Neurobiology of Disease

Resolving TRPV1- and TNF-α-Mediated Spinal Cord Synaptic Plasticity and Inflammatory Pain with Neuroprotectin D1

Chul-Kyu Park,[1] Ning Lü,[1] Zhen-Zhong Xu,[1] Tong Liu,[1] Charles N. Serhan,[2] and Ru-Rong Ji[1]

[1]Sensory Plasticity Laboratory, Pain Research Center, Department of Anesthesiology, Perioperative and Pain Medicine, and [2]Center for Experimental Therapeutics and Reperfusion Injury, Harvard Institutes of Medicine, Department of Anesthesiology, Perioperative and Pain Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts 02115

Mechanisms of inflammatory pain are not fully understood. We investigated the role of TRPV1 (transient receptor potential subtype V1) and TNF-α, two critical mediators for inflammatory pain, in regulating spinal cord synaptic transmission. We found in mice lacking *Trpv1* the frequency but not the amplitude of spontaneous EPSCs (sEPSCs) in lamina II neurons of spinal cord slices is reduced. Further, C-fiber-induced spinal long-term potentiation (LTP) *in vivo* is abolished in *Trpv1* knock-out mice. TNF-α also increases sEPSC frequency but not amplitude in spinal outer lamina II (lamina IIo) neurons, and this increase is abolished in *Trpv1* knock-out mice. Single-cell PCR analysis revealed that TNF-α-responding neurons in lamina IIo are exclusively excitatory (vGluT2$^+$) neurons. Notably, neuroprotectin-1 (NPD1), an anti-inflammatory lipid mediator derived from ω-3 polyunsaturated fatty acid (docosahexaenoic acid), blocks TNF-α- and capsaicin-evoked sEPSC frequency increases but has no effect on basal synaptic transmission. Strikingly, NPD1 potently inhibits capsaicin-induced TRPV1 current ($IC_{50} = 0.4$ nM) in dissociated dorsal root ganglion neurons, and this IC_{50} is ≈500 times lower than that of AMG9810, a commonly used TRPV1 antagonist. NPD1 inhibition of TRPV1 is mediated by GPCRs, since the effects were blocked by pertussis toxin. In contrast, NPD1 had no effect on mustard oil-induced TRPA1 currents. Spinal injection of NPD1, at very low doses (0.1–10 ng), blocks spinal LTP and reduces TRPV1-dependent inflammatory pain, without affecting baseline pain. NPD1 also reduces TRPV1-independent but TNF-α-dependent pain hypersensitivity. Our findings demonstrate a novel role of NPD1 in regulating TRPV1/TNF-α-mediated spinal synaptic plasticity and identify NPD1 as a novel analgesic for treating inflammatory pain.

Introduction

Molecular and cellular mechanisms of inflammatory pain have been extensively studied but are not fully understood (Hucho and Levine, 2007). After tissue injury sensitization of primary sensory neurons (peripheral sensitization) is initiated by inflammatory mediators (Hucho and Levine, 2007; Basbaum et al., 2009; Gold and Gebhart, 2010). Heat pain is in part mediated by the transient receptor potential subtype V1 (TRPV1) expressed by C-fiber nociceptors in the dorsal root ganglion (DRG) and trigeminal ganglion (Caterina et al., 1997; Tominaga et al., 1998). TRPV1 is upregulated in DRG neurons after persistent inflammation (Ji et al., 2002) and essential for the development of inflammatory heat hyperalgesia (Caterina et al., 2000; Davis et al., 2000). The proinflammatory cytokine TNF-α is also required for the genesis of inflammatory pain (Choi et al., 2010; Zhang et al., 2011). In DRG neurons TNF-α increases TRPV1 sensitivity (Jin and Gereau, 2006; Constantin et al., 2008) and induces spontaneous discharge (Schäfers et al., 2003). Persistent nociceptive input also enhances synaptic transmission in the spinal cord dorsal horn, i.e., central sensitization, which is critical for the induction and maintenance of inflammatory pain (Woolf and Salter, 2000; Ji et al., 2003; Kuner, 2010). Application of capsaicin (CAP) and TNF-α to spinal cord slices evokes a profound increase in the frequency of spontaneous EPSCs (sEPSCs) in lamina II neurons, as a result of presynaptic glutamate release (Yang et al., 1998; Kawasaki et al., 2008; Xu et al., 2010).

Current treatments of inflammatory pain are limited by potential side effects (Sommer and Birklein, 2010). The novel mediator neuroprotectin D1 (NPD1) is biosynthesized from ω-3 fatty acid docosahexaenoic acid (DHA) and was first identified in resolving inflammatory exudates and murine brain (Serhan et al., 2002; Hong et al., 2003) as well as in experimental stroke, where it displays potent protective actions (Marcheselli et al., 2003). Given the potent protective actions of this DHA-derived mediator it was coined neuroprotectin D1 (Mukherjee et al., 2004; Lukiw et al., 2005). The NPD1/PD1 complete stereochemistry,

Received May 16, 2011; revised Aug. 26, 2011; accepted Aug. 27, 2011.

Author contributions: C.-K.P., Z.-Z.X., T.L., and R.-R.J. designed research; C.-K.P., N.L., Z.-Z.X., and T.L. performed research; C.-K.P., N.L., Z.-Z.X., and T.L. analyzed data; R.-R.J. and C.N.S. wrote the paper.

The work was supported in part by US National Institutes of Health Grants R01-DE17794, NS54932 to R.-R.J., R01-GM38765 and P01-GM095467 to C.N.S., and R01-NS67686 to R.-R.J. and C.N.S. We thank the Resolvyx Pharmaceuticals Inc. for providing NPD1 and Dr. Philip J. Vickers for helpful discussion.

C.N.S. is an inventor on patents [resolvins] assigned to BWH and licensed to Resolvyx Pharmaceuticals. C.N.S. is a scientific founder of Resolvyx Pharmaceuticals and owns equity in the company. C.N.S.'s interests were reviewed and are managed by the Brigham and Women's Hospital and Partners HealthCare in accordance with their conflict of interest policies.

Correspondence should be addressed to Ru-Rong Ji, Department of Anesthesiology, Brigham and Women's Hospital, 75 Francis Street, Medical Research Building, Room 502, Boston, MA 02115. E-mail: rrji@zeus.bwh.harvard.edu.

DOI:10.1523/JNEUROSCI.2443-11.2011

 0270-6474/11/3115072-14$15.00/0

Table 1. List of DNA primer sequences designed for single-cell RT-PCR

Target gene (Product length)[a]	Outer primers	Inner primers	GenBank no.
TRPV1 (273 bp, 203 bp)			
Forward	TGATCATCTTCACCACGGCTG	AAGGCTTGCCCCCCTATAA	NM_001001445.1
Reverse	CCTTGCGATGGCTGAAGTACA	CACCAGCATGAACAGTGACTGT	
GAPDH (367 bp, 313 bp)			
Forward	AGCCTCGTCCCGTAGACAAAA	TGAAGGTCGGTGTGAACGAATT	XM_001473623.1
Reverse	TTTTGGCTCCACCCCTTCA	GCTTTCTCCATGGTGGTGAAGA	
TNFR1 (359 bp, 301 bp)			
Forward	CTCAGGCAGTGTCTCAGTTGCA	CGGAAAGAAATGTCCCAGGTG	NM_011609.4
Reverse	AACAGCACCGCAGTACCTGAGT	GGTTTGTGACATTTGCAAGCG	
TNFR2 (393 bp, 281 bp)			
Forward	AACGGCTTGGTACTGTACTTGC	TGATGACATCGGTTGAAAGGC	NM_011610.3
Reverse	AGACGTGTTCCTTCCCCTCTAC	TAACAAGTCGGCCCCAAGTGT	
vGluT2 (267 bp, 199 bp)			
Forward	TCATTGCTGCACTCGTCCACT	TTGCCTCAGGAGAGAAACAACC	NM_080853.3
Reverse	GCGCACCTTCTTGCACAAA	TCTTCCTTTTTCTCCCAGCCG	

[a]Numbers in parentheses indicate sizes of PCR products obtained from outer and inner primers, respectively.

potent anti-inflammatory actions and 10,17-docosatriene structure were confirmed by total organic synthesis and established as 10*R*,17*S*-dihydroxy-docosa-4*Z*,7*Z*,11*E*,13*E*,15*E*,19*Z*-hexaenoic acid (Serhan et al., 2006). The prefix *Neuro* denotes the location of PD1 formation and actions *in vivo*: within neural tissues NPD1 exhibits potent neuroprotective actions in experimental brain damage, oxidative-stressed retinal pigment epithelial cells, and human brain cells exposed to β-amyloid peptides (Lukiw et al., 2005; Bazan et al., 2010; Palacios-Pelaez et al., 2010). Along these lines in the immune system, eosinophils produce PD1 *in vivo* during resolution of inflammation where it promotes resolution, and NPD1/PD1 is also produced in murine joints during the resolution of Lyme disease (Blaho et al., 2009; Yamada et al., 2011). PD1 is also renoprotective (Hassan and Gronert, 2009), regulates adipokines in obesity (Gónzalez-Périz et al., 2009), and is produced by and acts on stem cells (Yanes et al., 2010).

The role of NPD1 in regulating synaptic transmission and pain has yet to be examined. Our results demonstrated that NPD1 is a highly potent endogenous inhibitor of TRPV1 (IC_{50} = 0.4 nM). NPD1 blocked both TRPV1 and TNF-α-mediated spinal cord synaptic transmission and long-term potentiation (LTP). In addition, spinal administration of NPD1, at very low doses (0.1–10 ng), inhibited TRPV1- and TNF-α-dependent inflammatory pain.

Materials and Methods

Animals and pain models. Knock-out mice lacking *Trpv1* (*Trpv1*$^{-/-}$), *Tnfr1* (*Tnfrsf1a*$^{-/-}$), and *Tnfr2* (*Tnfrsf1b*$^{-/-}$) and C57BL/6 background WT control mice were purchased from Jackson Laboratories and bred in the Thorn Research Building Animal Facility of Harvard Medical School. *Trpv1*$^{-/-}$, *Tnfrsf1a*$^{-/-}$, and *Tnfrsf1b*$^{-/-}$ mice were viable and showed no developmental defects. Young mice (4–6 weeks, C57BL/6) were used for electrophysiological studies in spinal cord slices and DRG neurons to obtain high quality recordings. Of note, DRG neurons and spinal cord circuit are fully developed by postnatal age of 2 weeks (Fitzgerald, 2005). Adult CD1 mice (male, 8–10 weeks) were used for behavioral and pharmacological studies. All the animal procedures were approved by the Animal Care Committee of Harvard Medical School. To produce acute and persistent inflammatory pain, diluted formalin (5%, 20 μl) or complete Freund's adjuvant (CFA, 20 μl, 1 mg/ml, Sigma) was injected into the plantar surface of a hindpaw.

Drugs and administration. TNF-α was purchased from R&D Systems. Capsaicin, allyl isothiocyanate (AITC), capsazepine, CNQX, and SQ22536 were obtained from Sigma. H89 and U0126 were obtained from Calbiochem. AMG9810 and pertussis toxin were purchased from Tocris Bioscience. RvD1 and DHA were purchased from Cayman Chemical. NPD1 was a kind gift from Resolvyx Pharmaceuticals Inc. NPD1/PD1 was initially isolated in exudates from the resolution phase of self-limited acute inflammation (Serhan et al., 2002). After the full structural elucidation, NPD1/PD1 physical and anti-inflammatory properties were confirmed by total organic synthesis (Serhan et al., 2006). NPD1 and TNF-α were prepared in PBS. For intrathecal injection, spinal cord puncture was made with a 30G needle between the L5 and L6 level to deliver reagents (10 μl) to the CSF (Hylden and Wilcox, 1980).

Spinal cord slice preparation and patch-clamp recordings. As we previously reported (Zhang et al., 2011), a portion of the lumbar spinal cord (L4-L5) was removed from mice (4–6 weeks old) under urethane anesthesia (1.5–2.0 g/kg, i.p.) and kept in preoxygenated ice-cold Krebs' solution. Transverse slices (600 μm) were cut on a vibrating microslicer. The slices were perfused with Krebs' solution (8–10 ml/min) that was saturated with 95% O_2 and 5% CO_2 at 36 ± 1°C for at least 1–3 h before experiment. The Krebs' solution contains (in mM): 117 NaCl, 3.6 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 1.2 NaH_2PO_4, 25 $NaHCO_3$, and 11 glucose.

The whole-cell patch-clamp recordings were made from outer lamina II (lamina IIo) neurons in voltage-clamp mode. Patch pipettes were fabricated from thin-walled, borosilicate, glass-capillary tubing (1.5 mm outer diameter, World Precision Instruments). After establishing the whole-cell configuration, neurons were held at the potential of −70 mV to record sEPSCs. The resistance of a typical patch pipette is 5–10 MΩ. The internal solution contains (in mM): 135 potassium gluconate, 5 KCl, 0.5 $CaCl_2$, 2 $MgCl_2$, 5 EGTA, 5 HEPES, 5 ATP-Mg. Membrane currents were amplified with an Axopatch 200B amplifier (Molecular Devices) in voltage-clamp mode. Signals were filtered at 2 kHz and digitized at 5 kHz. Data were stored with a personal computer using pCLAMP 10 software and analyzed with Mini Analysis (Synaptosoft Inc.).

