Research Paper

PAIN 165 (2024) 202–215

PAIN®

OPEN

Bradykinin receptor expression and bradykinin-mediated sensitization of human sensory neurons

Jiwon Yi[a,b], Zachariah Bertels[a], John Smith Del Rosario[a], Allie J. Widman[a], Richard A. Slivicki[a], Maria Payne[a], Henry M. Susser[a], Bryan A. Copits[a], Robert W. Gereau IV[a,c,d,*]

Abstract

Bradykinin is a peptide implicated in inflammatory pain in both humans and rodents. In rodent sensory neurons, activation of B1 and B2 bradykinin receptors induces neuronal hyperexcitability. Recent evidence suggests that human and rodent dorsal root ganglia (DRG), which contain the cell bodies of sensory neurons, differ in the expression and function of key GPCRs and ion channels; whether bradykinin receptor expression and function are conserved across species has not been studied in depth. In this study, we used human DRG tissue from organ donors to provide a detailed characterization of bradykinin receptor expression and bradykinin-induced changes in the excitability of human sensory neurons. We found that B2 and, to a lesser extent, B1 receptors are expressed by human DRG neurons and satellite glial cells. B2 receptors were enriched in the nociceptor subpopulation. Using patch-clamp electrophysiology, we found that acute bradykinin increases the excitability of human sensory neurons, whereas prolonged exposure to bradykinin decreases neuronal excitability in a subpopulation of human DRG neurons. Finally, our analyses suggest that donor's history of chronic pain and age may be predictors of higher B1 receptor expression in human DRG neurons. Together, these results indicate that acute bradykinin-induced hyperexcitability, first identified in rodents, is conserved in humans and provide further evidence supporting bradykinin signaling as a potential therapeutic target for treating pain in humans.

Keywords: Bradykinin, Human dorsal root ganglia, Sensory neurons, Pain

1. Introduction

Bradykinin (BK) is a potent inflammatory mediator released during tissue injury and inflammation.[5,19,21,36] Bradykinin is associated with pathogenesis of inflammatory pain in both humans and rodents: Intradermal injection of BK in healthy human volunteers or intraplantar injection of BK in rodents can lead to local inflammation and cutaneous thermal hyperalgesia.[37,55] Systemic and local administration of B1 or B2 receptor antagonists can be antinociceptive in neuropathic, visceral, inflammatory, and other rodent pain models.[11,18,20,43,45,65] In addition, patients with painful disorders such as arthritis, endometriosis, and myalgia exhibit elevated levels of BK in damaged tissue or plasma compared with healthy controls, with the level of kinin correlating with pain degree or disease progression.[5,7,19,23,33,42,46]

Bradykinin-mediated hyperalgesia is believed to occur, in part, through the activation of G_q-coupled B1 and B2 receptors expressed on sensory neurons. In rodent dorsal root ganglion (DRG) neurons in vitro, acute application of BK can induce inward current, action potential firing, hyperexcitability, and TRPV1 sensitization.[6,9,10,13,32,38,40,41,60] Although B2 is the predominant BK receptor subtype in the DRG of uninjured animals,[13] B1 receptors can be synthesized de novo after formalin injection, nerve injury, or exposure to inflammatory growth factors, thereby further contributing to BK-mediated pain.[13,30,43,63,65] In addition, satellite glial cells in the DRG and trigeminal ganglia express BK receptors and may contribute to BK's effects on neuronal physiology in the periphery.[8,22] Together, these preclinical studies indicate that BK receptor activation on sensory neurons is integral to development or maintenance of pain.

