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Characterization and pharmacological modulation of noci-responsive deep dorsal horn neurons across diverse rat models of pathological pain

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McGaraughty S, Chu KL, Xu J. Characterization and pharmacological modulation of noci-responsive deep dorsal horn neurons across diverse rat models of pathological pain. *J Neurophysiol* 120: 1893–1905, 2018. First published August 1, 2018; doi:10.1152/jn.00325.2018.—This overview compares the activity of wide dynamic range (WDR) and nociceptive specific (NS) neurons located in the deep dorsal horn across different rat models of pathological pain and following modulation by diverse pharmacology. The data were collected by our group under the same experimental conditions over numerous studies to facilitate comparison. Spontaneous firing of WDR neurons was significantly elevated (>3.7 Hz) in models of neuropathic, inflammation, and osteoarthritic pain compared with naive animals (1.9 Hz) but was very low (<0.5 Hz) and remained unchanged in NS neurons. WDR responses to low-intensity mechanical stimulation were elevated in neuropathic and inflammation models. WDR responses to high-intensity stimuli were enhanced in inflammatory (heat) and osteoarthritis (mechanical) models. NS responses to high-intensity stimulation did not change relative to control in any model examined. Several therapeutic agents reduced both evoked and spontaneous firing of WDR neurons (e.g., TRPV1, TRPV3, $Na_v1.7$, $Na_v1.8$, P2X7, P2X3, H_3), other targets affected neither evoked nor spontaneous firing of WDR neurons (e.g., H_4, TRPM8, KCNQ2/3), and some only modulated evoked (e.g, ASIC1a, $Ca_v3.2$) whereas others decreased evoked but affected spontaneous activity only in specific models (e.g., TRPA1, CB2). Spontaneous firing of WDR neurons was not altered by any peripherally restricted compound or by direct administration of compounds to peripheral sites, although the same compounds decreased evoked activity. Compounds acting centrally were effective against this endpoint. The diversity of incoming/modulating inputs to the deep dorsal horn positions this group of neurons as an important intersection within the pain system to validate novel therapeutics.

NEW & NOTEWORTHY Data from multiple individual experiments were combined to show firing properties of wide dynamic range and nociceptive specific spinal dorsal horn neurons across varied pathological pain models. This high-powered analysis describes the sensitization following different forms of injury. Effects of diverse pharmacology on these neurons is also summarized from published and unpublished data all recorded under the same conditions to facilitate comparison. This comprehensive overview describes the function and utility of these neurons.

dorsal horn; inflammation; neuropathic; nociceptive specific; osteoarthritis; wide dynamic range

INTRODUCTION

The dorsal horn of the spinal cord is a key nodal point for the transmission of somatosensory information, including pain. Unmyelinated small-diameter C-fibers, myelinated medium diameter Aδ fibers, and myelinated large-diameter Aβ fibers transmit somatosensory signals to the superficial and deep dorsal horn neurons forming mono- and polysynaptic inputs to projection and local interneurons (Christensen and Perl 1970; Todd 2010; Woolf and Fitzgerald 1986). Interactions with autonomic nerves can influence the input from these primary somatosensory afferent neurons (Drummond 2013). The activity of the spinal neurons is also modulated from ipsilateral and contralateral spinal spites as well as from descending output from the brain (Heinricher 2016; McGaraughty and Henry 1997; Ossipov et al. 2014; Todd 2010). Within the dorsal horn itself, there is direct and indirect communication between laminae as well as from elements in the local microenvironment to influence activity (Petitjean et al. 2012; Tsuda 2018). Functionally, neurons can be divided into those that respond to high-threshold noxious stimulation only (nociceptive specific), in a graded manner to low- and high-threshold stimulation (wide dynamic range), and to low-threshold non-noxious stimulation only (low threshold). Superficial laminae (lamina I and II) neurons are predominantly nociceptive specific (NS), and deeper neurons (lamina V and VI) are predominantly wide dynamic range (WDR); however, both classes of neurons are found in each region (Khasabov et al. 2002; Willis and Coggeshall 2004).

Dorsal horn neurons located in the deep laminae (i.e., V and VI), particularly WDR neurons, have been studied extensively by several groups, including our own. Because this region receives diverse direct and indirect inputs from peripheral afferents that convey somatosensory signals spanning the low- to high-threshold spectrum, the activity of these afferent neurons can then relay both nociceptive and non-nociceptive information to the central nervous system as well as signals associated with "sensitized" states (i.e., hyperalgesia/allodynia) following an injury. These sensitized states are also associated with functional and reorganizational changes that result in the processing of inputs from nociceptors as well non-nociceptors (e.g., Aβ fibers) in the spinal cord (Baba et al. 1999; Hsieh et al. 2015; Neumann et al. 1996). From a drug discovery perspective, the deep dorsal horn neurons are ideally situated based on diversity of inputs (peripheral, intraspinal, local, supraspinal) to determine whether novel pharmacology

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can modulate normal and/or pathological somatosensory signals. Indeed, modulation of these neurons was considered by our group as a critical path for pharmacology associated with primary afferent and spinal cord targets, and programs could be paused without demonstration of modulation. The study of pain-associated behaviors in animals is difficult, as there are many reasons why an animal may or may not respond to tactile or thermal stimulation (pain is only one reason), and thus the evaluation of these neurons represented an objective means to "peer into" the pain system.

The purpose of this review is to compare and contrast evoked and spontaneous firing of WDR and NS neurons located in the deep dorsal horn across different animal models of pathological pain. The effects of diverse pharmacologies on the activity of these neurons was also compared. These data are accumulated from our group over multiple individual studies (both published and unpublished) and are collapsed to examine population activity and highlight trends. With the acknowledgment that these neurons have been intensely studied by several groups for decades, the neuronal data collected for this review were recorded under the same conditions across studies and thus facilitates comparison across the groups of interest.

METHODS

Animal models. Animal handling and experimental protocols for all experiments contained in this review were approved by AbbVie/Abbott's Institutional Animal Care and Use Committee and conducted in accordance with the ethical principles for pain-related animal research of the American Pain Society. Male Sprague-Dawley rats (250–400 g; Charles River Laboratories) were used for all experiments and housed in a temperature controlled room with a 12:12-h day-night cycle. Food and water were available ad libitum.

Models of osteoarthritis: monoiodoacetate and medial meniscal tear. Two models of osteoarthritis (OA) were studied. In both models, the animals were anesthetized with 5% isoflurane, followed by 2.5% maintenance during the model induction procedure. In one model, monosodium iodoacetate (MIA; 3 mg, 50 μl) was injected into the intra-articular (ia) space through the infrapatella ligament of the right knee. MIA was dissolved in 0.9% sterile saline and administered using a 29-gauge needle. Sham MIA-OA animals received an ia injection of saline. In the second model, the medial meniscal tear (MMT) model, the medial collateral ligament was exposed by blunt dissection and transected to reflect the meniscus toward the femur. The meniscus was then cut through the full thickness at its narrowest point to simulate a complete tear. For sham animals, the medial collateral ligament was exposed but not transected. For both models, electrophysiological experiments were conducted 21–25 days after surgery.

Model of neuropathic pain: spinal nerve ligation. In isoflurane-anesthetized rats, either a unilateral tight ligation (5-0 black braided silk) of the L5 and L6 spinal nerves [spinal nerve ligation (SNL)] or sham surgery was performed on rats 14–18 days before electrophysiological experiments. On the day of neuronal recording, all SNL animals were tested for the development of mechanical allodynia. Individual animals were habituated to an elevated wire mess cage for 30 min before allodynia testing. Animals that did not withdraw to a 6-g von Frey hair stimulation of the ipsilateral hind paw (8 s cutoff) were excluded from electrophysiological experiments.

Models of inflammatory pain: carrageenan and complete Freund's adjuvant. Subchronic inflammatory hyperalgesia was induced in rats following the injection of complete Freund's adjuvant (CFA; 50%, 150 μl) into the plantar surface of the right hind paw 2 days before electrophysiological testing. Acute inflammatory hyperalgesia was induced by injection of 1 mg of carrageenan (in 100 μl of saline) into the right hind paw 2 h before electrophysiological evaluation.

Electrophysiological protocol for all experiments. On the day of neuronal recording, all animals were initially anesthetized with pentobarbital (50 mg/kg ip). Catheters were placed into the left and right external jugular veins. A laminectomy was performed to remove vertebral segments T12-L3 for naive, sham SNL, SNL, carrageenan, and CFA rats and T9-L2 for OA and OA-sham rats to record from spinal neurons that receive input from either the right hind paw or knee, respectively. The activity of neurons with paw-receptive fields was also recoded for MMT and MIA animals. In some rats, a laminectomy was performed at the T9-T10 level to permit transection of the spinal cord with a curved scalpel, which eliminated descending modulation of the recorded WDR neuron. Neuronal recording began $\geq$90 min after the transection. All animals were then secured in a stereotaxic apparatus (Kopf Instruments, Tujunga, CA) supported by clamps attached to the vertebral processes on either side of the exposure site. The exposed area of the spinal cord was first enveloped by agar and then filled with mineral oil. A stable plane of anesthesia was maintained throughout the experiment by a continuous infusion of propofol at a rate of 8–12 $mg \cdot kg^{-1} \cdot h^{-1}$ iv. Body temperature was kept at ~37°C by placing the animals on a circulating water blanket. Platinum-iridium microelectrodes (Frederick Haer, Brunswick, ME) were used to record extracellular activity of WDR or NS spinal dorsal horn neurons. Spike waveforms were monitored on an oscilloscope throughout the experiment, digitized (32 points), and then stored for offline analysis (SciWorks; Datawave Technologies, Longmont, CO) to ensure that the unit under study was unambiguously discriminated throughout the experiment. Experimenters were not blinded to the conditions since the readout was an objective measurement (spike counts) by the software, and each individual study was independently conducted by two or three experimenters to demonstrate inherent reproducibility.

No stimulation was given during the first 5 min of the experiment. This activity was used to represent the baseline spontaneous firing of the neuron. Neurons were then characterized to determine classification (see below). After characterization, three baseline "evoked" responses, separated by 5 min each, to specific stimulation (see below) of the neuronal receptive field (RF) were recorded. Spontaneous and evoked neuronal activity was then measured 1 (intra-DRG only), 5, 15, 25, and 35 min after systemic injection (iv injection over 6–7 min) of the test compound or vehicle. In most experiments, only one neuron per experiment was recorded. In rare cases, two easily distinguished neurons were simultaneously recorded on one electrode, and the activity of both neurons was kept for analysis.

During the characterization period, each neuron was evaluated to confirm WDR or NS response patterns. WDR neurons were defined as those neurons that responded in a graded manner to both low- and high-intensity stimulation of the neuronal receptive field. NS neurons were defined as those neurons that responded only to high-intensity (noxious) stimulation. For models with a hind paw neuronal receptive field (SNL, CFA, carrageenan, MIA, MMT), the ipsilateral hind paw was (in order) gently tapped, brushed, given a noxious pinch with forceps, and stimulated with a 10-g von Frey hair for 2–3 s. If we were searching for thermal-responsive neurons, the hind paw was stimulated with a heat stimulus (see below) to confirm responsiveness. For models with a knee neuronal receptive field (MMT, MIA), neurons were characterized by responses to manual gentle rubbing, and a 300-g von Frey was applied to the knee for 2–3 s. Only neurons that specifically responded to knee joint stimulation, without responding to pinch stimulation of the surrounding skin/tissue, were kept for recordings in MMT and MIA studies.

Only one form of test stimulation was given in each specific experiment. The test stimulus comprised of one of the following: *1*) a low-intensity 10-g von Frey hair applied for 15 s to the hind paw, *2*) a high-intensity noxious pinch (50 g) with a 22-mm mini bulldog clamp applied for 10 s to the hind paw, *3*) a high-intensity 300-g von

Frey hair applied for 10 s to the knee, or *4*) immersion of the hind paw in 49 ± 3°C water for 10 s. The thermal stimulus was a glass reservoir filled with noncirculating water. The reservoir was the inner section of a double-walled glass-tempering beaker. The temperature of the stimulus was maintained by water circulating through the enclosed external chamber and was feedback regulated by a flow-through heater (Polyscience, Niles, IL). The total number of spikes during stimulus presentation were counted and used as the measure of evoked firing.