Spinal cord LTP recordings in anesthetized mice. Mice were anesthetized with urethane (1.5 g/kg, i.p.). The trachea was cannulated to allow mechanical ventilation, if necessary. PBS (0.5–1 ml, i.p.) was injected before surgery and every 2 h after surgery to maintain electrolyte balance. A laminectomy was performed at vertebrae T13-L1 to expose the lumbar enlargement, and the left sciatic nerve was exposed for bipolar electrical stimulation. The vertebral column was firmly suspended by rostral and caudal clamps on the stereotaxic frame. The exposed spinal cord and the sciatic nerve were covered with paraffin oil. Colorectal temperature was kept constant at 37–38°C by a feedback-controlled heating blanket. Following electrical stimulation of the sciatic nerve, the field potentials were recorded in the ipsilateral L4-L5 spinal cord segments with glass microelectrodes, 100–300 μm from the surface of the cord. *In vivo* LTP was recorded as previously reported (Chu et al., 2010) with some modifications for mice. After recording stable responses following test stimuli (2×

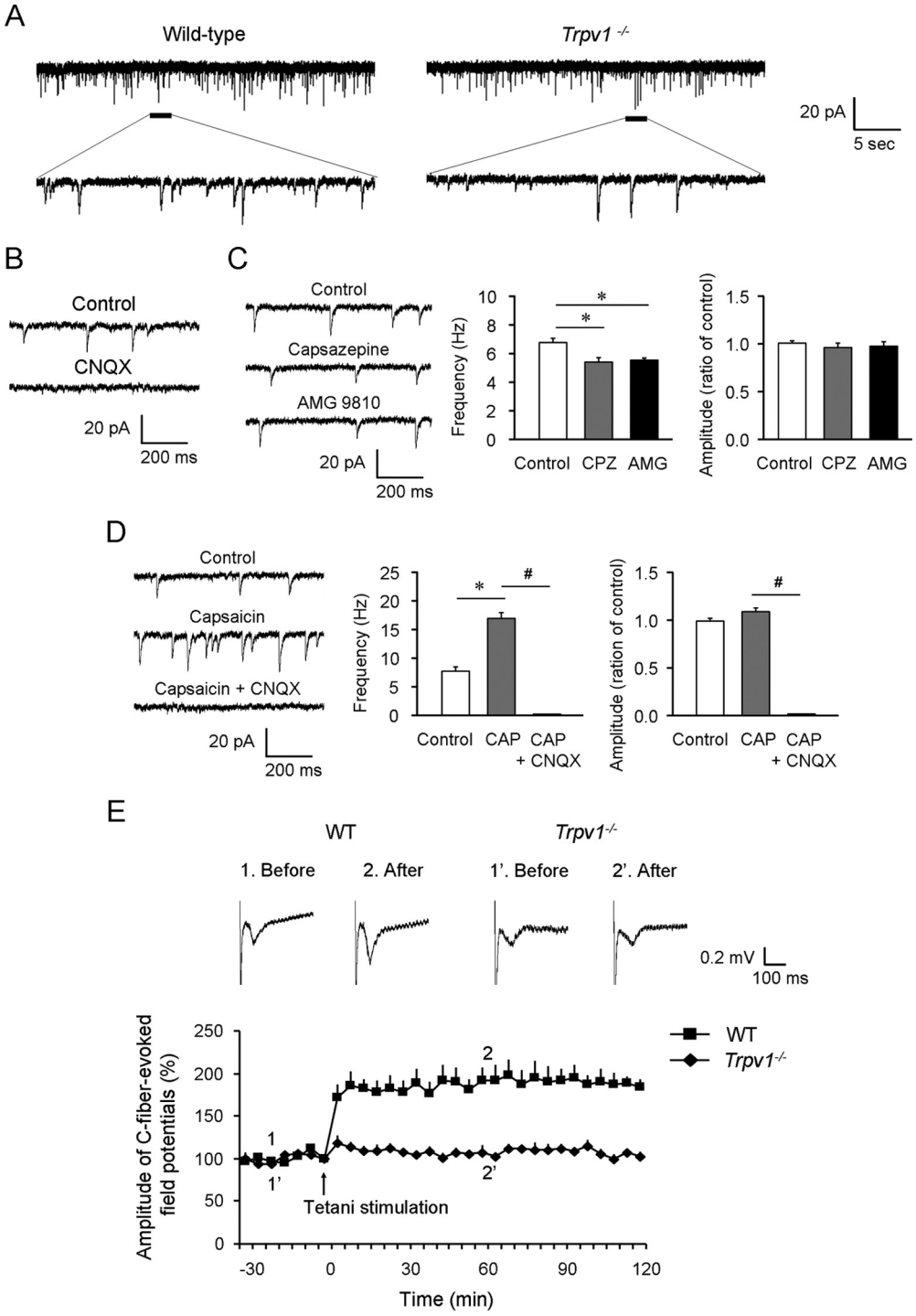

Figure 1. *Trpv1* $^{-/-}$ mice exhibit reduced spontaneous EPSC frequency and failed LTP induction in the spinal cord. ***A–D***, Patch-clamp recordings of sEPSCs in lamina IIo neurons of spinal cord slices. ***A***, sEPSCs traces of WT and *Trpv1* $^{-/-}$ mice. Note a reduction in the frequency but not amplitude of sEPSCs in *Trpv1* $^{-/-}$ mice. $n = 8$. ***B***, sEPSCs in WT mice are blocked by CNQX (20 μM). $n = 5$. ***C***, sEPSCs frequency but not amplitude in WT mice are reduced by capsazepine (10 μM) and AMG9810 (3 μM). $^{*}p < 0.05$, $n = 7$ (*t* test). ***D***, CAP (0.5 μM) increases sEPSCs frequency but not amplitude, which is blocked by CNQX (20 μM). $^{*}p < 0.05$, $^{\#}p < 0.05$, $n = 5$ (*t* test). ***E***, LTP of C-fiber-evoked field potentials is induced by tetani (4 trains of tetanic stimulation) in the spinal cord dorsal horn of anesthetized WT mice but not in *Trpv1* $^{-/-}$ mice. $^{*}p < 0.05$ (two-way ANOVA, KO vs WT, $n = 5$). Top: Traces of C-fiber-evoked field potentials in the dorsal horn of WT mice and *Trpv1* $^{-/-}$ mice before (1 and 1′) and after conditioning tetanic stimulation (2 and 2′). All the data shown are mean ± SEM.

C-fiber threshold, 0.5 ms, every 5 min) for >40 min, conditioning tetanic stimulation (5× C-fiber threshold, 100 Hz, 1 s duration, 4 trains, and 10 s interval) was delivered to the sciatic nerve for inducing LTP of C-fiber-evoked field potentials. For intrathecal drug delivery, a PE5 catheter was inserted at L5-L6 level via lumbar puncture.

Whole-cell patch-clamp recordings in cultured DRG neurons. DRGs were removed aseptically from mice (4–6 weeks) and incubated with collagenase (1.25 mg/ml, Roche)/dispase-II (2.4 U/ml, Roche) at 37°C for 90 min, then digested with 0.25% trypsin for 8 min at 37°C, followed by 0.25% trypsin inhibitor. Cells were mechanically dissociated with a flame-polished Pasteur pipette in the presence of 0.05% DNase I (Sigma). DRG cells were plated on glass coverslips and grown in a Neurobasal defined medium (with 2% B27 supplement, Invitrogen) with 5 μM AraC and 5% carbon dioxide at 36.5°C. DRG neurons were grown for 24 h before use.

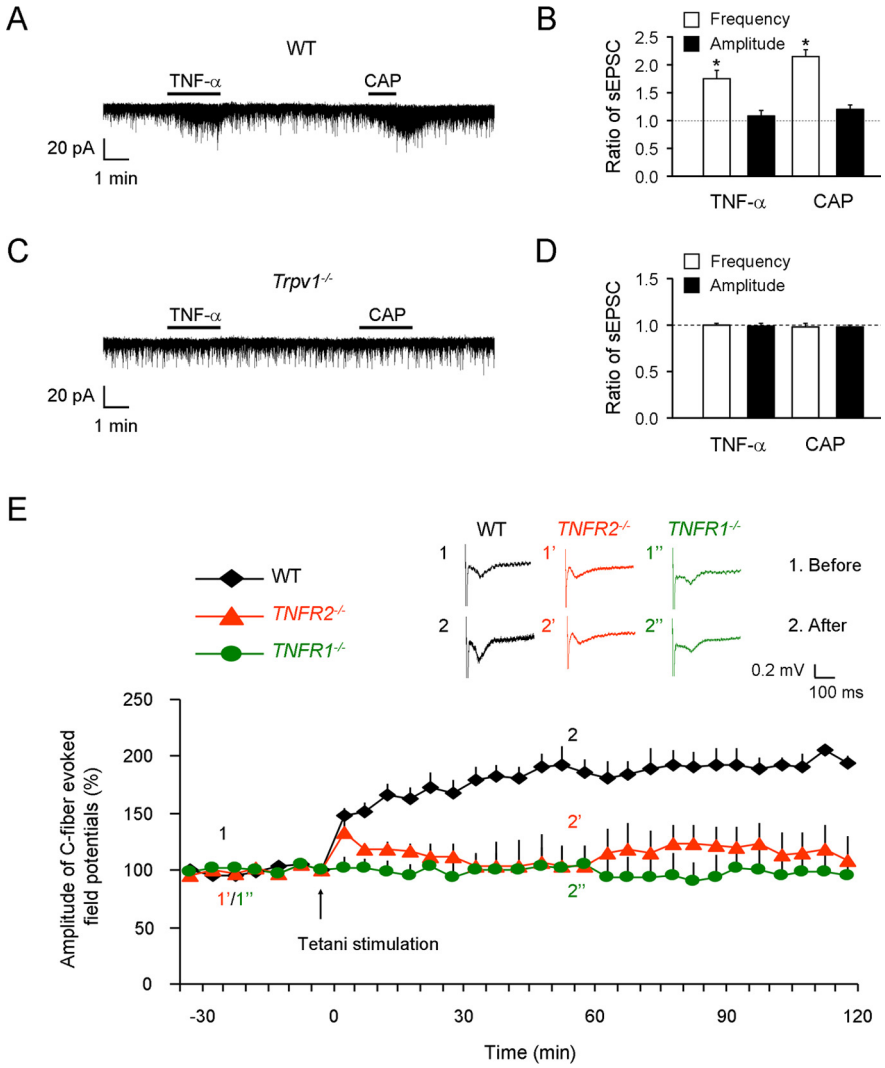

Figure 2. TNF-α increases spontaneous EPSC frequency in the spinal cord of WT mice but not in *Trpv1*$^{-/-}$ mice. ***A–D***, Patch-clamp recordings of sEPSCs in lamina IIo neurons of spinal cord slices. ***A***, sEPSCs traces in WT mice showing sEPSC increases after superfusion of TNF-α (10 ng/ml) and capsaicin (100 nM). Note the same neurons respond to both TNF-α and capsaicin. ***B***, Frequency and amplitude of sEPSCs. TNF-α and capsaicin increase frequency but not amplitude of sEPSCs. $*p < 0.05$ (compared with pretreatment baseline, $n = 8$, *t* test). ***C***, sEPSCs traces in spinal cord slices of *Trpv1*$^{-/-}$ mice showing no sEPSC increases after superfusion of TNF-α and capsaicin. ***D***, Frequency and amplitude of sEPSCs before and after TNF-α and capsaicin treatment in *Trpv1*$^{-/-}$ mice. $n = 5$. ***E***, LTP of C-fiber-evoked field potentials by tetani (4 trains of tetanic stimulation) is induced in the dorsal horn of anesthetized WT mice ($n = 5$) but impaired in *Tnfr2*$^{-/-}$ mice ($n = 5$) and abolished in *Tnfr1*$^{-/-}$ mice ($n = 6$). $*p < 0.05$ (two-way ANOVA, WT vs KO). Top traces are C-fiber-evoked field potentials in the dorsal horn of WT and KO mice before (1, 1′, 1″) and after conditioning tetanic stimulation (2, 2′ and 2″). All the data shown are mean ± SEM.

Whole-cell voltage- and current-clamp recordings were performed at room temperature to measure currents and action potentials, respectively, with Axopatch-200B amplifier (Molecular Devices). The patch pipettes were pulled from borosilicate capillaries (Chase Scientific Glass Inc.). When filled with the pipette solution, the resistance of the pipettes was 4~5 MΩ. The recording chamber (300 μl) was continuously superfused (2~3 ml/min). Series resistance was compensated for (> 80%), and leak subtraction was performed. Data were low-pass-filtered at 2 kHz, sampled at 10 kHz. The pClamp8 (Molecular Devices) software was used during experiments and analysis. The pipette solution for voltage-clamp experiments was composed of (in mM): 126 K-gluconate, 10 NaCl, 1 $MgCl_2$, 10 EGTA, 2 NaATP, and 0.1 MgGTP, adjusted to pH 7.4 with KOH, osmolarity 295–300 mOsm. Ca^{2+}-free extracellular solution contained 0 mM $CaCl_2$ and 2 mM EGTA for chelation of ambient Ca^{2+}. Extracellular solution for voltage-clamp experiments contained the following (in mM): 140 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 10 glucose, adjusted to pH 7.4 with NaOH and osmolarity to 300–310 mOsm. Voltage-clamp experiments were performed at a holding potential of −60 mV.

