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Targeting TLR4-lipid rafts reverses hyper-excitability in pain-associated human dorsal root ganglia

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CONFLICT OF INTEREST STATEMENT

Miller YI and Yaksh TL are inventors listed in patent applications related to the topic of this paper and scientific co-founders of Raft Pharmaceuticals LLC. The terms of this arrangement have been reviewed and approved by the University of California San Diego, in accordance with its conflict-of-interest policies. No other authors have any competing interests to declare.

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AUTHOR CONTRIBUTIONS

YL, MLU, HE, JMNP and MH were involved in study design, conducting whole cell and IHC experiments, analyzing data and writing of the manuscript. CBB, LBF acquired and assisted in analysis of the data in the IHC studies and contributed to writing of the manuscript. RYN, CET, GC, NACM and JPC were involved in patient recruitment, collection of clinical data, tissue recovery, and writing of the manuscript. GB, TLY and YIM, were involved in the synthesis and purification of AIBP, study design, data interpretation and writing of the manuscript. PMD was involved in study design, data analysis and interpretation, writing of the manuscript and management of the overall study.

ABSTRACT

Cholesterol-rich lipid rafts are organizing platforms for many excitatory ion channels and receptors in neurons and for inflammatory receptors like toll-like receptor 4 (TLR4) in immune cells. Human dorsal root ganglion (hDRG) neurons that express nociceptor markers also express high levels of TLR4. Apolipoprotein A-I binding protein (AIBP) binds to TLR4 and promotes cholesterol depletion and lipid raft disruption in cells where TLR4 is expressed. The current study conducted with male and female hDRG demonstrates that TLR4-lipid rafts are expressed with higher density in pain- versus non-pain-associated tissues and localized to neurons expressing transient receptor potential vanilloid 1 (TRPV1) typically expressed by nociceptors. This increased density of TLR4-lipid rafts results in increased excitability of hDRG TRPV1 receptors. Additionally, whole cell recordings show that disruption of TLR4-rafts with AIBP inhibited pain-associated ectopic action potential generation and evoked neuronal excitation in hDRG neurons. AIBP treatment impacted several action potential dynamics specifically in cells with on-going ectopic action potentials rather than non-spontaneously active neurons. Finally, AIBP is shown to be localized with satellite cells in human DRG and its expression increases along with glutamine synthetase in pain-associated hDRG. Given that TLR4-raft expression in DRG neurons is largely confined to nociceptors, a TLR4-raft specific therapeutic such as AIBP may have potential as a novel pain therapeutic with a low-side effect profile.

Key Words: AIBP, radiculopathy, neuropathic pain, patient

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SIGNIFICANCE STATEMENT

Lipid rafts are critical regulators of numerous neurological processes and disorders and are shown here to increase in density in pain-associated human dorsal root ganglia. Selective disruption of lipid rafts in toll-like receptor 4-expressing nociceptors significantly reduced the excitability of these neurons and suppressed ectopic, pain-associated action potential discharges. The agent used to disrupt lipid rafts, apolipoprotein A-I binding protein, localizes to macrophages and satellite glial cells and engages an endogenous pain relief mechanism with an expected limited side effect profile. This work reveals a novel mechanism underlying the generation of chronic pain in patients and identifies a potential therapeutic strategy for its management.

INTRODUCTION

Cholesterol- and sphingolipid-rich membrane domains termed lipid rafts are small (10-200 nm), dynamic, heterogeneous structures that play key roles in multiple cell systems (Pike 2006). A subtype of lipid rafts in which toll-like receptor 4 (TLR4) is expressed, the TLR4-raft, is localized in microglia and DRG macrophages (Miller, Navia-Pelaez et al. 2020). Toll-like receptor 4 (TLR4) is activated by the well-known pro-inflammatory agent lipopolysaccharide (LPS) and plays a central role in immune responses (Byrd-Leifer, Block et al. 2001). Previous studies have demonstrated that tissue inflammation, nerve injury or direct TLR4 activation with LPS result in a clustering of lipid rafts in macrophage/microglia cell membranes (Ruysschaert and Lonez 2015). This leads to decreased membrane fluidity, increased membrane thickness, and increased raft concentration of numerous ion channels and transmitter receptors (Edidin 2003, Pike 2003), supporting an increase in nociceptive responsiveness. We recently showed that apolipoprotein A-I binding protein (AIBP) binds to TLR4, leading to selective cholesterol depletion from activated microglia and macrophages (Woller, Choi et al. 2018, Navia-Pelaez, Choi et al. 2021). TLR4 is highly expressed in small nociceptive, but not large, non-nociceptive afferents in rodents (Li, Zhang et al. 2014, Li, Adamek et al. 2015), and TLR4 co-localizes with multiple excitatory voltage- and ligand-gated receptors, such as transient receptor potential vanilloid 1 (TRPV1), in the same plasma membrane lipid raft subdomains of nociceptive DRG neurons (Navia-Pelaez, Borges Paes Lemes et al. 2023). Additionally, intrathecal administration of AIBP reverses allodynia in mouse models of neuropathic pain known to be regulated by TLR4 signaling, including chemotherapy induced neuropathic pain (Navia-Pelaez, Choi et al. 2021), and studies in TLR4-knockout

rodents showed that AIBP was ineffective in behavioral and physiological measures of pain hyperexcitability, suggesting that AIBP can selectively alter the excitability of DRG nociceptors *via* TLR4 binding and disruption of the associated neuronal TLR4-rafts (Li, Uhelski et al. 2025). In a separate line of work focused on the human DRG (hDRG), we observed that a significant population of neurons isolated from dermatomes wherein patients are experiencing radicular neuropathic pain display on-going spontaneous action potential discharges (spontaneous activity, SA) when acutely cultured, a phenomenon rarely observed in DRG neurons isolated from non-pain dermatomes (North, Li et al. 2019). Moreover, SA in hDRG is suppressed with inhibitors of ion channels and receptors that commonly co-localize with TLR4 including voltage-gated sodium channel 1.7 (Nav1.7) and voltage gated calcium channel 3.2 (Cav3.2) (Li, Adamek et al. 2015, Li, Tatsui et al. 2017, Li, North et al. 2018). This suggests that human DRG nociceptors, like those in rodents, also express TLR4-rafts whose density may increase with the onset of inflammation or nerve injury, bringing numerous excitatory elements into close proximity to support increased neuronal SA and excitability and hence result in the development of chronic pain. To test this possibility, we compared the density and localization of lipid rafts in hDRG from pain-associated dermatomes and from those not associated with pain. We also tested whether AIBP alters the neurophysiological properties of quiescent and ectopically active human DRG neurons.