Although the mechanism underlying BK-induced pain and hyperalgesia has been extensively studied in rodent models, it is yet unclear whether the same mechanism translates across species to humans. Of note, an increasing number of studies have demonstrated that expression and function of key ion channels and receptors differ between human and rodent DRG.[12,16,44,51,54,56,57,61] This highlights the need for validating targets in human tissue to maximize the translational potential of preclinically identified analgesics. Whole-tissue transcriptomics shows that the B2 receptor is expressed in human DRG (hDRG),[51] and acute application of BK elicits spontaneous activity and induces hyperexcitability in a subset of hDRG neurons.[12] However, whether BK receptors are expressed

Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

[a] Department of Anesthesiology, Washington University Pain Center, Washington University School of Medicine, St. Louis, MO, United States, [b] Neuroscience Graduate Program, Division of Biology and Biomedical Sciences, Washington University School of Medicine, St. Louis, MO, United States, [c] Department of Neuroscience, Washington University, St. Louis, MO, United States, [d] Department of Biomedical Engineering, Washington University, St. Louis, MO, United States

*Corresponding Author. Address: Department of Anesthesiology, Washington University Pain Center, Washington University School of Medicine, 660 South Euclid Ave Campus Box 8054, St. Louis, 63110, MO, United States. Tel.: (314) 362-8312; fax: (314) 362-8334. E-mail: gereaur@wustl.edu (R. W. Gereau).

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.painjournalonline.com).

http://dx.doi.org/10.1097/j.pain.0000000000003013

among human nociceptor subpopulations and whether BK modulates all physiological subtypes of hDRG[53] is yet unknown.

In this study, we evaluated the expression of B1 (*BDKRB1*) and B2 (*BDKRB2*) receptors in hDRG tissue obtained postmortem from organ donors. We additionally examined the effects of acute and prolonged exposure to BK on the excitability of 2 physiologically distinct subpopulations of human sensory neurons. Finally, we assessed whether donors' pain history is associated with changes in BK receptor expression or functional modulation of neuronal excitability. We found that hDRG neurons express functional BK receptors, whose modulation of neuronal excitability is dependent on both the duration of BK exposure and physiological subtype of the sensory neuron. These findings suggest that BK-induced acute hyperexcitability is conserved from rodents to humans and provide further evidence of BK signaling as a viable analgesic target.

2. Methods

2.1. Human dorsal root ganglion extraction

Human dorsal root ganglion extraction and culture was performed as previously described.[62] In brief, T11-L5 DRGs were extracted from postmortem organ donors in collaboration with Mid-America Transplant. Donor's medical history, including the history of chronic pain, was determined based on Mid-America Transplant's interview with the donor's family (**Tables 1 and 2**). Both male and female donors were included in our study. Extraction was performed within 2 hours of aortic cross-clamp and hDRG samples were placed in ice-cold N-methyl-D-glucamine (NMDG)-based solution (93 mM NMDG, 2.5 mM KCl, 1.25 mM NaH_2PO_4, 30 mM $NaHCO_3$, 20 mM HEPES, 25 mM glucose, 5 mM ascorbic acid, 2 mM thiourea, 3 mM Na^+ pyruvate, 10 mM $MgSO_4$, 0.5 mM $CaCl_2$, and 12 mM N-acetylcysteine; adjusted to pH 7.3 using NMDG or HCl, and 300-310 mOsm using H_2O or sucrose) for transport. Extracted hDRG was cleaned and cultured in laboratory immediately after extraction.

2.2. Human dorsal root ganglion culture

Several thoracolumbar hDRGs were pooled for each culture. Following previously published protocol for hDRG culture,[62] hDRGs were first enzymatically dissociated with papain (Worthington, Lakewood, NJ) and collagenase type 2 (Sigma-Aldrich, Darmstadt, Germany), then mechanical dissociated by trituration. Dissociated DRGs were filtered using a 100 μm cell strainer (Fisher Scientific, Pittsburgh, PA) and cultured on collagen-coated glass coverslips in DRG media consisting of Neurobasal A medium (Gibco, Grand Island, NY), 5% fetal bovine serum (Gibco, Grand Island, NY), 1% penicillin or streptomycin (Corning, Corning, NY), Glutamax (Life Technologies, Carlsbad, CA), and B27 (Gibco, Grand Island, NY). Human dorsal root ganglia were used for experiments at days in vitro (DIV) 2 to 7.