Single-cell reverse-transcription PCR (RT-PCR). Single-cell RT-PCR was performed as previously described (Park et al., 2006; Liu et al., 2010). Briefly, following whole-cell patch-clamp recordings, spinal lamina II neurons or DRG neurons were harvested into patch pipettes with tip diameters of ~15–25 μm, gently put into reaction tubes containing reverse transcription reagents, and incubated for 1 h at 50°C (SuperScript III, Invitrogen). The cDNA products were used in separate PCR. The sequences of the primers used are shown in Table 1. The first round of PCR was performed in 50 μl of PCR buffer containing 0.2 mM dNTPs, 0.2 μM "outer" primers, 5 μl of RT product and 0.2 μl of platinum TaqDNA polymerase (Invitrogen). The protocol included an initial 5 min denaturizing step at 95°C followed by 40 cycles of 40 s denaturation at 95°C, 40 s annealing at 55°C, and 40 s elongation at 72°C. The reaction was completed with 7 min of final elongation. For the second round of amplification, the reaction buffer (20 μl) contained 0.2 mM dNTPs, 0.2 μM "inner" primers, 5 μl of the first round PCR products and 0.1 μl of platinum TaqDNA polymerase. The amplification procedure for the inner primers was the same as that of the first round. A negative control was obtained from pipettes that did not have cell contents but were submerged in the bath solution. The PCR products were displayed on ethidium bromide-stained agarose gels (1%).

Behavioral analysis. Animals were habituated to the testing environment daily for at least 2 d before baseline testing. Animals were put in plastic boxes for 30 min habituation before examination. Heat sensitivity was tested by radiant heat using Hargreaves (Hargreaves et al., 1988) apparatus (IITC Life Science Inc.) and expressed as paw withdrawal latency (PWL). The PWLs were adjusted to 9–12 s, with a cutoff of 20 s to prevent tissue damage. For capsaicin test, capsaicin was intrathecally (0.5 μg) or intraplantarly (5 μg, in a hindpaw) injected, and the time spent on nocifensive behavior (licking and flinching) was recorded. For formalin test, the time spent in flinching, licking, and lifting the affected paws was recorded every 5 min for 45 min. For testing mechanical sensitivity, mice were put in boxes on an elevated metal mesh floor and allowed 30 min for habituation before examination. The plantar surface of each hindpaw was stimulated with a series of von Frey hairs with logarithmically incrementing stiffness (0.02–2.56 g, Stoelting), presented perpendicular to the plantar surface. The 50% paw withdrawal threshold was determined using Dixon's up-down method (Dixon, 1980). The experimenters were blinded to genotypes and treatments.

Statistical analysis. All data were expressed as mean ± SEM. For electrophysiology in spinal cord slices, those cells showed >5% changes from the baseline levels during drug perfusion were regarded as responding ones. We collected the baseline recordings for 2 min and the recordings in the first 2 min of drug treatment for statistical analysis using paired or unpaired two-tailed Student's *t* test (Kawasaki et al., 2008). LTP data were tested using two-way ANOVA. Behavioral data were analyzed using Student's *t* test (two groups) or one-way ANOVA followed by *post hoc* Newman–Keuls test. The criterion for statistical significance was $p < 0.05$.

Results

TRPV1 contributes to excitatory synaptic transmission and LTP in the spinal cord

Neurons in the spinal cord lamina II (substantia gelatinosa) receive nociceptive input from C-fibers and play an important role in processing nociceptive information (Todd, 2010). In mice, TRPV1-expressing C-nociceptors are largely peptidergic and project to lamina I and lamina IIo in the spinal cord (Basbaum et al., 2009; Wang and Zylka, 2009). We used patch-clamp technique to record sEPSCs in lamina IIo of spinal cord slices. It is generally believed that (1) sEPSCs are mediated by glutamate AMPA/Kainate receptors and (2) changes in the frequency and amplitude of sEPSC are mediated by respective presynaptic and postsynaptic mechanisms (Yang et al., 1998; Engelman and MacDermott, 2004; Kawasaki et al., 2008). To determine the role of TRPV1 in spinal cord synaptic transmission, we first recorded sEPSC in WT and *Trpv1*$^{-/-}$ mice. The frequency of sEPSCs in WT mice was 7.1 ± 0.8 Hz. *Trpv1*$^{-/-}$ mice displayed a 38% reduction in the frequency of sEPSCs (4.4 ± 0.6 Hz, $p < 0.05$, vs WT, *t* test, Fig. 1*A*). In contrast, the amplitude of sEPSCs did not alter in *Trpv1* knock-out (KO) mice (14.0 ± 0.3 pA in WT group vs 14.0 ± 0.4 pA in KO group, $n = 6$, $p > 0.05$, *t* test, Fig. 1*A*). Of note, sEPSCs were completely blocked by CNQX (20 μM, Fig. 1*B*), confirming that sEPSCs are mediated by AMPA/Kainate glutamate receptors. The sEPSCs frequency but not amplitude were also significantly inhibited by the TRPV1 antagonists capsazepine (10 μM, 21% reduction, $p < 0.05$, *t* test, Fig. 1*C*) and AMG9810 (3 μM, 20% reduction, $p < 0.05$, *t* test, Fig. 1*C*) in WT mice, supporting a role of TRPV1 in spinal cord synaptic transmission.

Although TRPV1 antagonists significantly reduced sEPSC frequency, the effects were relatively moderate (~20% reduction). To exclude the contribution of vehicles to sEPSCs changes, we also tested the effects of 1% methanol (vehicle for capsazepine) and 1% DMSO (vehicle for AMG9810). We did not find any change in the frequency and amplitude of sEPSCs after the vehicles treatment ($p > 0.05$, *t* test). The sEPSC frequency of control (before treatment), 1% methanol, and 1% DMSO was 6.97 ± 0.12 Hz ($n = 5$), 6.98 ± 0.04 Hz ($n = 4$), and 6.99 ± 0.07 Hz ($n = 5$), respectively. The sEPSC amplitude of control, 1% methanol, and 1% DMSO was 14.04 ± 0.25 pA ($n = 5$), 14.07 ± 0.25 pA ($n = 4$), and 14.03 ± 0.23 pA ($n = 5$), respectively. Together, our data suggest that TRPV1 contributes to basal synaptic transmission in the spinal cord. However, the endogenous ligands for TRPV1 to control basal synaptic transmission under normal conditions remain to be identified.

We further investigated evoked sEPSCs by capsaicin, a C-fiber activator and a selective TRPV1 agonist. Capsaicin (0.5 μM) elicited marked increase in sEPSC frequency but not amplitude, which was completely blocked by CNQX (Fig. 1*D*). As expected, the capsaicin responses were lost in *Trpv1*$^{-/-}$ mice (data not shown).

LTP in the spinal cord dorsal horn is strongly implicated in genesis of chronic pain (Ruscheweyh et al., 2011). In anesthetized mice, spinal LTP was induced by tetanic stimulation (100 Hz, 1 s, 4 trains) in all C57/B6 WT mice, lasting for >2 h, with an amplitude increase of 91% at 1 h (Fig. 1*E*). Of note, LTP failed to be induced in *Trpv1* KO mice (Fig. 1*E*), indicating an essential role of TRPV1 in spinal LTP induction. Since TRPV1 is expressed by primary afferent terminals (Tominaga et al., 1998), our finding points to a presynaptic mechanism of spinal LTP.

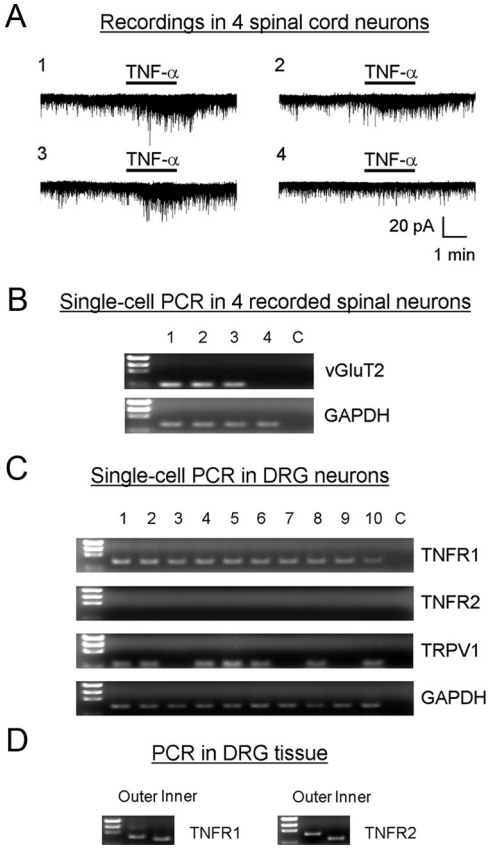

Figure 3. TNF-α increases sEPSC frequency in vGluT2-positive excitatory neurons in spinal cord slices. ***A***, sEPSCs traces in 4 lamina IIo neurons in spinal cord slices of WT mice showing sEPSC increases in 3 neurons after TNF-α (10 ng/ml) stimulation. ***B***, Single-cell PCR from the 4 lamina IIo neurons recorded in ***A*** showing that 3 TNF-α-responding neurons also express vGluT2. ***C***, Single-cell PCR in 10 small-sized DRG neurons (<25 μm) showing TRPV1 expression in 7 neurons, TNFR1 expression in all neurons, and no expression of TNFR2. c indicates negative control. GAPDH serves as internal control. ***D***, RT-PCR with both outer and inner primers showing positive bands for TNFR1 and TNFR2 mRNA in DRG tissues.

TNF-α increases sEPSC frequency via TRPV1 and contributes to spinal cord LTP induction

TNF-α plays a critical role in the development of inflammatory pain (Zhang et al., 2011) and increases TRPV1 sensitivity in DRG neurons (Nicol et al., 1997; Jin and Gereau, 2006; Constantin et al., 2008). We further investigated whether TNF-α would enhance spinal synaptic transmission via TRPV1. As previously reported (Kawasaki et al., 2008), perfusion of spinal cord slice with TNF-α (10 ng/ml) increased the frequency but not the amplitude of sEPSCs (Fig. 2*A*,*B*). Interestingly, all TNF-α-responding neurons also responded to capsaicin by displaying sEPSC frequency increase (Fig. 2*A*,*B*). In parallel, TNF-α-elicited sEPSC frequency increases were abolished in *Trpv1*$^{-/-}$ mice (Fig. 2*C*,*D*). Collectively, our data suggest that TNF-α might increase sEPSC frequency via TRPV1-mediated glutamate release from presynaptic terminals.

TNF-α signals through TNF receptor subtype 1 (TNFR1) and/or TNFR subtype 2 (TNFR2), and both TNFR1 and TNFR2 are required for eliciting central sensitization in the formalin test (Zhang et al., 2011). As expected, spinal cord LTP was abolished in *Tnfr1*$^{-/-}$ mice (Fig. 2*E*), in support of a previous study using a different LTP induction protocol (Zhong et al., 2010). Additionally, we found spinal cord LTP was also impaired in *Tnfr2*$^{-/-}$ mice (Fig. 2*E*).

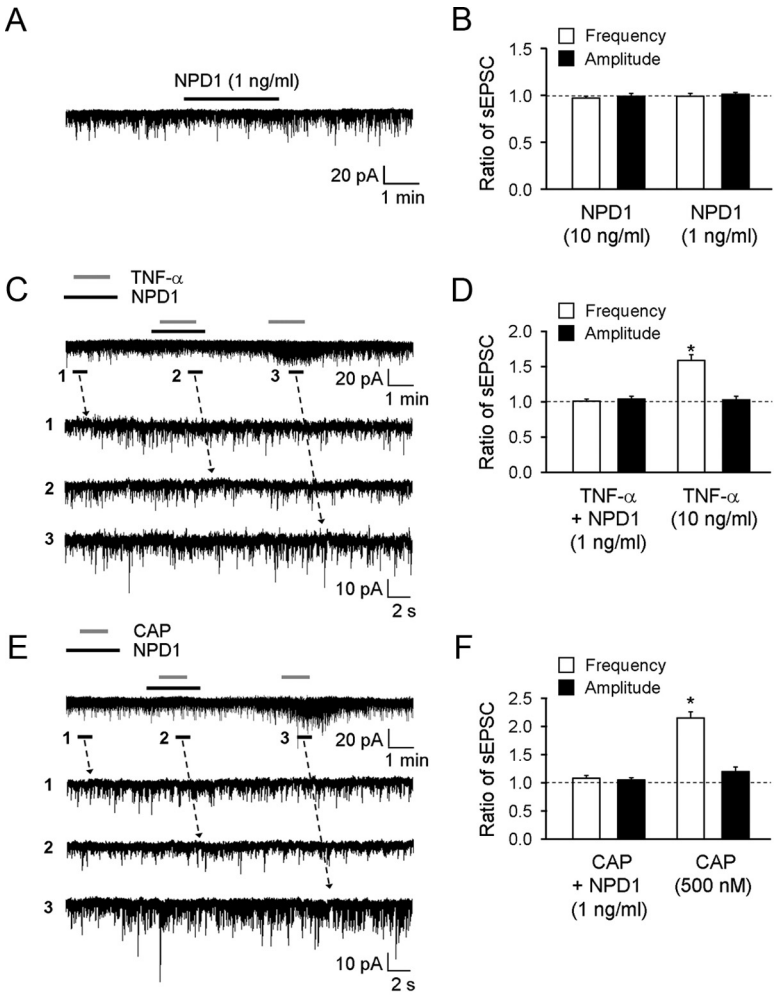

Figure 4. NPD1 blocks TRPV1- and TNF-α-evoked sEPSC frequency increases in lamina IIo neurons of spinal cord slices. ***A***, sEPSCs traces of WT mice before and after NPD1 perfusion (1 ng/ml). ***B***, Both the frequency and amplitude of sEPSCs in nontreated slices are not altered by NPD1 (1 and 10 ng/ml). $n = 8$. ***C***, sEPSCs traces of WT mice before and after TNF-α (10 ng/ml) and NPD1 perfusion (1 ng/ml). Trace 1, 2, and 3 are enlarged in lower rows and indicate recordings of baseline, TNF-α plus NPD1, and TNF-α plus vehicle, respectively. ***D***, NPD1 (1 ng/ml) blocks TNF-α-induced sEPSC frequency increase. $^*p < 0.05$ (vs pretreatment baseline, $n = 5$, *t* test). ***E***, sEPSCs traces in WT mice before and after CAP (0.5 μM) and NPD1 perfusion (1 ng/ml). Traces 1, 2, and 3 are enlarged in lower rows and indicate recordings of baseline, capsaicin plus NPD1, and capsaicin plus vehicle, respectively. ***F***, NPD1 (1 ng/ml) blocks capsaicin-induced sEPSC frequency increase. $^*p < 0.05$ (vs pretreatment baseline, $n = 5$, *t* test). All the data shown are mean ± SEM.