Site-specific injections. For intra-RF injections, 50 μl of vehicle or compound was slowly injected into the hind paw for 2 min, using a 30-gauge needle. For direct dorsal root ganglion (DRG) injections (intra-DRG) (McGaraughty et al. 2006), the compound or vehicle was injected over a period of 1 min in 1 μl onto the L4 or L5 DRG through an indwelling catheter attached to a 10-μl Hamilton syringe. If the intra-DRG injection of compound or vehicle was without effect on neuronal activity, 5% lidocaine (1 μl) was then infused onto the DRG. If evoked activity was unaffected by lidocaine, it was determined that the recorded spinal neuron did not receive direct/indirect input from the particular DRG and was not used for data analysis. For intraspinal injections (McGaraughty et al. 2006), a glass infusion pipette (outer diameter 75–80 μm) with an angled beveled tip was attached to the recording electrode in such a way that the tips were separated by ~300 μm laterally and by 30–100 μm dorsoventrally. The electrode and pipette were simultaneously lowered into the spinal tissue. The infusion pipette was attached to a 1 μl Hamilton syringe with a length of PE-50 tubing, and 0.2–0.5 μl of solution was delivered over a 2- to 6-min period. For supraspinal site or intracerebroventricular (icv) injections, the compound or vehicle was injected directly into the site (in 0.5 μl) or icv (in 10 μl) over a 2-min period through a 28-gauge stainless-steel cannula (Plastics One, Roanoke, VA) attached to a 2- or 25-μl Hamilton syringe via a length of PE 50 tubing.

Data analysis. The compiled data across experiments is shown as mean Hz ± SE. Data were analyzed for homogeneity of variance using Bartlett's test. If the group variances were significantly different, the groups were analyzed with a Welch's one-way ANOVA followed by a Games-Howell post hoc test to determine whether significance was achieved in the ANOVA. If the group variances were not significantly different, a standard one-way ANOVA was used followed by a Dunnett's post hoc test to determine whether significance was achieved in the ANOVA. The latter analysis was performed for the evoked heat responses of both WDR and NS neurons only. R and Prism software were used for statistical evaluation. A difference was considered significant if it reached a P value of ≤0.05.

RESULTS

Spontaneous and evoked firing of WDR neurons across models. A total of 2,552 WDR neurons were recorded in the deep dorsal horn, which was located 450–900 μM from the surface of the spinal cord. Table 1 [$F_{(12,260.2)} = 28.6$, $P < 0.0001$] and Fig. 1*A* breaks down the distribution of the spontaneous firing rate for these neurons across different animal models of pathological pain. The mean firing rates for WDR neurons recorded from SNL, CFA, carrageenan, MMT-OA, and MIA-OA rats were all significantly elevated versus neurons recorded from naive animals or from respective sham control animals. Sham groups did not differ from the naive group. The highest mean spontaneous firing (4.9 Hz) was recorded from OA-neurons (both models) with knee-receptive fields. Behaviorally, response thresholds in OA models are often evaluated following stimulation of the paw (Burston et al. 2016; Comi et al. 2017); therefore, in addition to recording from neurons with knee-receptive fields, neurons with a paw-receptive field were also examined in MMT-OA and MIA-OA animals. The mean spontaneous activity of WDR neurons with paw-receptive fields from both OA groups was not elevated relative to naive or sham groups and was significantly ($P < 0.05$, Games-Howell) lower than OA-neurons with knee receptive fields.

The firing rate distribution revealed that all groups of neurons have a range of firing rates, including low (0–2 Hz) and very high (>6 Hz) levels of spontaneous activity but that the WDR neurons from models of pathological pain have a higher percentage of neurons firing in the very high category compared with naive or sham controls (20.4–33.6 vs. 3.2–7.6%, respectively). Furthermore, neurons recorded from naive and sham groups tended to have a higher percentage of neurons firing at levels below 2 Hz than the pathological pain groups (61.7–78.5 vs. 18.8–43.4%, respectively). Of note, the group of SNL animals with spinal transection (removing descending

Table 1. *Spontaneous firing of WDR neurons across models*

	Mean Hz	0–2 Hz	2–4 Hz	4–6 Hz	>6 Hz
Naive (paw; $n = 327$)	1.9 ± 0.1 (1.7–2.1)	66.4% (217)	18.9% (62)	10.1% (33)	4.6% (15)
Sham SNL (paw; $n = 94$)	2.2 ± 0.2 (1.8–2.6)	61.7% (58)	25.5% (24)	7.4% (7)	5.3% (5)
SNL (paw; $n = 1,021$)	3.7 ± 0.1 (3.6–3.9)[a,b]	36.9% (377)	26.6% (272)	16.1% (164)	20.4% (208)
Transected SNL (paw; $n = 49$)	3.1 ± 0.3 (2.5–3.7)[a,e,h,i]	32.7% (16)	42.9% (21)	18.4% (9)	6.1% (3)
CFA (paw; $n = 484$)	4.2 ± 0.2 (3.8–4.5)[a,b,f]	30.6% (148)	26.9% (130)	21.3% (103)	21.3% (103)
Carrageenan (paw; $n = 32$)	4.7 ± 0.6 (3.5–5.8)[a,b]	18.8% (6)	31.3% (10)	18.8% (6)	31.3% (10)
Sham MMT (paw; $n = 31$)	1.8 ± 0.4 (1.1–2.5)[d]	67.7% (21)	25.8% (8)	3.2% (1)	3.2% (1)
MMT (paw; $n = 26$)	1.4 ± 0.3 (0.7–2.0)[d,e]	73.1% (19)	23.1% (6)	0.0% (0)	3.8% (1)
Sham MMT (knee; $n = 79$)	1.6 ± 0.3 (1.0–2.2)[d]	78.5% (62)	10.1% (8)	3.8% (3)	7.6% (6)
MMT (knee; $n = 122$)	4.9 ± 0.5 (3.9–6.0)[a,b,c,f,g]	43.4% (53)	17.2% (21)	5.7% (7)	33.6% (41)
MIA (paw; $n = 48$)	2.4 ± 0.4 (1.6–3.2)	66.6% (32)	14.6% (7)	8.3% (4)	10.4% (5)
Sham MIA (knee; $n = 27$)	1.7 ± 0.4 (0.8–2.5)[d]	74.1% (20)	14.8% (4)	3.7% (1)	7.4% (2)
MIA (knee; $n = 212$)	4.9 ± 0.4 (4.2–5.7)[a,b,c,g]	42.9% (91)	17.0% (36)	10.4% (22)	29.7% (63)

Values are means ± SE; 95% confidence interval (shown in parentheses beside the mean) and %neurons (n) are shown for each category of firing frequency; n = no. of neurons for that group, and "paw" and "knee" indicate receptive fields for recorded neurons. CFA, complete Freund's adjuvant; MIA, monosodium iodoacetate; MMT, medial meniscal tear; SNL, spinal nerve ligation; WDR, wide dynamic range. [a]$P < 0.05$ vs. naive; [b]$P < 0.05$ vs. sham SNL; [c]$P < 0.01$ vs. respective "paw" group; [d]$P < 0.01$ vs. CFA, SNL, and carrageenan; [e]$P < 0.05$ vs. MIA (knee); [f]$P < 0.05$ vs. MIA (paw); [g]$P < 0.01$ vs. sham MMT (knee), sham MMT (paw), and sham MIA (knee); [h]$P < 0.05$ vs. MMT (paw); [i]$P < 0.05$ vs. sham MMT (knee) using Games-Howell post hoc analysis. Each calculation is compiled from published (Boyce-Rustay et al. 2010; Brederson et al. 2018; Chu et al. 2004, 2011, 2015; El-Kouhen et al. 2006; Jarvis et al. 2007, 2014; McGaraughty et al. 2003, 2006, 2007, 2008a, 2008b, 2009, 2010, 2012, 2017; Surowy et al. 2008; Xu et al. 2012, 2014; Zhu et al. 2014) and unpublished data.

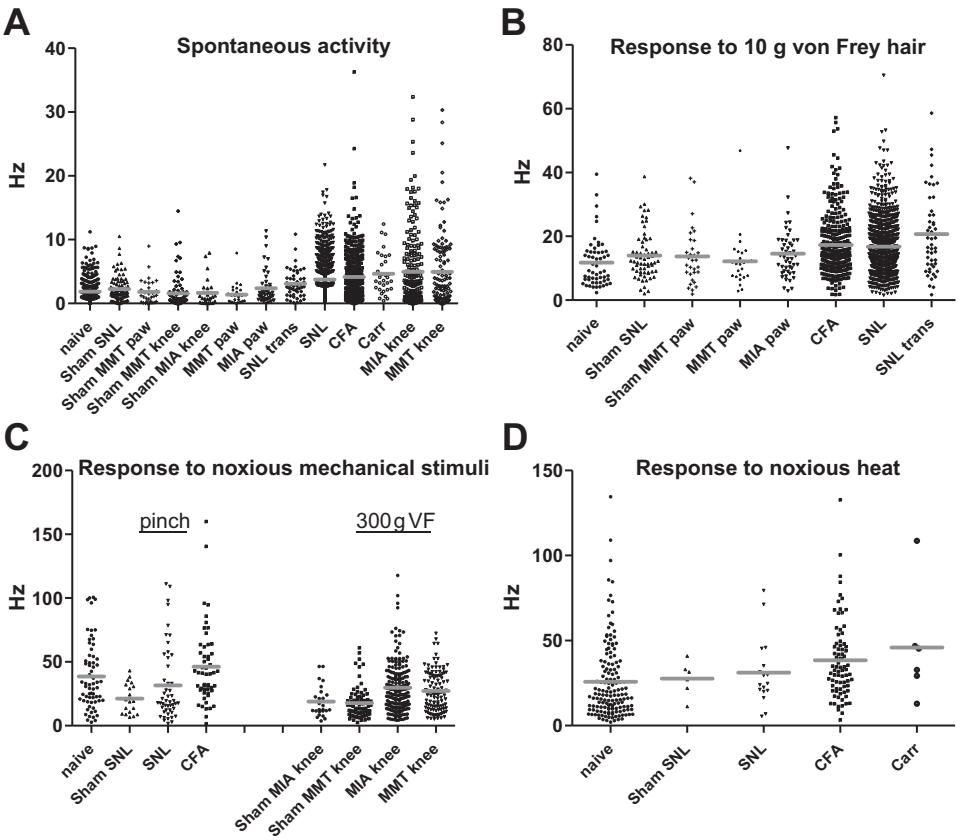

Fig. 1. Distribution of spontaneous and evoked firing for individual wide dynamic range (WDR) neurons. *A*: distribution of spontaneous firing for individual WDR neurons across models of pathological pain and control groups. *B–D*: distribution of WDR neuronal responses to a low-intensity 10-g von Frey hair applied for 15 s to the ipsilateral hind paw (*B*), a high-intensity noxious pinch with 22 mm mini bulldog clamp applied for 10 s to the ipsilateral hind paw or 300 g von Frey hair to the ipsilateral knee for 10 s [osteoarthritis (OA) models only] (*C*), and immersion of the ipsilateral hind paw in 49 ± 3°C water for 10 s (*D*). Each ● represents activity of individual neurons; gray bars indicates the mean firing rate. Carr, carrageenan; CFA, complete Freund's adjuvant; MIA, monosodium iodoacetate; MMT, medial meniscal tear; SNL trans, spinal nerve ligation animals with spinal transection; "paw" or "knee" indicate receptive field for recorded neurons in OA studies, otherwise receptive field is paw for all other models;.

modulation) had a mean level of spontaneous activity that was not significantly different from "intact" SNL or sham-SNL groups. However, the transected SNL group had more neurons (42.9%) firing in the 2- to 4-Hz range than any other group and did not have a high preponderance of neurons firing at a very high levels (6.1%).