METHODS

Study Approval

Written informed consent for participation, including use of tissue samples, was obtained from each patient or donor next of kin prior to inclusion. The protocol was reviewed and approved by the University of Texas MD Anderson Institutional Review Board and all experiments conform to relevant guidelines and regulations.

Study Demographics

Tissue samples from 31 patients and one organ donor were used in the study. The demographic details are summarized in Supplementary Table 1 and show that 23 patients were males while 8 patients and the organ donor were female. No clear differences emerged based on sex, so all data is pooled throughout.

Human dorsal root ganglion neuron preparation

Human DRG neurons were prepared as described previously (Li, Adamek et al. 2015, Li, North et al. 2018). Briefly, each surgical patient donor was undergoing treatment that necessitated ligation of one or more spinal nerve roots to facilitate tumor resection or spinal reconstruction. For the organ donor, a section of the thoracic and lumbar spine was removed upon completion of organ collection and DRG were dissected free. All excised DRG were immediately immersed in cold (~4°C) sterile balanced salt solution in 50 mL centrifuge tubes, which were sealed and placed on ice for transport to the laboratory. For patient tissue samples, each ganglion was then carefully dissected from the surrounding connective tissues and sectioned into parts. One section was immersed into 4% paraformaldehyde and later processed for immunohistochemical (IHC) staining as described below. Another section of the DRG was dissociated to allow whole-cell

recording. This section was further cut into several small ~1 mm^2 sections using a surgical scalpel and placed in a Petri-dish with 2 mL DMEM/F12 containing trypsin, collagenase, and DNase and shaken for 20 min in a heated (37°C) orbital shaking incubator. The digestion solution was then collected and put into 15 mL centrifuge tubes with 10 mL blocking solution (DMEM/F12 with 10% horse serum) while the remaining tissue was again submerged in 2 mL of fresh digestion solution. This process was repeated until the sections were fully digested. Tubes with combined digestion and blocking solution were centrifuged at 23°C and 180G for 5 min. The supernatant was removed, and the cell pellet re-suspended with 3 mL of culture media (DMEM/F12 with 10% horse serum) and then filtered through a 100-micron cell strainer. The resulting cell suspension was collected and centrifuged at 23°C and 180G for 5 min. The supernatant was again removed, and the final cell pellet re-suspended with culture media. Cells were plated on poly-L-lysine-coated glass sheets and held in culture dishes with culture media until used (Li, North et al. 2018). For the organ donor DRG, whole ganglia were dissected and cut into small ~1 mm^2 sections and dissociated as described above. A subset of two organ donor DRG were incubated with interleukin-6 (IL-6, 2pg/ml) for 48h and three were incubated with oncostatin M (OSM, 2ng/ml) for 72h prior to use in the whole cell studies to induce hyperexcitability and on-going action potentials (Uhelski, Gorur et al. 2022).

Whole-cell recording in dissociated DRG neurons

Briefly, 24-72h after plating, dissociated DRG neurons were transferred to a recording chamber perfused with extracellular solution containing 117 mM NaCl, 3.6 mM KCl, 1.2 mM $NaH_2PO_4 \cdot H_2O$, 2.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, 25 mM $NaHCO_3$, and 11 mM glucose adjusted to pH 7.4 with NaOH. Glass-micropipettes (5–8 MΩ) were prepared and were

filled with an internal solution with 125 mM KCl, 15 mM K-gluconate, 5 mM Mg-ATP, 0.5 mM Na_2GTP, 5 mM HEPES, 2 mM $MgCl_2$, 5 mM EGTA, and 0.5 mM $CaCl_2$ adjusted to pH 7.4 with KOH. Whole cell configuration was achieved on DRG neurons with a diameter between 30 and 50 µm that were then held at 0 pA to record the presence or absence of spontaneous action potentials for 5 min. Neurons were then held at 0 pA, and action potentials were evoked using a series of 500-ms depolarizing current injections in 10-pA steps starting from -50 pA. The current that induced the first action potential was defined as 1X rheobase. Only neurons with a resting membrane potential more negative than -40mV, stable baseline recordings, and evoked spikes that overshot 0 mV were used for further experiments and analysis. Series resistance (Rs) was compensated to above 70%. All recordings were made at room temperature (25 - 26°C).

Intracellular Calcium Imaging

DRG cultures were incubated at 37 °C for 40 min with the calcium indicator dye Fura-2 AM (2 µM; Molecular Probes, Life Technologies). Neurons were then transferred to a recording chamber on a Nikon Eclipse microscope and continuously perfused with oxygenated extracellular solution, as described in the whole-cell studies above. Intracellular calcium concentration was measured using the 340/380 nm ratio on neurons with a diameter between 30 and 50µm, with signals captured and analyzed using NIS-Elements AR software (Nikon). After a 10 min baseline recording, capsaicin (1 µM) was applied to activate TRPV1 channels (Li, Adamek et al. 2015, Li, Uhelski et al. 2025). Responses were quantified as the maximum change in the 340/380 ratio from baseline, with an increase of ≥5% considered a positive response, thereby classifying DRG cells as TRPV1+.

Immunohistochemistry

DRG segments were fixed in 4% paraformaldehyde for 12 hours and cryoprotected in 20% sucrose followed by 30% sucrose solution. Frozen DRG sections (12 μm) were cut using a cryostat. DRG sections were mounted on gelatin-coated glass slides (Southern Biotech, Birmingham, AL). After blocking in 5% normal donkey serum and 0.2% Triton X-100 in PBS for 1 hour at room temperature, the sections were incubated overnight at 4°C in 1% normal donkey serum and 0.2% Triton X-100 in PBS containing primary antibodies against the following targets: Cholera toxin subunit B (CTxB, C22841, 1:500, Thermo Fisher Scientific Inc.), TRPV1 (rabbit anti-human, ACC-030, 1:500, Alomone, Israel), Glutamine Synthetase (GS, rabbit anti-human, ab49873, 1:1,000, Abcam, Cambridge, MA), and CD68 (mouse anti-human, 514H12, 1:500, Bio-Rad Laboratories, Inc. Hercules, CA). We have tested a large number of commercially available anti-AIBP antibodies and found them not suitable for immunohistochemistry of human or mouse tissues. Thus, we developed in-house mouse anti-human AIBP monoclonal antibodies A7 and BE-1(Choi, Agatisa-Boyle et al. 2021, Navia-Pelaez, Choi et al. 2021) and tested their utility and specificity using cells and tissues from AIBP knockout mice. We found that A7 and BE-1 recognize different epitopes on the AIBP molecule, and their mix at 1:2 ratio provides the optimal intensity and selectivity for IHC applications. We have previously reported staining of human (and mouse) lung tissue with the A7/BE-1 cocktail (Miller, Pham et al. 2022) .(Navia-Pelaez, Choi et al. 2021, Miller, Pham et al. 2022)). After the sections were washed, they were incubated with Cy3- or FITC-conjugated secondary antibodies overnight at 4°C. To perform the proximity ligation assay (PLA), the DuoLink PLA Multicolor Reagent Pack (Sigma-Aldrich) was used in

combination with the DuoLink PLA Multicolor Probemaker Kit-Red to conjugate TRPV1 and cholera toxin subunit B (CTxB) antibodies to oligonucleotide probes, enabling detection of TRPV1–CTxB proximity, according to the manufacturer's protocol (Navia-Pelaez, Borges Paes Lemes et al. 2023). Sections were viewed with use of a fluorescence microscope (Eclipse E600; Nikon). For a given experiment, all images were taken using identical acquisition parameters with experimenters blinded to patient clinical information.