2.3. RNAScope in situ hybridization

Lumbar hDRG not used for culture were fixed in 4% paraformaldehyde overnight, transferred to solution containing 30% sucrose for 10 days or until the tissue sank to the bottom of the container, embedded in OCT in a cryomold, flash-frozen, and stored at −80˚C until use. Twelve micrometer sections were prepared from fixed-frozen hDRG tissue, mounted on Superfrost Plus slides (Fisher, Pittsburgh, PA), and stored at −20˚C or −80˚C with desiccant until used.

Slides containing hDRG sections were dehydrated using 50%, 70%, and 100% ethanol and treated with Protease IV and hydrogen peroxide (ACDBio, Minneapolis, MN). The following probes from ACDBio were used: BDKRB1 (#424321), BDKRB2 (#424331-C2), AND SCN10A (#406291-C3). RNAScope was performed following manufacturer's protocol. At the end of RNAScope, TrueBlack Autofluorescence Quencher (Biotium, Fremont, CA) was applied to sections as directed by manufacturer protocol. Slides were subsequently washed ×3 with PBS. DAPI was applied to the slides immediately before or after the application of TrueBlack. Slides were mounted with coverslips using ProLong Gold Antifade Mountant (Invitrogen, Waltham, MA) and stored at −20˚C. Slides were imaged within 3 days.

2.4. Immunohistochemistry

For experiments involving dual in situ hybridization–immunohistochemistry, immunohistochemistry was performed after RNAScope. After the final HRP signal was developed, slides

Table 1

Demographic information and medical history of donors included in physiology experiments.

Donor ID	Age	Sex	Race	BMI	COD	Pain Hx
No pain Hx						
210,328	53	M	White	30.3	Anoxia	
210,505	51	F	White	33.5	Anoxia	
210,608	12	M	White	20	Anoxia	
210,629	23	M	White	21.4	Head trauma	
210,713	28	M	White	27.6	Anoxia	
210,827	27	M	Undisclosed	18.7	Head trauma	
211,007	23	M	White	24.2	Head trauma	
211,019	23	F	White	35.4	Head trauma	
211,222	17	M	Black	22.6	Anoxia	
220,717	31	M	White	21.57	Anoxia	
230,202	33	M	White	21.68	Stroke	
Pain hx						
210,519	56	F	Black	23.1	Anoxia	Leg pain and scleroderma
210,625	53	M	White	24.2	Stroke	Chronic back pain
210,824	39	M	White	24.1	Head trauma	Arthritis and headaches
211,004	53	M	Black	36.5	Anoxia	Painful right knee arthritis

Table 2

Demographic information and medical history of donors included in RNAScope experiments.

Donor ID	Age	Sex	Race	COD	Level	Pain Hx
No pain Hx						
20,210,329	53	M	White	Anoxia	L3	
20,210,706	45	M	White	Anoxia	L1	
20,211,222	17	M	Black	Anoxia	L4	
Pain Hx						
20,210,404	23	M	White	Head trauma	L1	Chronic joint pain
20,210,813	32	M	White	Head trauma	L2	Frequent joint pain in hands
20,220,916	58	F	Unknown	Stroke	Lumbar (unspecified)	Painful degenerative disc disease

were washed 2 times in PBS-T (PBS with 0.1% Tween-20), then incubated in blocking buffer (10% donkey serum in PBS-T) for 1 hour at room temperature in the dark. Slides were then incubated in primary antibody (rabbit anti-FABP7; Invitrogen, Waltham, MA) diluted 1:500 in blocking buffer overnight at 4°. The next day, slides were washed 3 times in PBS-T and incubated in secondary antibody (goat anti-rabbit 647; Invitrogen, Waltham, MA) diluted 1:1000 in PBS for 2 hours at room temperature. Slides were washed 3 times in PBS before application of DAPI, treatment with TrueBlack, and mounting as described above.

