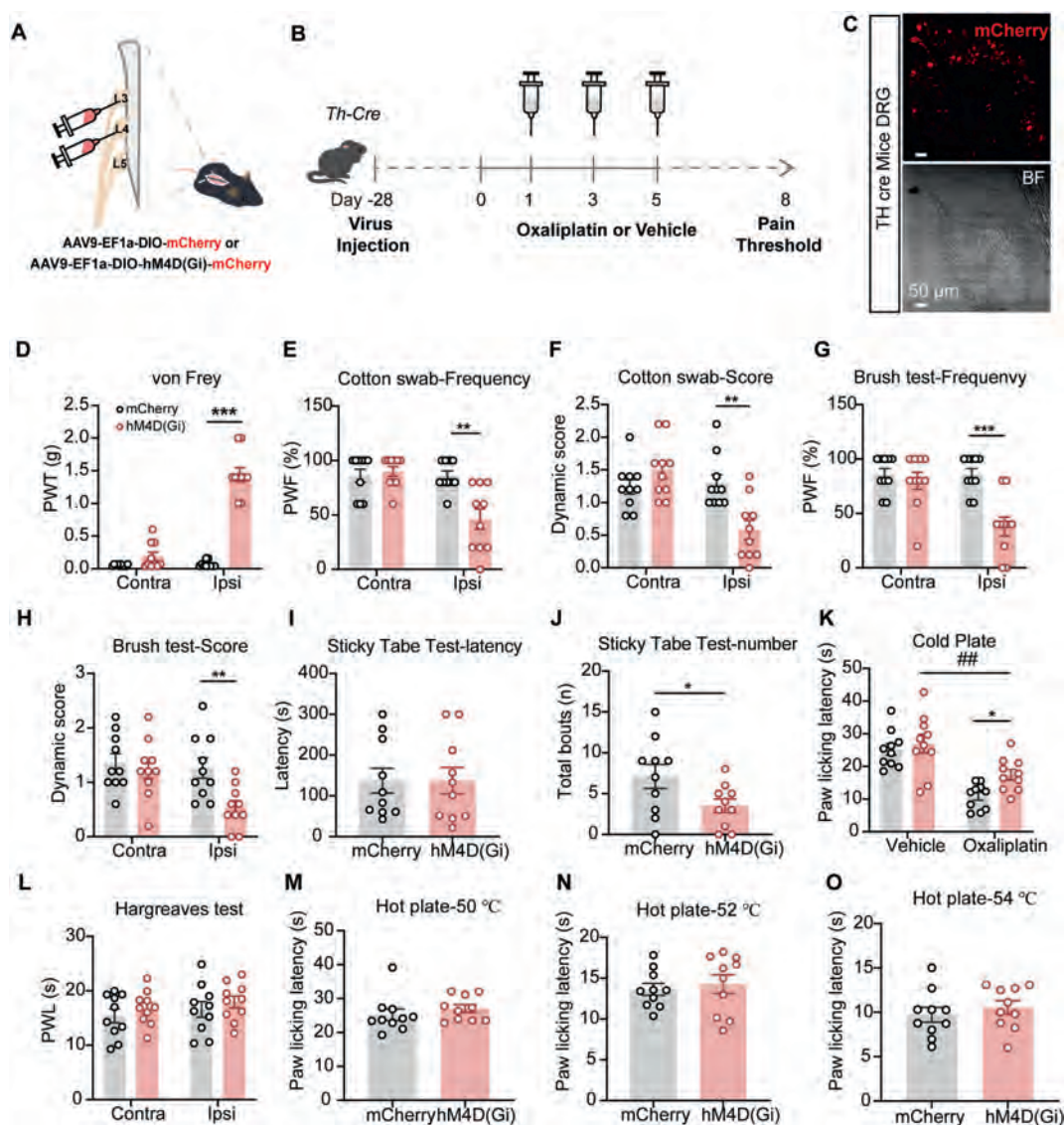


Figure 2 Chemogenetic inhibition of TH⁺ neurons in the DRG attenuates oxaliplatin-induced mechanical allodynia. (A) Schematic representation of chemogenetic viral vector injection targeting the DRG in TH-Cre transgenic mice. (B) Experimental timeline illustrating key procedures: viral vector injection, OIPN model establishment, and behavioral assessments. (C) Verification of viral infection in the mouse DRG. Scale bars: 50 μ m. (D) Quantitative analysis of paw withdrawal thresholds (PWT) in response to von Frey filament stimulation in OIPN mice treated with clozapine-N-oxide (CNO), comparing mCherry control and hM4D(Gi) groups ($n = 10$ per group). Statistical significance determined by Mann–Whitney U test. (E, F) Quantitative assessment of paw withdrawal frequency (PWF) (E) and dynamic score (F) in response to cotton swab stimulation in OIPN mice treated with CNO ($n = 10$ per group). Statistical analysis performed using Mann–Whitney U test or Student’s unpaired t test. (G, H) Quantitative evaluation of PWF (G) and dynamic score (H) in response to light brush stimulation in OIPN mice treated with CNO ($n = 10$ per group). Statistical analysis was performed using Mann–Whitney U test or Student’s unpaired t test. (I, J) Analysis of adhesive tape response latency (I) and removal attempts (J) in OIPN mice treated with CNO ($n = 10$ per group). Statistical analysis performed using Mann–Whitney U test or Student’s unpaired t test. (K) Paw licking latency in response to cold plate stimulation in mice treated with CNO ($n = 10$ per group). Statistical analysis performed using two-way ANOVA with Sidak’s *post hoc* test. (L–O) Quantitative analysis of thermal withdrawal latencies following Hargreaves test (L) and hot plate test (M–O) ($n = 10$ per group). Data presented as mean $\pm$ SEM. Statistical analysis performed using Student’s unpaired t test or Mann–Whitney U test. All data presented as mean $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. mCherry control, ## $P < 0.01$ vs. Vehicle.

and Supporting Information Fig. S2A–S2K), validating the feasibility of this approach without confounding normal sensory modulation. In the OIPN model, the chemogenetic inhibition of TH⁺ neurons *via* CNO administration in the hM4D(Gi) group significantly reduced ipsilateral mechanical hypersensitivity and tactile allodynia compared with those in the mCherry control

group, as evidenced by increased von Frey thresholds and attenuated behavioral responses to innocuous mechanical stimulation, including cotton swab and dynamic brush tests (Fig. 2D–H). In the sticky tape test, mice in the hM4D(Gi) group exhibited fewer attempts to remove adhesive tape, although the initiation latency of tape removal was comparable to that of mice in the mCherry

control group (Fig. 2I and J). These findings collectively demonstrated that the functional silencing of TH⁺ neurons modulated tactile sensory processing, diminished mechanical sensitivity, and reversed oxaliplatin-induced mechanical allodynia, highlighting the critical role of this neuronal subpopulation in OIPN pathogenesis. The cold plate test revealed that chemogenetic inhibition of TH⁺ neurons in hM4D(Gi)-expressing mice partially attenuated oxaliplatin-induced cold hypersensitivity but failed to fully restore nociceptive thresholds to physiological levels (Fig. 2K). This incomplete phenotypic rescue suggested that oxaliplatin-driven cold allodynia involves mechanisms in addition to TH⁺ neuronal hyperexcitability and that other sensory neuron populations may also be involved. These findings align with prior reports demonstrating A-fiber hyperexcitability and enhanced cold-sensitive TRPM8/TRPA1 channels activity in oxaliplatin induced cold allodynia pathogenesis^{39–44}. Moreover, in noxious thermal tests, including the Hargreaves test and hot plate test, there was no significant difference in thermal withdrawal latency following TH⁺ neuron silencing (Fig. 2L–O). Taken together, these results demonstrated that the selective suppression of TH⁺ neuronal hyperexcitability in the DRG preferentially ameliorated oxaliplatin-induced mechanical hyperalgesia and tactile allodynia.

3.4. Kv4.3 channels are highly expressed in TH⁺ neurons in the mouse DRG

To investigate the potential mechanisms underlying the involvement of TH⁺ neurons in oxaliplatin-induced mechanical allodynia, and given the observed alterations in the firing patterns of TH⁺ neurons following oxaliplatin treatment, we initially focused on the major ion channel composition responsible for action potentials in DRG TH⁺ neurons, with particular emphasis on voltage-gated ion channels. In 2019, Zheng et al.³⁵ conducted transcriptomic profiling of major mouse DRG subtypes using deep RNA sequencing and revealed that Kv4.3 was uniquely and highly expressed in C-LTMRs. Electrophysiological studies further demonstrated that A-type potassium currents (I_A) mediated by Kv4 channels constitute the primary component of outward potassium currents in these neurons. Additionally, previous studies have shown that Nav1.7, Nav1.8, Nav1.9, and Cav3.2 channels which are enriched in C-LTMRs, are involved in the regulation of pain sensation by these neurons^{35,45–49}. To further elucidate the enrichment characteristics of voltage-gated ion channel genes in distinct neuronal subpopulations of the DRG, we obtained preprocessed gene expression data of DRG neurons from the XSpeciesDRGAtlas database (<http://research-pub.gene.com/XSpeciesDRGAtlas/>). The standardized datasets integrated in this database were derived from a publicly available single-cell RNA sequencing (scRNA-seq) resource (GSE201654)⁵⁰. The original data have undergone bioinformatics processes including quality control, cell clustering, and gene expression quantification, thereby providing a standardized data foundation for subsequent analyses of gene enrichment specific to different subpopulations. Our analysis confirmed the findings of Zheng et al.³⁵, who demonstrated that *Kcnd3* (encoding the Kv4.3 channel) is highly expressed specifically within the C-LTMR subpopulation (Supporting Information Fig. S3A). This cross-validation between independent datasets strengthens the reliability of this expression pattern, suggesting the robustness of this molecular signature in C-LTMRs. However, this result contradicts previous reports that Kv4.3 channels are predominantly distributed in iB4⁺ neurons and coexpressed with the nociceptor marker TRPV1^{51–56}, as C-LTMRs

neither bind iB4 nor express TRPV1¹⁴. To address this discrepancy, we performed immunofluorescence staining of frozen tissue sections coupled with fluorescence confocal microscopy to systematically determine the expression patterns of Kv4.3 channels across distinct neuronal subpopulations within the DRG (Fig. 3 and Fig. S3B). We utilized the following neuronal markers: neurofilament heavy chain (NFH) for myelinated large neurons, calcitonin gene-related peptide (CGRP) for peptidergic small neurons, parvalbumin (PV) for proprioceptive neurons, isolectin B4 (iB4) for nonmyelinated nonpeptidergic small neurons, and TH for C-LTMRs. Our analysis revealed distinct coexpression patterns of Kv4.3 channels with these markers: TH, iB4, CGRP, NFH, and PV were detected in 51.8%, 41.3%, 11.1%, 7.8%, and 0.5% of Kv4.3⁺ cells, respectively (Fig. 3A and C). The experimental data revealed a distinct pattern of Kv4.3 channel distribution across neuronal subpopulations, with preferential localization in TH⁺ neurons (C-LTMRs) and iB4⁺ neurons (nonpeptidergic unmyelinated neurons). Strikingly, 96.4% of TH⁺ neurons expressed Kv4.3, in contrast to only 38.0% of iB4⁺ neurons (Fig. 3B). Further quantification of Kv4.3 fluorescence intensity in colabeled neurons revealed significantly higher Kv4.3 protein levels in TH⁺ neurons than in their iB4⁺ counterparts (Fig. 3D). While minimal Kv4.3 expression was observed in the CGRP⁺ (peptidergic nociceptors) and NFH⁺ (myelinated large neurons) subpopulations, Kv4.3 was virtually undetectable in PV⁺ (proprioceptive) neurons (Fig. 3B and C). In contrast, other voltage-gated ion channels, including Kv4.1, Nav1.7, Nav1.8, Nav1.9, and Cav3.2, although abundantly expressed in TH⁺ neurons, demonstrated a more widespread distribution across various neuronal subtypes (Fig. S3A).