Table 2 and Fig. 1, *B–D*, show the firing rates of WDR neurons in response to different stimuli across models. The WDR neuronal response to application of a low-threshold mechanical stimulus for 15 s (10-g von Frey hair) to the ipsilateral hind paw was significantly elevated [$F_{(7,146.1)} = 6.9$, $P < 0.0001$] in SNL, SNL-transected, and CFA animals compared with the naive group. The 10-g von Frey evoked firing from SNL rats with a spinal transection was also elevated ($P < 0.05$, Games-Howell) compared with sham-SNL rats but not the SNL rats. Responses to low-intensity stimulation of the hind paw were not enhanced in either model of OA compared with control groups.

The overall Welch's ANOVA was significant for the WDR neuronal responses to a 10-s high-intensity pinch (22-mm mini-bulldog clamp) of the ipsilateral hind paw [$F_{(3,88.6)} = 10.3$, $P < 0.0001$]. However, the post hoc analysis revealed no

Table 2. *Evoked firing of WDR neurons across models*

	10 g VF (paw), Hz	Noxious pinch (paw), Hz	300 g VF (knee), Hz	Heat (49 ± 3°C) (paw), Hz
Naive (paw)	11.7 ± 1.01 (9.7–13.8, $n = 56$)	38.6 ± 3.2[e] (32.1–45.1, $n = 65$)		25.8 ± 1.8 (22.1–29.4, $n = 149$)
Sham SNL (paw)	14.0 ± 0.9 (12.1–15.8, $n = 64$)	21.3 ± 2.7[a,d] (15.6–27.0, $n = 19$)		26.9 ± 5.0 (17.0–38.4, $n = 5$)
SNL (paw)	16.8 ± 0.3[a] (16.2–17.3, $n = 964$)	31.7 ± 4.2[e] (23.3–40.0, $n = 53$)		31.7 ± 5.1 (21.0–41.4, $n = 16$)
Transected SNL (paw)	20.7 ± 1.8[a,b,c] (17.0–24.4, $n = 56$)			
CFA (paw)	17.3 ± 0.6[a,b] (16.2–18.4, $n = 298$)	46.2 ± 4.2[e] (37.8–54.6, $n = 53$)		38.6 ± 2.6[a] (33.2–43.6, $n = 78$)
Carrageenan (paw)				48.6 ± 16.2 (11.3–80.7, $n = 5$)
Sham MMT (paw)	13.7 ± 1.5 (10.4–16.9, $n = 31$)			
MMT (paw)	12.2 ± 1.6 (8.8–15.5, $n = 26$)			
Sham MMT (knee)			17.7 ± 1.4 (14.9–20.5, $n = 79$)	
MMT (knee)			27.0 ± 1.4[f] (24.5–29.9, $n = 107$)	
MIA (paw)	14.6 ± 1.2 (12.2–16.9, $n = 48$)			
Sham MIA (knee)			18.9 ± 2.4 (13.8–23.9, $n = 25$)	
MIA (knee)			29.7 ± 1.3[f] (27.2–32.3, $n = 212$)	

Values are means ± SE; 95% confidence interval (shown in parentheses beside the mean) and group size (n) for each type of stimulus; "paw" and "knee" indicate receptive fields for recorded neurons. CFA, complete Freund's adjuvant; MIA, monosodium iodoacetate; MMT, medial meniscal tear; VF, von Frey; WDR wide dynamic range; [a]$P < 0.01$ vs. naive group; [b]$P < 0.05$ vs. sham SNL; [c]$P < 0.05$ vs. MMT (paw); [d]$P < 0.01$ vs. CFA; [e]$P < 0.05$ vs. respective 10-g VF group; [f]$P < 0.05$ vs. sham MMT (knee) and sham MIA (knee) using Games-Howell or Dunnett's (heat only) post hoc analysis. Each calculation is compiled from published (Boyce-Rustay et al. 2010; Brederson et al. 2018; Chu et al. 2004, 2011, 2015; El-Kouhen et al. 2006; Jarvis et al. 2007, 2014; McGaraughty et al. 2003, 2006, 2007, 2008a, 2008b, 2009, 2010, 2012, 2017; Surowy et al. 2008; Xu et al. 2012, 2014; Zhu et al. 2014) and unpublished data.

significant differences between CFA, SNL, and naive groups, although the CFA group had the highest response rate. WDR neurons from SNL rats also did not respond significantly different to the noxious pinch stimulus compared with sham SNL animals but tended to be higher than the sham SNL group, firing ~10 Hz greater. Sham SNL animals had significantly lower ($P < 0.01$, Games-Howell) pinch-evoked activity than naive rats. The WDR responses to pinch stimulation were significantly greater than responses to 10-g von Frey when compared within the respective naive, CFA, and SNL groups [$F_{(7,147)} = 20.1$, $P < 0.0001$].

The responses of WDR neurons to 10-s application of a 300-g von Frey hair to the ipsilateral knee from MIA-OA and MMT-OA rats were significantly greater compared with their respective sham animals [$F_{(3,106)} = 16.2$, $P < 0.0001$].

The CFA group of WDR neurons was the only group that had significantly greater [$F_{(4,252)} = 4.8$, $P < 0.0001$] responses to immersion of the ipsilateral hind paw in noxious hot water for 10 s compared with neurons from the naive group. There was no difference between SNL animals and the control groups. Although WDR neurons recorded from carrageenan-inflamed rats appeared to respond stronger to the heat stimulus, this was not significantly different from neurons from naive rats and was likely due to the small group size.

Pharmacological modulation of WDR neurons. Table 3 reviews the effects of diverse pharmacologies on spontaneous and evoked activity of WDR neurons in different models of pathological pain. These pharmacologies include several TRP, Na_v, Ca_v, and P2X channels, with TRPV1 antagonists the most explored across different models. The majority of the studies were run in the SNL model of neuropathic pain. A trend that stands out is that compounds that were peripherally restricted never affected the spontaneous firing WDR neurons in any model, although evoked firing was modulated, and that CNS penetrant compounds affecting the same mechanism could now modulate spontaneous firing. Thus, peripherally restricted TRPV1, TRPV3, mixed $Na_v1.7/Na_v1.8$, selective $Na_v1.7$, and P2X3 antagonists/blockers did not decrease the spontaneous firing of WDR neurons, but their centrally acting counterparts did. Consistent with these observations, site-specific applications of compounds to the peripheral receptive field or to the DRG for these same mechanisms also did not affect the spontaneous firing of WDR neurons, but importantly, these injections reduced the evoked firing of the same neurons (Table 4). A similar lack of modulation of spontaneous activity (but still decreased evoked responses) was also observed with CB2 antagonists, mixed Ca_v blockers, and lidocaine when specifically delivered to peripheral sites. It is notable that across all mechanisms evaluated, direct delivery of the test compounds to the spinal cord and/or supraspinal sites decreased both spontaneous and evoked firing.

H_3 receptor agonists and TRPV3 receptor antagonists modulated the spontaneous firing of WDR neurons only through descending modulation, as there was no effect with spinal or

Table 3. *Pharmacological modulation of WDR neurons across models*

	Naive (e)	CFA (s/e)	Carr (s/e)	SNL (s/e)	Sham-SNL (s/e)	MIA-OA (s/e)	Sham-MIA (s/e)	MMT-OA (s/e)
TRPV1 antagonist†	+	+/+		x/x		+/+	x/x	+/+
TRPV1 antagonist PR†	+	x/+				x/+		
TRPV3 antagonist†	+			+/+				+/+
TRPV3 antagonist PR†	+			x/+				
TRPA1 antagonist†	+	+/+		x/+		x/+	x/+	
TRPM8 antagonist*		x/x				x/x		x/x
CB2 agonist†			x/+	+/+	x/x	+/+	x/x	
$Na_v1.7/Na_v1.8$ blocker*	+			+/+		+/+		
$Na_v1.7/Na_v1.8$ blocker PR*				x/+		x/+		
$Na_v1.7$ blocker*	+			+/+		+/+		+/+
$Na_v1.7$ blocker PR*						x/+		
$Na_v1.8$ blocker†	+			+/+				
Ca_v mixed blocker†	x	+/+		+/+		+/+		
$Ca_v3.2$ blocker†		x/+						
P2X7 antagonist†				+/+	x/+			
P2X3 antagonist†		+/+		+/+		+/+		
P2X3 antagonist PR†				x/+		x/+		
H3 antagonist†				+/+	x/x			
H4 antagonist*		x/x	x/x	x/x				
ASIC1a blocker*		x/+						
KCNQ2/3 opener*				x/x				
LPAR1 antagonist*				+/+				
LPAR3 antagonist*				+/+				
α2b NA agonist†	x			x/x				
mGluR1 antagonist†				+/+				
5-HT7 antagonist*		x/+						
ROCK inhibitor†				+/+	x/x			
NNR – α7 PAM*				x/x				
NNR – α7 agonist*				x/x		x/+		
NSAIDS*		+/+				+/+		+/+
Duloxetine†				x/x				

All compounds were administered intravenously. +, Significant decrease; e, evoked firing; PR; peripherally restricted; s/e, spontaneous/evoked firing; WDR, wide dynamic range; x, no effect. Effect on evoked activity could be related to any of the stimuli described in Table 2. Compiled from †published data (Boyce-Rustay et al., 2010; Brederson et al., 2018; Chu et al., 2004, 2011, 2015; El-Kouhen et al., 2006; Jarvis et al., 2007, 2014; McGaraughty et al., 2003, 2006, 2007, 2008a, 2008b, 2009, 2010, 2012, 2017; Surowy et al., 2008; Xu et al., 2012, 2014; Zhu et al., 2014) and *unpublished data.

Table 4. *Pharmacological modulation of WDR neurons via site specific injections*

	Intra-RF (s/e)	Intra-DRG (s/e)	Intraspinal (s/e)	Intracerebroventricular or Supraspinal Site (s/e)
TRPV1 antagonist (CFA)†	x/+	x/+	+/+	+/+
TRPV3 antagonist (SNL)†	x/+	x/+	x/x	+/+
CB2 agonist (SNL)†	x/x	x/+	+/+	NT
$Na_v1.7/Na_v1.8$ blocker (SNL)*	x/+	x/+	+/+	NT
$Na_v1.8$ blocker (SNL)†	x/+	x/+	+/+	NT
Cav mixed blocker (SNL)†	x/+	NT	+/+	NT
H4 antagonist (CFA)*	NT	NT	x/x	NT
H3 antagonist (SNL)†	NT	NT	x/x	+/+
Lidocaine (CFA)†	NT	x/+	+/+	NT

CFA, complete Freund's adjuvant DRG, dorsal root ganglion; +, significant decrease, NT, not tested; RF, receptive field; s/e, spontaneous/evoked firing; x, no effect; SNL, spinal nerve ligation; WDR, wide dynamic range. Compiled from †published data (McGaraughty et al., 2003, 2006, 2008a, 2008b, 2009, 2012, 2017; Surowy et al. 2008; Xu et al. 2014) and *unpublished data.

peripheral injection of these compounds (Tables 3 and 4). Additionally, spinal transection totally eliminated the effects of systemic delivered H_3 and TRPV3 compounds on spontaneous activity (not shown in the table) (McGaraughty et al. 2012, 2017). TRPV1 antagonists acted at both spinal and supraspinal sites to modulate WDR spontaneous activity, whereas other mechanisms (CB2, $Na_v1.7/1.8$, Ca_v) modulated this firing primarily through a spinal mode of action.

There were a few mechanisms, specifically, H_4, TRPM8, α2b NA, KCNQ2/3, and duloxetine, that did not modulate either evoked or spontaneous firing of WDR neurons in the models evaluated. In the cases of the specific α2b NA receptor agonist and duloxetine, these compounds were shown to modulate nociceptive-related neurons in the medial prefrontal cortex (Chu et al. 2015).