TNF-α increases sEPSC frequency in spinal excitatory neurons via TRPV1

Spinal cord lamina II neurons are not well characterized, although excitatory neurons dominate the lamina II (Santos et al., 2007; Todd, 2010). Lamina II contains 4 types of neurons: islet, central, radial, and vertical neurons (Grudt and Perl, 2002; Lu and Perl, 2005). It appears that all islet neurons are inhibitory and most vertical neurons are excitatory (Todd, 2010). GAD65/67-GFP mice are useful to identify inhibitory neurons but not excitatory neurons, since GAD-GFP does not label all inhibitor neurons (Daniele and MacDermott, 2009; Yasaka et al., 2010). We combined patch-clamp recording and single-cell PCR to define whether the neurons we recorded are excitatory ones, using vGluT2 as a marker (Cheng et al., 2004). An early study used single-cell PCR to characterize nicotine receptor transcripts in recorded spinal cord neurons (Cordero-Erausquin et al., 2004). To improve the sensitivity and selectivity of single-cell PCR, we performed two rounds of PCRs using two different sets of primers (outer and inner primers, Table 1), as we recently demonstrated (Park et al., 2006; Liu et al., 2010). Figure 3*A* shows that 3 of 4 lamina IIo neurons responded to TNF-α showing increases in sEPSC frequency. Consistently, these 3 neurons also expressed vGluT2 (Fig. 3*B*). However, negative control showed no signal for vGluT2 (Fig. 3*B*). Also see below (Fig. 5) for analyses in additional 6 neurons.

To provide a cellular substrate for TNF-α regulation of TRPV1, we also conducted single-cell PCR to characterize the colocalization of TRPV1 and TNF receptors (TNFR1 and TNFR2) in small-sized C-fiber neurons (<25 μm) in DRG cultures. Among 10 small neurons we collected, 7 of them expressed TRPV1, but all of them expressed TNFR1 (Fig. 3*C*), suggesting that TNFR1 is ubiquitously expressed in DRG neurons. However, DRG neurons did not express TNFR2 (Fig. 3*C*), despite the presence of TNFR2 transcript in DRG tissues (Fig. 3*D*). Thus it is conceivable to postulate that TNF-α acts on primary afferent central terminals to increase glutamate release via TRPV1 activation. We postulate that these TRPV1$^+$ terminals in lamina IIo form synapses with vGluT2$^+$ excitatory neurons, which in turn connect to lamina I projection neurons as part of the ascending pain pathway (Todd, 2010). Although TNFR2 was not detected in DRG neurons, we cannot exclude the possibility that functional TNFR2 (e.g., different splicing form of TNFR2) may be present in central terminals to mediate TNF-α signaling.

NPD1 blocks TRPV1- and TNF-α-evoked enhancement in synaptic transmission

To define the role of NPD1 in pain control, we first examined the action of NPD1 on basal and evoked synaptic transmission in the spinal cord. Notably, NPD1 did not alter basal synaptic transmission: both the frequency and amplitude of sEPSCs were not affected by NPD1 perfusion at low and high concentrations [1 and 10 ng/ml (3 and 30 nM); Figure 4*A*, *B*)]. Of interest, at a low concentration, NPD1 (1 ng/ml) completely blocked the sEPSC frequency increase by TNF-α (Fig. 4*C*,*D*) and capsaicin (Fig. 4*E*, *F*). The actions of NPD1 were rapidly washed away during perfusion and sEPSC increases were induced by TNF-α and capsaicin (Fig. 4*C*,*E*). These results suggested that NPD1 can potently abolish TNF-α- and TRPV1-evoked spinal cord synaptic plasticity (sEPSC frequency increases). But unlike TRPV1 antagonists (Fig. 1*D*), NPD1 did not modulate basal synaptic transmission (Fig. 4*A*, *B*), because NPD1 is not a direct blocker of the TRPV1 channels (see Fig. 7*A*).

Single-cell PCR analyses in additional 6 recorded lamina IIo neurons indicated that 4 neurons responded to TNF-α with sEPSC frequency increases and these 4 neurons also expressed vGluT2 (Fig. 5*A*, *B*), confirming that only excitatory neurons in

lamina IIo display sEPSC frequency increase following TNF-α stimulation. Further, NPD1 treatment prevented sEPSC frequency increase by TNF-α (Fig. 5*A*,*B*).

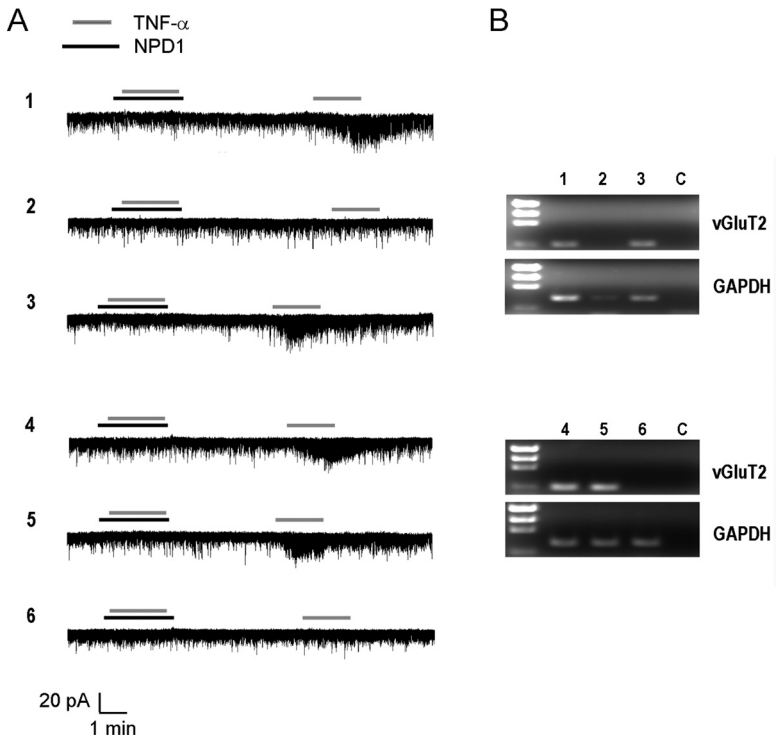

Figure 5. NPD1 prevents TNF-α-induced sEPSC frequency increase in vGluT2-positive excitatory neurons in spinal cord slices. ***A***, sEPSCs traces in 6 lamina IIo neurons in spinal cord slices of WT mice showing sEPSC increases in 4 neurons after TNF-α (10 ng/ml) stimulation, which is prevented by NPD1 (1 ng/ml). ***B***, Single-cell PCR from the 6 lamina IIo neurons recorded in ***A*** showing that 4 TNF-α-responding neurons also express vGluT2. c, Negative control. GAPDH serves as internal control.

NPD1 potently inhibits TRPV1 but not TRPA1 currents in dissociate DRG neurons

Since TNF-α increases sEPSC frequency via TRPV1, it is tempting to postulate that NPD1 modulates synaptic transmission via regulating TRPV1 activity. To directly test this hypothesis, we examined the actions of NPD1 on TRPV1 currents in dissociated DRG neurons, which were induced by low concentration of capsaicin (CAP, 100 nM) to minimize TRPV1 desensitization following multiple applications of capsaicin. NPD1 elicited a dose-dependent inhibition of TRPV1 currents: TRPV1 currents were completely blocked by 10, 1, and 0.5 ng/ml NPD1 (Fig. 6*A–C*), partly inhibited by 0.3 and 0.1 ng/ml NPD1 (Fig. 6*D*,*E*), but not affected by 0.05 ng/ml NPD1 (Fig. 6*F*). Strikingly, NPD1 inhibited TRPV1 currents with a very low IC_{50}, 0.36 nM (i.e., 0.13 ng/ml, Fig. 6*G*). For a comparison AMG9810 inhibited TRPV1 currents with IC_{50} = 163 nM, which is ≈500 times higher than that of NPD1 (Fig. 6*H*). In addition, current-clamp recording revealed that NPD1 (1 ng/ml) also potently blocked capsaicin-induced action potentials (Fig. 6*I*).

We also examined whether NPD1 would modulate TRPA1 current, induced by mustard oil AITC (300 μM). Of interest, NPD1, even at the highest concentration (10 ng/ml), failed to inhibit TRPA1 current (Fig. 6*J*), and the ratio of the second TRPA1 current amplitude vs the first current amplitude was 1.0 ± 0.02 ($p > 0.05$, $n = 12$, *t* test).

NPD1 inhibits TRPV1 currents in dissociate DRG neurons via Gαi-coupled GPCRs and associated signaling pathways

Neurons are known to express receptors for lipid mediators (Bito et al., 1992; Hucho and Levine, 2007). Our results suggest that primary sensory neurons express functional receptors for NPD1. Although NPD1-specific receptors are not identified at the recombinant level, these anti-inflammatory-proresolving mediators are known to signal via GPCRs (Serhan et al., 2008). Labeled NPD1 specifically binds to neurons and human leukocytes (Marcheselli et al., 2010). Notably, pretreatment of DRG neurons with pertussis toxin (PTX, 0.5 μg/ml) for 18 h completely blocked NPD1's inhibitory effects on capsaicin-induced TRPV1 currents (Fig. 7*A*), and the ratio of the second TRPV1 current amplitude vs the first current amplitude was 1.0 ± 0.03 ($p > 0.05$, $n = 12$, *t* test). This finding suggests that NPD1 could act on Gαi-coupled GPCR to modulate TRPV1 activity.

Since Gαi-coupled GPCRs are known to inhibit the adenylyl cyclase (AC) and protein kinase A (PKA) pathway, we further tested whether NPD1 would modulate TRPV1 activity via the AC/PKA pathway. Capsaicin not only activates TRPV1 but also activates Ca^{2+}-dependent signaling transduction pathway, such as AC/PKA and the extracellular signal-regulated kinase (ERK) pathways (Zhuang et al., 2004). Perfusion of DRG neurons with AC inhibitor SQ22536 (1 and 10 μM) and PKA inhibitor H89 (1 and 10 nM) each suppressed CAP-induced currents, in a dose-dependent manner (Fig. 7*B*), suggesting a critical role of the AC/PKA pathway in regulating TRPV1 activity in our recording setting. Notably, the residual TRPV1 currents after SQ22536 and H89 treatment were completely blocked by NPD1 (Fig. 7*B*). Of note, PKA has been shown to modulate TRPV1 activity through direct phosphorylation of TRPV1 (Bhave et al., 2002), involving the scaffolding protein A-kinase anchoring protein (AKAP). AKAP can mediate TRPV1 thermal hyperalgesia through PKA phosphorylation of TRPV1 (Jeske et al., 2008; Schnizler et al., 2008).

ERK activation in DRG neurons was implicated in TRPV1 regulation and heat hyperalgesia (Zhuang et al., 2004). The ERK pathway can be activated by cAMP through exchange proteins activated by cAMP (Epac) pathway (Eijkelkamp et al., 2010) or by PKA (Kawasaki et al., 2004). ERK can also be inhibited by activation of the Gαi-coupled resolvin E1 receptor ChemR23, which has also been shown to block TRPV1 signaling (Xu et al., 2010). Perfusion of DRG neurons with U0126 (1 and 10 μM), a selective inhibitor of the ERK kinase, MEK, suppressed TRPV1 currents in a dose-dependent manner (Fig. 7*B*). Thus, it is likely that NPD1 might block TRPV1 signaling via inhibiting the AC, PKA, and ERK signaling pathways.