Drug Administration

Modified AIBP (RFT1081) (Miller, Pham et al. 2022) was developed by Raft Pharmaceuticals and custom produced by Selvita, Inc. (Cambridge, MA). In brief, RFT1081 was expressed in a baculovirus/insect cell system to ensure post-translational modification and endotoxin-free preparation and purified by affinity chromatography using a Ni-NTA agarose column, followed by ion exchange chromatography and buffer replacement. The product was >90% pure, with no detectable aggregates (HPLC-SEC) and residual endotoxin <0.2EU/mg. Storage-stability study of AIBP for up to 6 months at -80°C or for 1 week at 4°C did not show any loss of its titer or purity. AIBP was diluted in sterile extracellular solution to the desired concentration of 0.1mg/ml. This test concentration was determined in earlier studies (Woller, Choi et al. 2018). After identification of spontaneously active neurons in current clamp mode, AIBP was bath applied in the external solution until the APs stopped firing (2 - 26 minutes). Peak AIBP effect was defined as the last minute of AIBP application. This was followed by a washout period lasting 5-10 minutes. During the washout period, we expected action potentials to reoccur, as neurons are typically washed for the same duration as their AIBP treatment.

The neuron that required 26 minutes to cease firing was washed for 25 minutes, while the other 10 of 11 neurons were washed for 5–10 minutes. For sensitization of organ donor cultures, human recombinant IL-6 (206-IL, R&D Systems) was reconstituted in sterile PBS at 20 mg/mL and diluted to 2 pg/mL in culture media. Human recombinant OSM (H6541, Sigma) was reconstituted in sterile PBS at 500 ng/mL and diluted to 2 ng/mL in culture media. Capsaicin (Sigma) was prepared in a stock solution of 100% ethanol and diluted to 1 µM in extracellular solution and applied to activate TRPV1 channels. TRPV1 has been widely studied in pain mechanisms. It is expressed in subsets of nociceptors and is activated by a variety of noxious stimuli, including capsaicin—the active component of chili peppers—as well as protons, heat above 42 °C, and a family of endogenous lipids associated with inflammation (Caterina, Schumacher et al. 1997, Nagy, Friston et al. 2014).

Experimental Design and Statistical Analyses

All statistical analyses were performed with consideration of the heterogeneity inherent in human dorsal root ganglion (DRG) neuron preparations. Given the biological variability of these samples, particularly in human DRG cultures, nonparametric tests were used appropriately to account for deviations from normality and small sample sizes. Sample sizes (e.g., number of cells, animals, or donors) are reported in figure legends. Data are expressed as the mean ± standard error of the mean (SEM). Analyses were conducted using Prism 9 software (GraphPad Software, San Diego, CA). Group differences in immunostaining intensity (Figs. 1, 3, and 6) were assessed using two-tailed, unpaired t-tests with Welch's correction. For electrophysiological experiments, data were analyzed using the Mann-Whitney *U* test (Fig. 2, 3), Wilcoxon matched-pairs signed-rank test (Fig.

4) or Paired t-test (Fig. 5), as appropriate for the experimental design. A significance threshold of $p < 0.05$ was used for all statistical comparisons.

Data availability

Deidentified data available upon request.

RESULTS

Increased expression of Lipid rafts and TRPV1 in pain-associated versus non-pain human DRG neurons

DRG were collected from patients whose clinical data is summarized in Supplemental Table 1. Patient samples were characterized by radiographic analysis of nerve root compression and a history of associated dermatomal radicular/neuropathic pain, resulting in two categories: patients presenting with only axial spine pain and no nerve root, DRG or spinal cord compression (Non-Pain; Supplementary Fig. 1a), and patients with tumors compressing the nerve root or DRG and producing radicular pain in the associated dermatome (Pain; Supplementary Fig. 1b). Fixed sections of hDRG from non-pain and pain-associated dermatomes (non-pain and pain DRG, respectively) were stained with CTxB to detect the presence of lipid rafts and their expression in relation to TRPV1 neurons was determined. CTxB staining was diffuse and appeared in small patches in non-pain DRG (Fig 1a, in green). TRPV1 expression in non-pain DRG (Fig. 1b, in red) was pronounced in some neurons but more frequently observed as diffuse or weak staining in medium and small-diameter neurons. The co-localization of these (Fig. 1c, yellow), while present, was not very intense or frequent. The cell outlined by the white box in Fig. 1c is shown at magnified scale in Fig. 1d where only low intensity

speckled co-localization can be seen because the lipid rafts are small and diffuse in non-pain DRG. In contrast, CTxB staining in DRG from pain-associated dermatomes was highly pronounced as shown in Fig. 1e (green). TRPV1 staining was also markedly elevated (Fig. 1f, red); and co-localization of lipid rafts to TRPV1+ cell profiles was prominent (Fig. 1g, yellow). The cell outlined by the white box in Fig.1g is shown at higher magnification in Fig. 1h where the area of co-localization for this cell appears along the cell profile margins consistent with the location of the cell membrane. The grouped IHC quantitative data shown in the scatter and box plots in Fig. 1i indicate that the results in the representative images did indeed reflect the population sample and that the increases in both CTxB (green) and TRPV1 (red) in the pain DRG group (filled boxes) were statistically significant compared to the non-pain DRG (open boxes). Proximity Ligation Assay (PLA) was used to confirm co-localization of TRPV1 to CTxB-lipid rafts and quantify the level of their expression. As shown in Fig. 1j the CTxB/TRPV1 PLA signal was significantly increased in pain-associated DRGs (filled bar) compared to non-pain–associated DRGs (open bars, $t(8.63) = 2.43$, $p = 0.039$). This indicates that TRPV1+ lipid rafts are increased in pain-associated DRG of pain patients.