Additionally, we performed RNAscope *in situ* hybridization on DRG tissues from wild-type (WT) mice (Fig. 4A). This highly sensitive and specific technique, combined with immunofluorescence labeling of neuronal markers, was used to further delineate the distribution of *Kcnd3*, the gene encoding the Kv4.3 channel, across neuronal subpopulations. *Kcnd3* transcripts were detected in approximately 63.2% of neurons, with predominant expression in small-diameter DRG neurons (Fig. 4B). Substantial colocalization was observed with TH⁺ and iB4⁺ neuronal populations, and expression levels were markedly higher in TH⁺ neurons compared with other neuronal subtypes (Fig. 4C–E).

These findings collectively established that Kv4.3 channels were selectively enriched in TH⁺ and iB4⁺ neuronal populations, with particularly pronounced expression in TH⁺ neurons. This pattern further implied that the Kv4.3 channel may play a prominent role in modulating the excitability of TH⁺ neurons.

3.5. Oxaliplatin administration modulates A-type potassium currents mediated by Kv4.3 channels in TH⁺ neurons

Kv4 channels, which can be activated at subthreshold membrane potentials, mediate transient outward I_A currents that counteract depolarizing inward currents, delay membrane depolarization, and serve as a critical “braking” mechanism to mitigate neuronal hyperexcitability during repetitive firing^{52,57–59}. Given this functional significance, we examined whether oxaliplatin-induced pathological alterations in the firing patterns of TH⁺ neurons result from the dysregulation of I_A currents mediated by Kv4 channels. To specifically identify TH⁺ neurons, we utilized *TH-Cre-Ai9* transgenic mice, in which TH⁺ neurons were genetically labeled with tdTomato fluorescence. Acutely dissociated DRG neurons from these mice were subjected to whole-cell voltage-clamp

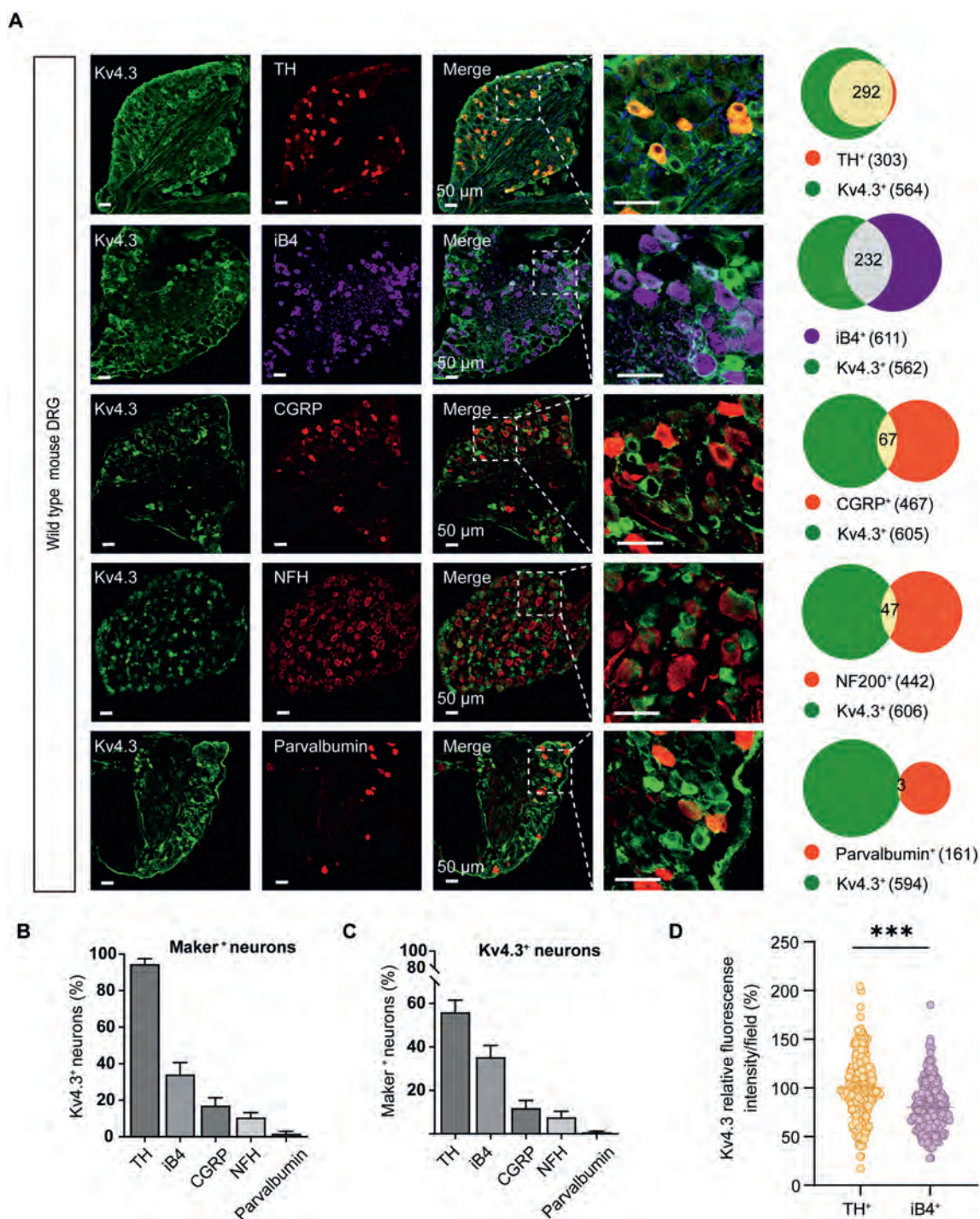


Figure 3 Kv4.3 channels exhibit high expression levels in TH⁺ neurons of DRG. (A) Representative immunofluorescence images demonstrating the colocalization of Kv4.3 (green) with neuronal markers, including TH (red), iB4 (magenta), CGRP (red), NFH (red), and parvalbumin (red) in the DRGs of wild-type mice. The results of quantitative analysis of marker coexpression are presented in a pie chart. Scale bars represent 50 μ m. (B) Quantitative analysis of the percentage of Kv4.3-positive neurons that co-express each specific marker, presented as bar graphs. (C) Quantitative analysis of the percentage of neurons expressing each specific marker that are colabeled with Kv4.3, presented as bar graphs. (D) Summary data illustrating the relative fluorescence intensity of Kv4.3 in colabeled neuronal populations, with the fluorescence intensity of Kv4.3 in TH⁺ neurons was taken as 100%. Statistical analysis performed using Mann–Whitney U test. All data presented as mean $\pm$ SEM. ****P* < 0.001 vs. TH⁺ neurons.