Spontaneous and evoked firing of NS neurons across models. A total of 254 NS neurons were recorded in the deep dorsal horn. Table 5 and Fig. 2*A* break down the distribution of the spontaneous firing rate for these neurons across different animal models of pathological pain. The overall Welch's ANOVA was significant [$F_{(6,49.3)} = 5.8$, $P < 0.00013$], but this was due to the lower spontaneous firing in the carrageenan group versus both the SNL and CFA groups ($P < 0.05$, Games-Howell). The mean firing rates for NS neurons were 0.5 Hz or lower, and no group was significantly different from the naive group. The SNL and OA groups did not differ from their respective sham controls. The vast majority of NS neurons (86.7–100%) had spontaneous discharge rates of <2 Hz, and no group of NS neurons fired at >6 Hz.

Table 6 and Fig. 2, *B* and *C*, summarize the responses of NS neurons to different noxious evoked stimuli across models. The evoked responses to noxious pinch [$F_{(2,31.1)} = 2.5$, $P = 0.1$] and heat [$F_{(3,61)} = 0.5$, $P = 0.7$] did not differ between naive and models of pathological pain. There were no naive animals examined to evaluate NS neurons with knee-receptive fields.

Pharmacological modulation of NS neurons. Due to the very low spontaneous activity, pharmacological modulation was evaluated only for evoked activity from NS neurons. The pharmacological effects on NS neurons (Table 7) mirrored that of WDR neurons. Compounds for mechanisms (i.e., TRPV1, TRPV3, P2X7, P2X3, ASIC1a, 5-HT7, and α7 NNR) that decreased WDR firing in one or more models also decreased NS firing in the same models, and compounds that did not alter WDR activity (i.e., H_4, TRPM8, α2b NA, KCNQ2/3, and duloxetine) also did not alter NS activity.

DISCUSSION

We evaluated the activity of WDR and NS neurons located in the deep spinal dorsal horn to get a better understanding of their function following pain-associated injuries and to determine whether diverse pharmacologies modulate this key element of the pain pathway. These neurons were chosen as a primary focus since they can be modulated directly or indirectly by C, Aβ, or Aδ primary somatosensory neurons, by outflow from the upper lamina neurons, by supraspinal descending modulation, or by contralateral and heterosegmental spinal inputs as well as by elements in the local microenvironment such glial cells (Christensen and Perl 1970; Heinricher 2016; McGaraughty and Henry 1997; Ossipov et al., 014; Petitjean et al. 2012; Todd 2010; Woolf and Fitzgerald 1986). Thus, although the direct site of action for a particular test compound/target mechanism may not be in the deep dorsal horn (although direct action at this site is also a possibility), if

Table 5. *Spontaneous firing of NS neurons across models*

	Mean Hz	0–2 Hz	2–4 Hz	4–6 Hz	>6 Hz
Naive (paw; $n = 32$)	0.3 ± 0.1 (−0.007–0.6)	93.8% (30)	6.2% (2)	0% (0)	0% (0)
Sham SNL (paw; $n = 13$)	0.3 ± 0.1 (0.05–0.5)	100% (13)	0% (0)	0% (0)	0% (0)
SNL (paw; $n = 48$)	0.5 ± 0.1* (0.2–0.7)	93.8% (45)	4.2% (2)	2.1% (1)	0% (0)
CFA (paw; $n = 87$)	0.4 ± 0.1* (0.2–0.6)	95.4% (83)	2.2% (2)	2.2% (2)	0% (0)
Carrageenan (paw; $n = 13$)	0.05 ± 0.02 (0.003–0.09)	100% (13)	0% (0)	0% (0)	0% (0)
Sham MIA (knee; $n = 7$)	0.5 ± 0.3 (−0.2–1.3)	86.7% (6)	14.3% (1)	0% (0)	0% (0)
MIA (knee; $n = 54$)	0.4 ± 0.1 (0.1–0.58)	94.4% (51)	3.7% (2)	1.9% (1)	0% (0)

Values are means ± SE; 95% confidence interval (shown in parentheses beside the mean) and %neurons (n) are shown for each category of firing frequency; n = no. of neurons for that group, and "paw" and "knee" indicate receptive field for recorded neurons. CFA, complete Freund's adjuvant; MIA, monosodium iodoacetate; NS, nociceptive specific; SNL, spinal nerve ligation. *$P < 0.05$ vs. carrageenan using Games-Howell post hoc analysis. Each calculation is compiled from published (Chu et al. 2011, 2015; Jarvis et al. 2014; McGaraughty et al. 2007, 2008a, 2010, 2017; Xu et al. 2012) and unpublished data.

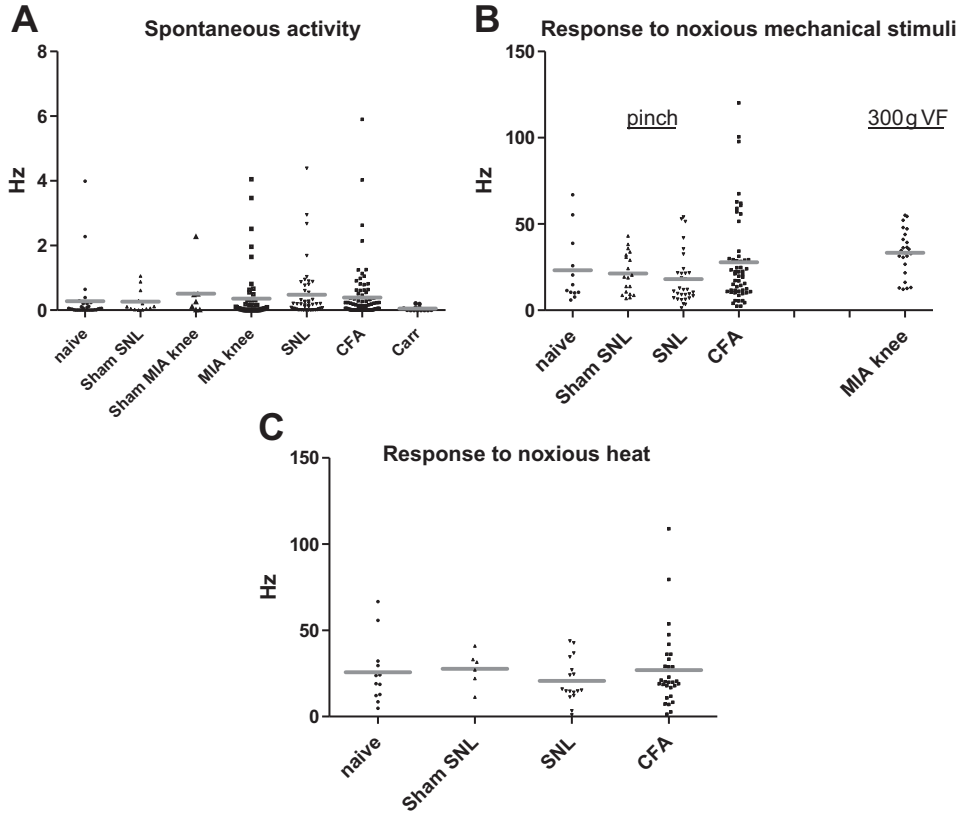

Fig. 2. Distribution of spontaneous and evoked firing for individual nociceptive specific (NS) neurons *A*: distribution of spontaneous firing for individual NS neurons across models of pathological pain and control groups. *B* and *C*: distribution of NS neuronal responses to a high-intensity noxious pinch with 22 mm mini bulldog clamp applied for 10 s to the ipsilateral hind paw or 300-g von Frey hair to the ipsilateral knee for 10 s (MIA-OA model only; *B*), and immersion of the ipsilateral hind paw in 49 ± 3°C water for 10 s (*C*). Each ● indicates activity of individual neurons; gray bar indicates the mean firing rate. "Knee" indicates receptive field for recorded neurons in osteoarthritis (OA) studies; otherwise receptive field is paw for all other models; Carr, carrageenan. CFA, complete Freund's adjuvant; MIA, monosodium iodoacetate.

the compound acts at one or more of these other sites it is likely to affect the firing of the studied neurons, particularly WDR neurons. It is an important node that can give an initial glimpse into whether the mechanism of interest is modulating the pain system and can lead to more detailed interrogations, including of other sites. Of course, there are pain targets that may not directly or indirectly affect these spinal neurons and instead utilize a tangential node, and this was observed in our studies as well (e.g., an α2b NA receptor agonist acting through prefrontal cortex neurons), but the diversity of incoming/modulating inputs to the deep dorsal horn positions this group of neurons as an important intersection within the pain system.

Pain is difficult to assess in rodents since they have adapted mechanisms to hide injury or weakness from predators (Arras et al. 2007). Measuring drug effects on behavioral "pain" readouts in rodents can be subjective, lending itself to unintentional bias, and influenced by a variety of factors. Thus, although changes to rodent "pain" behaviors after drug administration may be due to direct modulation of the nociceptive system, it is also possible that changes in behaviors are the result of unintended effects such as shifting of attention or anxiety, altering cardiovascular function or motor activity, or compromised health (Gabriel et al. 2010; Hoybergs et al. 2008; Munro et al. 2007; Pinho et al. 2011; Richardson and McNally 2003). Recording the activity of WDR and NS neurons provides an objective means to study nociceptive modulation that may be less influenced by some of these factors, particularly in the anesthetized animal. As an example, administration of an H_4 receptor antagonist was reported to be anti-allodynic and anti-hyperalgesic in models of neuropathic and inflammatory pain (Hsieh et al. 2010), but the same compound at comparable exposures did not affect the spontaneous or evoked firing of WDR and NS neurons in the same models. These findings were difficult to reconcile since H_4 receptors are expressed in the DRG and spinal dorsal horn (Strakhova et al. 2009). However, recent studies with global H_4-knockout mice demonstrated that these animals possess a hypersensitive nociceptive phenotype and are anxiogenic (Sanna et al. 2017). Additionally, supraspinal administration of an H_4 agonist was anti-allodynic (Sanna et al. 2015). At a fundamental level, the observed anti-allodynia/hyperalgesia effects of the H_4 antagonist in the study by Hsieh et al. (2010) were not mediated through the deep dorsal

Table 6. *Evoked firing of NS neurons across models*

	Noxious pinch (paw), Hz	300 g VF (knee), Hz	Heat (49 ± 3°C) (paw) Hz
Naive (paw)	23.2 ± 5.8 (10.4–35.9, n = 12)		22.9 ± 5.1 (13.8–37.5; n = 11)
SNL (paw)	18.1 ± 2.7 (12.6–23.6, n = 31)		20.6 ± 3.1 (14.1–27.2; n = 17)
CFA (paw)	27.9 ± 3.5 (20.9–34.8, n = 55)		27.8 ± 4.1 (18.5–35.3; n = 29)
MIA (knee)		32.3 ± 2.7 (27.6–38.9, n = 23)	

Values are means ± SE; 95% confidence interval (shown beside the mean), and group size (n) for each type of stimulus across models. NS, nociceptive specific "Paw" or "knee" indicate receptive field for recorded neurons. Each calculation is compiled from published (Chu et al. 2011, 2015; Jarvis et al. 2014; McGaraughty et al. 2007, 2008a, 2010, 2017; Xu et al. 2012) and unpublished data.