Increased responses to capsaicin in pain versus non-pain human DRG neurons

Given that pain DRGs have increased expression of TRPV1 and lipid raft density, the next issue was whether this had functional consequences. To test this possibility, neurons from non-pain and pain DRG were challenged in two different experiments with a minimally effective concentration of capsaicin. Whole cell current clamp experiments on non-pain DRG neurons determined this to be one-minute exposure to 1mm

capsaicin as 2 of 8 cells showed failed to show any response and the remaining 6 only produced short bursts of evoked action potential with mild membrane depolarization as shown in Fig. 2a. In contrast, all of the neurons from pain DRG showed marked membrane depolarization and extended trains of action potential discharges (Fig. 2b). The grouped data summarized in the scatter and bar plots in Fig. 2c confirmed that the responses of the neurons from pain DRG (red bar) were significantly increased compared to the non-pain DRG neurons (Fig**.** 2c; Mann-Whitney U test, U=0, p = 0.0007). Calcium imaging was used to build upon these initial whole cell findings and like in the whole -cell studies it was observed that capsaicin was less effective in inducing responses from non-pain DRG neurons and those responding showed more muted responses than in neurons from pain DRG. A representative example of calcium imaging in a non-pain DRG neuron to capsaicin administration is shown in Fig. 2d. Each colored line shows the response of a single neuron; and it can be seen that 2 of the 6 neurons in this sample showed no response to capsaicin (note the green and light blue traces) while the responses shown by the remaining cells were relatively small and slow. In contrast, the representative set of traces from pain DRG neurons shown in Fig. 2e reveals that all four of these neurons responded with much faster and exaggerated increases in intracellular calcium as reflected by the larger change in 340/380 ratio. The scatter and bar graphs in Fig. 2f summarize the grouped data for the capsaicin responders in both groups and show that intracellular calcium intensity was significantly increased in neurons from pain DRG (red bar) compared to those from non-pain DRG (Fig**.** 2f**;** Mann-Whitney U test, U = 31, p = 0.0089). Overall, one minute administration of 1mm capsaicin induced an increase in 340/380 ratio above 5% in only 9 of 19

neurons from non-pain patients, while evoking a response above 5% change in 18 of 21 pain DRG neurons. These findings suggest that spinal cord/nerve root compression enhances TRPV1-mediated excitability in human DRG neurons. That said, we also noted that the capsaicin-evoked calcium responses in neurons from our cancer patient cohort were weaker than those reported in healthy organ donors (Lesnak, Schaub et al. 2025). This observation suggests the possibility that age-related and health status impacts hDRG neuronal responsiveness at TRPV1. Many of these cells also notably expressed lipofuscin that may also have quenched some of the fluorescence intensity.

Functional Regulation of TRPV1 by TLR4-lipid rafts

Given the positive functional impact of increased expression of TRPV1+ lipid rafts on TRPV1 signaling in human pain DRG neurons like we had also shown in rodents (Li, Adamek et al. 2015, Li, Uhelski et al. 2025), the next step was to determine whether disruption of lipid rafts would reverse this using AIBP, a specific disruptor of TLR4–lipid rafts (Woller, Choi et al. 2018, Navia-Pelaez, Borges Paes Lemes et al. 2023). Whole-cell patch clamp experiments on 5 pain DRG neurons showed that the same one-minute application of 1mm capsaicin could reliably yield reproducible sharp, large amplitude inward currents as shown by the representative analog recording shown in Fig. 2g and the grouped data shown in the scatter line plot in Fig. 2h (each dot-line pair is a single neuron). The same protocol was used to test the effects of AIBP on capsaicin-evoked currents in DRG neurons from one organ donor, one pain patient, and one non-pain patient (6 neurons in total). Capsaicin (1 μM) alone again induced a sharp, large-amplitude inward current as shown on the left side of the analog recording in Fig. 2i. However, application of capsaicin with AIBP resulted in a markedly reduced

capsaicin-evoked current in all neurons tested, independent of the pain state associated with the DRG (Fig. 2i, right side). The grouped data summarized in the scatter line plots in Fig. 2j reveal that AIBP significantly reduced the responses of the second capsaicin application (blue dots) compared to the first (red dots, Wilcoxon matched-pairs signed rank test, W = -21.00, p = 0.0312, the mean values are 59.47 and 16.81, and the SEM values are 20.36 and 12.05 for the capsaicin-only group and the capsaicin + AIBP co-treatment group, respectively), consistent with our findings in rat neurons (Li, Uhelski et al. 2025). This finding indicates that, regardless of the pain state of the DRG, TRPV1 is incorporated into TLR4-lipid rafts, and disruption of the lipid rafts with AIBP results in attenuated TRPV1 activation.

Effects of Unilateral Compression on Membrane Lipid Rafts and Excitability in Human DRG Neurons

An important extension and further validation of the findings shown in Figure 3 was possible using a unique set of bilateral DRG patient samples (5 pairs) where one side was affected by root compression and associated radicular neuropathic pain while the contralateral side was unaffected. IHC was used to first determine whether lipid rafts and TRPV1 were increased on the pain side DRG versus the contralateral non-pan side. A representative example of the results is shown in Fig. 3a. Lipid rafts visualized using CTxB (green) were found to be increased in the compression and pain-associated DRG (Fig. 3ai) versus unaffected and non-pain DRG (Fig. 3aii). TRPV1 (red) was similarly increased on the pain side (Fig. 3aiii) compared to the non-pain contralateral side (Fig. 3aiv). Notably, the pain DRG ipsilateral to the compression exhibited a higher prevalence of lipid rafts that colocalized with TRPV1 staining (yellow, Fig. 3av), while

levels of co-expression were low on the non-pain side (Fig. 3avi). The scatter box plots in Fig.3b show that the representative findings just described were consistent across all samples a significantly different for both CTxB (green, t (6.1) = 3.33, p= 0.016) and TRPV1 (red, t (7.4) = 4.59, p=0.002) in the pain DRG (filled boxes) compared to the non-pain DRG (open boxes). The MRI image shown in Figure 3c is from the patient donor whose DRG were used for the representative IHC (Fig. 3a) and shows the typical presentation where tumor (red outline) is seen impinging upon the DRG and spinal cord on the left, which corresponds to the side unilaterally affected by radicular pain as illustrated on the inset of the body outline where the area of radiating pain is shown also in red. Representative whole cell recordings from DRG neurons from the same patient donor are shown in Fig. 3d and e where we confirm previous reports from our group (North, Li et al. 2019) that neurons sourced from the pain-associated ipsilateral DRGs frequently exhibited spontaneous action potential discharges in culture (Fig. 3d, red, 5/5 neurons in this sample) that is only rarely found on the contralateral side (Fig. 3e, blue, 0/4 neurons in this sample). The grouped whole cell data is shown in the scatter plots in Fig. 3f where it was found that DRG neurons originating from the ipsilateral pain side (red) displayed a significantly lower rheobase (the minimum current required to induce an action potential) and more depolarized resting membrane potential (RMP) compared to neurons from the contralateral side (blue, Mann-Whitney $U = 0$, $p = 0.0159$, and $U = 1$, $p = 0.0317$, two-tailed) (Fig. **3f**). Rheobase and RMP in contralateral patient DRG were not different from what we have reported for organ donor DRG (Li, Uhelski et al. 2024). These data show that compression-induced neuropathic pain and neuronal

hyperexcitability is likely associated with increased lipid raft content, indicating a possible role of lipid rafts in the development of this hyperexcitability.