2.5. Imaging

After RNAScope, slides were imaged using Leica DM6b system at 20x magnification. The entire hDRG section was imaged and merged using Leica Application Suite X (LAS-X, v.3.7, Leica Microsystems, Wetzlar, Germany). Acquisition settings were kept consistent throughout imaging across different experimental days. Acquisition settings for each fluorescence channel were as follows: DAPI, 50 ms exposure, 2.1 gain, FIM 55%; L5, 300 ms exposure, 2.5 gain, FIM 55%; TXR, 500 ms exposure, 3.0 gain, FIM 55%; and Y5 500 ms exposure, 2.0 gain, FIM 55%.

2.6. Image analysis

For analysis, 2 to 3 hDRG sections per donor were chosen at random, although preference was given to sections that did not have extensive artifacts from sectioning and mounting, such as tear in section or bubbles. Dorsal root ganglion cell body profiles were visually identified using autofluorescence and DAPI fluorescence from the satellite glial cells surrounding neuronal cell bodies using methods described previously.[57] Identified cell bodies were manually segmented using the freehand ROI tool in ImageJ (NIH). The number of cells per section varied between sections, ranging from 100 to 900. For each cell, its area was measured using ImageJ and equivalent diameter was calculated using the formula, $\text{diameter} = 2 \times \sqrt{\text{Area}/\Pi}$. After segmentation, cells were manually classified as "positive" or "negative" for each gene of interest based on the presence of RNA puncta in the soma. Fluorescent signal that appeared across all 3 channels were considered as background autofluorescence and not included in analysis. For each experiment, any adjustment to the brightness and contrast was kept consistent across all sections and donors used for that experiment. The proportion of neurons expressing the gene of interest was calculated by dividing the sum of all "positive" neurons in all the sections for each donor by the sum of all neurons in all sections for that specific donor.

2.7. Patch-clamp electrophysiology

At DIV 2 to 7, coverslips containing cultured primary hDRG neurons were transferred to a recording chamber. Experiments were conducted in external solution containing (in mM): 145 NaCl, 3 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 7 glucose, and 10 HEPES, adjusted to pH 7.3 with NaOH. Whole-cell patch-clamp recordings were made using fire-polished borosilicate glass pipettes with 2 to 3.5 MΩ resistance. The pipettes were filled with K-gluconate–based internal solution consisting of the following (in mM): 120 K-gluconate, 5 NaCl, 3 $MgCl_2$, 0.1 $CaCl_2$, 10 HEPES, 1.1 EGTA, 4 Na_2ATP, 0.4 Na_2GTP, 15 Na_2phosphocreatine, adjusted to pH 7.3 with KOH and HCl, and 290 mOsm with sucrose. All experiments were conducted at room temperature. External solution was perfused continuously using gravity-fed bath perfusion at a flow rate of 1-2mL/min.

Recordings were made using a MultiClamp 800B amplifier and a Digidata 1550B digitizer (Molecular Devices, San Jose, CA). Data were sampled at 10 kHz. Electrophysiology data were compiled using ClampFit (v.11.1, Molecular Devices, San Jose, CA). Series resistance was kept below 15 MΩ in all current-clamp recordings.

After a stable whole-cell configuration was achieved, membrane excitability was assessed in the current-clamp mode from a holding potential of −60 mV, with the exception of resting membrane potential. Resting membrane potential was assessed using a gap-free recording with no current injection. Cells whose resting membrane potentials were below −75 mV or above −35 mV were considered unhealthy and excluded from analysis. A small number of neurons displayed spontaneous activity at rest that prevented us from measuring their resting potential. These spontaneously active neurons were included for analysis if they required less than 500 pA of negative holding current to be held at −60 mV. Small to medium-sized neurons with soma diameter ≤60 μm were selected for patch-clamp experiments, as the cut-off captures more than half of *SCN10A*-expressing nociceptors in hDRG while excluding the proprioceptors and nonnociceptive subpopulations of Aβ neurons in the hDRG.[57,61] Although some Aβ nociceptors may have been excluded from our study as a result, the 60 μm cut-off was chosen to minimize the likelihood that nonnociceptive subpopulations would be included in our sample.