Table 7. *Pharmacological modulation of evoked firing of NS neurons across models*

	Naive	CFA	Carr	SNL	MIA-OA
TRPV1 antagonist†	+	+			+
TRPV1 antagonist PR†					+
TRPV3 antagonist†	+				
TRPA1 antagonist†	+	+			
TRPM8 antagonist*					x
$Ca_v3.2$ blocker†	x				
P2X7 antagonist†				+	
P2X3 antagonist†		+			
H4 antagonist*			x		
ASIC1a blocker*		+			
KCNQ2/3 opener†				x	
α2b NA agonist†				x	
5-HT7 antagonist*		+			
NNR – α7 agonist*					+
duloxetine†				x	

All compounds were administered intravenously. Effects on evoked firing are shown only as spontaneous firing was too low to accurately quantify a pharmacological effect. +, Significant decrease, Carr, carrageenan; CFA, complete Freund's adjuvant; PR, peripherally restricted; SNL, spinal nerve ligation; x, no effect. Effect on evoked activity could be related to any of the stimuli described in Table 2. Compiled from †published (Chu et al., 2011, 2015; Jarvis et al., 2014; McGaraughty et al., 2007, 2008a, 2010, 2017; Xu et al., 2012);and *unpublished data.

horn neurons, but it is unclear how mechanistically the efficacy was achieved. The effect may have been mediated through a supraspinal anxiogenic action, through a peripheral action on the immune system, and/or through a different set of spinal neurons that may independently mediate itch/irritant properties of the stimuli (Butler and Finn 2009; Gutzmer et al. 2011; Huang et al. 2018). Nonetheless, without a clear understanding of its mechanism of action, H_4 receptor antagonists did not proceed further as a therapeutic target for pathological pain in our group. TRPM8 receptor antagonism was another mechanism that did not affect the firing of WDR and NS neurons across multiple models of pathological pain, and this lack of effect is consistent with a behavioral report using different antagonists (Lehto et al. 2015). However, cold stimulation was not examined by our team, which is likely the key modality to evaluate for this mechanism (Patel et al. 2014). In contrast, other teams have reported that TRPM8 receptor antagonists were efficacious against mechanical allodynia in rodent models (De Caro et al. 2018; Salat and Filipek 2015), but it is unclear how this effect was induced since these studies did not determine the pathways/sites that were modulated by the antagonists.

Evoked stimuli were used to evaluate WDR and NS responses across the different models and as such could be compared with behavioral assays that used similar testing approaches. The evoked endpoints were also important to gauge whether novel pharmacology could modulate heightened somatosensory sensitivity, particularly mechanical allodynia, which was a focal point for our team. Compared with naive animals, responses of WDR neurons to a non-noxious mechanical stimulus (10-g von Frey hair) were elevated in the SNL neuropathic and CFA inflammation models, which likely corresponds to behavioral mechanical allodynia. Interestingly, although there was an increase in the responses of WDR neurons to the 10-g von Frey hair in SNL rats (16.8 Hz) relative to sham SNL animals (14 Hz), this was not a significant difference. The lack of significance is meaningful, as this computation was robustly powered with large sample sizes (e.g., 964 WDR neurons from SNL rats). A comparable lack of difference in evoked responses between SNL and their shams have been reported by other groups as well (Elmes et al. 2004; Sagar et al. 2005; Suzuki et al. 2000). The WDR neuronal response to the von Frey stimulation in SNL sham rats was slightly greater than naive animals (11.7 Hz) but was also not significant. Thus, the sham responses were between SNL and naive rats but not significantly different from either. These data imply that the sham surgery, which does not induce frank damage to the afferent neurons, may induce a low-level sensitivity possibly linked to an injury at a distal sight (i.e., skin incision/muscle retraction), but it is not a profound sensitizer.

WDR responses to high-intensity (noxious) thermal and mechanical stimulation in SNL animals were not different from naive animals. In contrast, CFA-inflamed (thermal) as well as MMT- and MIA-OA (mechanical) models demonstrated enhanced responses, relative to naive or sham animals, to high-threshold stimuli at the site of injury, which likely corresponds to the occurrence of primary hyperalgesia in these models. It is noteworthy that WDR neurons with paw-receptive fields in both models of OA did not fire at a higher rate relative to sham or naive rats after low-threshold stimulation of the paw. Behavioral studies in these models, particularly the MIA-OA model (Burston et al. 2016; Comi et al. 2017; Mapp et al. 2013), often use stimulation of the paw as an assessment of secondary allodynia/hyperalgesia. Although this behavioral hypersensitivity did not translate into enhanced evoked responses of WDR neurons, the spontaneous firing of these neurons with hind paw-receptive fields was elevated in the MIA-OA model compared with naive animals.

The relevance or translatability of these "evoked" endpoints to human pain conditions has been debated, particularly since the primary reason to seek medical attention is due to spontaneous or nonevoked pain (Backonja and Stacey 2004; Birklein et al. 2000). The spontaneous firing of WDR but not NS neurons was elevated relative to the control groups across the osteoarthritic, neuropathic, and inflammatory models examined and likely reflects injury-related sensitization of this class of spinal neurons, which has been reported by multiple groups (Cata et al. 2008; Chapman et al. 1998; Elmes et al. 2004; Kitagawa et al. 2005; Liu and Walker 2006; Pertovaara et al. 2001; Pitcher and Henry 2008; Sagar et al. 2005; Simone et al. 2008; Sotgiu et al. 2009; Suzuki and Dickenson 2006; Tabo et al. 1999). The firing rate distribution shows that individual WDR neurons across the pathological models spontaneously discharge at a range of rates, including very low ones ($<$2 Hz), but compared with the non-injury groups, these models have a higher proportion of neurons firing at the higher end of the spectrum ($>$6 Hz). This ongoing discharge at rates higher than normal from these intensity-driven neurons may indicate that there is unevoked or "nagging" discomfort in the animal. Thus, there was a focus on identifying targets that could reduce spontaneous firing in addition to evoked firing. Several targets were associated with reducing both evoked and spontaneous firing (e.g., TRPV1, TRPV3, $Na_v1.7$, $Na_v1.8$, P2X7, P2X3, and H_3), other targets affected neither evoked or spontaneous firing (e.g., H_4, TRPM8, KCNQ2/3), and some only modulated evoked (e.g, ASIC1a, $Ca_v3.2$) whereas others decreased WDR spontaneous activity only in specific models (e.g., TRPA1,

CB2). Although there have been recent advances in the assessment of behavioral state in rodent models of pain (Andrews et al. 2012; King et al. 2009; Leys et al. 2013; Miyagi et al. 2011), examining the spontaneous firing of WDR neurons still represents a useful gauge of ongoing pain, and its relationship to clinical outcomes has been reported previously (Suzuki and Dickenson 2006). We also observed that compounds that either reduced or had no effect on the spontaneous firing of WDR neurons similarly affected a behavioral model of non-evoked pain (i.e., chronic pain-induced sleep disturbance) (Leys et al. 2013 and Leys LJ, unpublished observations). Thus, we used this neuronal assessment in conjunction with other readouts (e.g., behavior, cellular assays) to aid in our identification of compounds/targets to advance into clinical testing for disorders with tactile allodynia and spontaneous pain. The neuronal piece added an objective in vivo evaluation of the pharmacological actions on fundamental biology that is preserved across species and could help guide the modalities and readouts to be evaluated in the clinic (Baron et al. 2017; Sikandar et al. 2013). These were used to help guide our clinical studies on TRPV1-, H_3-, and $Ca_v3.2$-related compounds (Rowbotham et al. 2011, Serra et al. 2015), and the outcomes were consistent with the actions on WDR neurons.

One of the unexpected and consistent results across the different pharmacologies and models was that the spontaneous firing of WDR neurons was not altered by any peripherally restricted compound or by direct administration of compounds to peripheral sites (i.e., receptive field or intra-DRG). This lack of effect occurred even though the same compounds, in the same experiments, decreased the evoked activity of WDR neurons. This was found for TRPV1, TRPV3, and P2X3 receptor antagonists, $Na_v1.7/1.8$ and Ca_v channel blockers, and CB2 receptor agonists. Additionally, when these compounds were injected centrally, either intra-spinally or supraspinally, an effect on both evoked and spontaneous firing was now detected. Thus, it would appear that the elevated spontaneous firing of WDR neurons in the deep dorsal horn was primarily a central event at the time of recording and that a single injection to dampen peripheral inputs was not sufficient to decrease the heightened ongoing activity. Of course, ectopic/sensitized peripheral inputs to the spinal cord were likely critical to initiation of the elevated firing (Ji and Woolf 2001). Because the current studies were typically performed days to weeks after the initial injury, there may be a need for repeated injections of the therapeutic agents over time to dampen "maintenance" inputs to the spinal cord and in turn reduce abnormally high spontaneous firing of WDR neurons (Haroutounian et al. 2014; Ji and Woolf 2001; Sotgiu et al. 1994). This phenomenon was also observed with peripheral versus central administration of lidocaine (McGaraughty et al. 2006), which is in contrast to another group that applied lidocaine to the DRG in spinally transected neuropathic rats and subsequently attenuated the ongoing firing of WDR neurons (Pitcher and Henry 2008). However, the discrepancy in outcome may be explained by a high volume of injection relative to our technique (1500-fold difference: 1.5 ml vs. 1 μl), which may have led to dispersion of the solution into the spinal cord itself to reduce the spontaneous discharges. It is well accepted that lidocaine plasters can dampen many aspects of neuropathic pain, including the spontaneous pain element (Casale et al. 2014; Rehm et al. 2010). The onset of action for these plasters in placebo-controlled studies is typically reported to be days or weeks after repeated application of the patch (Rehm et al. 2010; Casale et al. 2014). At minimum, this collection of data on the modulation of WDR spontaneous firing implies that compounds acting centrally would be more efficient at affecting this endpoint and that local and descending systems contribute to the abnormally elevated firing of WDR neurons (Heinricher 2016; Vera-Portocarrero et al. 2006).

Unlike WDR neurons, the firing of NS neurons did not appear to be sensitized in the neuropathic, inflammation, or osteoarthritic models studied, as neither the evoked nor spontaneous firing differed from control groups. The spontaneous firing was very low ($<$0.5 Hz) across these models. It is possible that although the individual neuron firing rates did not change, other pathological features like receptive field expansion that affect populations of nociceptive neurons may still trigger the ascent of "aversive" information to supraspinal sites (Chu et al. 2004; Suzuki et al. 2000). It is also possible that the recorded cells were a subgroup of NS neurons that do not become sensitized after injury, but there may be other subclasses that do become sensitized and then respond to lower-threshold somatosensory inputs (Pitcher and Cervero 2010; Randich et al. 1997). However, at the time of neuronal characterization in the electrophysiological study, the phenotypic change would have likely already occurred, and these neurons would then be classified as WDR based on their response profile. Phenotypic changes to nociceptive neurons have been reported in both the deep and superficial dorsal horn (Keller et al. 2007; Neumann et al. 1996; Randich et al. 1997; Urch et al. 2003). The phenotypic switch of neurons in the superficial laminae can also influence the response properties of deeper laminae neurons (Suzuki et al. 2002). Thus, under the conditions examined, there was a group of NS neurons in the deep dorsal horn that appeared to transmit nociceptive signaling without indication of sensitization under pathological conditions.

The strength of this review is also its weakness. The neuronal firing properties and the response to the different pharmacologies could be directly compared, as all of the recording conditions, including experimenters, were the same. It is uncertain how variances to these conditions, particularly the choice of anesthetic, may affect the readouts. However, the observations on changes to neuronal firing rates and efficacy related to some of pharmacological targets have been reported by other groups using different recording paradigms (e.g., see Elmes et al. 2004; Luo et al. 2008; Patel et al. 2015; Rahman and Dickenson 2015; Sagar et al. 2005; Sharp et al. 2006; Tsuruoka et al. 2008). Additionally, although anesthesia can keep in-check variables such as unintended behavioral stress, it introduces other pharmacology that may interfere with the test therapeutics, and most choices of anesthetics interact with more than one mechanism. The key is to keep a steady (not too far on deep end) anesthetic state so that the baseline and post-drug measurements are recorded in the animal at a comparable depth of anesthesia. Continuous infusion of short-acting anesthetic agents such as methohextital, propofol, or inhalents (e.g., isoflurane) are ideal for maintaining this state. Bolus injections of anesthetics with moderate half-lives are more challenging to keep at a steady state and to predict the degree of pharmacokinetic decay once a viable neuron in the class of interest is identified after variable and often lengthy

searching times. Thus, the timing of the bolus injection may influence study outcome. Propofol was used in the current set of experiments, and there are benefits and limitations associated with this agent (Barter et al. 2008; Brammer et al. 1993; Kim et al. 2007; Patten et al. 2001; Takazawa et al. 2009). Despite every attempt to keep the anesthetic state stable within individual animals, the state may vary between animals and may be a contributing factor to variable baseline firing rates across neurons. A limitation of in vivo electrophysiology from a drug discovery perspective is that it is a low-throughput technique, but in combination with other approaches it provides strong validation and mechanistic understanding of the targets investigated.