Effects of AIBP administration on excitability of human DRG neurons

Cultured human DRG neurons are normally quiescent except when isolated from dermatomes associated with ongoing pain, or after incubation with inflammatory cytokines (primed) (Kandasamy and Price 2015) such as IL-6 or Oncostatin M (OSM). Under these sensitizing conditions, spontaneous action potential discharges (spontaneous activity, SA) by the neurons are commonly observed (North, Li et al. 2019). To examine whether the assembly of lipid rafts contributes to SA and hyperexcitability of pain side or sensitized DRG neurons in culture, we treated SA cells with AIBP to specifically disrupt TLR4-lipid rafts while recording spike frequency using whole cell patch clamp. We recorded from 15 neurons, 10 of which were from pain-associated DRGs (radicular neuropathic pain or axial nociceptive pain), and 5 were from healthy donor DRGs primed with IL-6 or OSM. The representative analog recording in Fig. 4a is from a pain patient donor DRG, while that in Fig. 4b is a sensitized neuron from an organ donor. In both cases the application of AIBP (0.1 mg/ml, indicated by the line and label above each trace) suppressed on-going discharges. Termination of AIBP application allowed resumption of action potential firing, although the firing frequency was reduced compared to pre-treatment conditions. Another representative example of SA from a pain patient donor DRG is shown in Fig.4c, that demonstrates no change in SA over the recording interval was observed with administration of PBS in which AIBP was dissolved. The summarized grouped data is shown in the scatter plot in Fig. 4d. The patient donor DRG neurons treated with AIBP are shown by the circles, while sensitized organ donor DRG

are shown in the squares, and the pain patient DRG treated with vehicle are shown in the diamonds. The time points sampled are coded by shading of the shapes where black is the baseline data, the dark gray is at 2 min of AIBP (vehicle) application, light gray is at maximum effect for AIBP (fixed at 10 min for vehicle as detailed below) and the open shape is at 5 min following washout (fixed at 30 min for vehicle). Each data point is the mean across 2 minute segments taken just prior to AIBP (vehicle) application (baseline), beginning two minutes after AIBP (vehicle) was started (2 minute), immediately after termination of AIBP (peak effect), and beginning 5 minutes after termination of AIBP. As shown, AIBP significantly suppressed SA in 11/11 of the DRG neurons (Wilcoxon matched-pairs signed rank test, W = -45.0, p = 0.0039). Meanwhile, vehicle treatment had no effect on SA in the four cells where this negative control was run. Given that the recordings were from small-sized neurons (35–45 µm) (North, Li et al. 2019) across multiple sensitization paradigms this supports a generalized effect of AIBP on SA independent of the provoking stimuli and is consistent with the voltage clamp findings (Fig. 2i, j), where AIBP reduced capsaicin-induced inward currents regardless of whether these were from patient pain DRG, no pain DRG or organ donors. It should also be noted that neurons from the same ganglion of an individual patient required similar treatment times to reach cessation of spontaneous activity, suggesting intra-patient consistency. However, the overall time required for AIBP to abolish spontaneous activity varied considerably across neurons, ranging from 2 to 26 minutes, with a mean onset of effect of 9 minutes. Notably, only one neuron required 26 minutes to cease firing, whereas the majority (10 of 11) ceased within 2–10 minutes. Hence, the rationale for the time points selected for the measurement of SA rate in the vehicle treated group in this set of experiments and those

to be described next. Perhaps the variability across patients but consistency within individual ganglia underscores the potential clinical importance of tailoring treatment strategies to individual patients.

Effects of AIBP on action potential properties of human DRG neurons

The scatter and line plots in Fig. 5 and Table 1 show the data for the effects of AIBP on action potential (AP) properties of hDRG neurons. The representative analog recordings and symbols in green in Fig. 5a show the data for cells that had on-going SA that AIBP suppressed included in Fig.4, while more comprehensive grouped AP data for these cells are shown in the top three lines (top section) of Table 1. The APs used for this analysis were the last prior to beginning application of AIBP and the last during application of AIBP with waveform properties analyzed according to previously published methods (North, Odem et al. 2022). The baseline analog recordings in Fig. 5a are shown in the trace using dotted lines at the top, while recordings after AIBP treatment are shown in solid lines in the lower trace. As shown, AIBP had little effect on APs in SA cells. Most cells like that in the analog recording showed a small hyperpolarization of resting membrane potential (RMP), but this was not statistically significant. However, statistically significant increases in rheobase and rise time and statistically significant decrease in AP overshoot were observed in these cells.

The remainder of the data in Fig.5 and the second two sections of Table 1 are for cells that did not have SA. A uniform treatment duration of 10 minutes was used in these experiments based on the results shown in Fig. 4, where the mean time for AIBP to suppress spontaneous APs was 9 minutes. The effects of AIBP on AP properties were not uniform for non-SA cells, even among neurons within the same ganglion. Two

patterns of effects were observed for non-SA cells as shown in the analog recordings in Fig. 5b and c. Baseline recordings are again shown in dotted lines in the traces at the top, while recordings after a 10 min exposure to AIBP are shown in solid lines in the lower traces. In the first group shown in red AIBP had only modest effects on 11 of 18 non-SA cells where less than 4 parameters of the AP waveform were altered. The representative cell in Fig. 5b showed hyperpolarization of RMP and suppression of repeated spiking that was evoked at rheobase. The scatter and line plots show that other cells in this group also showed hyperpolarization of RMP, but others also showed modest depolarization of RMP, such that no significant change was observed overall. Similarly, the effects on rheobase and AP amplitude were variable. The only statistically significant changes in this group were a reduction in AP overshoot and slowing of AP rise time. In contrast, the non-SA cell shown in blue in Fig. 5c exhibited pronounced effects of AIBP with more than 4 altered AP waveform parameters, including depolarization of the RMP, an attenuation of spike amplitude, and an increase in the AP rise time. Pronounced effects of AIBP were observed in 7 of the 18 non-SA neurons studied in this experiment. Significant change in RMP, rheobase, AP amplitude, AP overshoot and AP rise time were observed for cells showing pronounced effects of AIBP. Finally, the bottom section of Table 1 shows the group AP waveform data for cells where the AIBP vehicle (PBS) was administered. These cells were also used in Fig. 4 that had on-going SA where PBS was applied. Again, the last AP prior to PBS application and first following its termination were used to extract AP parameters. No parameters showed any change from baseline in this group. Taken together, these

findings suggest that AIBP exerts heterogenous effects on neurons, possibly reflecting differences in lipid raft composition or variability in ion channel expression.