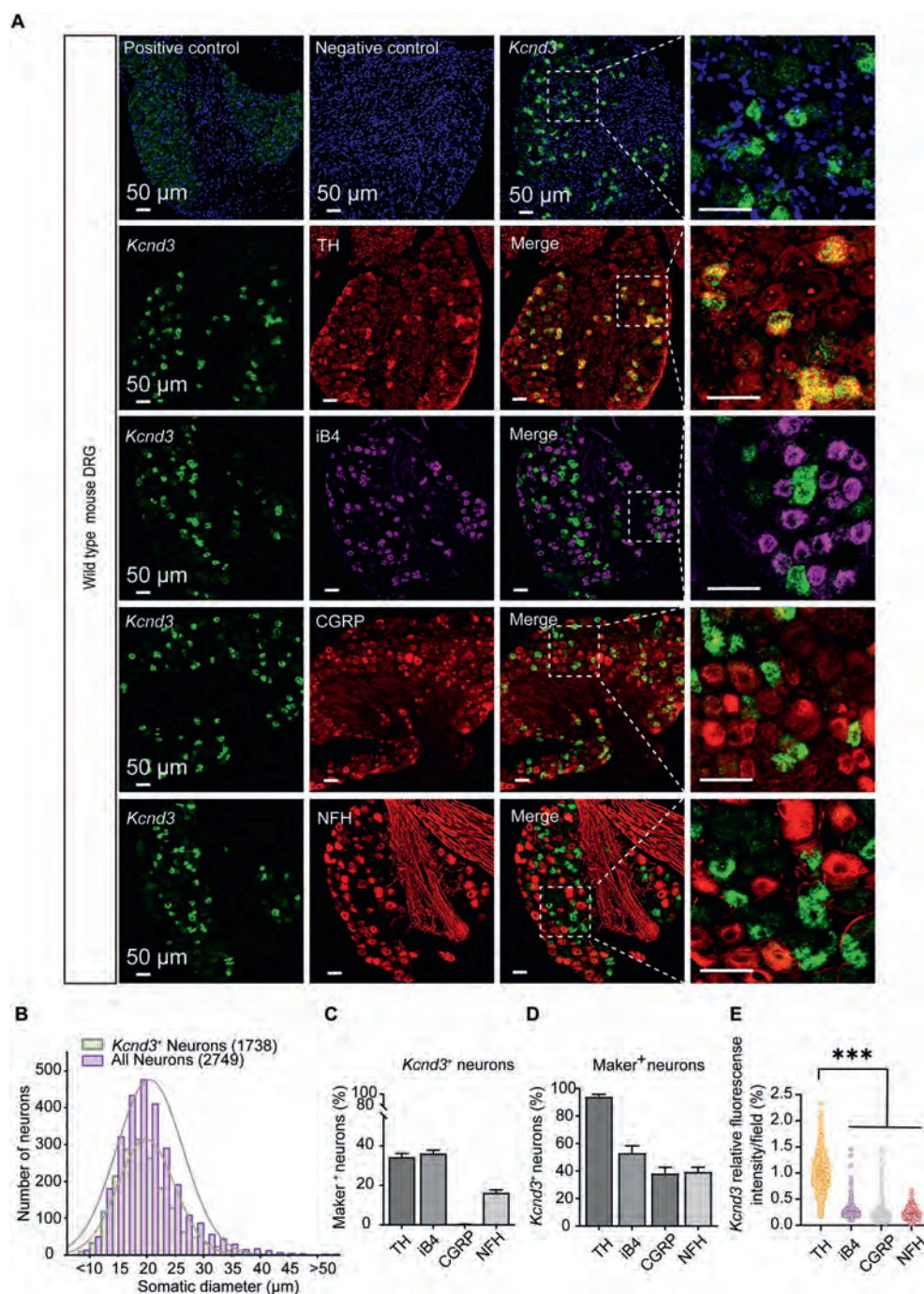


Figure 4 *Kcnd3* exhibits high expression levels in TH⁺ neurons of DRG. (A) Representative RNAscope combined with immunofluorescence co-detection images of mouse DRG with probes for *Kcnd3* (green), *Ppib* (positive control, green), *DapB* (negative control, green) and antibodies against TH (red), iB4 (magenta), CGRP (red) and NFH (red). Scale bars represent 50 μ m. (B) Distributions of *Kcnd3* in mouse DRG neurons. (C) Quantitative analysis of the percentage of neurons expressing each specific marker that are colabeled with *Kcnd3*, presented as bar graphs. (D) Quantitative analysis of the percentage of *Kcnd3*-expressing neurons that co-express each specific marker, presented as bar graphs. (E) Summary data illustrating the relative fluorescence intensity of *Kcnd3* in colabeled neuronal populations, with the fluorescence intensity of *Kcnd3* in TH⁺ neurons was taken as 100%. Statistical analysis performed using Kruskal–Wallis H test followed by Bonferroni's *post hoc* test. All data presented as mean $\pm$ SEM. *** P < 0.001 vs. TH⁺ neurons.

electrophysiology to assess the effects of oxaliplatin treatment on I_A currents (Fig. 5A). Briefly, *TH-Cre-Ai9* mice received intraperitoneal injections of oxaliplatin or 5% glucose vehicle control every other day for a total of three injections, with electrophysiological recordings conducted 8–14 days after the initial

administration. Neurons were selectively isolated from L3–L5 DRGs for recordings. To isolate and record I_A currents, we employed a two-step voltage protocol established in prior studies (Fig. 5B)^{29,60}. The pharmacological application of 4-aminopyridine (4-AP, 5 mmol/L) significantly suppressed the

amplitude of I_A currents in recorded neurons, confirming the specificity and successful isolation of I_A currents under our experimental conditions (Fig. 5B). Our results revealed that, compared with vehicle treatment, oxaliplatin treatment significantly reduced both the peak amplitude and current density of I_A currents in TH⁺ neurons (Fig. 5C). Additionally, peak I_A currents were markedly attenuated at all depolarizing potentials above 10 mV in TH⁺ neurons following oxaliplatin administration (Fig. 5D). Unlike in the control groups, oxaliplatin-induced neuropathy did not significantly affect the I_A amplitude or current density in TH[−] neurons (Fig. 5C and D). We next examined whether oxaliplatin treatment alters the biophysical properties of the A-type potassium channels in TH⁺ neurons. Our results indicated that while oxaliplatin did not affect the voltage-dependent activation of I_A (V_{50} shifted from -9.0 ± 1.3 mV to -11.4 ± 1.4 mV), it induced a hyperpolarizing shift of approximately 18.9 mV in the steady-state inactivation curve (V_{50} shifted from -43.5 ± 1.7 mV to -62.5 ± 3.4 mV) (Fig. 5E–G). Given that the I_A current mediated by the Kv4.3 channel constitutes the major outward potassium current in TH⁺ neurons (C-LTMRs)³⁵, we further assessed the recovery kinetics of total outward potassium currents using a double-pulse protocol with variable interpulse intervals. Oxaliplatin significantly slowed the recovery of the total outward potassium current, with the time constant increasing from 31.5 ± 3.6 ms to 65.4 ± 5.3 ms in TH⁺ neurons (Fig. 5H). Furthermore, we recorded I_A currents in TH⁺ neurons at both short-term (1–3 days) and long-term (14–21 days) time points following oxaliplatin administration. We found that I_A currents remained unchanged in the short term but was significantly reduced in the long term compared with the control group (Supporting Information Fig. S4A–S4B). These results indicate that the decrease in the I_A current occurred after the clear manifestation of pain behaviors and is sustained over an extended period.

The amplitude of the currents is governed by both the intrinsic gating kinetics of ion channels and the density of functional channels on the plasma membrane, the latter being further modulated by gene transcription and protein synthesis. Considering that Kv4 channels in DRG are composed of Kv4.1 and Kv4.3 subunits⁵⁹, we initially assessed the mRNA and protein expression levels of these channels in the DRG. As shown in Fig. 6A and B, oxaliplatin treatment selectively downregulated both the mRNA and protein expression of Kv4.3 channels, but had a minimal effect on Kv4.1 channels. To further investigate the cell type-specific molecular changes induced by oxaliplatin, we conducted immunofluorescence staining to quantify Kv4.3 and Kv4.1 channel protein levels in TH⁺ neurons. Consistent with our Western blot results, the mean fluorescence intensity of Kv4.3 channels in TH⁺ neurons was significantly lower in the oxaliplatin-treated group than in the control group, whereas the Kv4.1 channel fluorescence intensity remained unchanged between the two groups (Fig. 6C and D). To determine whether the functional expression of Kv4.3 channels on the plasma membrane was also reduced, we further examined their surface expression and observed a significant decrease in membrane-localized Kv4.3 channel in oxaliplatin group (Fig. 6E). The repressor element 1-silencing transcription factor (REST), also known as neuronal restrictive silencer factor (NRSF), is a transcriptional repressor of numerous neuronal genes. Upregulation of REST in peripheral sensory neurons has been implicated in the development of neuropathic pain^{61–63}. Previous studies indicated that nerve injury induced downregulation of Kv4.3 mRNA is partly mediated by

REST binding to the promoter region of *Kcnd3*, leading to transcriptional repression through epigenetic mechanisms⁶⁴. We therefore assessed nuclear REST protein levels and found a significant increase in DRG of oxaliplatin-treated mice, a finding consistent with earlier work by Pereira et al.⁶⁵ (Fig. 6F). These results suggested that oxaliplatin may downregulate Kv4.3 expression through REST-dependent transcriptional silencing. Extending our analysis to peripheral cutaneous nerve terminals, we observed a similar reduction in Kv4.3 channel fluorescence intensity within TH⁺ neurons in the oxaliplatin-treated group, which was consistent with the findings in DRG neurons (Supporting Information Fig. S5A and S5B).

Given that C-LTMRs also highly express Nav1.7, Nav1.8, Nav1.9, and Cav3.2 channels, we evaluated the mRNA and protein expression levels of Nav1.7, Nav1.8, Nav1.9, and Cav3.2 in the mouse DRG and found that oxaliplatin treatment did not significantly alter the expression of these ion channels compared with that of ion channels in vehicle-treated controls (Supporting Information Fig. S6A–S6D). In addition to the voltage-gated ion channels mentioned above, M-type potassium channels (Kv7.2/Kv7.3) and HCN channels have also been implicated in various neuropathic pain models, where they regulate neuronal excitability and contribute to pain pathogenesis^{66–72}. Furthermore, the mechanosensitive Piezo2 channel, essential for light touch sensation and serving as a key “touch receptor” in mechanical transduction^{73,74}. We therefore detected whether functional alterations in these channels might contribute to the hyperexcitability of TH⁺ neurons in oxaliplatin-induced neuropathic pain. We first characterized the enrichment of these channels across different DRG neuronal subtypes in rodents using the XSpeciesDRGAtlas database (<http://research-pub.gene.com/XSpeciesDRGAtlas/>)⁵⁰. As shown in Supporting Information Fig. S7A, *Piezo2* was highly enriched in C-LTMRs, *Hcn1* was moderately expressed, while both *Kcnq2* and *Kcnq3* showed low abundance, with *Kcnq2* being slightly higher than *Kcnq3*. We then examined the protein expression of Piezo2, Kv7.2, and HCN1 and evaluated changes in their immunofluorescence intensity within TH⁺ neurons. Our results indicated that oxaliplatin treatment did not significantly affect the expression of these channels (Supporting Information Fig. S7B–S7G).

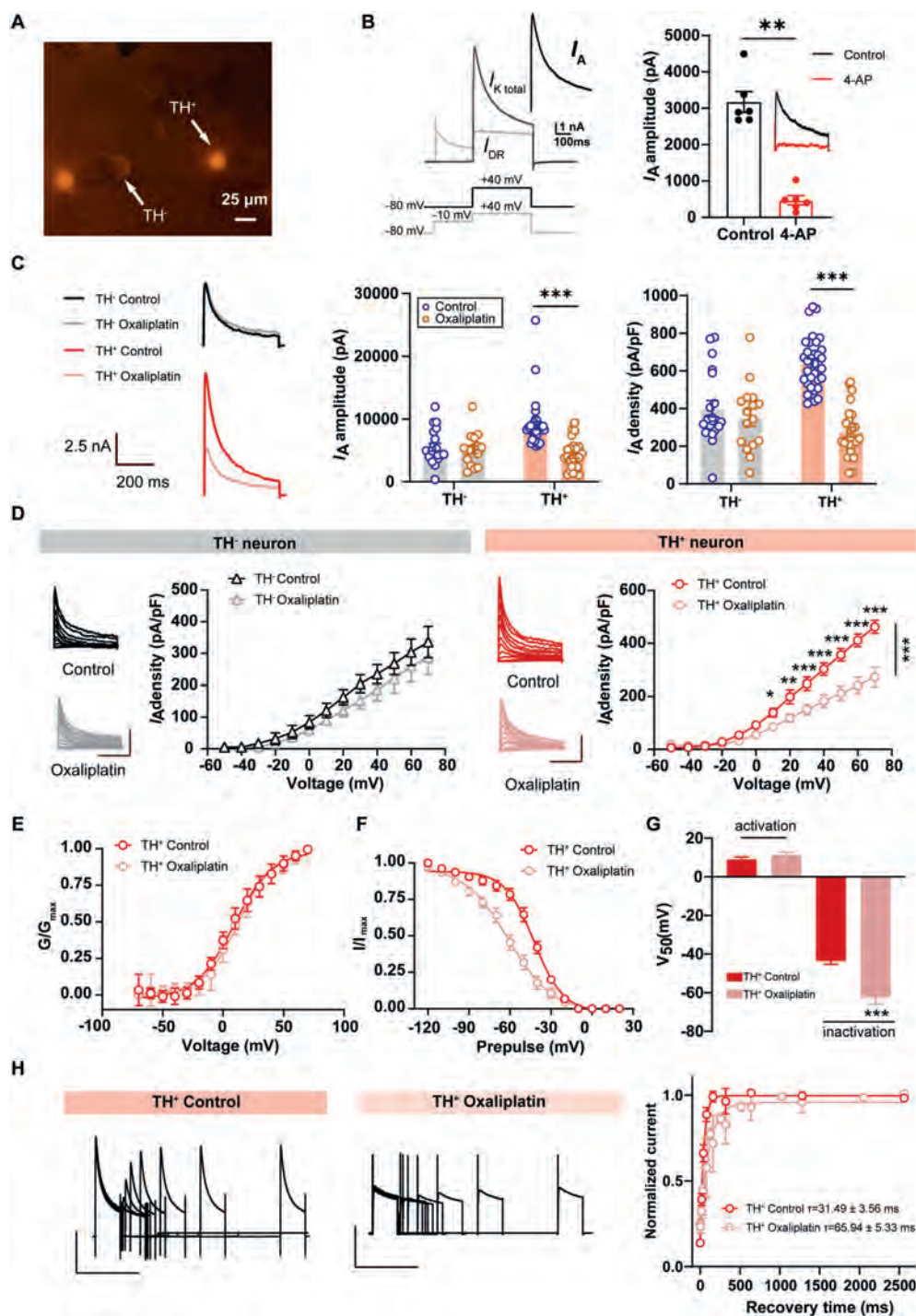
In summary, our findings demonstrated that oxaliplatin selectively impaired Kv4.3 channel function in TH⁺ neurons, through both reduced surface expression and altered biophysical properties.