Active and passive membrane properties were analyzed using Easy Electrophysiology (Easy Electrophysiology Ltd., London, United Kingdom). Resting membrane potential was continuously monitored throughout the experiment. Input–output relationship was determined by counting the number of action potentials generated by 1-second long current steps from 50 pA to 2 nA (Δ50 pA). Rheobase was defined as the minimum amount of current step required to evoke an action potential.

Action potential kinetics were analyzed from the first action potential evoked at rheobase. Threshold was defined as membrane voltage when dV/dt = 10. Action potential (AP) rise time and decay time were defined as time from 10% to 90% of rising phase or of the falling phase, respectively. Half-width was measured as the time between 50% of the rise and decay phase of the action potential. AP amplitude was measured from the AP threshold to peak. Passive membrane properties, including input resistance and voltage sag, were measured using 1-second long hyperpolarizing current steps from −250 to −50 pA (Δ50 pA) from a holding potential of −60 mV. No significant differences in rheobase, input resistance, resting membrane potential, and action potential threshold were detected in hDRG neurons across DIV 2 to 7, when all recordings were conducted.

2.8. Acute bradykinin treatment

Stocks of BK (Sigma-Aldrich, Darmstadt, Germany; 100 μM) were prepared in 0.1 mM acetic acid (vehicle) and stored at −20°C until use. Solutions containing working concentration of BK (100 nM) or vehicle were prepared on the day of experimentation in external solution.

Measures of membrane excitability were assessed both before and during BK treatment. Resting membrane potential was continuously assessed for 1 minute before BK application and during the first 3 minutes of acute BK application. Human dorsal root ganglion neuron was considered depolarized by BK if the change in membrane potential was greater than 1 mV after 3 minutes of BK treatment; the neuron was considered hyperpolarized if the magnitude of hyperpolarization was greater than 1 mV. The threshold of $|\Delta V|>1$ mV was set based on a series of control experiments using various vehicle solutions, including the 0.01 mM acetic acid used in this study, that found that hDRG and mouse DRG neurons display, on average, <1 mV membrane potential fluctuation (mean = 0.89 mV) over the course of 2- to 3-min long vehicle application.

2.9. Prolonged bradykinin treatment

For experiments assessing the effects of prolonged BK exposure, coverslips containing hDRG neurons were incubated overnight with DRG media containing either 100 nM BK or equivalent concentration of vehicle (0.01 mM acetic acid). After overnight treatment, coverslips of hDRG neurons were removed from conditioned media. Membrane excitability was assessed as described above in external solution not containing BK.

2.10. Classification of firing phenotype

Cells were categorized as single or repetitively spiking. Cells that fired no more than one action potential in response to depolarizing current steps from 50 pA to 2 nA (Δ50 pA) and at 2-3x rheobase were categorized as "single spiking," similar to the "rapidly adapting" clusters described in rat DRG.[3,48] Cells which fired more than one action potential across multiple stepwise current injections were categorized as "repetitive-spiking" neurons, similar to the "nonadapting" clusters identified in rat DRG.[3,48] Repetitive-spiking neurons were further categorized into adapting and nonadapting clusters based on the presence of spike frequency adaptation. A cell was classified as "repetitive adapting" if the cell displayed an inverse U-shaped input–output curve of spike number vs current injected, whereas cells displaying a linearly increasing input–output curve with no spike frequency adaptation were classified as "repetitive nonadapting."