AIBP expression in Human DRG

AIBP is a secreted protein known to be expressed by various cell types in the rodent DRG, including neurons and macrophages (Fang and Miller 2019). Having shown that AIBP can influence human DRG neuron excitability when applied exogenously, we next sought to localize endogenous AIBP expression in human DRG tissue using immunohistochemistry. Fig. 6a displays a wide low-power magnification overview of a cross-section from a pain-associated, whole human DRG, with immunoreactivity for AIBP observed in small cellular profiles that are especially concentrated near blood vessels but also scattered through the tissue. This staining pattern and morphology is very similar to that of the macrophage marker, CD68, shown in a low-power wide overview of the DRG in Fig. 6b. As well, the patterns of macrophage infiltration into pain-associated human DRG is very similar to that of rats with paclitaxel-induced peripheral neuropathy (Zhang, Li et al. 2016). The areas of Figs. 6a and b within the white boxes are shown at higher magnification in Figs. 6 c and d. Unfortunately, both the antibodies used here were raised in mice which precluded more direct co-localization. We next compared AIBP expression in relation to satellite glia which surround and encapsulate neurons in the DRG. Images at 20X from a patient with unilateral radicular neuropathic pain show that AIBP (**e**, in red) appears in faint annular cell profiles consistent with satellite cells comparison in the non-pain DRG (**e**). Staining for glutamine synthetase (GS, **f**, in green) appears in the same annular pattern expected in satellite cells; and the merged images show some of these profiles are co-

localized (**g**, in yellow). Representative images from the pain side show that AIBP (**h**) and GS (**i**) are increased compared to the non-pain side and as shown in **j** the co-localization is now more pronounced. The scatter and box plots bar in **k** quantifying fluorescence intensity across all cells studied indicate that both AIBP (red) and GS (green) were significantly increased in pain-associated DRG (filled boxes) compared to the contralateral non-pain DRG (open boxes). (Fig. **6e-j**). AIBP expressions across all sections appeared in annular cell profiles consistent with satellite cells and showed significant overlap with glutamine synthetase expression (GS). Direct co-localization of these markers is shown in yellow in both Fig. **6g** and **j**. Quantification of the fluorescence intensity data revealed that both AIBP and GS staining were significantly elevated in DRGs from pain-associated dermatomes (AIBP: $t(8.3) = 9.83$, $p < 0.0001$; GS: $t(6.2) = 6.44$, $p = 0.001$) (Fig. **6k**).

DISCUSSION

One notable finding from this study is the increased density of lipid rafts in pain-associated human dorsal root ganglia (DRGs) compared to non-pain DRGs. In many quiescent cell types, lipid rafts are small, dynamic structures (on the nanometer scale) that continuously form, dissolve, and reform over milliseconds to seconds (Pike 2009, Lingwood and Simons 2010). However, upon cell activation, raft clustering increases, and they persist for a longer duration of minutes or more (Miller, Navia-Pelaez et al. 2020). Lipid rafts form due to the self-assembly of cholesterol and sphingolipids, creating solid platforms within the otherwise fluid plasma membrane (Simons and Ikonen 1997, Sviridov and Miller 2020). These platforms facilitate the formation and activation of protein complexes (Sviridov and Miller 2020), such as Toll-like receptor 4

(TLR4), which only initiates downstream signaling after forming a homodimer within the cholesterol-rich lipid rafts. The localization of TLR4 and other proteins to lipid rafts is mediated by specific cholesterol-binding motifs and post-translational modifications (e.g., GPI anchors, myristic acid tails, cysteine acylation) (Levental, Grzybek et al. 2010, Aicart-Ramos, Valero et al. 2011, Katritch, Cherezov et al. 2013, Ruysschaert and Lonez 2015, Fantini, Epand et al. 2019, Bongarzone and Givogri 2021). Upon activation, proteins such as TLR4 undergo oligomerization, leading to the formation of larger lipid raft clusters (Sezgin, Levental et al. 2017). For instance, in microglial cells stimulated by lipopolysaccharide (LPS), TLR4 dimers and enlarged lipid rafts persist on the cell surface for up to 15 minutes before being internalized (Woller, Choi et al. 2018). However, in chronic conditions such as chemotherapy-induced neuropathy, these processes can persist for up to six weeks (Navia-Pelaez, Choi et al. 2021), suggesting prolonged raft-mediated signaling even in the absence of ligands (Miller, Navia-Pelaez et al. 2020).

Another key finding is that disrupting lipid rafts in TLR4-expressing neurons using the protein AIBP significantly reduces the excitability of these neurons. This suggests a potential novel approach to modulate the activity of human sensory neurons, particularly nociceptors, which express TLR4 and other markers like TRPV1 and Nav1.7. The historical approach to achieve cholesterol depletion through the use of β-cyclodextrins induces broad and complex effects due to a lack of specificity, resulting in functional disruption of multiple channels and receptors (Levitan, Singh et al. 2014, Miller, Navia-Pelaez et al. 2020, Navia-Pelaez, Choi et al. 2021). AIBP, encoded by the *APOA1BP* gene (aka *NAXE)*, however, specifically targets TLR4-expressing cells by binding to

556 TLR4 and promoting local cholesterol depletion, thus selectively disrupting lipid rafts in
557 these cells (Ritter, Buechler et al. 2002, Marbaix, Noël et al. 2011, Shumilin,
558 Cymborowski et al. 2012). In both microglia and macrophages, AIBP-mediated
559 disruption of TLR4-rafts reduces raft size and causes their dispersal (Woller, Choi et al.
560 2018, Navia-Pelaez, Choi et al. 2021). Disruption of TLR4-lipid rafts in neurons would
561 result in dispersal of excitatory elements known to often localize with TLR4 and so
562 reduce neuronal excitability consistent with the suppression of SA seen here. As well,
563 since AIBP suppresses ectopic activity in DRG neurons from both naïve patients and
564 donors pre-treated with inflammatory cytokines such as IL-6 and OSM, which have
565 been linked to neuropathic pain (Andratsch, Mair et al. 2009, Uhelski, Gorur et al. 2022),
566 AIBP may suppress pathological neuronal hyperexcitability from numerous causes
567 aside from cancer-related nerve compression including other injuries or inflammation
568 known to elevate pro-inflammatory cytokines in the DRG. Given that TLR4 is inly
569 expressed in nociceptors, it is possible that even though small diameter (Li, Zhang et al.
570 2014, Li, Adamek et al. 2015) (Navia-Pelaez, Borges Paes Lemes et al. 2023), the cells
571 showing little response to AIBP may either have only low levels of TLR4 expression or
572 not have been nociceptors.