3.6. Functional inhibition of Kv4.3 channels reduces the pain threshold in mice

Previous investigations have demonstrated that Kv4.3 channel functionality can be pharmacologically modulated through specific agonists and antagonists^{58,75}. To evaluate the therapeutic potential of Kv4.3 channels in OIPN, we employed 4-AP as a pharmacological inhibitor to examine its effects on pain-related behaviors. The experimental design included two distinct drug administration protocols. In the first approach, we implemented a localized drug delivery system utilizing a DRG cannula for the acute administration of either 4-AP (5 mmol/L) or vehicle control (0.9% saline) directly to the lumbar DRG (Fig. 7A). This method allows localized delivery of small-volume compounds directly to DRG without affecting the spinal cord^{30,31}. Furthermore, the cannula implantation itself did not alter baseline pain thresholds in mice (Supporting Information Fig. S8). Specifically, a

microinjection cannula was surgically implanted above the L4 DRG in murine subjects, followed by a 7-day postoperative recovery period. Pharmacological agents (4 μ L) were administered *via* microsyringe, with subsequent behavioral assessments for mechanical allodynia, cold hypersensitivity, and thermal hyperalgesia conducted 10 min postinjection. As expected, compared with the vehicle, the pharmacological inhibition of Kv4.3 channels significantly reduced mechanical withdrawal thresholds and enhanced tactile sensitivity (Fig. 7B–F), whereas no significant alterations were observed in the response to tape application (Fig. 7G). Additionally, nociceptive responses to cold and thermal

stimuli remained unaltered following 4-AP administration (Fig. 7H–L). Building upon our previous observation of the reduced Kv4.3 channel protein expression in the peripheral nerve terminals of OIPN mice, we further investigated the functional consequences of peripheral Kv4.3 channel suppression. This was achieved through the intraplantar administration of 4-AP to target Kv4.3 channels localized on hindpaw nerve terminals (Supporting Information Fig. S9A). Behavioral analyses revealed that peripheral Kv4.3 channel inhibition recapitulated the pain hypersensitivity phenotypes observed with intra-DRG administration, including mechanical hyperalgesia and tactile allodynia



(Fig. S9B–S9G), while maintaining unaltered responses to thermal and cold stimuli (Fig. S9H–S9L). Collectively, these findings suggest that the activity of Kv4.3 channels may serve as a critical endogenous mechanism for counteracting mechanical allodynia.

Considering the nonspecific pharmacological actions of 4-AP as a broad-spectrum potassium channel antagonist, we implemented a genetic approach to systematically investigate the functional contribution of Kv4.3 channels (encoded by *Kcnd3*) within TH⁺ neurons in oxaliplatin-induced mechanical allodynia. To achieve cell type-specific gene silencing, we engineered an adeno-associated virus (AAV) construct under the transcriptional control of the TH promoter, incorporating a short hairpin RNA (shRNA) sequence targeting *Kcnd3*. This AAV vector was unilaterally injected into the L3 and L4 DRGs of wild-type (WT) mice, enabling the conditional suppression of endogenous *Kcnd3* expression specifically in TH⁺ neurons (Fig. 8A). The efficacy of *Kcnd3* knockdown was quantitatively validated through qPCR analyses of mRNA levels and Western blot assessment of Kv4.3 protein expression (Fig. 8B and C). Compared with the AAV-scramble control groups, *Kcnd3*-knockdown (KD) group presented significant decreases in baseline mechanical withdrawal thresholds ipsilateral to the injection site, accompanied by increased tactile sensitivity to cotton swab, light brush, and adhesive tape stimuli (Fig. 8D–J). In contrast, the cold and thermal baseline thresholds remained unaltered, except a slight reduction in thermal latency at 54 °C (Fig. 8K–O). Subsequent oxaliplatin administration in *Kcnd3*-knockdown mice induced cold hyperalgesia but did not further potentiate mechanical hypersensitivity (Supporting Information Fig. S10A–S10L). These data provide compelling evidence that Kv4.3 channels in TH⁺ neurons play a critical role in mediating the oxaliplatin-induced sensitization of mechanosensory pathways, whereas their involvement in thermosensory processing appears to be either secondary or context dependent.

3.7. The pharmacological manipulation of Kv4.3 channels modulates pain behaviors in OIPN model mice

Building upon these findings, we subsequently investigated the therapeutic efficacy of NS5806, a selective activator of Kv4.2/Kv4.3 channel subtypes. To assess whether the NS5806-mediated

potentiation of Kv4.3 currents could mitigate neuronal hyperexcitability in neuropathic pain, we performed whole-cell current-clamp recordings using acutely dissociated small-diameter TH⁺ DRG neurons from OIPN model mice. The acute bath application of NS5806 (10 μmol/L) significantly attenuated pathological hyperexcitability in TH⁺ neurons, as evidenced by several electrophysiological parameters: increased rheobase current for AP generation, elevated AP threshold, prolonged latency to the first spike, reduced AP half-width, and decreased firing frequency (Fig. 9A–G). We further evaluated the *in vivo* analgesic effects of NS5806 in OIPN model mice. On Days 14, 15, and 16 following the initial oxaliplatin injection, OIPN mice received daily intraperitoneal injections of NS5806 (2.5 mg/kg) or vehicle control (Fig. 9H). NS5806 treatment transiently alleviated mechanical hypersensitivity and tactile allodynia on the day of administration, with a time-dependent reduction in analgesic efficacy following treatment cessation (Fig. 9I–O). However, NS5806 had no significant effect on cold hypersensitivity or thermal nociception, as indicated by the unchanged withdrawal latencies in the cold plate and Hargreaves tests (Fig. 9P and Q). Importantly, in OIPN mice with prior *Kcnd3* knockdown in TH⁺ neurons, NS5806 administration failed to rescue mechanical allodynia (Supporting Information Fig. S11A–S11L), confirming that its analgesic effects were mechanistically dependent on Kv4.3 channel potentiation. These findings collectively demonstrated that Kv4.3 channels represent a selective therapeutic target for alleviating mechanical allodynia in neuropathic conditions such as OIPN, with pharmacological activation providing rapid but modality-specific analgesia.

4. Discussion

This study revealed that TH⁺ neurons in the DRG, which are identified as C-LTMRs expressing Kv4.3 channels, play a pivotal role in OIPN, particularly in the pathogenesis of mechanical allodynia. This conclusion was substantiated by the following evidence. (1) Oxaliplatin preferentially enhanced intrinsic excitability and selectively altered firing patterns in TH⁺ neurons while sparing TH[−] nociceptors. (2) The chemogenetic inhibition of TH⁺ neurons reversed oxaliplatin-induced mechanical allodynia but exhibited limited efficacy against cold allodynia. (3) Kv4.3

Figure 5 Oxaliplatin specifically downregulates I_A currents in TH⁺ neurons. (A–D) Electrophysiological assessment of I_A current density in TH⁺ and TH[−] neurons from the DRGs of *TH-Cre-Ai9* mice. (A) Schematic representation of whole-cell patch-clamp recordings performed using Tomato fluorescence-positive (TH⁺) and Tomato fluorescence-negative (TH[−]) neurons. Scale bar: 25 μm. (B) Left: Voltage-clamp protocol for isolating I_A currents using a two-step protocol. Right: Representative current traces (black: Control, red: 4-AP; Scale bar: 1 nA and 200 ms) and summary data demonstrating the reduction in I_A amplitude induced by 4-AP (5 mmol/L). $n = 6$, 6 cells. Statistical analysis was performed using the Mann–Whitney U test. (C) Left: Representative I_A current traces in DRG neurons from control and oxaliplatin-treated *TH-Cre-Ai9* mice. Scale bars: 200 ms and 2.5 nA. Middle and right: Summary data of I_A current amplitude and density in TH[−] ($n = 20$, 16 cells) and TH⁺ ($n = 34$, 25 cells) neurons from control and OIPN model mice. Statistical significance was determined using the Mann–Whitney U test or Student's unpaired t test. (D) Representative I_A current traces and current–voltage (I – V) curves for TH[−] ($n = 13$, 13 cells) and TH⁺ ($n = 19$, 19 cells) neurons from control and OIPN model mice. Group difference was analyzed using two-way ANOVA followed by Bonferroni's *post hoc* test. (E) The voltage-dependent activation curve of I_A in TH⁺ neurons ($n = 16$, 16 cells). Voltage-dependent activation was measured with voltage commands ranging from −70 to +70 mV (400 ms, in 10 mV increment) and the curve was fitted by the Boltzmann equation. (F) The steady-state voltage-dependent inactivation curve of I_A in TH⁺ neurons ($n = 14$, 14 cells). Steady-state voltage-dependent inactivation of I_A was determined by varying a 150 ms conditioning prepulse from −120 to +20 mV followed by a 500 ms voltage step pulse to +40 mV and the curve was fitted by the Boltzmann equation. (G) Summary data of V50 of the activation and inactivation curves. Statistical analysis performed using Mann–Whitney U test. (H) Left: representative current traces of the total outward potassium currents in TH⁺ neurons from control group and oxaliplatin-treated group. Right: recovery from inactivation curves of the total outward potassium currents which were fitted by the single-exponential equation. $n = 13$, 17 cells. Scale bars: 1 s and 10 nA. All data are presented as mean ± SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. Control.