In vivo electrophysiological recording is one of the oldest techniques used in the investigation of neuronal activity from the spinal dorsal horn (Christensen and Perl 1970; Skinner and Willis 1970; Tasaki 1952; Wall 1959, 1960). Although the equipment has advanced (e.g., from chart and reel-to-reel recorders to computer-based spike wave discrimination), the basic premise and its value remain the same. It is an objective and translatable technique that provides mechanistic understanding of the pain system and how it can be modulated. For pain research, its major strength lies in that the neurons can be functionally identified by relevant external stimulation. With the identification of subgroups of neurons that respond to stimulation along the low- to high-threshold spectrum and change their discharge properties following various forms of injury, a greater understanding of normal and pathological pain has been gained. Although an animal cannot respond to the question, "Are you feeling pain?" as a human would, recording of nociceptive neurons in vivo provides an insight into this state (Maixner et al. 1986; Sikandar et al. 2013). It is with this basic premise that this systems approach was used by our group to assess the utility and potential efficacy of novel therapeutics for pathological pain.

DISCLOSURES

All authors are employees or former employees of AbbVie. This study was sponsored by AbbVie. AbbVie contributed to the study design, research, and interpretation of data, writing, reviewing, and approval of the publication. The authors declare no other competing financial interests.

AUTHOR CONTRIBUTIONS

S.M., K.L.C., and J.X. conceived and designed research; S.M., K.L.C., and J.X. performed experiments; S.M., K.L.C., and J.X. analyzed data; S.M., K.L.C., and J.X. interpreted results of experiments; S.M. prepared figures; S.M. drafted manuscript; S.M., K.L.C., and J.X. edited and revised manuscript; S.M., K.L.C., and J.X. approved final version of manuscript.

REFERENCES

Andrews N, Legg E, Lisak D, Issop Y, Richardson D, Harper S, Pheby T, Huang W, Burgess G, Machin I, Rice AS. Spontaneous burrowing behaviour in the rat is reduced by peripheral nerve injury or inflammation associated pain. *Eur J Pain* 16: 485–495, 2012. doi:10.1016/j.ejpain.2011.07.012.

Arras M, Rettich A, Cinelli P, Kasermann HP, Burki K. Assessment of post-laparotomy pain in laboratory mice by telemetric recording of heart rate and heart rate variability. *BMC Vet Res* 3: 16, 2007. doi:10.1186/1746-6148-3-16.

Baba H, Doubell TP, Woolf CJ. Peripheral inflammation facilitates Abeta fiber-mediated synaptic input to the substantia gelatinosa of the adult rat spinal cord. *J Neurosci* 19: 859–867, 1999. doi:10.1523/JNEUROSCI.19-02-00859.1999.

Backonja MM, Stacey B. Neuropathic pain symptoms relative to overall pain rating. *J Pain* 5: 491–497, 2004. doi:10.1016/j.jpain.2004.09.001.

Baron R, Maier C, Attal N, Binder A, Bouhassira D, Cruccu G, Finnerup NB, Haanpää M, Hansson P, Hüllemann P, Jensen TS, Freynhagen R, Kennedy JD, Magerl W, Mainka T, Reimer M, Rice AS, Segerdahl M, Serra J, Sindrup S, Sommer C, Tölle T, Vollert J, Treede RD. Peripheral neuropathic pain: a mechanism-related organizing principle based on sensory profiles. *Pain* 158: 261–272, 2017. doi:10.1097/j.pain.0000000000000753.

Barter LS, Mark LO, Jinks SL, Carstens EE, Antognini JF. Immobilizing doses of halothane, isoflurane or propofol, do not preferentially depress noxious heat-evoked responses of rat lumbar dorsal horn neurons with ascending projections. *Anesth Analg* 106: 985–990, 2008. doi:10.1213/ane.0b013e318163f8f3.

Birklein F, Riedl B, Sieweke N, Weber M, Neundörfer B. Neurological findings in complex regional pain syndromes–analysis of 145 cases. *Acta Neurol Scand* 101: 262–269, 2000. doi:10.1034/j.1600-0404.2000.101004262x./.

Boyce-Rustay JM, Simler GH, McGaraughty S, Chu KL, Wensink EJ, Vasudevan A, Honore P. Characterization of Fasudil in preclinical models of pain. *J Pain* 11: 941–949, 2010. doi:10.1016/j.jpain.2009.12.014.

Brammer A, West CD, Allen SL. A comparison of propofol with other injectable anaesthetics in a rat model for measuring cardiovascular parameters. *Lab Anim* 27: 250–257, 1993. doi:10.1258/002367793780745354.

Brederson JD, Chu KL, Xu J, Nikkel AL, Markosyan S, Jarvis MF, Edelmayer R, Bitner RS, McGaraughty S. Characterization and comparison of rat monosodium iodoacetate and medial meniscal tear models of osteoarthritic pain. *J Orthop Res* 36: 2109–2117, 2018. doi:10.1002/jor.23869.

Burston JJ, Mapp PI, Sarmad S, Barrett DA, Niphakis MJ, Cravatt BF, Walsh DA, Chapman V. Robust anti-nociceptive effects of monoacylglycerol lipase inhibition in a model of osteoarthritis pain. *Br J Pharmacol* 173: 3134–3144, 2016. doi:10.1111/bph.13574.

Butler RK, Finn DP. Stress-induced analgesia. *Prog Neurobiol* 88: 184–202, 2009. doi:10.1016/j.pneurobio.2009.04.003.

Casale R, Di Matteo M, Minella CE, Fanelli G, Allegri M. Reduction of painful area as new possible therapeutic target in post-herpetic neuropathic pain treated with 5% lidocaine medicated plaster: a case series. *J Pain Res* 7: 353–357, 2014. doi:10.2147/JPR.S65398.

Cata JP, Weng HR, Dougherty PM. Behavioral and electrophysiological studies in rats with cisplatin-induced chemoneuropathy. *Brain Res* 1230: 91–98, 2008. doi:10.1016/j.brainres.2008.07.022.

Chapman V, Suzuki R, Dickenson AH. Electrophysiological characterization of spinal neuronal response properties in anaesthetized rats after ligation of spinal nerves L5-L6. *J Physiol* 507: 881–894, 1998. doi:10.1111/j.1469-7793.1998.881bs.x.

Christensen BN, Perl ER. Spinal neurons specifically excited by noxious or thermal stimuli: marginal zone of the dorsal horn. *J Neurophysiol* 33: 293–307, 1970. doi:10.1152/jn.1970.33.2.293.

Chu KL, Chandran P, Joshi SK, Jarvis MF, Kym PR, McGaraughty S. TRPV1-related modulation of spinal neuronal activity and behavior in a rat model of osteoarthritic pain. *Brain Res* 1369: 158–166, 2011. doi:10.1016/j.brainres.2010.10.101.

Chu KL, Faltynek CR, Jarvis MF, McGaraughty S. Increased WDR spontaneous activity and receptive field size in rats following a neuropathic or inflammatory injury: implications for mechanical sensitivity. *Neurosci Lett* 372: 123–126, 2004. doi:10.1016/j.neulet.2004.09.025.

Chu KL, Xu J, Frost J, Li L, Gomez E, Dart MJ, Jarvis MF, Meyer MD, McGaraughty S. A-1262543, a selective α_{2B} adrenoceptor agonist, and duloxetine modulate nociceptive neurons in the medial prefrontal cortex, but not spinal cord in neuropathic rats. *Eur J Pain* 19: 649–660, 2015. doi:10.1002/ejp.586.

Comi E, Lanza M, Ferrari F, Mauri V, Caselli G, Rovati LC. Efficacy of CR4056, a first-in-class imidazoline-2 analgesic drug, in comparison with naproxen in two rat models of osteoarthritis. *J Pain Res* 10: 1033–1043, 2017. doi:10.2147/JPR.S132026.

De Caro C, Russo R, Avagliano C, Cristiano C, Calignano A, Aramini A, Bianchini G, Allegretti M, Brandolini L. Antinociceptive effect of two novel transient receptor potential melastatin 8 antagonists in acute and chronic pain models in rat. *Br J Pharmacol* 175: 1691–1706, 2018. doi:10.1111/bph.14177.

Drummond PD. Sensory-autonomic interactions in health and disease. *Handb Clin Neurol* 117: 309–319, 2013. doi:10.1016/B978-0-444-53491-0.00024-9.

El-Kouhen O, Lehto SG, Pan JB, Chang R, Baker SJ, Zhong C, Hollingsworth PR, Mikusa JP, Cronin EA, Chu KL, McGaraughty SP, Uchic ME, Miller LN, Rodell NM, Patel M, Bhatia P, Mezler M, Kolasa T, Zheng G-Z, Fox GB, Stewart AO, Decker MW, Moreland RB, Brioni JD, Honore P. Blockade of mGluR1 receptor results in analgesia and disruption of motor and cognitive performances: effects of A-841720, a novel non-competitive mGluR1 receptor antagonist. *Br J Pharmacol* 149: 761–774, 2006. doi:10.1038/sj.bjp.0706877.

Elmes SJ, Jhaveri MD, Smart D, Kendall DA, Chapman V. Cannabinoid CB2 receptor activation inhibits mechanically evoked responses of wide dynamic range dorsal horn neurons in naïve rats and in rat models of inflammatory and neuropathic pain. *Eur J Neurosci* 20: 2311–2320, 2004. doi:10.1111/j.1460-9568.2004.03690.x.

Gabriel AF, Paoletti G, Della Seta D, Panelli R, Marcus MA, Farabollini F, Carli G, Joosten EA. Enriched environment and the recovery from inflammatory pain: Social versus physical aspects and their interaction. *Behav Brain Res* 208: 90–95, 2010. doi:10.1016/j.bbr.2009.11.015.

Gutzmer R, Gschwandtner M, Rossbach K, Mommert S, Werfel T, Kietzmann M, Baeumer W. Pathogenetic and therapeutic implications of the histamine H4 receptor in inflammatory skin diseases and pruritus. *Front Biosci (Schol Ed)* S3: 985–994, 2011. doi:10.2741/s203.

Haroutounian S, Nikolajsen L, Bendtsen TF, Finnerup NB, Kristensen AD, Hasselstrøm JB, Jensen TS. Primary afferent input critical for maintaining spontaneous pain in peripheral neuropathy. *Pain* 155: 1272–1279, 2014. doi:10.1016/j.pain.2014.03.022.

Heinricher MM. Pain modulation and the transition from acute to chronic pain. *Adv Exp Med Biol* 904: 105–115, 2016. doi:10.1007/978-94-017-7537-3_8.

Hoybergs YM, Biermans RL, Meert TF. The impact of bodyweight and body condition on behavioral testing for painful diabetic neuropathy in the streptozotocin rat model. *Neurosci Lett* 436: 13–18, 2008. doi:10.1016/j.neulet.2008.02.051.

Hsieh GC, Chandran P, Salyers AK, Pai M, Zhu CZ, Wensink EJ, Witte DG, Miller TR, Mikusa JP, Baker SJ, Wetter JM, Marsh KC, Hancock AA, Cowart MD, Esbenshade TA, Brioni JD, Honore P. H4 receptor antagonism exhibits anti-nociceptive effects in inflammatory and neuropathic pain models in rats. *Pharmacol Biochem Behav* 95: 41–50, 2010. doi:10.1016/j.pbb.2009.12.004.