Spinal delivery of NPD1 abrogates spinal cord LTP

Because (1) TRPV1 and TNF-α signaling were required for the induction of spinal LTP, and (2) NPD1 inhibited TRPV1 and TNF-α-evoked sEPSC increase, we hypothesized that NPD1 could also inhibit the induction of spinal LTP. As we expected, spinal administration of NPD1 (10 ng) via intrathecal (i.t.) route,

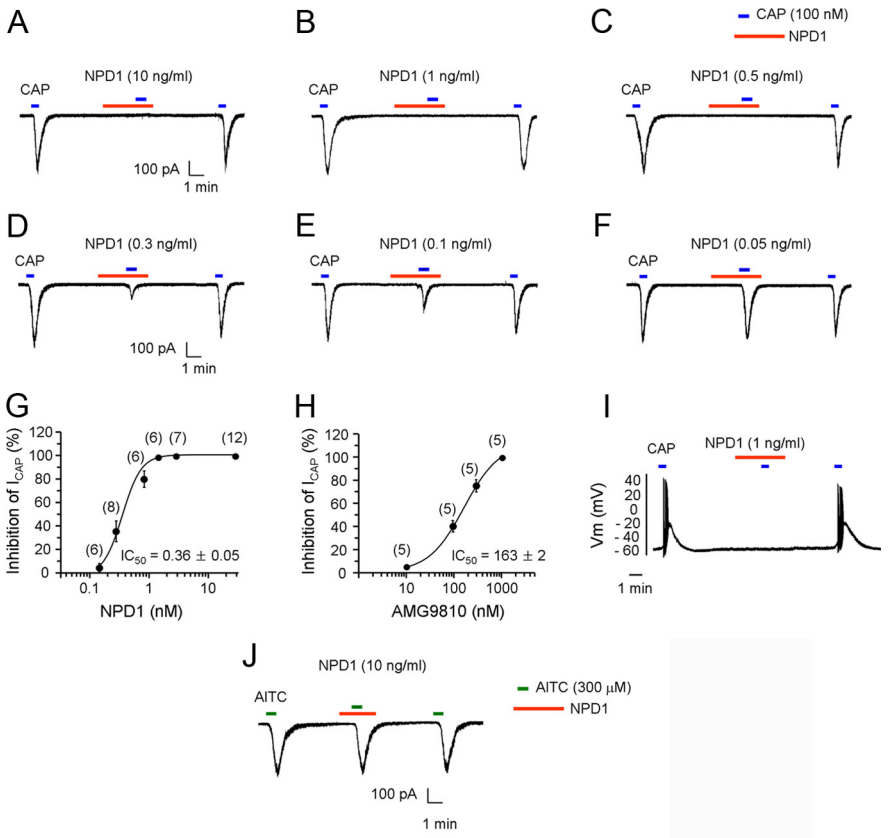

Figure 6. NPD1 potently inhibits TRPV1 but not TRPA1 current in dissociate DRG neurons. ***A–F***, Voltage-clamp recordings showing dose-dependent inhibition of capsaicin (100 nM)-induced TRPV1 currents by NPD1 in small-sized DRG neurons. ***G***, ***H***, Dose–response curve of NPD1 (***G***)- and AMG9810 (***H***)-induced inhibition of TRPV1 currents. Note the IC_{50} (0.36 nM ≈ 0.13 ng/ml; molecular weight of NPD1 = 360.5) is very low. ***I***, Current-clamp recording showing blockade of capsaicin-induced action potentials by NPD1 (1 ng/ml). ***J***, Actions of NPD1 (10 ng/ml) on AITC (300 μM)-induced TRPA1 currents. Note that NPD1 does not inhibit TRPA1 currents. $n = 12$.

10 min before tetanic stimulation, completely prevented the induction of spinal LTP in all mice we tested ($n = 5$, Fig. 8*A*). To further determine whether the established spinal LTP could be reversed by NPD1, we injected NPD1 (10 ng, i.t.) 2 h after the LTP induction. In vehicle (PBS)-treated animals, the amplitude of field potential was slightly increased after the tetanic stimulation (2–4 h vs 0–2 h, Fig. 8*B*). Strikingly, LTP was rapidly reversed by intrathecal NPD1 (Fig. 8*B*). However, intrathecal administration of the TRPV1 antagonist AMG 9810 (20 nmol), 2 h after the LTP induction, had no effect on established LTP (Fig. 8*C*), although this dose (20 nmol) via intrathecal route should inhibit heat hyperalgesia (Yu et al., 2008). Thus, TRPV1 is only important for the induction but not the maintenance of spinal LTP.

NPD1 reduces TRPV1-dependent inflammatory pain but not baseline pain

Given the actions of NPD1 in regulating synaptic plasticity and TRPV1 activity, we further postulated that NPD1 could attenuate TRPV1-mediated inflammatory pain. To this end, we tested the actions of NPD1 in several different pain conditions that are mediated by TRPV1. First, we tested CFA-induced inflammatory heat hyperalgesia, which is abrogated in *Trpv1*$^{-/-}$ mice (Caterina et al., 2000; Davis et al., 2000). Intraplantar injection of CFA into a hindpaw-induced robust heat hyperalgesia, a reduction of paw withdrawal latency, which was reduced by post-treatment of NPD1 (10 ng, i.t., Fig. 9*A*). Notably, NPD1 elicited a rapid attenuation of heat hyperalgesia within 20 min, and this anti-hyperalgesic effect lasted 2 h and recovered at 3 h (Fig. 9*A*). Even a lower dose of NPD1 (1 ng, i.t.) heat hyperalgesia was transiently reduced for 40 min (Fig. 9*B*).

Next, we investigated intrathecal TNF-α-induced heat hyperalgesia, which is abolished in *Trpv1*$^{-/-}$ mice (Xu et al., 2010). TNF-α (20 ng, i.t.) elicited a transient heat hyperalgesia recovering after 24 h, which was prevented by NPD1 (10 ng, i.t., Fig. 9*C*).

Although NPD1 potently reduced inflammatory heat hyperalgesia, NPD1 did not alter baseline pain in naive mice, even at a high dose (10 ng, i.t., Fig. 9*D*), consistent with the result that NPD1 did not impact basal synaptic transmission (Fig. 4*B*). Neither did peripheral administration of NPD1 via intraplantar route (i.pl.)—even at a very high dose (200 ng)—alter the heat pain sensitivity in naive mice (Fig. 9*E*). Thus, NPD1 does not act as a classic analgesic (e.g., morphine). Instead, NPD1 plays a unique role in "normalization" of inflammatory pain by bringing the hyperactive state back to the normal state. Of note, basal heat pain sensitivity in Hargreaves test did not change either in *Trpv1*$^{-/-}$ mice (Xu et al., 2010).

Intrathecal capsaicin is also known to elicit spontaneous pain (Xu et al., 2010). Intrathecal NPD1 (10 ng, i.t.) suppressed capsaicin (500 ng, i.t.)-induced nocifensive licking behavior by 75% (Fig. 9*F*). The seconds spent on licking were 175.1 ± 13.7 s and 47.5 ± 9.6 s for vehicle and NPD1-treated mice, respectively ($p < 0.05$, $n = 7$, *t* test).

We further examined intraplantar capsaicin-induced nocifensive behavior. NPD1 at the dose of 20 and 200 ng (i.pl.) reduced intraplantar capsaicin-induced pain by 35% and 53%, respectively. The seconds spent on licking were 157.6 ± 7.0 s, 102.3 ± 6.4 s, and 73.8 ± 12.2 s for vehicle, NPD1–20 ng, and NPD1–200 ng group, respectively ($p < 0.05$, $n = 5–8$, *t* test).

Finally, we investigated the TRPA1-induced pain. NPD1 treatment, even at a high dose (200 ng), did not affect AITC-induced licking/flinching behavior. The seconds spent on spontaneous pain were 125.3 ± 9.3 s and 119.7 ± 5.7 s for vehicle and NPD1-treated mice, respectively ($p > 0.05$, $n = 6$, *t* test, Fig. 9*F*). Together, our data suggest that NPD1 also differently regulates TRPV1 and TRPA1-mediated evoked pain (heat hyperalgesia) and spontaneous pain.

NPD1 inhibits TRPV1-independent but TNF-α-dependent inflammatory pain

Intraplantar injection of diluted formalin (5%) induces characteristic two-phase spontaneous pain behaviors (licking and flinching). While the first-phase pain is a result of direct sensitization of TRPA1 in nociceptor terminals (McNamara et al., 2007), the second-phase pain may result from central sensitization (Xu et al., 2010). Notably, the second-phase pain is reduced in *Tnfr*$^{-/-}$ mice but not in *Trpv1*$^{-/-}$ mice (Xu et al., 2010; Zhang et al., 2011). Preemptive injection of NPD1, at very low doses (0.1–10 ng,

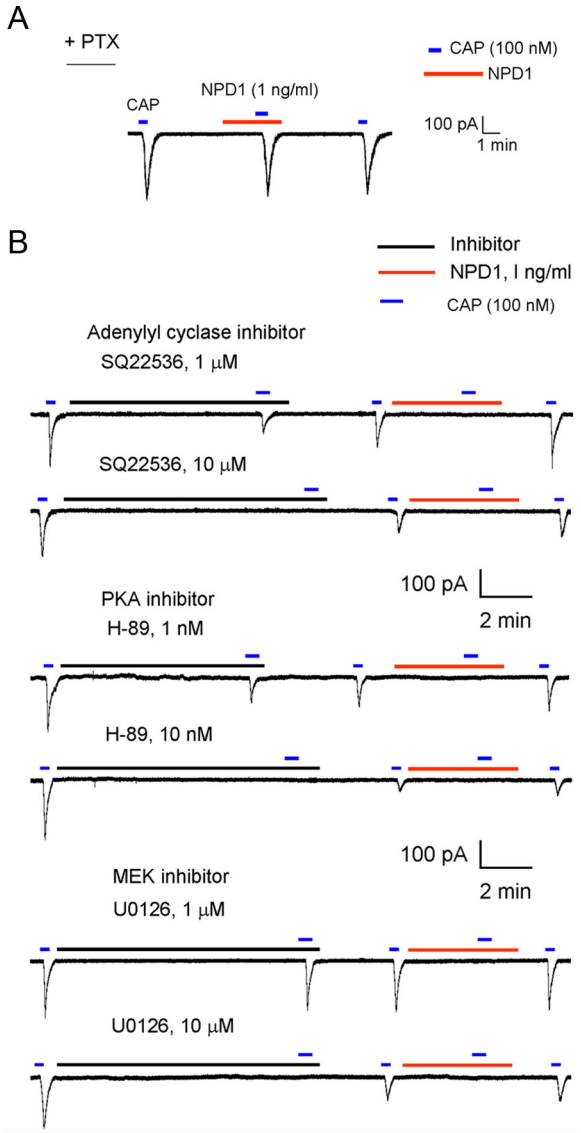

Figure 7. Effects of NPD1 on TRPV1 currents after pretreatment of pertussis toxin (PTX) and effects AC, PKA, and MEK inhibitor on TRPV1 currents in dissociate DRG neurons. ***A***, Effects of PTX on NPD1 inhibition of TRPV1 current. Note that PTX pretreatment (0.5 μg/ml, 18 h) abolishes NPD1's inhibitory effects on TRPV1 currents. $n = 11$. ***B***, Effects of adenylyl cyclase inhibitor SQ22536 (1 and 10 μM), PKA inhibitor H89 (1 and 10 nM), MEK inhibitor U0126 (1 and 10 μM), and NPD1 (1 ng/ml) on capsaicin-induced currents.

i.t.), reduced the formalin-evoked pain in the second-phase but not in the first-phase in a dose-dependent manner (Fig. 10*A*,*B*). Intrathecal TNF-α also elicited TRPV1-independent mechanical allodynia (Xu et al., 2010), a reduction in paw withdrawal threshold, which was more persistent than TNF-α-evoked heat hyperalgesia, recovering after 5 d (Fig. 10*C*). NPD1 treatment (10 ng, i.t.) partially prevented the TNF-α-evoked mechanical allodynia (Fig. 10*C*).

Discussion

TRPV1 contributes to inflammatory pain via regulating spinal cord synaptic transmission and LTP in presynaptic terminals

Our findings demonstrated an important role of TRPV1 in regulating basal and evoked synaptic transmission as well as LTP in the spinal cord. A brief stimulation of TRPV1 induces prolonged elevation of presynaptic $[Ca^{2+}]$ levels and concomitant enhancement of glutamate release at sensory synapses, involving presynaptic mitochondria (Medvedeva et al., 2008). Spinal activation of mGluR5 elicits heat hyperalgesia via functional coupling with TRPV1 at presynaptic terminals (Kim et al., 2009). Our patch-clamp recordings in lamina IIo neurons showed that sEPSC frequency but not amplitude was reduced in *Trpv1*$^{-/-}$ mice and further in WT mice treated with TRPV1 antagonists. Furthermore, induction of spinal LTP was abolished in *Trpv1*$^{-/-}$ mice (Fig. 1). However, TRPV1 was not required for the maintenance of spinal LTP (Fig. 8*C*). Since anatomical and functional TRPV1 is expressed in primary afferent terminals in the superficial spinal cord (Tominaga et al., 1998; Ji et al., 2002; Medvedeva et al., 2008; Kim et al., 2009; Cavanaugh et al., 2011), our data highlight a presynaptic mechanism of spinal cord LTP, although postsynaptic mechanisms of spinal LTP (e.g., activation of NMDA and NK-1 receptors) are well documented (Ruscheweyh et al., 2011). Presynaptic control of spinal LTP was also implicated in a recent study showing that ablation of TRPV1-expressing central terminals blocked opioid-induced spinal LTP (Zhou et al., 2010). Notably, recent studies demonstrated a role of postsynaptic TRPV1 in the induction of long-term depression (LTD) in the dentate gyrus and nucleus accumbens (Chávez et al., 2010; Grueter et al., 2010). Thus, TRPV1 can regulate both LTP and LTD, depending on the regions (spinal cord vs brain) and synaptic sites (presynaptic vs postsynaptic). Although TRPV1 currents were recorded in postsynaptic neurons in the dentate gyrus and nucleus accumbens, postsynaptic TRPV1 expression in these brain regions and spinal cord was not well established (Chávez et al., 2010; Grueter et al., 2010; Cavanaugh et al., 2011).

It is conceivable that TRPV1 mediates inflammatory pain via both peripheral and central mechanisms. After tissue injury, TRPV1 in peripheral terminals of nociceptors could be sensitized by various inflammatory mediators, e.g., bradykinin, NGF, and TNF-α (Chuang et al., 2001; Zhuang et al., 2004; Jin and Gereau, 2006). The present study also points to a central role of TRPV1 in pain control. Functional coupling of TRPV1 with mGluR5 (Kim et al., 2009) and TNF-α (see next section) as well as generation of endogenous lipid ligands under stress conditions (Patwardhan et al., 2010) could activate and sensitize TRPV1 in central terminals to release neurotransmitters (Fig. 11). It is important to emphasize that CNS penetration is critical for TRPV1 antagonists to produce broad-spectrum analgesia (Cui et al., 2006).