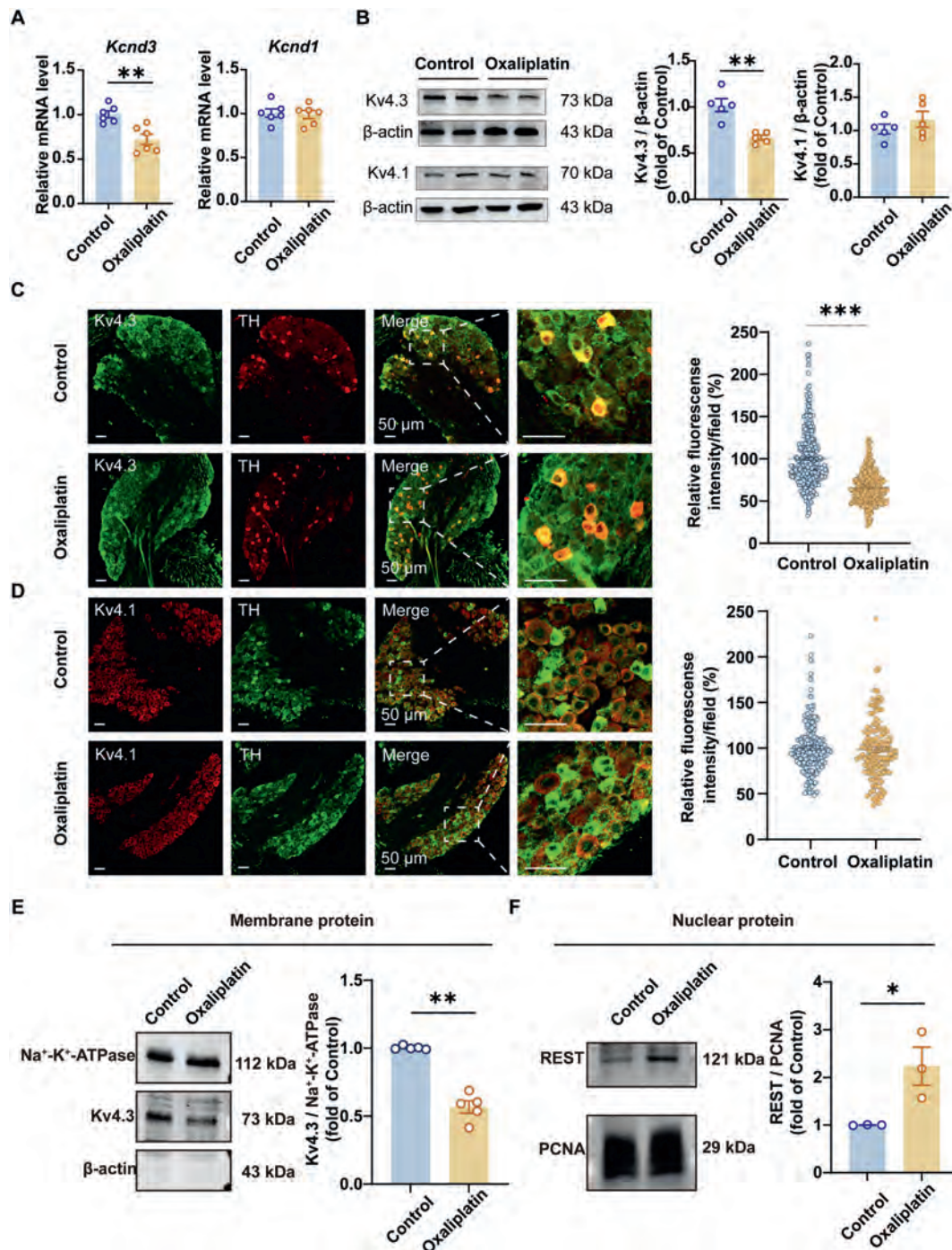


Figure 6 Oxaliplatin specifically downregulates Kv4.3 channel expression in TH⁺ neurons. (A, B) Relative mRNA (A) and protein (B) expression levels of Kv4.3 and Kv4.1 in L3–L5 DRGs from control and oxaliplatin-treated mice. $n = 6$ per group in (A). $n = 5$ per group in (B). Statistical significance was determined using Student's unpaired t test. (C) Left: Representative immunofluorescence images of Kv4.3 (green) and TH (red) in L3–L5 DRGs collected 8 days after the first dose of oxaliplatin. Scale bar: 50 μ m. Right: Quantification of the relative fluorescence intensity of Kv4.3 in TH⁺ cells. Statistical significance was determined using the Mann–Whitney U test. (D) Left: Representative immunofluorescence images of Kv4.1 (red) and TH (green) in L3–L5 DRGs collected 8 days after the first dose of oxaliplatin. Scale bar: 50 μ m. Right: Quantification of relative fluorescence intensity of Kv4.1 in TH⁺ cells. Statistical significance was determined using the Mann–Whitney U test. (E) Relative membrane protein expression level of Kv4.3 in L3–L5 DRGs from control and oxaliplatin-treated mice. $n = 5$ per group. Statistical significance was determined using the Mann–Whitney U test. (F) Relative nuclear protein expression level of REST in L3–L5 DRGs from control and oxaliplatin-treated mice. $n = 3$ per group. Statistical significance was determined using Student's unpaired t -test. All data are presented as mean $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. Control.

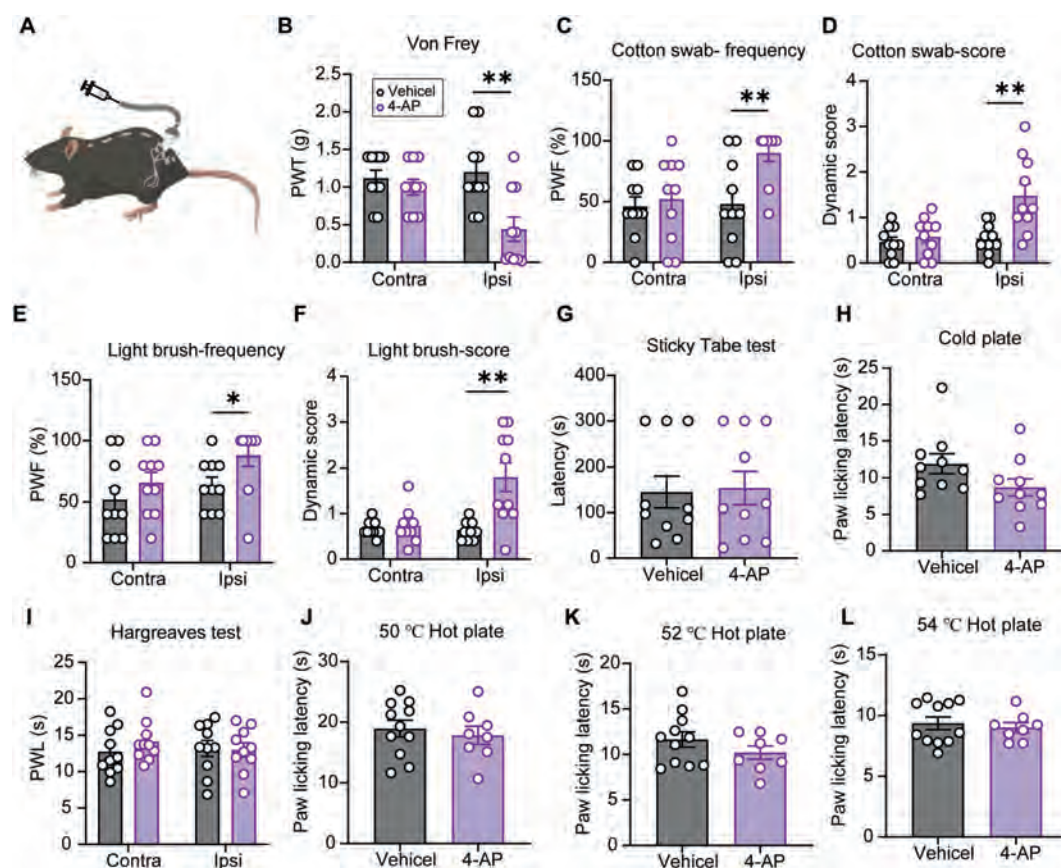


Figure 7 The pharmacological blockade of Kv4 channels potentiates mechanical allodynia. (A) Schematic representation of cannula-mediated drug administration to the DRG. (B–G) The pharmacological inhibition of Kv4.3 channels using 4-AP in the DRG significantly enhanced mechanical hypersensitivity in mice compared with vehicle-treated controls, as demonstrated by multiple behavioral assays: von Frey test (B), cotton swab test (C, D), light brush test (E, F), and sticky tape test (G) ($n = 10$ per group). Statistical significance was assessed using Mann–Whitney U test. (H–L) Nociceptive responses to cold stimuli, as evaluated by the cold plate test (H), and thermal sensitivity, as assessed by the Hargreaves test (I) and hot plate test (J–L), showed no significant alterations following 4-AP administration ($n = 10$ and 10 for cold plate test and Hargreaves test; $n = 11$ and 8 for hot plate test). Statistical analyses were performed using Mann–Whitney U test or Student’s unpaired t -test. All the data are presented as mean $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$ vs. Vehicle.

channels were predominantly distributed in TH⁺ and iB4⁺ neurons, with significantly greater protein density in TH⁺ neurons than in other DRG subpopulations. (4) Oxaliplatin treatment selectively suppressed the function of Kv4.3 channels in TH⁺ neurons. (5) Pharmacological and genetic modulation approaches revealed that Kv4.3 channels in TH⁺ neurons specifically regulated mechanical pain. (6) The pharmacological activation of Kv4.3 channels using a selective opener had analgesic effects on oxaliplatin-induced mechanical allodynia. Collectively, these findings indicated that the downregulation of Kv4.3 channels in C-LTMRs altered the firing patterns of this DRG subpopulation, driving mechanical allodynia in OIPN. This study provides a novel therapeutic strategy for treating OIPN, particularly symptoms related to mechanical allodynia, through the targeted potentiation of Kv4.3 channels.