Hsieh MT, Donaldson LF, Lumb BM. Differential contributions of A- and C-nociceptors to primary and secondary inflammatory hypersensitivity in the rat. *Pain* 156: 1074–1083, 2015. doi:10.1097/j.pain.0000000000000151.

Huang K, Hu DD, Bai D, Wu ZY, Chen YY, Zhang YJ, Lv X, Wang QX, Zhang L. Persistent extracellular signal-regulated kinase activation by the histamine H4 receptor in spinal neurons underlies chronic itch. *J Invest Dermatol* 138: 1843–1850, 2018. doi:10.1016/j.jid.2018.02.019.

Jarvis MF, Honore P, Shieh C-C, Chapman M, Joshi S, Zhang X-F, Kort M, Carroll W, Marron B, Atkinson R, Thomas J, Liu D, Krambis M, Liu Y, McGaraughty S, Chu K, Roeloffs R, Zhong C, Mikusa JP, Hernandez G, Gauvin D, Wade C, Zhu C, Pai M, Scanio M, Shi L, Drizin I, Gregg R, Matulenko M, Hakeem A, Gross M, Johnson M, Marsh K, Wagoner PK, Sullivan JP, Faltynek CR, Krafte DS. A-803467, a potent and selective Nav1.8 sodium channel blocker, attenuates neuropathic and inflammatory pain in the rat. *Proc Natl Acad Sci USA* 104: 8520–8525, 2007. doi:10.1073/pnas.0611364104.

Jarvis MF, Scott VE, McGaraughty S, Chu KL, Xu J, Niforatos W, Milicic I, Joshi S, Zhang Q, Xia Z. A peripherally acting, selective T-type calcium channel blocker, ABT-639, effectively reduces nociceptive and neuropathic pain in rats. *Biochem Pharmacol* 89: 536–544, 2014. doi:10.1016/j.bcp.2014.03.015.

Ji RR, Woolf CJ. Neuronal plasticity and signal transduction in nociceptive neurons: implications for the initiation and maintenance of pathological pain. *Neurobiol Dis* 8: 1–10, 2001. doi:10.1006/nbdi.2000.0360.

Keller AF, Beggs S, Salter MW, De Koninck Y. Transformation of the output of spinal lamina I neurons after nerve injury and microglia stimulation underlying neuropathic pain. *Mol Pain* 3: 27, 2007. doi:10.1186/1744-8069-3-27.

Khasabov SG, Rogers SD, Ghilardi JR, Peters CM, Mantyh PW, Simone DA. Spinal neurons that possess the substance P receptor are required for the development of central sensitization. *J Neurosci* 22: 9086–9098, 2002. doi:10.1523/JNEUROSCI.22-20-09086.2002.

Kim J, Yao A, Atherley R, Carstens E, Jinks SL, Antognini JF. Neurons in the ventral spinal cord are more depressed by isoflurane, halothane, and propofol than are neurons in the dorsal spinal cord. *Anesth Analg* 105: 1020–1026, 2007. doi:10.1213/01.ane.0000280483.17854.56.

King T, Vera-Portocarrero L, Gutierrez T, Vanderah TW, Dussor G, Lai J, Fields HL, Porreca F. Unmasking the tonic-aversive state in neuropathic pain. *Nat Neurosci* 12: 1364–1366, 2009. [Erratum in *Nat Neurosci* 13: 1033, 2010.] doi:10.1038/nn.2407.

Kitagawa J, Kanda K, Sugiura M, Tsuboi Y, Ogawa A, Shimizu K, Koyama N, Kamo H, Watanabe T, Ren K, Iwata K. Effect of chronic inflammation on dorsal horn nociceptive neurons in aged rats. *J Neurophysiol* 93: 3594–3604, 2005. doi:10.1152/jn.01075.2004.

Lehto SG, Weyer AD, Zhang M, Youngblood BD, Wang J, Wang W, Kerstein PC, Davis C, Wild KD, Stucky CL, Gavva NR. AMG2850, a potent and selective TRPM8 antagonist, is not effective in rat models of inflammatory mechanical hypersensitivity and neuropathic tactile allodynia. *Naunyn Schmiedebergs Arch Pharmacol* 388: 465–476, 2015. doi:10.1007/s00210-015-1090-9.

Leys LJ, Chu KL, Xu J, Pai M, Yang HS, Robb HM, Jarvis MF, Radek RJ, McGaraughty S. Disturbances in slow-wave sleep are induced by models of bilateral inflammation, neuropathic, and postoperative pain, but not osteoarthritic pain in rats. *Pain* 154: 1092–1102, 2013. doi:10.1016/j.pain.2013.03.019.

Liu C, Walker JM. Effects of a cannabinoid agonist on spinal nociceptive neurons in a rodent model of neuropathic pain. *J Neurophysiol* 96: 2984–2994, 2006. doi:10.1152/jn.00498.2006.

Luo H, Xu IS, Chen Y, Yang F, Yu L, Li GX, Liu FY, Xing GG, Shi YS, Li T, Han JS, Wan Y. Behavioral and electrophysiological evidence for the differential functions of TRPV1 at early and late stages of chronic inflammatory nociception in rats. *Neurochem Res* 33: 2151–2158, 2008. doi:10.1007/s11064-008-9751-4.

Maixner W, Dubner R, Bushnell MC, Kenshalo DR Jr, Oliveras JL. Wide-dynamic-range dorsal horn neurons participate in the encoding process by which monkeys perceive the intensity of noxious heat stimuli. *Brain Res* 374: 385–388, 1986. doi:10.1016/0006-8993(86)90435-X.

Mapp PI, Sagar DR, Ashraf S, Burston JJ, Suri S, Chapman V, Walsh DA. Differences in structural and pain phenotypes in the sodium monoiodoacetate and meniscal transection models of osteoarthritis. *Osteoarthritis Cartilage* 21: 1336–1345, 2013. doi:10.1016/j.joca.2013.06.031.

McGaraughty S, Chu KL, Bitner RS, Martino B, El Kouhen R, Han P, Nikkel AL, Burgard EC, Faltynek CR, Jarvis MF. Capsaicin infused into the PAG affects rat tail flick responses to noxious heat and alters neuronal firing in the RVM. *J Neurophysiol* 90: 2702–2710, 2003. doi:10.1152/jn.00433.2003.

McGaraughty S, Chu KL, Brown BS, Zhu CZ, Zhong C, Joshi SK, Honore P, Faltynek CR, Jarvis MF. Contributions of central and peripheral TRPV1 receptors to mechanically evoked and spontaneous firing of spinal neurons in inflamed rats. *J Neurophysiol* 100: 3158–3166, 2008a. doi:10.1152/jn.90768.2008.

McGaraughty S, Chu KL, Cowart MD, Brioni JD. Antagonism of supraspinal histamine H_3 receptors modulates spinal neuronal activity in neuropathic rats. *J Pharmacol Exp Ther* 343: 13–20, 2012. doi:10.1124/jpet.112.194761.

McGaraughty S, Chu KL, Dart MJ, Yao BB, Meyer MD. A CB(2) receptor agonist, A-836339, modulates wide dynamic range neuronal activity in neuropathic rats: contributions of spinal and peripheral CB(2) receptors. *Neuroscience* 158: 1652–1661, 2009. doi:10.1016/j.neuroscience.2008.11.015.

McGaraughty S, Chu KL, Faltynek CR, Jarvis MF. Systemic and site-specific effects of A-425619, a selective TRPV1 receptor antagonist, on wide dynamic range neurons in CFA-treated and uninjured rats. *J Neurophysiol* 95: 18–25, 2006. doi:10.1152/jn.00560.2005.

McGaraughty S, Chu KL, Namovic MT, Donnelly-Roberts DL, Harris RR, Zhang X-F, Shieh C-C, Wismer CT, Zhu CZ, Gauvin DM, Fabiyi AC, Honore P, Gregg RJ, Kort ME, Nelson DW, Carroll WA, Marsh K, Faltynek CR, Jarvis MF. P2X7-related modulation of pathological nociception in rats. *Neuroscience* 146: 1817–1828, 2007. doi:10.1016/j.neuroscience.2007.03.035.

McGaraughty S, Chu KL, Perner RJ, Didomenico S, Kort ME, Kym PR. TRPA1 modulation of spontaneous and mechanically evoked firing of spinal neurons in uninjured, osteoarthritic, and inflamed rats. *Mol Pain* 6: 14, 2010. doi:10.1186/1744-8069-6-14.

McGaraughty S, Chu KL, Scanio MJC, Kort ME, Faltynek CR, Jarvis MF. A selective Nav1.8 sodium channel blocker, A-803467 [5-(4-chlorophenyl-N-(3,5-dimethoxyphenyl)furan-2-carboxamide], attenuates spinal neuronal activity in neuropathic rats. *J Pharmacol Exp Ther* 324: 1204–1211, 2008b. doi:10.1124/jpet.107.134148.

McGaraughty S, Chu KL, Xu J, Leys L, Radek RJ, Dart MJ, Gomtsyan A, Schmidt RG, Kym PR, Brederson JD. TRPV3 modulates nociceptive

signaling through peripheral and supraspinal sites in rats. *J Neurophysiol* 118: 904–916, 2017. doi:10.1152/jn.00104.2017.

McGaraughty S, Henry JL. Effects of noxious hindpaw immersion on evoked and spontaneous firing of contralateral convergent dorsal horn neurons in both intact and spinalized rats. *Brain Res Bull* 43: 263–267, 1997. doi:10.1016/S0361-9230(97)00002-6.

Miyagi M, Ishikawa T, Kamoda H, Orita S, Kuniyoshi K, Ochiai N, Kishida S, Nakamura J, Eguchi Y, Arai G, Suzuki M, Aoki Y, Toyone T, Takahashi K, Inoue G, Ohtori S. Assessment of gait in a rat model of myofascial inflammation using the CatWalk system. *Spine* 36: 1760–1764, 2011. doi:10.1097/BRS.0b013e3182269732.

Munro G, Erichsen HK, Mirza NR. Pharmacological comparison of anticonvulsant drugs in animal models of persistent pain and anxiety. *Neuropharmacology* 53: 609–618, 2007. doi:10.1016/j.neuropharm.2007.07.002.

Neumann S, Doubell TP, Leslie T, Woolf CJ. Inflammatory pain hypersensitivity mediated by phenotypic switch in myelinated primary sensory neurons. *Nature* 384: 360–364, 1996. doi:10.1038/384360a0.

Ossipov MH, Morimura K, Porreca F. Descending pain modulation and chronification of pain. *Curr Opin Support Palliat Care* 8: 143–151, 2014. doi:10.1097/SPC.0000000000000055.

Patel R, Gonçalves L, Leveridge M, Mack SR, Hendrick A, Brice NL, Dickenson AH. Anti-hyperalgesic effects of a novel TRPM8 agonist in neuropathic rats: a comparison with topical menthol. *Pain* 155: 2097–2107, 2014. doi:10.1016/j.pain.2014.07.022.

Patel R, Rutten K, Valdor M, Schiene K, Wigge S, Schunk S, Damann N, Christoph T, Dickenson AH. Electrophysiological characterization of activation state-dependent Ca(v)2 channel antagonist TROX-1 in spinal nerve injured rats. *Neuroscience* 297: 47–57, 2015. doi:10.1016/j.neuroscience.2015.03.057.

Patten D, Foxon GR, Martin KF, Halliwell RF. An electrophysiological study of the effects of propofol on native neuronal ligand-gated ion channels. *Clin Exp Pharmacol Physiol* 28: 451–458, 2001. doi:10.1046/j.1440-1681.2001.03469.x.

Pertovaara A, Wei H, Kalmari J, Ruotsalainen M. Pain behavior and response properties of spinal dorsal horn neurons following experimental diabetic neuropathy in the rat: modulation by nitecapone, a COMT inhibitor with antioxidant properties. *Exp Neurol* 167: 425–434, 2001. doi:10.1006/exnr.2000.7574.