2.11. Viability assay

Cultured hDRG neurons were treated with either 100 nM BK, 200 mM KCl, or vehicle (0.1 mM acetic acid) overnight (18-24 hours). After overnight treatment, plated DRGs were harvested by scraping the coverslip with p1000 pipette tips. Cell suspension was collected and spun down at 1000 rpm for 3 minutes. Supernatant was discarded, and the pellet was resuspended in 20 to 50 μL of fresh HBSS + HEPES buffer. Calcein-AM (Invitrogen, Waltham, MA) stock was prepared using DMSO according to manufacturer's instructions. A 10 μM working solution of Calcein-AM was prepared in HBSS+H. The cell suspension (10 μL) was diluted with equal volume of Calcein-AM and loaded onto a hemocytometer. The cells were imaged using Leica DM6b system with LAS-X (v.3.7, Leica Microsystems, Wetzlar, Germany) at 20x. The presence of green fluorescence was used to distinguish between live (fluorescent) and dead (nonfluorescent) cells. The viability ratio was calculated by dividing the number of live cells over the total number of cells (sum of live and dead cells).

2.12. Pain history assessment

Donors' pain history was assessed by reviewing the social and medical history of the donor as documented on the United Network for Organ Sharing (UNOS) records. Donors whose UNOS records contained key words such as "constant pain," "frequent pain," "joint pain," or diseases associated with pain (eg, scleroderma, arthritis) were designated as having a history of chronic or persistent pain (**Tables 1 and 2**).

3. Statistics

Statistical analyses were performed using GraphPad Prism 9 (GraphPad Software Inc, Boston, MA). For each data set analyzed, normality of residuals was tested using the Shapiro–Wilk test to inform the use of parametric or nonparametric tests. To assess statistical differences between 2 groups, *t* test (paired or unpaired; as indicated in text), Mann–Whitney tests, or Wilcoxon tests were performed. To assess statistical differences across multiple groups or time points, one-way or two-way repeated measures ANOVAs with Geisser–Greenhouse correction and Sidak post hoc test, Friedman test with Geisser–Greenhouse correction and Dunn post hoc test, or restricted maximum likelihood (REML) mixed effects analysis with Geisser–Greenhouse correction and Sidak post hoc tests were used. For select tests with significant results as defined by $P < 0.05$, effect size was calculated.[29] All data are represented as mean ± SEM. Detailed statistics are reported in Supplementary Tables S1-31, available at http://links.lww.com/PAIN/B904.

4. Results

4.1. Bradykinin receptors are expressed in human sensory neurons

Previously, a whole-tissue transcriptomics study of hDRG has found that B2 and, to a lesser extent, B1 receptors are expressed in the hDRG.[51] To characterize the distribution pattern of BK receptors in hDRG subpopulations, we performed RNAScope in situ hybridization for *BDKRB1* and *BDKRB2*, the genes for B1 and B2 receptors, respectively, on DRG samples from donors without pain history (**Fig. 1A, B**; **Table 2**). Consistent with the findings in whole-tissue transcriptomics study of human and mouse DRG,[51] *BDKRB1* was detected at low levels and in a small fraction