TNF-α elicits spinal synaptic plasticity and inflammatory pain partly via functional coupling with TRPV1

It is well known that TNF-α generates pathological pain via peripheral actions (Sommer and Kress, 2004). Several lines of evidence also point to central actions of TNF-α in pain sensitization. First, TNF-α is induced in spinal cord glial cells (e.g., microglia) in chronic pain conditions (Ji and Suter, 2007; Zhou et al., 2008). Second, intrathecal injection of TNF-α produces heat hyperalgesia and mechanical allodynia (Fig. 10*C*). Third, intrathecal TNF-α inhibitor etanercept attenuates inflammatory pain (Choi et al., 2010). In particular, TNF-α can powerfully modulate synaptic transmission in the spinal cord (Kawasaki et al., 2008; Zhang et al., 2010). Perfusion of spinal cord slices with TNF-α dramatically increased the sEPSC frequency (Fig. 2*A*,*B*).

One interesting finding is *Trpv1*$^{-/-}$ mice exhibited no sEPSC frequency increase following TNF-α stimulation (Fig. 2*C*,*D*), suggesting that TRPV1 is required for TNF-α-induced glutamate release at presynaptic terminals (Fig. 11). TNF-α was shown to increase capsaicin sensitivity in DRG neurons (Nicol et al., 1997; Jin and Gereau, 2006; Constantin et al., 2008). Our finding further suggests that TNF-α may serve as an endogenous activator of

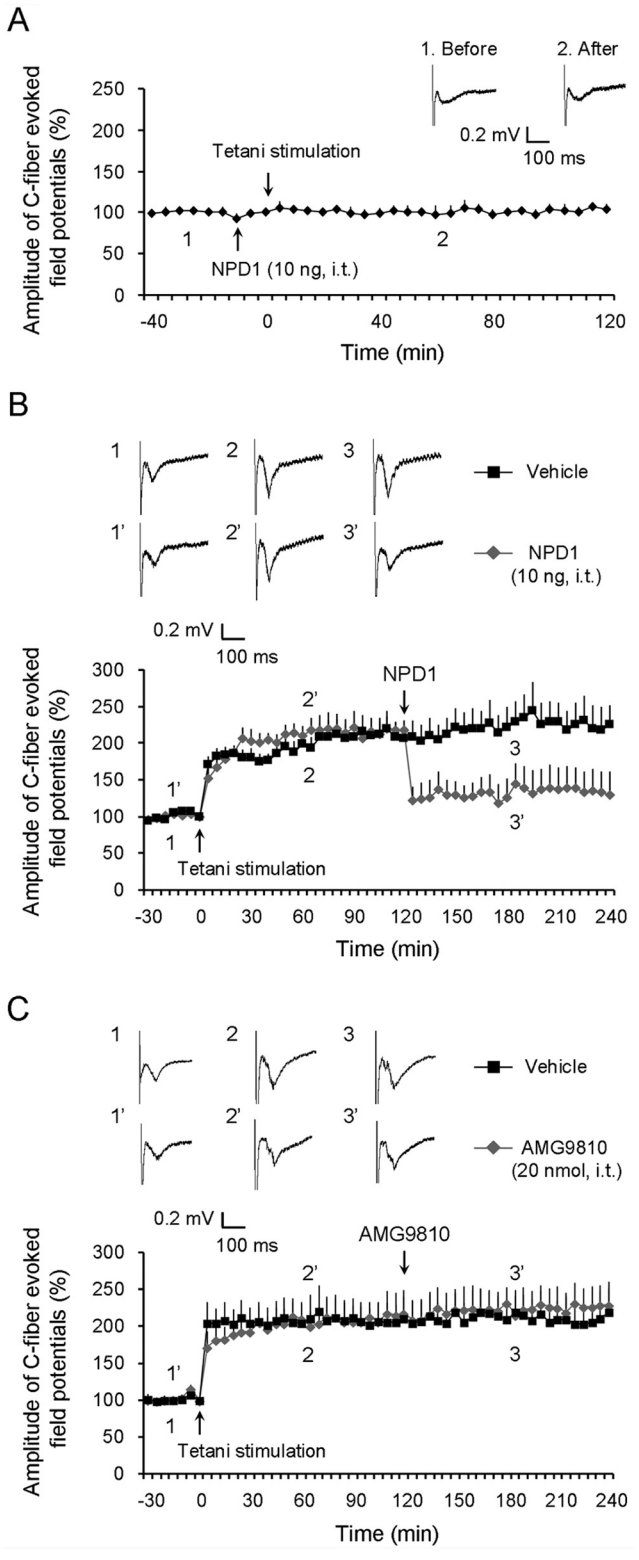

Figure 8. Prevention and reversal of spinal LTP by intrathecal NPD1 and no reversal of spinal LTP by intrathecal AMG9810. ***A***, Prevention of LTP of C-fiber-evoked field potentials in the dorsal horn of anesthetized WT mice by NPD1 (10 ng, i.t.). Top traces are C-fiber-evoked field potentials in the dorsal horn in NPD1-treated mice before (1) and after (2) conditioning tetanic stimulation. $n = 5$ mice. ***B***, Reversal of LTP of C-fiber-evoked field potentials in the dorsal horn of anesthetized WT mice by NPD1 (10 ng, i.t.), administrated 2 h after LTP induction. Top traces are C-fiber-evoked spinal field potentials in vehicle- and NPD1-treated mice before LTP induction (1 and 1′), after LTP induction (2 and 2′), and after NPD1 treatment (3 and 3′). $^*p < 0.05$ (vehicle vs NPD1, two-way ANOVA, $n = 5$). ***C***, No reversal of LTP of C-fiber-evoked field potentials in the dorsal horn of anesthetized WT mice by AMG9810 (20 nmol, i.t.), administrated 2 h after LTP induction by tetani (4 trains of tetanic stimulation). Top traces are C-fiber-evoked spinal field potentials in vehicle- and NPD1-treated mice before LTP induction (1 and 1′), after LTP induction (2 and 2′), and after NPD1 treatment (3 and 3′). $n = 5$ mice for AMG9801 and 4 mice for vehicle (10% DMSO). All the data shown are mean ± SEM.

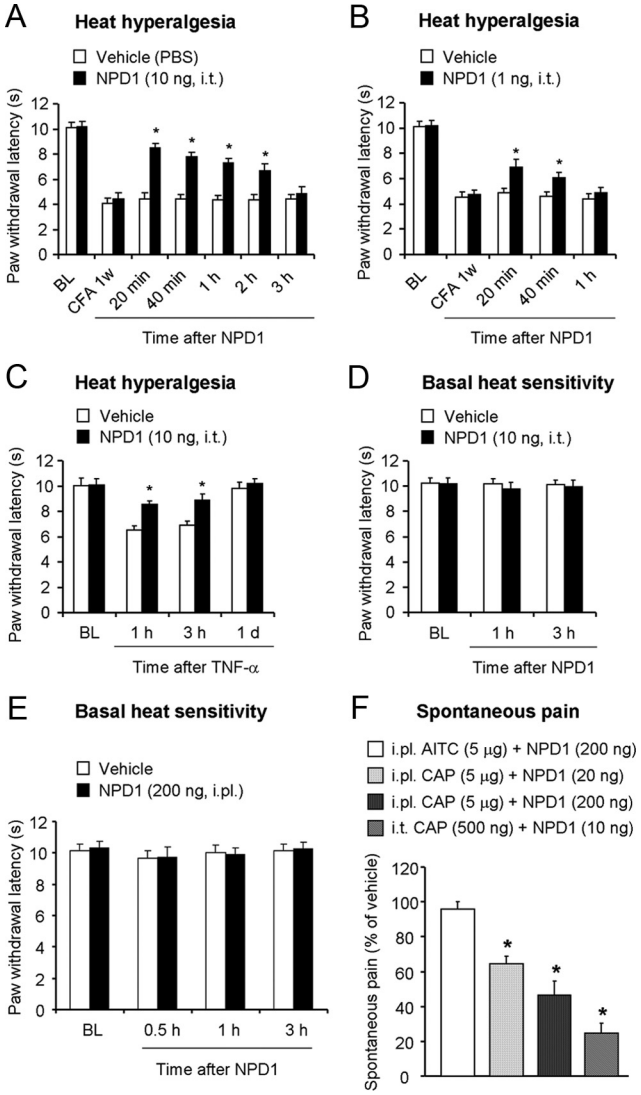

Figure 9. Intrathecal injection of NPD1 reduces TRPV1-dependent inflammatory pain but not baseline pain. ***A***, ***B***, CFA-induced heat hyperalgesia on day 7 is rapidly reduced by intrathecal post-treatment of 10 ng of NPD1 (***A***) and 1 ng of NPD1 (***B***). $^*p < 0.05$ (PBS vehicle vs NPD1, $n = 6$, *t* test). ***C***, Heat hyperalgesia evoked by TNF-α (20 ng, i.t.) is prevented by NPD1 (10 ng, i.t.). $p < 0.05$ (vehicle vs NPD1, $n = 6$, *t* test). ***D***, ***E***, Baseline heat sensitivity in naive mice is not altered by NPD1 either after intrathecal injection (10 ng, i.t., $n = 5$, D) or after intraplantar injection (200 ng, i.pl., $n = 5$, E). ***F***, Spontaneous pain induced by intrathecal capsaicin (500 ng, i.t.) and intraplantar capsaicin (5 μg, i.pl.) but not by intraplantar AITC (5 μg) is reduced by NPD1. The spontaneous pain was measured by seconds mice spent on licking and flinching behavior, and the data are expressed as percentage of vehicle. $^*p < 0.05$, $n = 5$–8, *t* test, compared with corresponding vehicles. All the data shown are mean ± SEM.

TRPV1 to evoke glutamate release in central terminals of DRG neurons (Fig. 11). As in *Trpv1*$^{-/-}$ mice, spinal LTP is abolished in *Tnfr1*$^{-/-}$ mice and impaired in *Tnfr2*$^{-/-}$ mice, indicating an importance of TNF-α signaling in spinal long-term synaptic plasticity.

Another interesting finding is TNF-α only increased sEPSC frequency in excitatory neurons: all the TNF-α-responding neurons recorded in lamina IIo expressed vGluT2, a marker for excitatory neurons. We postulate that TNF-α induces glutamate release at presynaptic sties of the first-order synapses in the ascending pain pathway (Fig. 11). Although several groups of neurons with different morphology have been characterized in the lamina II, these morphological characterizations cannot define whether a neuron is excitatory (Todd, 2010). GABAergic neurons in lamina II were shown to receive C-fiber input (Daniele and MacDermott, 2009), but GAD65/67-GFP staining dose not label all the inhibitory neurons. Our results showed that single-cell PCR is a sensitive and selective method to define chemical features of the recorded neurons in the spinal cord circuitry. Although our data support a presynaptic regulation of TNF-α, we should not rule out that TNF-α may also drive inflammatory pain via postsynaptic mechanisms, such as inducing trafficking and surface expression of AMPA receptors (Beattie et al., 2002; Stellwagen et al., 2005; Choi et al., 2010) and increasing NMDA-induced currents in spinal lamina II neurons (Kawasaki et al., 2008).

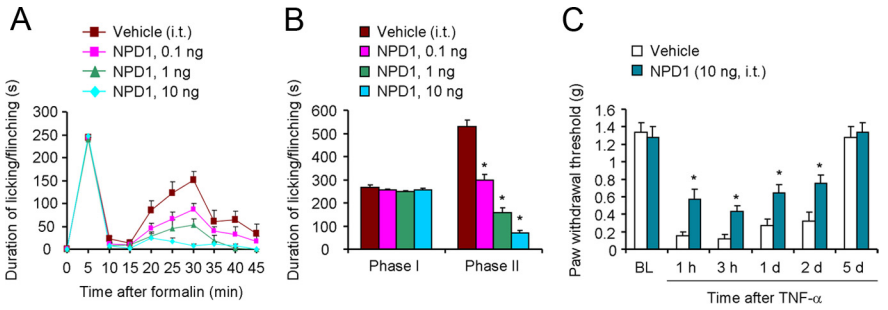

Figure 10. Intrathecal injection of NPD1 reduces TRPV1-independent but TNF-α-dependent inflammatory pain. ***A***, Time course of formalin-induced spontaneous pain (licking, lifting, and flinching) in mice treated with vehicle (PBS) and NPD1 (0.1, 1, and 10 ng, i.t.). ***B***, Formalin-induced spontaneous pain in the first-phase (0–10 min) and second-phase (10–45 min) in mice treated with vehicle (PBS) and NPD1 (0.1, 1, and 10 ng, i.t.). $p < 0.05$ (vehicle vs NPD1, $n = 5–8$, one-way ANOVA followed by *post hoc* Newman–Keuls test). ***C***, Mechanical allodynia evoked by TNF-α (20 ng, i.t.) is partially prevented by NPD1 (10 ng, i.t.). $p < 0.05$ (vehicle vs NPD1, $n = 5$, *t* test). All the data shown are mean ± SEM.