Petitjean H, Rodeau JL, Schlichter R. Interactions between superficial and deep dorsal horn spinal cord neurons in the processing of nociceptive information. *Eur J Neurosci* 36: 3500–3508, 2012. doi:10.1111/j.1460-9568.2012.08273.x.

Pinho D, Morato M, Couto MR, Marques-Lopes J, Tavares I, Albino-Teixeira A. Does chronic pain alter the normal interaction between cardiovascular and pain regulatory systems? Pain modulation in the hypertensive-monoarthritic rat. *J Pain* 12: 194–204, 2011. doi:10.1016/j.jpain.2010.06.009.

Pitcher GM, Henry JL. Governing role of primary afferent drive in increased excitation of spinal nociceptive neurons in a model of sciatic neuropathy. *Exp Neurol* 214: 219–228, 2008. doi:10.1016/j.expneurol.2008.08.003.

Pitcher MH, Cervero F. Role of the NKCC1 co-transporter in sensitization of spinal nociceptive neurons. *Pain* 151: 756–762, 2010. doi:10.1016/j.pain.2010.09.008.

Rahman W, Dickenson AH. Osteoarthritis-dependent changes in antinociceptive action of Nav1.7 and Nav1.8 sodium channel blockers: An in vivo electrophysiological study in the rat. *Neuroscience* 295: 103–116, 2015. doi:10.1016/j.neuroscience.2015.03.042.

Randich A, Meller ST, Gebhart GF. Responses of primary afferents and spinal dorsal horn neurons to thermal and mechanical stimuli before and during zymosan-induced inflammation of the rat hindpaw. *Brain Res* 772: 135–148, 1997. doi:10.1016/S0006-8993(97)00883-4.

Rehm S, Binder A, Baron R. Post-herpetic neuralgia: 5% lidocaine medicated plaster, pregabalin, or a combination of both? A randomized, open, clinical effectiveness study. *Curr Med Res Opin* 26: 1607–1619, 2010. doi:10.1185/03007995.2010.483675.

Richardson R, McNally GP. Effects of an odor paired with illness on startle, freezing, and analgesia in rats. *Physiol Behav* 78: 213–219, 2003. doi:10.1016/S0031-9384(02)00974-5.

Rowbotham MC, Nothaft W, Duan WR, Wang Y, Faltynek C, McGaraughty S, Chu KL, Svensson P. Oral and cutaneous thermosensory profile of selective TRPV1 inhibition by ABT-102 in a randomized healthy volunteer trial. *Pain* 152: 1192–1200, 2011. doi:10.1016/j.pain.2011.01.051.

Sagar DR, Kelly S, Millns PJ, O'Shaughnessey CT, Kendall DA, Chapman V. Inhibitory effects of CB1 and CB2 receptor agonists on responses of DRG neurons and dorsal horn neurons in neuropathic rats. *Eur J Neurosci* 22: 371–379, 2005. doi:10.1111/j.1460-9568.2005.04206.x.

Sałat K, Filipek B. Antinociceptive activity of transient receptor potential channel TRPV1, TRPA1, and TRPM8 antagonists in neurogenic and neuropathic pain models in mice. *J Zhejiang Univ Sci B* 16: 167–178, 2015. doi:10.1631/jzus.B1400189.

Sanna MD, Ghelardini C, Thurmond RL, Masini E, Galeotti N. Behavioural phenotype of histamine H_4 receptor knockout mice: focus on central neuronal functions. *Neuropharmacology* 114: 48–57, 2017. doi:10.1016/j.neuropharm.2016.11.023.

Sanna MD, Stark H, Lucarini L, Ghelardini C, Masini E, Galeotti N. Histamine H4 receptor activation alleviates neuropathic pain through differential regulation of ERK, JNK, and P38 MAPK phosphorylation. *Pain* 156: 2492–2504, 2015. doi:10.1097/j.pain.0000000000000319.

Serra J, Duan WR, Locke C, Solà R, Liu W, Nothaft W. Effects of a T-type calcium channel blocker, ABT-639, on spontaneous activity in C-nociceptors in patients with painful diabetic neuropathy: a randomized controlled trial. *Pain* 156: 2175–2183, 2015. doi:10.1097/j.pain.0000000000000249.

Sharp CJ, Reeve AJ, Collins SD, Martindale JC, Summerfield SG, Sargent BS, Bate ST, Chessell IP. Investigation into the role of P2X(3)/P2X(2/3) receptors in neuropathic pain following chronic constriction injury in the rat: an electrophysiological study. *Br J Pharmacol* 148: 845–852, 2006. doi:10.1038/sj.bjp.0706790.

Sikandar S, Ronga I, Iannetti GD, Dickenson AH. Neural coding of nociceptive stimuli-from rat spinal neurones to human perception. *Pain* 154: 1263–1273, 2013. doi:10.1016/j.pain.2013.03.041.

Simone DA, Khasabov SG, Hamamoto DT. Changes in response properties of nociceptive dorsal horn neurons in a murine model of cancer pain. *Sheng Li Xue Bao* 60: 635–644, 2008.

Skinner RD, Willis WD. Spinal cord potentials produced by ventral cord volleys in the cat. *Exp Neurol* 27: 305–317, 1970. doi:10.1016/0014-4886(70)90223-2.

Sotgiu ML, Biella G, Riva L. A study of early ongoing activity in dorsal horn units following sciatic nerve constriction. *Neuroreport* 5: 2609–2612, 1994. doi:10.1097/00001756-199412000-00051.

Sotgiu ML, Valente M, Storchi R, Caramenti G, Biella GE. Cooperative N-methyl-D-aspartate (NMDA) receptor antagonism and mu-opioid receptor agonism mediate the methadone inhibition of the spinal neuron pain-related hyperactivity in a rat model of neuropathic pain. *Pharmacol Res* 60: 284–290, 2009. doi:10.1016/j.phrs.2009.04.002.

Strakhova MI, Nikkel AL, Manelli AM, Hsieh GC, Esbenshade TA, Brioni JD, Bitner RS. Localization of histamine H4 receptors in the central nervous system of human and rat. *Brain Res* 1250: 41–48, 2009. doi:10.1016/j.brainres.2008.11.018.

Surowy CS, Neelands TR, Bianchi BR, McGaraughty S, El-Kouhen R, Han P, Chu KL, McDonald HA, Vos M, Niforatos W, Bayburt EK, Lee C-H, Gomtsyan A, Honore P, Sullivan JP, Jarvis MF, Faltynek CR. (R)-(5-tert-Butyl-2,3-dihydro-1H-inden-1-yl)-3-(1H-indazol-4-yl)-urea (ABT-102) blocks polymodal activation of transient receptor potential vanilloid 1 receptors in vitro and heat-evoked firing of spinal dorsal horn neurons in vivo. *JPET* 326: 879–888, 2008. doi:10.1124/jpet.108.138511.

Suzuki R, Dickenson AH. Differential pharmacological modulation of the spontaneous stimulus-independent activity in the rat spinal cord following peripheral nerve injury. *Exp Neurol* 198: 72–80, 2006. doi:10.1016/j.expneurol.2005.10.032.

Suzuki R, Kontinen VK, Matthews E, Williams E, Dickenson AH. Enlargement of the receptive field size to low intensity mechanical stimulation in the rat spinal nerve ligation model of neuropathy. *Exp Neurol* 163: 408–413, 2000. doi:10.1006/exnr.2000.7371.

Suzuki R, Morcuende S, Webber M, Hunt SP, Dickenson AH. Superficial NK1-expressing neurons control spinal excitability through activation of descending pathways. *Nat Neurosci* 5: 1319–1326, 2002. doi:10.1038/nn966.

Tabo E, Jinks SL, Eisele JH Jr, Carstens E. Behavioral manifestations of neuropathic pain and mechanical allodynia, and changes in spinal dorsal horn neurons, following L4-L6 dorsal root constriction in rats. *Pain* 80: 503–520, 1999. doi:10.1016/S0304-3959(98)00243-7.

Takazawa T, Furue H, Nishikawa K, Uta D, Takeshima K, Goto F, Yoshimura M. Actions of propofol on substantia gelatinosa neurones in rat spinal cord revealed by in vitro and in vivo patch-clamp recordings. *Eur J Neurosci* 29: 518–528, 2009. doi:10.1111/j.1460-9568.2008.06607.x.

Tasaki I. Properties of myelinated fibers in frog sciatic nerve and in spinal cord as examined with micro-electrodes. *Jpn J Physiol* 3: 73–94, 1952. doi:10.2170/jjphysiol.3.73.

Todd AJ. Neuronal circuitry for pain processing in the dorsal horn. *Nat Rev Neurosci* 11: 823–836, 2010. doi:10.1038/nrn2947.

Tsuda M. Modulation of pain and itch by spinal glia. *Neurosci Bull* 34: 178–185, 2018. doi:10.1007/s12264-017-0129-y.

Tsuruoka M, Maeda M, Hayashi B, Liu L, Inoue T. NSAID loxoprofen inhibits high threshold or wide dynamic range neuronal responses in the rat at different time-courses. *Pharmacol Rep* 60: 156–162, 2008.

Urch CE, Donovan-Rodriguez T, Dickenson AH. Alterations in dorsal horn neurones in a rat model of cancer-induced bone pain. *Pain* 106: 347–356, 2003. doi:10.1016/j.pain.2003.08.002.

Vera-Portocarrero LP, Zhang ET, Ossipov MH, Xie JY, King T, Lai J, Porreca F. Descending facilitation from the rostral ventromedial medulla maintains nerve injury-induced central sensitization. *Neuroscience* 140: 1311–1320, 2006. doi:10.1016/j.neuroscience.2006.03.016.

Wall PD. Repetitive discharge of neurons. *J Neurophysiol* 22: 305–320, 1959. doi:10.1152/jn.1959.22.3.305.

Wall PD. Cord cells responding to touch, damage, and temperature of skin. *J Neurophysiol* 23: 197–210, 1960. doi:10.1152/jn.1960.23.2.197.

Willis WD, Coggeshall RE. *Sensory mechanisms of the spinal cord* (3rd ed.). New York: Kluwer Academic/Plenum Press, 2004, p. 295–303.

Woolf CJ, Fitzgerald M. Somatotopic organization of cutaneous afferent terminals and dorsal horn neuronal receptive fields in the superficial and deep laminae of the rat lumbar spinal cord. *J Comp Neurol* 251: 517–531, 1986. doi:10.1002/cne.902510407.

Xu J, Chu KL, Brederson J-D, Jarvis MF, McGaraughty S. Spontaneous firing and evoked responses of spinal nociceptive neurons are attenuated by blockade of P2X3 and P2X2/3 receptors in inflamed rats. *J Neurosci Res* 90: 1597–1606, 2012. doi:10.1002/jnr.23042.

Xu J, Chu KL, Zhu CZ, Niforatos W, Swensen A, Searle X, Lee L, Jarvis MF, McGaraughty S. A mixed Ca^{2+} channel blocker, A-1264087, utilizes peripheral and spinal mechanisms to inhibit spinal nociceptive transmission in a rat model of neuropathic pain. *J Neurophysiol* 111: 394–404, 2014. doi:10.1152/jn.00463.2013.

Zhu CZ, Vortherms TA, Zhang M, Xu J, Swensen AM, Niforatos W, Neelands T, Milicic I, Lewis LG, Zhong C, Gauvin D, Mikusa J, Zhan C, Pai M, Roderwald V, Chu KL, Cole EE, Bespalov A, Searle XB, McGaraughty S, Bitner RS, Jarvis MF, Bannon AW, Joshi SK, Scott VE, Lee L. Mechanistic insights into the analgesic efficacy of A-1264087, a novel neuronal Ca2+ channel blocker that reduces nociception in rat preclinical pain models. *J Pain* 15: 387.e1–387.e14, 2014. doi:10.1016/j.jpain.2013.12.002.