The OIPN murine model developed in this study, established in the absence of tumor burden and consistent with previously reported protocols for CIPN modeling^{26,76,77}, demonstrated consistent neurological manifestations that closely recapitulated the clinical phenotype observed in patients receiving oxaliplatin-based chemotherapy, as evidenced by the development of mechanical allodynia and cold hypersensitivity (Fig. S1A–S1H). These findings confirmed the successful

establishment of a reliable preclinical model, thus providing a robust platform for investigating the molecular mechanisms underlying CIPN pathogenesis and for evaluating potential therapeutic targets in subsequent pharmacological studies. In clinical practice, cancer patients undergoing chemotherapy often experience two distinct types of pain: neuropathic pain induced by chemotherapeutic agents, and cancer-related pain directly mediated by the tumor microenvironment through mechanisms such as neural infiltration, bone metastasis with nerve compression, and release of inflammatory factors⁷⁸. Cancer-related pain typically presents as persistent dull pain accompanied by spontaneous breakthrough episodes, which is phenotypically distinct from chemotherapy-induced neuropathic pain. It is important to recognize that administration of oxaliplatin alone models only neuropathic pain resulting from chemical injury and fails to fully recapitulate the complex clinical scenario where cancer-related pain and chemotherapy-induced neuropathy coexist. Although rodent CIPN models incorporating tumor burdens may be considered more clinically relevant, their practical and ethical challenges should not be underestimated⁷⁷. A cohort study has revealed that 57.7% of colorectal cancer patients still experience polyneuropathy and neuropathic pain 4–5 years after oxaliplatin treatment, with a

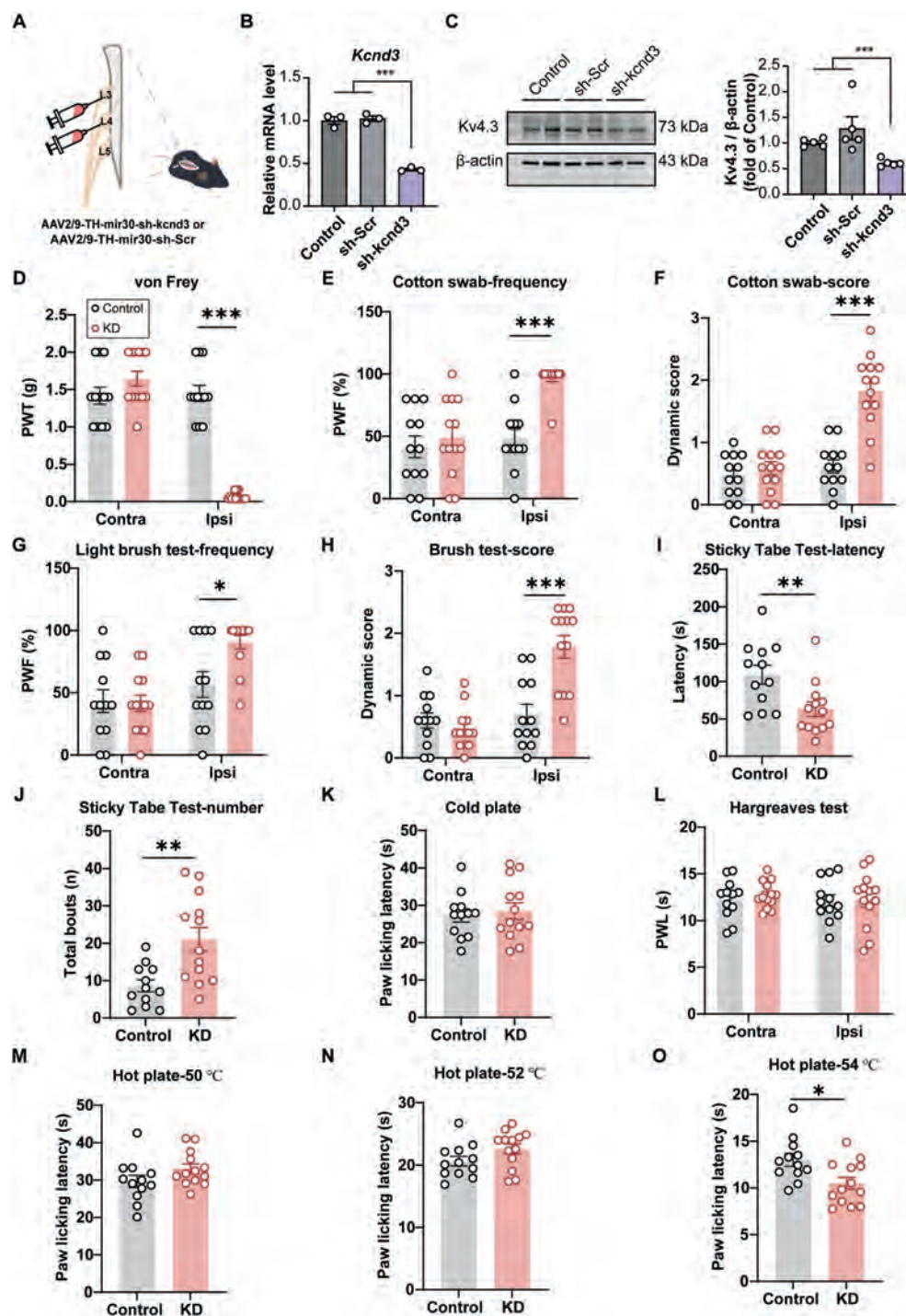


Figure 8 Kv4.3 knockdown in TH⁺ DRG neurons reduces the mechanical threshold in mice. (A) Schematic representation of localized virus injection into L3–L4 DRGs. (B, C) The knockdown efficiency of Kv4.3 in DRG neurons was validated using quantitative reverse transcription PCR (qRT-PCR; B, *n* = 3 per group) and Western blot analysis (C, *n* = 5 per group). Statistical significance was assessed by one-way ANOVA followed by Tukey's *post hoc* test. (D–J) Kv4.3 knockdown in TH⁺ neurons significantly enhanced pain-related behaviors in response to mechanical stimuli, as evaluated using von Frey filaments (D), cotton swabs (E, F), a light brush (G, H), and adhesive tape (I, J). *n* = 12 in Control, *n* = 13 in KD. Statistical significance was determined using Mann–Whitney U test or unpaired Student's *t*-test. (K–O) Behavioral responses to noxious cold stimuli, assessed using a cold plate (K), and thermal stimuli, evaluated using the Hargreaves test (I) and hot plate tests (L–O). *n* = 12 in Control, *n* = 13 in KD. Statistical significance was determined using unpaired Student's *t*-test. All data are presented as mean $\pm$ SEM. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 vs. Control. ###*P* < 0.001 vs. sh-Scr.

significantly higher prevalence compared to chemotherapy-naïve controls⁷⁹, suggesting that persistent pain in these patients is primarily attributable to chemotherapy-induced neuropathic pain. Since chemotherapy is typically administered following surgical tumor resection to eliminate potential micrometastases, modeling CIPN using chemotherapy alone represents a clinically relevant approach. Accordingly, the present study specifically focuses on the neurotoxic effects induced by the chemotherapeutic agent itself.

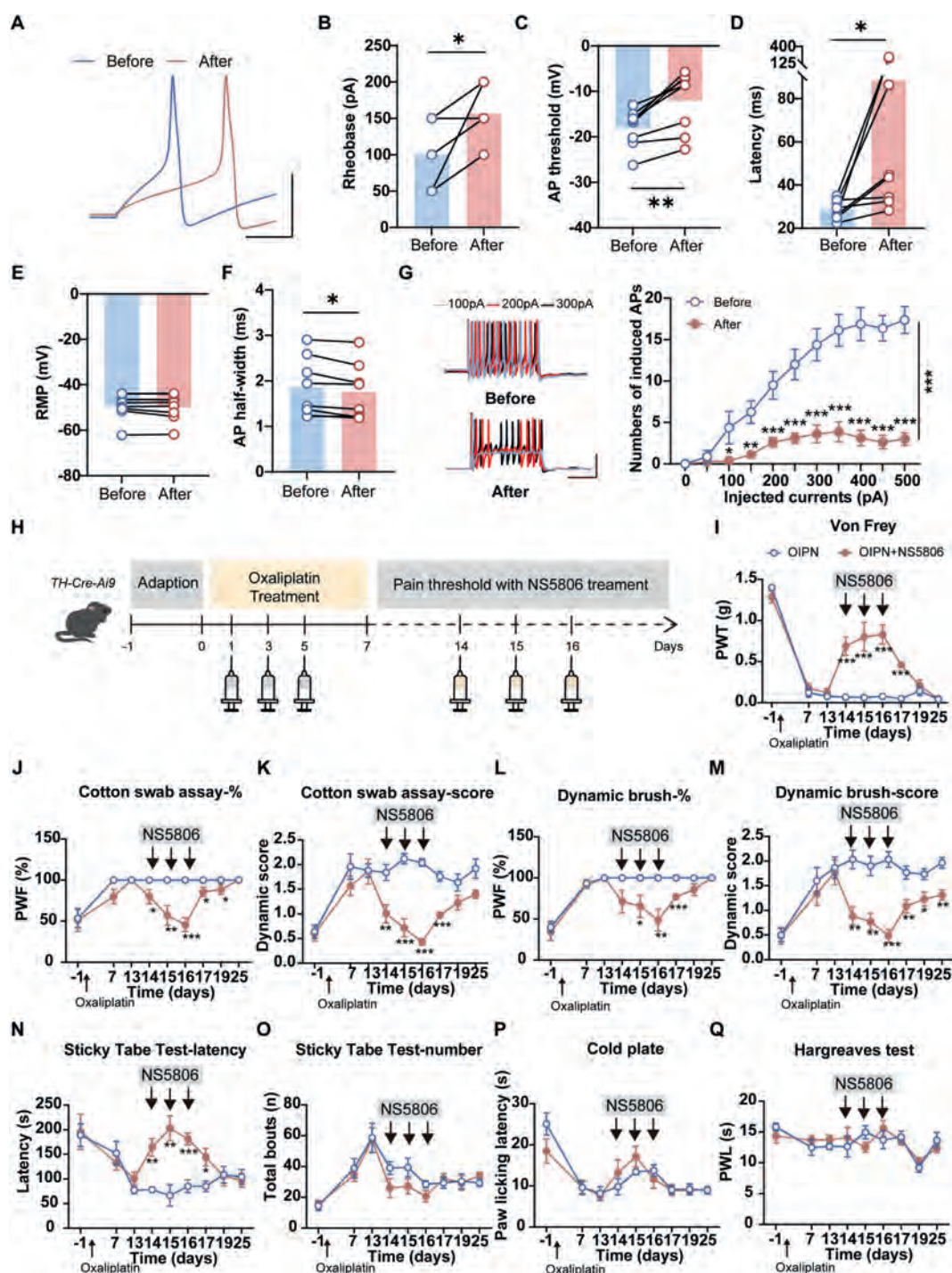
Substantial evidence indicates that peripheral sensitization is a central mechanism underlying peripheral sensitization as a pivotal mechanism in oxaliplatin-induced neuropathic pain, involving the dysregulated expression and altered channel kinetics of various ion channels, including transient receptor potential (TRP) channels (TRPM8, TRPA1 and TRPV1)^{80–85}, two-pore domain potassium channels (TREK1 and TRAAK)⁸⁶, hyperpolarization-activated cyclic nucleotide-gated channels (HCN1)⁸⁶, and voltage-gated sodium channels (Nav1.6)⁸⁷. However, many studies have focused on elucidating cold-evoked hyperalgesia, and the molecular and cellular mechanisms underlying the pathological conversion of innocuous mechanical stimuli (*e.g.*, light touch) into pain signals in oxaliplatin-induced neuropathy remain poorly characterized. In this study, we focused on C-LTMRs, a subset of unmyelinated small-diameter DRG neurons specifically labeled with TH, which are known to mediate pleasant tactile sensations under physiological conditions^{13,14}. Notably, C-LTMRs lack the expression of canonical nociceptor markers. Specifically, C-LTMRs do not express CGRP, tropomyosin receptor kinase A (TrkA), or TRPV1 (markers of peptidergic nociceptors)^{14,88}, or bind iB4 (a marker of nonpeptidergic nociceptors)^{14,88}. Furthermore, C-LTMRs do not express Mas-related G protein-coupled receptors (MrgprA1, MrgprA3, MrgprA4, MrgprB4, or MrgprC11), which are receptors characteristic of nonpeptidergic nociceptive subpopulations^{14,89}. Intriguingly, the majority of TH⁺ neurons coexpress c-Ret and GFR α 2, which are molecular markers characteristic of nonpeptidergic nociceptors^{14,88}, suggesting that C-LTMRs may constitute a distinct class of “silent” or dormant nociceptors. Additionally, these neurons project peripheral afferents to the inner lamina II of the spinal dorsal horn¹³, a region implicated in injury-induced persistent pain⁹⁰. This anatomical localization implies that C-LTMRs possess the capacity to transform physiologically innocuous tactile stimuli into pathological mechanical allodynia under nociceptive conditions. Our electrophysiological analyses revealed significant differences between TH⁺ and TH[−] neurons under physiological conditions. TH⁺ neurons exhibited a characteristic delayed firing pattern, manifested by prolonged latency to first AP initiation (Fig. 1C and F), a result that is consistent with previous findings by Zheng *et al.*³⁵ Furthermore, TH⁺ neurons demonstrated a lower rheobase under physiological conditions (Fig. 1G), potentially enhancing their sensitivity to innocuous mechanical stimuli, which is consistent with their proposed role in light touch perception. Notably, oxaliplatin selectively abolished the delayed firing pattern in TH⁺ neurons and induced profound hyperexcitability in this neuronal subpopulation. This hyperexcitable state was characterized by (1) depolarized RMP (Fig. 1E), (2) a reduced AP threshold (Fig. 1H), and (3) enhanced responsiveness to submaximal current injections, as evidenced by increased AP firing frequency at low-to-moderate stimulation intensities (Fig. 1M). This excitability remodeling enables TH⁺ neurons to transduce innocuous mechanical stimuli into nociceptive signals, potentially resulting in oxaliplatin-induced mechanical allodynia. Compared