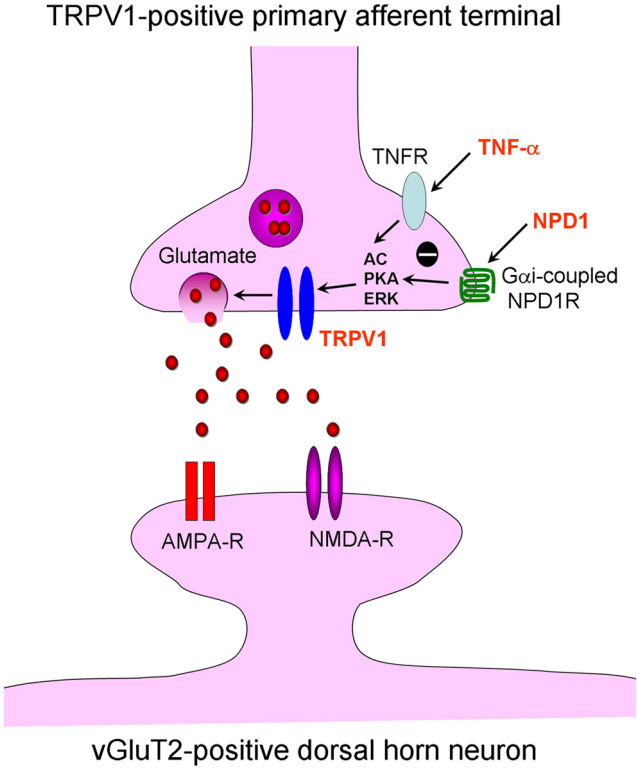

Figure 11. Working hypothesis for NPD1-mediated inhibition of spinal cord synaptic plasticity. TNF-α increases excitatory synaptic transmission (sEPSC frequency) via TRPV1 activation and glutamate release in spinal cord lamina IIo neurons, involving possible activation of the AC, PKA, and ERK pathways. This event only occurs in vGluT2-expressing excitatory neurons. Primary afferent central terminals not only express TRPV1 and TNF receptors, but also express Gαi-coupled GPCRs for NPD1. Activation of the NPD1 receptors inhibits TRPV1 and glutamate release by inhibiting the AC, PKA, and ERK pathways. In addition, NPD1 may also normalize synaptic plasticity and inflammatory pain via TRPV1-independent mechanisms, such as inhibition of NMDA receptor hyperactivity in postsynaptic neurons.

NPD1 is a potent TRPV1 inhibitor and blocks spinal synaptic plasticity and inflammatory pain via TRPV1- and TNF-α-dependent mechanisms

NPD1 is biosynthesized from ω-3 fatty acid DHA and is anti-inflammatory-proresolving as well as exhibits potent neuroprotective role in several neurodegenerative conditions (Bazan et al., 2010). NPD1 is also renoprotective (Hassan and Gronert, 2009) and promotes resolution of inflammation (Gónzalez-Périz et al., 2009; Yamada et al., 2011). NPD1/PD1 activates inflammation-resolution programmers dampening further neutrophil infiltration to the site and also promotes the removal of apoptotic phagocytes, cellular debris and microbes by both neutrophils and macrophages during acute inflammation (Schwab et al., 2007). We demonstrated herein several novel roles of NPD1 in regulating synaptic transmission, TRPV1 activity, and inflammatory pain. Intrathecal injection of NPD1, at very low doses (0.1–10 ng, i.t.), effectively reduced inflammatory pain symptoms in mouse models of acute pain (formalin model) and persistent pain (CFA model). This dose range is even slightly lower than that of RvE1 (0.3–10 ng, i.t.) for the same models (Xu et al., 2010). A dramatic reduction in the formalin-induced second-phase pain by NPD1 supported a central role of NPD1 for pain control. Our results clearly demonstrated that NPD1 could effectively reduce TRPV1-mediated inflammatory pain, including intrathecal or intraplantar capsaicin-induced spontaneous behavior as well as CFA/TNF-α-induced evoked pain (heat hyperalgesia). NPD1 also abrogated TRPV1-independent inflammatory pain, i.e., formalin-elicited second-phase pain and TNF-α-induced mechanical allodynia. It is noteworthy that NPD1 very rapidly attenuated CFA-induced inflammatory pain within 20 min. This rapid inhibition of pain by NPD1 could be a result of inhibiting TRPV1- and TNF-α-evoked enhancement of excitatory synaptic transmission (sEPSC frequency).

Of note NPD1 not only prevented the induction of spinal LTP, but also reversed the established spinal LTP, without affecting basal synaptic transmission. Neither did NPD1 alter basal pain perception in normal conditions. These results suggest a unique role of NPD1 in resolving abnormal synaptic and pain sensitivity. Since the maintenance of spinal LTP and second phase formalin pain are TRPV1-independent (Fig. 8*C*) (Xu et al., 2010), NPD1 may also reduce inflammatory pain via other mechanisms, such as inhibition of postsynaptic NMDA receptor hyperactivity as resolvin E1 (Xu et al., 2010).

It is very striking that NPD1 blocked capsaicin-induced TRPV1 currents in DRG neurons at very low concentrations. The IC_{50} of NPD1 (0.36 nM) for TRPV1 inhibition is ~500 times lower than that of AMG9810, a widely used TRPV1 antagonist. The signaling mechanisms of NPD1 are largely unknown, but NPD1's action on TRPV1 is likely to be mediated by activation of specific pertussis toxin-sensitive/Gαi-coupled GPCRs and subsequent inhibition of the AC, PKA, and ERK signaling pathways (Fig. 11) Thus, lipid mediators not only act as endogenous activators of TRPV1 (Hwang et al., 2000; Patwardhan et al., 2009) but may also serve as endogenous inhibitors of TRPV1.

Clinical relevance

Inflammatory pain, associated with arthritis, lower back injury, and surgery is a growing health problem worldwide. Current treatments for inflammatory pain such as opioids and COX-2 inhibitors are limited by side effects, such as respiratory depression, sedation, and constipation after acute opioid treatment and addiction after chronic opioid treatment, as well as cardiovascular defects after long-term treatment of COX-2 inhibitors (Sommer and Birklein, 2010). Given an important role of TNF-α in regulating spinal cord synaptic plasticity, anti-TNF-α treatment should be effective in alleviating clinical pain associated with inflammation. Indeed, a recent study using functional imaging in patients with rheumatoid arthritis reported that neutralization of TNF-α rapidly (within 24 h) inhibited pain responses in the CNS but slowly (2 weeks) reduced joint inflammation (Hess et al., 2011). However, sustained inhibition of TNF-α may cause infection due to immune suppression. Given an important role of TRPV1 in peripheral sensitization (Basbaum et al., 2009) and central sensitization, TRPV1 antagonists are promising for treating inflammatory pain (Cui et al., 2006) but may also cause hyperthermia (Gavva, 2008). In sharp contrast, endogenous lipid mediators derived from ω-3 fish oils such as NPD1 and resolvins should display a wide safety range. Although NPD1 effectively inhibited TNF-α and TRPV1 signaling at very low concentrations, it acts via GPCR receptors and therefore, should not tonically inhibit the functions of these key signaling molecules, reducing the risk of side effects. Given the remarkable anti-hyperalgesic efficacy of NPD1, neuroprotectins and their metabolically stable analogues and mimetics may be useful for the resolution of inflammatory pain, by inhibiting abnormal synaptic and pain sensitivity but leaving normal pain transmission intact.

References

Basbaum AI, Bautista DM, Scherrer G, Julius D (2009) Cellular and molecular mechanisms of pain. Cell 139:267–284.

Bazan NG, Calandria JM, Serhan CN (2010) Rescue and repair during photoreceptor cell renewal mediated by docosahexaenoic acid-derived neuroprotectin D1. J Lipid Res 51:2018–2031.

Beattie EC, Stellwagen D, Morishita W, Bresnahan JC, Ha BK, Von Zastrow M, Beattie MS, Malenka RC (2002) Control of synaptic strength by glial TNFalpha. Science 295:2282–2285.

Bhave G, Zhu W, Wang H, Brasier DJ, Oxford GS, Gereau RW 4th (2002) cAMP-dependent protein kinase regulates desensitization of the capsaicin receptor (VR1) by direct phosphorylation. Neuron 35:721–731.

Bito H, Nakamura M, Honda Z, Izumi T, Iwatsubo T, Seyama Y, Ogura A, Kudo Y, Shimizu T (1992) Platelet-activating factor (PAF) receptor in rat brain: PAF mobilizes intracellular Ca^{2+} in hippocampal neurons. Neuron 9:285–294.

Blaho VA, Buczynski MW, Brown CR, Dennis EA (2009) Lipidomic analysis of dynamic eicosanoid responses during the induction and resolution of Lyme arthritis. J Biol Chem 284:21599–21612.

Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D (1997) The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 389:816–824.

Caterina MJ, Leffler A, Malmberg AB, Martin WJ, Trafton J, Petersen-Zeitz KR, Koltzenburg M, Basbaum AI, Julius D (2000) Impaired nociception and pain sensation in mice lacking the capsaicin receptor. Science 288:306–313.

Cavanaugh DJ, Chesler AT, Jackson AC, Sigal YM, Yamanaka H, Grant R, O'Donnell D, Nicoll RA, Shah NM, Julius D, Basbaum AI (2011) Trpv1 reporter mice reveal highly restricted brain distribution and functional expression in arteriolar smooth muscle cells. J Neurosci 31:5067–5077.

Chávez AE, Chiu CQ, Castillo PE (2010) TRPV1 activation by endogenous anandamide triggers postsynaptic long-term depression in dentate gyrus. Nat Neurosci 13:1511–1518.

Cheng L, Arata A, Mizuguchi R, Qian Y, Karunaratne A, Gray PA, Arata S, Shirasawa S, Bouchard M, Luo P, Chen CL, Busslinger M, Goulding M, Onimaru H, Ma Q (2004) Tlx3 and Tlx1 are post-mitotic selector genes determining glutamatergic over GABAergic cell fates. Nat Neurosci 7:510–517.

Choi JI, Svensson CI, Koehrn FJ, Bhuskute A, Sorkin LS (2010) Peripheral inflammation induces tumor necrosis factor dependent AMPA receptor trafficking and Akt phosphorylation in spinal cord in addition to pain behavior. Pain 149:243–253.

Chu YX, Zhang Y, Zhang YQ, Zhao ZQ (2010) Involvement of microglial P2X7 receptors and downstream signaling pathways in long-term potentiation of spinal nociceptive responses. Brain Behav Immun 24:1176–1189.

Chuang HH, Prescott ED, Kong H, Shields S, Jordt SE, Basbaum AI, Chao MV, Julius D (2001) Bradykinin and nerve growth factor release the capsaicin receptor from PtdIns(4,5)P2-mediated inhibition. Nature 411:957–962.

Constantin CE, Mair N, Sailer CA, Andratsch M, Xu ZZ, Blumer MJ, Scherbakov N, Davis JB, Bluethmann H, Ji RR, Kress M (2008) Endogenous tumor necrosis factor alpha (TNFalpha) requires TNF receptor type 2 to generate heat hyperalgesia in a mouse cancer model. J Neurosci 28:5072–5081.

Cordero-Erausquin M, Pons S, Faure P, Changeux JP (2004) Nicotine differentially activates inhibitory and excitatory neurons in the dorsal spinal cord. Pain 109:308–318.

Cui M, Honore P, Zhong C, Gauvin D, Mikusa J, Hernandez G, Chandran P, Gomtsyan A, Brown B, Bayburt EK, Marsh K, Bianchi B, McDonald H, Niforatos W, Neelands TR, Moreland RB, Decker MW, Lee CH, Sullivan JP, Faltynek CR (2006) TRPV1 receptors in the CNS play a key role in broad-spectrum analgesia of TRPV1 antagonists. J Neurosci 26:9385–9393.

Daniele CA, MacDermott AB (2009) Low-threshold primary afferent drive onto GABAergic interneurons in the superficial dorsal horn of the mouse. J Neurosci 29:686–695.

Davis JB, Gray J, Gunthorpe MJ, Hatcher JP, Davey PT, Overend P, Harries MH, Latcham J, Clapham C, Atkinson K, Hughes SA, Rance K, Grau E, Harper AJ, Pugh PL, Rogers DC, Bingham S, Randall A, Sheardown SA (2000) Vanilloid receptor-1 is essential for inflammatory thermal hyperalgesia. Nature 405:183–187.

Dixon WJ (1980) Efficient analysis of experimental observations. Annu Rev Pharmacol Toxicol 20:441–462.

Eijkelkamp N, Wang H, Garza-Carbajal A, Willemen HL, Zwartkruis FJ, Wood JN, Dantzer R, Kelley KW, Heijnen CJ, Kavelaars A (2010) Low nociceptor GRK2 prolongs prostaglandin E2 hyperalgesia via biased cAMP signaling to Epac/Rap1, protein kinase Cepsilon, and MEK/ERK. J Neurosci 30:12806–12815.

Engelman HS, MacDermott AB (2004) Presynaptic ionotropic receptors and control of transmitter release. Nat Rev Neurosci 5:135–145.

Fitzgerald M (2005) The development of nociceptive circuits. Nat Rev Neurosci 6:507–520.

Gavva NR (2008) Body-temperature maintenance as the predominant function of the vanilloid receptor TRPV1. Trends Pharmacol Sci 29:550–557.

Gold MS, Gebhart GF (2010) Nociceptor sensitization in pain pathogenesis. Nat Med 16:1248–1257.

Gónzalez-Périz A, Horrillo R, Ferré N, Gronert K, Dong B, Morán-Salvador E, Titos E, Martínez-Clemente M, López-Parra M, Arroyo V, Clària J (2009) Obesity-induced insulin resistance and hepatic steatosis are alle-

viated by omega-3 fatty acids: a role for resolvins and protectins. FASEB J 23:1946–1957.