573 Consistent with the potential impact on multiple excitatory elements to suppress
574 neuronal SA and hyperexcitability, AIBP affected the configuration of action potentials in
575 DRG neurons. As just noted, TLR4-lipid rafts are known to interact with various
576 channels, including $Na_V1.7$, N-methyl-D-aspartate, TRPV1, and $Ca_V3.2$. Depletion of
577 membrane cholesterol can reduce the activity of inwardly rectifying K^+ channels (Levitan
578 2009), voltage-gated K^+ channels, and Ca^{2+} channels (Bowles, Heaps et al. 2004,

Toselli, Biella et al. 2005). Voltage-gated Na^+ and transient receptor potential channels are similarly inhibited by cholesterol removal, suggesting that TLR4-lipid rafts play a central role in organizing ion channels and regulating nociceptor excitability (Lundbaek, Birn et al. 2004, Lundbaek, Birn et al. 2005, Liu, Huang et al. 2006, Lundbaek, Koeppe et al. 2010). Interestingly, the effect of AIBP on action potentials was not uniform across neurons, indicating possible heterogeneity in TLR4 expression or lipid raft composition. As shown in Figure **5**, in over half of the neurons tested (11 of 18), AIBP had little effect on the action potential waveform (≤4 parameters altered), likely because these cells were TLR4-negative. In the remaining neurons (7 of 18), AIBP significantly altered action potential properties, including reducing amplitude and widening duration. In this sample, there was no clear association between the source of the cells (pain versus no-pain DRGs) and the effects of AIBP on action potential (AP) waveform. An interesting possibility is that the baseline density or composition of lipid rafts may vary between these groups. Similarly, no clear distinction was observed between patient and donor-derived neurons, as both groups had neurons that fell into either category. This raises questions about which channels might be affected to alter AP waveform properties. The physical properties of the membrane lipid bilayer, such as membrane curvature and bilayer elasticity, can influence the energetic cost of channel gating (Lundbaek, Birn et al. 2004). For example, cholesterol depletion can alter membrane elasticity, and Na_V channels have been shown to be regulated by these properties (Lundbaek, Birn et al. 2005). A key finding in this study was that AIBP reduced amplitude and widened the AP duration in human DRG neurons (e.g., Fig. **5b**), an effect linked to the role of $Na_V1.7$ in shaping the action potential upstroke (Cummins, Dib-Hajj et al. 2004, Yang, Wang et al.

2004, Cox, Reimann et al. 2006). Another significant observation was the alteration in resting membrane potential (RMP) and suppression of repetitive AP discharges, which are closely tied to T-type calcium channel activity. These low-voltage-activated calcium channels are triggered by minor membrane depolarization and play a role in various after-depolarizing potentials (ADP), after-hyperpolarization (AHP), and rebound depolarization (Huguenard 1996, Perez-Reyes 2003, Todorovic and Jevtovic-Todorovic 2006). Calcium-channel blockers reduce ADP, AHP, and LTS (low-threshold spikes) without affecting early sodium spikes. The AHP is dependent on ADP amplitude and is sensitive to external calcium depletion, as it results from Ca^{2+}-activated K^{+} currents (IKCa). The LTS, activated at sub-threshold potentials, is influenced by T-type Ca^{2+}-channel blockers, leading to sodium action potential generation when hyperpolarization decreases. The current study found no clear link between cell origin (pain versus no-pain DRGs or patient versus donor) and the effects of AIBP on AP waveform in human DRG neurons.

A fourth significant finding is the localization of AIBP to satellite glial cells and macrophages in the DRG. This suggests that, like endorphins and enkephalins, AIBP may also have a key biological role as endogenous pain-control signal and have a limited side effect profile. Macrophage infiltration into the DRG has been observed in pain conditions, including chemotherapy-induced peripheral neuropathy (Peters, Jimenez-Andrade et al. 2007, Zhang, Li et al. 2016). These macrophages can exhibit either pain-promoting or pain-resolving phenotypes, depending on the context (Coussens and Werb 2002, Mosesson, Mills et al. 2008, Krukowski, Eijkelkamp et al. 2016). The CD68+ macrophage cohort is typically associated with cells demonstrating a

pro-inflammatory phenotype (Peters, Jimenez-Andrade et al. 2007, Zhang, Li et al. 2016). In satellite glial cells, one of the strongest transcriptional changes observed during neuropathic pain is related to cholesterol biosynthesis, which may influence the production of AIBP (Jager, Pallesen et al. 2022). AIBP is endogenously expressed by satellite glial cells surrounding neurons or recruited from circulating macrophages, providing a physiological mechanism for the intrinsic regulation of neuronal excitability. However, it is unclear how the pain-facilitating roles of macrophages and satellite cells align with the inhibitory effects of AIBP on DRG neuron signaling.

In summary, the ability of AIBP to disrupt lipid rafts specifically in TLR4-expressing cells offers a promising avenue for therapeutic intervention in pain conditions as TLR4 is expressed in each of the three key cellular elements that support neuropathic pain (macrophages, microglia and nociceptors), but not other neural cellular elements. Although TLR4 is expressed on hepatocytes and endothelial cells, TLR4 activation is pathogenic and not engaged in normal physiological processes and so AIBP should have low systemic toxicity, but this will require further study. Further investigation is also needed to fully understand the broader impact of AIBP on specific ion channels, the duration of its effect, and the use of repeated dosing strategies to more fully develop its potential applications in treating chronic pain.

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Figure Legends

Figure 1. Increased Lipid raft and TRPV1 expression in pain-associated human DRG versus non-Pain Human DRG

DRGs from non-pain (**a-d**) and pain-associated segments (**e-h**) were immunohistochemically stained for CTxB (to visualize lipid rafts, green) and TRPV1 (red)**.** Co-localization of CTxB with TRPV1 (yellow, **c-h**) is seen to be increased in pain DRG compared to non-pain DRG in both low (**c, g**) and higher power views (**d, h**). In i**,** the bar graphs show the grouped data with significantly higher mean staining intensity for CTxB (green) and TRPV1 (red) in the pain patient group (filled boxes) vs the non-pain patient group (open boxes). Finally, in **j** the scatter and bar graphs show the grouped data for the CTxB–TRPV1 proximity ligation assay where the pain group (filled box) had significantly higher integrated density than the non-pain group. Welch's t-test * $p<0.05$, ** $p<0.01$. Scale bar = 100 µm (**a-c** and **e-g**). Scale bars = 10 µm (**d, h**).