with TH[−] neurons, TH⁺ neurons in the OIPN model presented a reduced rheobase and AHP amplitude, indicating the higher excitability in TH⁺ neurons. The decreased rheobase facilitated TH⁺ neuron responsiveness to weaker stimuli under pathological conditions, whereas the reduced AHP amplitude increased their signal integration capacity and potential for increased firing frequency (Fig. 1G and K). The chemogenetic inhibition of TH⁺ neuronal excitability markedly reduced oxaliplatin-induced tactile allodynia (Fig. 2), providing direct and robust evidence for the critical involvement of TH⁺ neurons in mediating mechanical allodynia signaling under pathological conditions. These findings substantiated the necessity of TH⁺ neurons in the neural circuitry underlying touch-evoked pain. In contrast, oxaliplatin exhibited minimal effects on TH[−] neuronal excitability, with alterations limited to AP threshold modulation and increased AP firing frequency only under high-intensity current stimulation (Fig. 1H and L). This observation suggested a potential role for TH[−] neurons in hyperalgesia (reduced thresholds to noxious stimuli) rather than allodynia (pain elicited by innocuous stimuli), which is consistent with previous findings by Wu *et al.*⁴³, who demonstrated that oxaliplatin preferentially enhanced excitability in iB4⁺ small-diameter DRG neurons, including C-LTMRs¹⁴. Collectively, these results highlight the distinct pathological role of TH⁺ neurons in oxaliplatin-induced mechanical allodynia. Although contributions from other sensory neuronal subpopulations cannot be ruled out, our data implicate that nonclassical nociceptors, such as C-LTMRs, may be involved in neuropathic pain through functional plasticity during peripheral sensitization.

Previous investigations have focused predominantly on the role of TRP channels in pain modulation under OIPN conditions^{80–83}. However, voltage-gated ion channels (VGICs) serve as the primary regulators of DRG functionality. While TRP channels generate local ligand-gated potentials, these signals are ultimately modulated by VGICs, which produce voltage-gated currents that critically regulate AP thresholds, firing patterns, and synaptic transmission. These fundamental processes are essential for the encoding and propagation of nociceptive signals^{35,83,91–93}. Consequently, we sought to investigate whether VGICs in C-LTMRs undergo functional modifications. By conducting a cross-analysis of the transcriptomic profile defining C-LTMR neurons as described by Zheng *et al.*³⁵ and an independent publicly available single-cell RNA-seq dataset (GSE201654)⁵⁰, we identified the significant and consistent enrichment of Kv4.3 channel (*Kcnd3* gene) expression specifically within the C-LTMR subpopulation (Fig. S3A). This finding, however, contrasts with previous studies indicating that Kv4.3 channels are predominantly localized in iB4⁺ neurons^{51–53} and coexpressed with the nociceptor marker TRPV1^{54–56}, given that C-LTMRs neither bind iB4 nor express TRPV1¹⁴. To investigate the differential expression patterns of Kv4.3 channels in the DRG, we conducted an immunofluorescence analysis (Fig. 3). Initial observations indicated predominant localization within TH⁺ and iB4⁺ neurons, with particularly high expression levels in TH⁺ populations. However, due to variations in staining background across different tissue sections from multiple mice processed in separate batches, as well as inherent limitations of using a mouse-derived anti-Kv4.3 antibody on mouse tissue—which resulted in unavoidable non-specific binding—the clarity of Kv4.3 channel distribution was compromised. To address these issues, we optimized the immunofluorescence protocol by using freshly prepared paraformaldehyde for fixation and performing co-staining on multiple sections from the same DRG of individual mice. While these

modifications notably improved staining quality (Fig. S3B), the resolution of Kv4.3 channel distribution remained suboptimal. To overcome these limitations, we performed RNAscope *in situ* hybridization on mouse DRG tissue (Fig. 4). This technique utilizes 20 pairs of ZZ probes that bind specifically to the target RNA, with each probe recognizing a unique sequence. Upon hybridization, enzyme-mediated amplification generates fluorescent puncta, each representing an individual RNA molecule, thereby ensuring high sensitivity and specificity⁹⁴. The inclusion of positive (*Ppib*) and negative (*DapB*) control probes further validated

the reliability of the results (Fig. 4A). RNAscope analysis revealed widespread distribution of *Kcnd3* transcripts, which were detected in 63.2% of DRG neurons, predominantly within small-diameter neurons. Notably, *Kcnd3* showed enriched expression in TH⁺ and iB4⁺ neuronal subpopulations (Fig. 4B and C). Although co-staining data with PV were not obtained, the characteristically large soma size (>35 μ m) of PV⁺ proprioceptive neurons suggested minimal *Kcnd3* expression in this group. Despite its broad distribution, *Kcnd3* exhibited a dichotomous expression pattern across neuronal subtypes, with the highest transcript abundance



observed in TH⁺ neurons—significantly exceeding levels in other populations (Fig. 4E). This indicates that Kv4.3 channels may play a particularly critical functional role in TH⁺ neurons. This discovery resolved previous discrepancies in the literature by establishing that Kv4.3 channels display expression-level heterogeneity across neuronal subtypes, suggesting potential functional specialization. Specifically, the enriched Kv4.3 expression in TH⁺ neurons may facilitate the precise encoding of low-threshold mechanical stimuli under physiological conditions, whereas the relatively lower expression in iB4⁺ nonpeptidergic nociceptors may modulate nociceptive signal processing during pathological states of hypersensitivity.

Kv4.3 channels, which are activated at subthreshold membrane potentials, mediate transient outward A-type potassium currents that counteract depolarizing inward ionic fluxes, delay AP initiation, and critically suppress neuronal hyperexcitability during repetitive firing by imposing a voltage-dependent “braking” mechanism on membrane excitability^{57–59}. Consequently, Kv4.3 channels are recognized as pivotal modulators of nociceptive signal transduction. Previous evidence indicates that Kv4.3 channel expression is downregulated in V2 trigeminal ganglion (V2TG) neurons under oxaliplatin-induced orofacial neuropathic pain⁹⁵. This observation prompts the question of whether Kv4.3 channel dysfunction similarly contributes to oxaliplatin-induced neuropathy in DRG neurons. In this study, we provide the first evidence that oxaliplatin selectively reduces I_A currents in TH⁺ neurons, a phenomenon specifically mediated by the downregulation of the expression of Kv4.3 channels rather than by Kv4.1 dysfunction (Figs. 5 and 6). This channel-specific impairment directly drives electrophysiological remodeling in TH⁺ neurons, as evidenced by hyperexcitability and altered firing patterns (Fig. 1). Our findings are consistent with prior work by Zheng et al.³⁵, who established through genetic ablation, pharmacological inhibition, and computational modeling that Kv4.3 loss-of-function shortened the prolonged latency of the first AP in C-LTMRs following current injection. Notably, oxaliplatin did not significantly affect Kv4.3 expression or I_A currents in TH[−] neurons (Fig. 5), indicating that OIPN pathology regulated Kv4.3 in a neuron subtype-specific manner. The subpopulation-specific modulation of Kv4.3 channels underscores their critical role in the TH⁺ mechanotransduction circuitry and highlights that they may serve as precision therapeutic targets for alleviating oxaliplatin-induced mechanical allodynia. Previous studies have consistently reported reduced Kv4.3 channel mRNA expression

and diminished I_A current density in various rodent models of neuropathic pain, including diabetic neuropathy⁹⁶, chronic constriction injury⁹⁷, and chronic infraorbital nerve constriction⁹⁸. Although these investigations did not elucidate the contribution of Kv4.3 dysfunction to excitability changes in specific neuronal subtypes, they collectively implicated Kv4.3 channel as a promising therapeutic target for neuropathic pain. The functional conservation of Kv4.3 across diverse etiologies further highlights its potential utility in addressing heterogeneous neuropathic conditions.

Clinically, oxaliplatin induces both acute and chronic peripheral neuropathy, with approximately 30%–40% of patients experiencing persistent CIPN even five years after chemotherapy cessation^{2,79}. We examined changes in I_A currents at short-term (Days 1–3) and longer-term (Days 15–21) intervals following oxaliplatin administration and observed that the reduction in I_A currents emerged after the establishment of overt neuropathic pain (Fig. S4). This suggests that decreased Kv4.3 channel function may contribute to the maintenance of long-term CIPN. In contrast, acute OIPN primarily involves myelinated A-fibers and is largely mediated by Nav1.6 channels⁹⁹.