Grudt TJ, Perl ER (2002) Correlations between neuronal morphology and electrophysiological features in the rodent superficial dorsal horn. J Physiol 540:189–207.

Grueter BA, Brasnjo G, Malenka RC (2010) Postsynaptic TRPV1 triggers cell type-specific long-term depression in the nucleus accumbens. Nat Neurosci 13:1519–1525.

Hargreaves K, Dubner R, Brown F, Flores C, Joris J (1988) A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. Pain 32:77–88.

Hassan IR, Gronert K (2009) Acute changes in dietary omega-3 and omega-6 polyunsaturated fatty acids have a pronounced impact on survival following ischemic renal injury and formation of renoprotective docosahexaenoic acid-derived protectin D1. J Immunol 182:3223–3232.

Hess A, Axmann R, Rech J, Finzel S, Heindl C, Kreitz S, Sergeeva M, Saake M, Garcia M, Kollias G, Straub RH, Sporns O, Doerfler A, Brune K, Schett G (2011) Blockade of TNF-alpha rapidly inhibits pain responses in the central nervous system. Proc Natl Acad Sci U S A 108:3731–3736.

Hong S, Gronert K, Devchand PR, Moussignac RL, Serhan CN (2003) Novel docosatrienes and 17*S*-resolvins generated from docosahexaenoic acid in murine brain, human blood, and glial cells. Autacoids in anti-inflammation. J Biol Chem 278:14677–14687.

Hucho T, Levine JD (2007) Signaling pathways in sensitization: toward a nociceptor cell biology. Neuron 55:365–376.

Hwang SW, Cho H, Kwak J, Lee SY, Kang CJ, Jung J, Cho S, Min KH, Suh YG, Kim D, Oh U (2000) Direct activation of capsaicin receptors by products of lipoxygenases: endogenous capsaicin-like substances. Proc Natl Acad Sci U S A 97:6155–6160.

Hylden JL, Wilcox GL (1980) Intrathecal morphine in mice: a new technique. Eur J Pharmacol 67:313–316.

Jeske NA, Diogenes A, Ruparel NB, Fehrenbacher JC, Henry M, Akopian AN, Hargreaves KM (2008) A-kinase anchoring protein mediates TRPV1 thermal hyperalgesia through PKA phosphorylation of TRPV1. Pain 138:604–616.

Ji RR, Suter MR (2007) p38 MAPK, microglial signaling, and neuropathic pain. Mol Pain 3:33.

Ji RR, Samad TA, Jin SX, Schmoll R, Woolf CJ (2002) p38 MAPK activation by NGF in primary sensory neurons after inflammation increases TRPV1 levels and maintains heat hyperalgesia. Neuron 36:57–68.

Ji RR, Kohno T, Moore KA, Woolf CJ (2003) Central sensitization and LTP: do pain and memory share similar mechanisms? Trends Neurosci 26:696–705.

Jin X, Gereau RW 4th (2006) Acute p38-mediated modulation of tetrodotoxin-resistant sodium channels in mouse sensory neurons by tumor necrosis factor-alpha. J Neurosci 26:246–255.

Kawasaki Y, Kohno T, Zhuang ZY, Brenner GJ, Wang H, Van Der Meer C, Befort K, Woolf CJ, Ji RR (2004) Ionotropic and metabotropic receptors, protein kinase A, protein kinase C, and Src contribute to C-fiber-induced ERK activation and cAMP response element-binding protein phosphorylation in dorsal horn neurons, leading to central sensitization. J Neurosci 24:8310–8321.

Kawasaki Y, Zhang L, Cheng JK, Ji RR (2008) Cytokine mechanisms of central sensitization: distinct and overlapping role of interleukin-1beta, interleukin-6, and tumor necrosis factor-alpha in regulating synaptic and neuronal activity in the superficial spinal cord. J Neurosci 28:5189–5194.

Kim YH, Park CK, Back SK, Lee CJ, Hwang SJ, Bae YC, Na HS, Kim JS, Jung SJ, Oh SB (2009) Membrane-delimited coupling of TRPV1 and mGluR5 on presynaptic terminals of nociceptive neurons. J Neurosci 29:10000–10009.

Kuner R (2010) Central mechanisms of pathological pain. Nat Med 16:1258–1266.

Liu T, Xu ZZ, Park CK, Berta T, Ji RR (2010) Toll-like receptor 7 mediates pruritus. Nat Neurosci 13:1460–1462.

Lu Y, Perl ER (2005) Modular organization of excitatory circuits between neurons of the spinal superficial dorsal horn (laminae I and II). J Neurosci 25:3900–3907.

Lukiw WJ, Cui JG, Marcheselli VL, Bodker M, Botkjaer A, Gotlinger K, Serhan CN, Bazan NG (2005) A role for docosahexaenoic acid-derived neuroprotectin D1 in neural cell survival and Alzheimer disease. J Clin Invest 115:2774–2783.

Marcheselli VL, Hong S, Lukiw WJ, Tian XH, Gronert K, Musto A, Hardy M, Gimenez JM, Chiang N, Serhan CN, Bazan NG (2003) Novel docosanoids inhibit brain ischemia-reperfusion-mediated leukocyte infiltration and pro-inflammatory gene expression. J Biol Chem 278:43807–43817.

Marcheselli VL, Mukherjee PK, Arita M, Hong S, Antony R, Sheets K, Winkler JW, Petasis NA, Serhan CN, Bazan NG (2010) Neuroprotectin D1/protectin D1 stereoselective and specific binding with human retinal pigment epithelial cells and neutrophils. Prostaglandins Leukot Essent Fatty Acids 82:27–34.

McNamara CR, Mandel-Brehm J, Bautista DM, Siemens J, Deranian KL, Zhao M, Hayward NJ, Chong JA, Julius D, Moran MM, Fanger CM (2007) TRPA1 mediates formalin-induced pain. Proc Natl Acad Sci U S A 104:13525–13530.

Medvedeva YV, Kim MS, Usachev YM (2008) Mechanisms of prolonged presynaptic Ca^{2+} signaling and glutamate release induced by TRPV1 activation in rat sensory neurons. J Neurosci 28:5295–5311.

Mukherjee PK, Marcheselli VL, Serhan CN, Bazan NG (2004) Neuroprotectin D1: a docosahexaenoic acid-derived docosatriene protects human retinal pigment epithelial cells from oxidative stress. Proc Natl Acad Sci U S A 101:8491–8496.

Nicol GD, Lopshire JC, Pafford CM (1997) Tumor necrosis factor enhances the capsaicin sensitivity of rat sensory neurons. J Neurosci 17:975–982.

Palacios-Pelaez R, Lukiw WJ, Bazan NG (2010) Omega-3 essential fatty acids modulate initiation and progression of neurodegenerative disease. Mol Neurobiol 41:367–374.

Park CK, Kim MS, Fang Z, Li HY, Jung SJ, Choi SY, Lee SJ, Park K, Kim JS, Oh SB (2006) Functional expression of thermo-transient receptor potential channels in dental primary afferent neurons: implication for tooth pain. J Biol Chem 281:17304–17311.

Patwardhan AM, Scotland PE, Akopian AN, Hargreaves KM (2009) Activation of TRPV1 in the spinal cord by oxidized linoleic acid metabolites contributes to inflammatory hyperalgesia. Proc Natl Acad Sci U S A 106:18820–18824.

Patwardhan AM, Akopian AN, Ruparel NB, Diogenes A, Weintraub ST, Uhlson C, Murphy RC, Hargreaves KM (2010) Heat generates oxidized linoleic acid metabolites that activate TRPV1 and produce pain in rodents. J Clin Invest 120:1617–1626.

Ruscheweyh R, Wilder-Smith O, Drdla R, Liu XG, Sandkühler J (2011) Long-term potentiation in spinal nociceptive pathways as a novel target for pain therapy. Mol Pain 7:20.

Santos SF, Rebelo S, Derkach VA, Safronov BV (2007) Excitatory interneurons dominate sensory processing in the spinal substantia gelatinosa of rat. J Physiol 581:241–254.

Schäfers M, Lee DH, Brors D, Yaksh TL, Sorkin LS (2003) Increased sensitivity of injured and adjacent uninjured rat primary sensory neurons to exogenous tumor necrosis factor-alpha after spinal nerve ligation. J Neurosci 23:3028–3038.

Schnizler K, Shutov LP, Van Kanegan MJ, Merrill MA, Nichols B, McKnight GS, Strack S, Hell JW, Usachev YM (2008) Protein kinase A anchoring via AKAP150 is essential for TRPV1 modulation by forskolin and prostaglandin E2 in mouse sensory neurons. J Neurosci 28:4904–4917.

Schwab JM, Chiang N, Arita M, Serhan CN (2007) Resolvin E1 and protectin D1 activate inflammation-resolution programmes. Nature 447:869–874.

Serhan CN, Hong S, Gronert K, Colgan SP, Devchand PR, Mirick G, Moussignac RL (2002) Resolvins: a family of bioactive products of omega-3 fatty acid transformation circuits initiated by aspirin treatment that counter proinflammation signals. J Exp Med 196:1025–1037.

Serhan CN, Gotlinger K, Hong S, Lu Y, Siegelman J, Baer T, Yang R, Colgan SP, Petasis NA (2006) Anti-inflammatory actions of neuroprotectin D1/protectin D1 and its natural stereoisomers: assignments of dihydroxy-containing docosatrienes. J Immunol 176:1848–1859.

Serhan CN, Chiang N, Van Dyke TE (2008) Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. Nat Rev Immunol 8:349–361.

Sommer C, Birklein F (2010) Fighting off pain with resolvins. Nat Med 16:518–520.

Sommer C, Kress M (2004) Recent findings on how proinflammatory cytokines cause pain: peripheral mechanisms in inflammatory and neuropathic hyperalgesia. Neurosci Lett 361:184–187.

Stellwagen D, Beattie EC, Seo JY, Malenka RC (2005) Differential regulation

of AMPA receptor and GABA receptor trafficking by tumor necrosis factor-alpha. J Neurosci 25:3219–3228.

Todd AJ (2010) Neuronal circuitry for pain processing in the dorsal horn. Nat Rev Neurosci 11:823–836.

Tominaga M, Caterina MJ, Malmberg AB, Rosen TA, Gilbert H, Skinner K, Raumann BE, Basbaum AI, Julius D (1998) The cloned capsaicin receptor integrates multiple pain-producing stimuli. Neuron 21:531–543.

Wang H, Zylka MJ (2009) Mrgprd-expressing polymodal nociceptive neurons innervate most known classes of substantia gelatinosa neurons. J Neurosci 29:13202–13209.

Woolf CJ, Salter MW (2000) Neuronal plasticity: increasing the gain in pain. Science 288:1765–1769.

Xu ZZ, Zhang L, Liu T, Park JY, Berta T, Yang R, Serhan CN, Ji RR (2010) Resolvins RvE1 and RvD1 attenuate inflammatory pain via central and peripheral actions. Nat Med 16:592–597.

Yamada T, Tani Y, Nakanishi H, Taguchi R, Arita M, Arai H (2011) Eosinophils promote resolution of acute peritonitis by producing proresolving mediators in mice. FASEB J 25:561–568.

Yanes O, Clark J, Wong DM, Patti GJ, Sánchez-Ruiz A, Benton HP, Trauger SA, Desponts C, Ding S, Siuzdak G (2010) Metabolic oxidation regulates embryonic stem cell differentiation. Nat Chem Biol 6:411–417.

Yang K, Kumamoto E, Furue H, Yoshimura M (1998) Capsaicin facilitates excitatory but not inhibitory synaptic transmission in substantia gelatinosa of the rat spinal cord. Neurosci Lett 255:135–138.

Yasaka T, Tiong SY, Hughes DI, Riddell JS, Todd AJ (2010) Populations of inhibitory and excitatory interneurons in lamina II of the adult rat spinal dorsal horn revealed by a combined electrophysiological and anatomical approach. Pain 151:475–488.

Yu L, Yang F, Luo H, Liu FY, Han JS, Xing GG, Wan Y (2008) The role of TRPV1 in different subtypes of dorsal root ganglion neurons in rat chronic inflammatory nociception induced by complete Freund's adjuvant. Mol Pain 4:61.

Zhang H, Nei H, Dougherty PM (2010) A p38 mitogen-activated protein kinase-dependent mechanism of disinhibition in spinal synaptic transmission induced by tumor necrosis factor-alpha. J Neurosci 30:12844–12855.

Zhang L, Berta T, Xu ZZ, Liu T, Park JY, Ji RR (2011) TNF-alpha contributes to spinal cord synaptic plasticity and inflammatory pain: distinct role of TNF receptor subtypes 1 and 2. Pain 152:419–427.

Zhong Y, Zhou LJ, Ren WJ, Xin WJ, Li YY, Zhang T, Liu XG (2010) The direction of synaptic plasticity mediated by C-fibers in spinal dorsal horn is decided by Src-family kinases in microglia: the role of tumor necrosis factor-alpha. Brain Behav Immun 24:874–880.

Zhou HY, Chen SR, Chen H, Pan HL (2010) Opioid-induced long-term potentiation in the spinal cord is a presynaptic event. J Neurosci 30:4460–4466.

Zhou Z, Peng X, Hao S, Fink DJ, Mata M (2008) HSV-mediated transfer of interleukin-10 reduces inflammatory pain through modulation of membrane tumor necrosis factor alpha in spinal cord microglia. Gene Ther 15:183–190.

Zhuang ZY, Xu H, Clapham DE, Ji RR (2004) Phosphatidylinositol 3-kinase activates ERK in primary sensory neurons and mediates inflammatory heat hyperalgesia through TRPV1 sensitization. J Neurosci 24:8300–8309.