Figure 2. Increased responses to capsaicin in pain associated versus non-pain human DRG neurons. Representative whole-cell recordings from non-pain (**a**) and pain-associated human DRG neurons (**b**) show that one minute application of a low concentration capsaicin (1 µM, indicated by the red line above each trace) produces only a brief weak response in a non-pain DRG neurons, but a robust response in pain-associated DRG neurons. The scatter and bar graph in **c** shows that the grouped quantification of AP frequency per minute was significantly increased in the pain-associated neurons (red bar) compared to the non-pain neurons (open bar). Representative traces from calcium imaging responses (each color line is from a separate neuron) to a one minute application of low concentration capsaicin (1 µM,

indicated by the red line above each trace) show muted responses in non-pain neurons (**d**) but strong responses in pain neurons (**e**). The scatter and bar graphs show the quantified group data that indicates that pain-associated neurons responding to capsaicin showed a significantly greater percent increase in 340/380 ratio (red bar) than non-pain neurons (open bar). The numbers over each bar indicate the numbers of cells in each group tested (denominator) and the numbers showing response to capsaicin (numerator). The representative voltage-clamp recording in **g** shows that the current evoked by a one-minute application of low concentration capsaicin (1 μM, indicated by the red line above each trace) is reproducible when applied a second time 10 minutes following the first; and this is supported by the grouped data shown in the scatter line plot in **h**. Finally, the representative voltage clamp recording in **i** shows that co-application of AIBP (0.1 mg/ml, indicated by the blue line above the trace) greatly reduces the response to the second application of capsaicin; and the grouped data in **j** show that this was a consistent finding and statistically significant. Mann-Whitney *U* test (**c**, **f**) and Non-parametric Wilcoxon matched pairs signed rank test (**h**, **j**). * $p<0.05$, ** $p<0.01$, *** $p<0.001$**.**

Figure 3. Lipid raft expression and excitability are increased in human DRG neurons ipsilateral to radicular pain compared to neurons from the contralateral side with no pain. The representative IHC images in **a** show staining for CTxB staining of lipid rafts (green) is increased in the DRG ipsilateral to root compression and associated radicular pain (**i**) compared to DRG on the contralateral side without pain (**ii**). Similarly, TRPV1 staining (red) is increased in the pain side DRG (**iii**) compared to the non-pain side DRG (**iv**). Finally, co-localization of CTxB with TRPV1 (yellow) is also

increased in the pain side DRG (**v**) compared to the non-pain side DRG (**vi**). The scatter and box plots in **b** indicate that the increase in CTxB (green) and TRPV1 (red) for the group of samples collected was significantly greater in the DRG ipsilateral to radicular pain (filled boxes) compared to that in DRG contralateral to pain (open boxes, Welch's t-test). The MRI shown in **c** is from the patient who donated the DRG used in the representative IHC shown in **a** and the representative whole cell recordings shown in **d** and **e.** The tumor margin area is outlined in red and can be seen compressing the left side DRG root. The inset body diagram shows the distribution of the pain area also in red. Typical on-going neuron discharge activity found in 5 of 5 DRG neurons recorded from this patient donor ipsilateral to pain is shown in **d**; and a typical silent recording found in the 4 DRG neurons recorded from this patient contralateral to pain is shown in **e**. Grouped data for these 9 neurons is shown in **f** and indicates that the pain associated neurons (red symbols) had significantly lower rheobase and more depolarized resting membrane potential (RMP) than non-pain associated neurons (blue symbols). Mann-Whitney *U* test. Scale bar=100 mm. * $p<0.05$, ** $p<0.01$.

Figure 4: AIBP suppresses on-going pain-associated discharges in human DRG neurons. Analog recordings showing representative effects of AIBP or vehicle on ongoing spontaneous action potential (SA) discharges in human DRG neurons. Shown in **a** is the effect of AIBP on on-going activity in DRG pain-associated dermatomes while in **b** the effect of AIBP is shown for organ-donor neurons with SA due to conditioning with oncostatin M. Shown in **c** is that vehicle treatment did not affect on-going activity. The period of AIBP (or vehicle) application is indicated by the solid black bar above the traces. The scatter and line plots in **d** show that SA was suppressed in all 11 neurons

888 treated with AIBP but not in vehicle-treated neurons. Neurons from patient samples
889 treated with AIBP are indicated by circles, donor-derived neurons treated with AIBP by
890 squares, and vehicle-treated neurons by diamonds. Statistical analysis was performed
891 using the Wilcoxon matched-pairs signed-rank test. ** $p < 0.01$.

892 **Figure 5: AIBP alters membrane and action potential properties more in SA than**
893 **non-SA human DRG neurons.** The analog recordings at the top of the figure show
894 representative effects APs from SA neurons (**a**, in green), in non-SA neurons where
895 AIBP had minimal effects (**b**, in red), and in non-SA cells where AIBP had more
896 pronounced effects as defined in the text (**c**, in blue). The traces in dotted lines show
897 the last action potential before AIBP treatment was begun and those in solid lines are
898 the last action potential at the conclusion of AIBP treatment. The grouped data are
899 shown in the scatter line plots at the right of the figure and include, respectively, resting
900 membrane potential (RMP), rheobase, AP amplitude, AP overshoot, and AP rising time.
901 Each symbol line pair represents an individual cell, with open and solid circles indicating
902 baseline and post-AIBP treatment, respectively. Non-parametric Wilcoxon matched
903 pairs signed rank test. * $p < 0.05$, ** $p < 0.01$.

904 **Figure 6. Endogenous AIBP expression in human DRG.** The IHC images at the top
905 are representative of 4X objective views from a radicular neuropathic pain patient that
906 shows AIBP positive cell profiles (**a**, in green) diffusely scattered through the tissue that
907 matches the pattern and cell profiles of CD68 positive cells (**b**, in red). The areas
908 outlined by the white boxes in **a** and **b** are shown at higher magnification in **c** and **d.**
909 The scale bar in all panels is 100 μm. The images in the remaining tissue sections (**e**-**j**)
910 are at 20X from a patient with unilateral radiculopathy, with the non-pain side in the

upper row (**e**-**g**) and the neuropathic pain side in the second row (**h**-**j**). AIBP is shown in red and glutamine synthetase (GS) is shown in green. The staining for both AIBP and GS is in somewhat faint annular cell profiles (**e, f**) consistent with satellite cells in the non-pain DRG, and the merged images show some of these profiles are co-localized (**g,** in yellow). Representative images from the pain side show that AIBP (**h**) and GS (**i**) are increased compared to the non-pain side and, as shown in **j,** the co-localization is now more pronounced. The scatter and box plots bar in **k** quantifying fluorescence intensity for the two groups indicate that both AIBP (red) and GS (green) were significantly increased in pain-associated DRG (filled boxes) compared to the contralateral non-pain DRG (open boxes). Welch's t-test. *** $p < 0.001$