Our study demonstrated that the downregulation of Kv4.3 channel function results from both reduced surface expression and alterations in its biophysical properties (Figs. 5 and 6). However, the upstream mechanisms underlying oxaliplatin-induced Kv4.3 suppression remain largely unclear. Accumulating evidence indicates that REST, a key transcriptional repressor in the nervous system, regulates the transcription of multiple ion channels and plays a critical role in the development of neuropathic pain^{61–63}. Previous work has shown that REST epigenetically silences Kv4.3 expression in DRG neurons following nerve injury⁶⁴. Here, we detected a significant upregulation of nuclear REST in DRG neurons after oxaliplatin treatment, leading us to hypothesize that REST may contribute to the suppression of Kv4.3 expression and function. This hypothesis is corroborated by earlier studies demonstrating that REST (also known as NRSF) and its epigenetic co-repressors, HDACs, are involved in oxaliplatin-induced downregulation of potassium channels including Kv4.3⁶⁵. Notably, genetic ablation of NRSF or pharmacological inhibition of class I HDACs reversed the reduction of *Kcnd3* expression⁶⁵. Future studies will be needed to further validate the role of REST in TH⁺ DRG neurons in oxaliplatin-induced mechanical allodynia, for example by selectively knocking down the expression of REST in TH⁺ neurons to determine whether this intervention can

Figure 9 The pharmacological modulation of Kv4.3 channels mitigates oxaliplatin-induced neuronal hyperexcitability and mechanical allodynia. (A–G) *In vitro* electrophysiological recordings were performed using acutely dissociated small-diameter TH⁺ DRG neurons from OIPN model mice. (A) Representative traces of evoked APs in TH⁺ neurons from the control and OIPN groups. Scale bars: 10 ms and 50 mV. (B–G) Quantitative analysis of electrophysiological parameters, including rheobase (B), AP threshold (C), latency (D), RMP (E), and AP half-width (F). $n = 8$ cells per group. Statistical significance was assessed using paired *t*-tests. (G) Representative traces and quantitative analysis of APs evoked by current injection in TH⁺ neurons. $n = 8$ cells per group. Statistical significance was assessed using two-way ANOVA followed by Bonferroni's *post hoc* test. The data in (B–G) are presented as mean $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. Before. (H) Schematic of the *in vivo* treatment protocol: OIPN mice received intraperitoneal injections of NS5806 (2.5 mg/kg) or vehicle on Days 14–16 following initial oxaliplatin administration. (I–O) Behavioral assessments revealed that NS5806 transiently alleviated mechanical hypersensitivity, as measured by von Frey filament testing (I), and tactile allodynia, evaluated *via* cotton swab (J, K) and light brush tests (L, M), in a time-dependent manner. The analgesic effects peaked on the day of administration and diminished post-treatment cessation. However, no improvement was observed in the response to adhesive tape (O). $n = 6$ in OIPN, $n = 7$ in OIPN + NS5806. Statistical significance was determined using two-way repeated-measures ANOVA followed by Bonferroni's *post hoc* test. (P, Q) NS5806 did not significantly alter cold hypersensitivity (cold plate test, P) or thermal nociception (Hargreaves test, Q), as withdrawal latencies remained unchanged. $n = 6$ in OIPN, $n = 7$ in OIPN + NS5806. Statistical significance was assessed using two-way repeated-measures ANOVA followed by Bonferroni's *post hoc* test. Data in (I–Q) are presented as mean $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. OIPN.

rescue Kv4.3 channel downregulation and alleviate mechanical allodynia.

Additionally, we explored the potential involvement of other ion channels—Nav1.7, Nav1.8, Nav1.9, and Cav3.2, which are highly expressed in C-LTMR neurons and implicated in their functional regulation^{35,45-49}—in oxaliplatin-induced mechanical allodynia. Prior studies have demonstrated that Nav1.7 is dispensable for sustained pain in oxaliplatin-induced neuropathy¹⁰⁰ and that the genetic ablation of Nav1.7, Nav1.8, or Nav1.9 fails to attenuate oxaliplatin-induced cold hypersensitivity, indicating their nonessential roles in this pathology⁸⁷. Although the expression of Cav3.2 channels are upregulated in the spinal cord of oxaliplatin-treated male and female mice, mechanical hypersensitivity persists in Cav3.2 knockout models⁴⁶. To further investigate their involvement, we conducted qPCR and Western blot analyses of Nav1.7, Nav1.8, Nav1.9, and Cav3.2 expression in DRG neurons (Fig. S6). Consistent with previous findings, oxaliplatin exerted minimal effects on the transcriptional or translational levels of these channels, reinforcing their limited contribution to oxaliplatin-induced mechanical allodynia. Previous studies have indicated that endothelial Piezo2 may act as a mechanotransducer responsible for stimulus-dependent hyperalgesia in oxaliplatin-induced mechanical pain¹⁰¹. Downregulation of Kv7.2 channels has been suggested to contribute to oxaliplatin-induced orofacial neuropathic pain¹⁰², while HCN1 channels in cold-sensitive DRG nociceptors play a key role in mediating cold hypersensitivity following acute oxaliplatin exposure⁸⁶. Given their putative contributions to OIPN, we further evaluated their expression in TH⁺ neurons using Western blot and immunofluorescence. Our results showed that oxaliplatin had minimal effects on their expression levels, highlighting the specific importance of Kv4.3 channel alterations within TH-positive neurons in this model of oxaliplatin-induced neuropathy (Fig. S7). These results collectively suggest that oxaliplatin may induce mechanical allodynia primarily through the downregulation of Kv4.3 expression or function in TH⁺ neurons, leading to reduced I_A currents, neuronal hyperexcitability, and aberrant mechanosensory signaling. This hypothesis was strongly supported by experimental evidence demonstrating that the pharmacological inhibition of Kv4.3 or localized DRG-specific knockdown of Kv4.3 in TH⁺ neurons recapitulated oxaliplatin-induced mechanical allodynia (Figs. 7 and 8, and Fig. S9).

These findings collectively indicate that Kv4.3 channel represent a promising therapeutic target for the clinical intervention of oxaliplatin-induced mechanical allodynia. To validate this hypothesis, we utilized NS5806, a pharmacological activator of Kv4.3 channels, to enhance their functional activity. NS5806 significantly attenuated hyperexcitability in TH⁺ neurons and reversed oxaliplatin-induced pain behaviors in OIPN models (Fig. 9). These results are consistent with those of previous studies demonstrating the potent analgesic effects of NS5806 in rat models of spinal nerve ligation (SNL)-induced neuropathic pain and carrageenan-induced inflammatory pain^{103,104}, thereby further supporting our experimental findings. Importantly, our results underscore the critical role of Kv4.3 channel, particularly those expressed in TH⁺ neuronal subpopulations, as an ideal pharmacological target for alleviating chemotherapy-induced peripheral mechanical hypersensitivity. This study not only elucidated a neuron subtype-specific mechanism underlying neuropathic pain but also provided a novel idea for developing precision analgesic strategies targeting molecularly defined sensory neuron subpopulations, thereby advancing personalized therapeutic

approaches for CIPN. Moreover, a recent study revealed that the C-LTMRs in DRG exhibits the most pronounced transcriptomic alterations following paclitaxel administration¹⁰⁵, suggesting that dysfunction of C-LTMRs may represent a key driver underlying peripheral neuropathy induced by various chemotherapeutic agents.

The present study revealed that the dysfunction of Kv4.3 channels in C-LTMRs is a principal mediator of oxaliplatin-induced mechanical allodynia. However, several limitations of this work should be acknowledged. In this study, we exclusively used male mice to investigate the role of Kv4.3 channels in C-LTMR-mediated oxaliplatin-induced neuropathic pain, and it remains uncertain whether these findings can be generalized to female mice. Our initial choice of male mice was aimed at avoiding additional variability potentially introduced by hormonal cycles. However, accumulating evidence indicates that sex differences may exist in pain perception^{106,107}. Epidemiological surveys have shown that women exhibit greater pain sensitivity¹⁰⁸. Moreover, one of the largest surveys of cancer survivors self-reporting the impact of CIPN identified sex as a significant predictor of CIPN severity, with female patients being more prone to CIPN¹⁰⁹. This suggests that sex-related differences may underlie the mechanisms of chemotherapy-induced neuropathic pain¹¹⁰. Estrogen has been widely recognized as a key modulator of pain¹¹¹, and previous studies have demonstrated that estrogen can downregulate Kv4.3 expression and regulate its subcellular localization in tissues such as the heart and uterine smooth muscle¹¹²⁻¹¹⁵. These findings raise the possibility that the estrogenic environment may influence baseline Kv4.3 levels or modify its response to stimuli such as drugs or injury. Therefore, the contribution of Kv4.3 channels to oxaliplatin-induced pain may differ between sexes and could be modulated by hormonal status. In future studies, we plan to investigate whether there are baseline differences in Kv4.3 channel expression between male and female DRGs, and to assess the functional changes of Kv4.3 channels in female mice before and after CIPN induction. These investigations will provide experimental evidence to explain the greater pain sensitivity observed in females and clarify whether the role of Kv4.3 channels in this model is sex-dependent. Such work is essential for a comprehensive understanding of the pathophysiology of chemotherapy-induced pain and for guiding the development of more targeted and sex-sensitive therapeutic strategies. Such work is essential for a comprehensive understanding of the pathophysiology of chemotherapy-induced pain and for developing targeted, sex-informed therapeutic strategies. Furthermore, the specific molecular mechanisms governing this regulatory process remain to be fully elucidated. Further investigations are warranted to determine whether oxaliplatin exerts direct regulatory effects on critical molecular targets within TH⁺ neurons, thereby modulating Kv4.3 expression or its posttranslational modifications, such as phosphorylation. Moreover, the clinical translation of Kv4.3-targeted interventions necessitates comprehensive safety assessments, given the ubiquitous expression of the channel in both the cardiac and central nervous systems, which may lead to potential off-target effects.

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Author contributions

Jiali Zhang: Conceptualization, Investigation, Formal analysis, Data Curation, Writing - Original Draft, Writing - Review & Editing, Methodology, Validation, Software. Xiangyu Wang: Conceptualization, Resources, Validation, Data Curation, Funding acquisition. Xingang Liu: Validation, Resources, Software, Formal analysis. Jincan Li, Kening Qiao, Jinglei Sun, Jiabao Zuo: Methodology, Software, Validation, Investigation. Weidong Zhao, Tenghui He, Xuedong Li, Zhengkun Guan: Validation, Formal analysis, Visualization. Yang Zhang, Yanfang Xu, Qingzhong Jia: Conceptualization, Supervision, Project administration, Funding acquisition, Writing - Original Draft, Writing - Review & Editing.

Conflicts of interest

The authors declare no competing interests.

Appendix A. Supporting information

Supporting information to this article can be found online at <https://doi.org/10.1016/j.apsb.2025.12.005>.

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