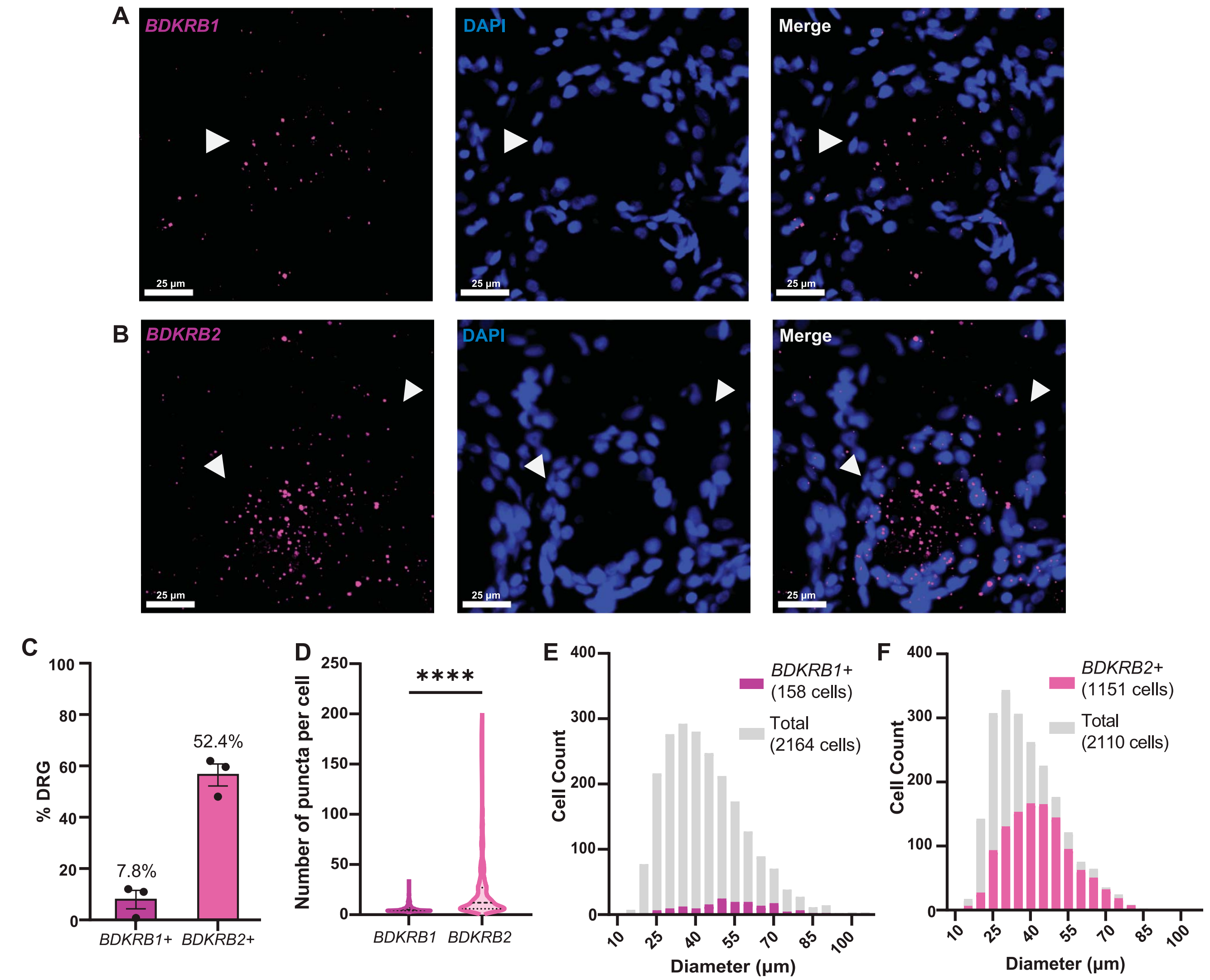

Figure 1. B1 and B2 bradykinin receptors are expressed by human sensory neurons. (A) Representative image of *BDKRB1* expression in hDRG assessed using RNAScope; scale bar, 25 μm. Arrowheads mark *BDKRB1*-expressing hDRG neuron. (B) Representative image of *BDKRB2* expression in hDRG assessed using RNAScope; scale bar, 25 μm. Arrowheads mark *BDKRB2*-expressing neurons. (C) *BDKRB1* and *BDKRB2* expression in hDRG averaged across donors (N = 3 donors, 2-4 sections, 323-1044 cells per donor). (D) Count of RNA puncta per cell in *BDKRB1*+ (left; n = 405) or *BDKRB2*+ (right; n = 655) hDRG neurons; ****$P < 0.0001$, Mann–Whitney test. (E) Size distribution of *BDKRB1*-expressing neurons (purple) compared with all DRG neurons (grey; n = 2164 total neurons, 158 *BDKRB1*+ neurons from 3 donors). (F) Size distribution of *BDKRB2*-expressing neurons (magenta) compared with all DRG neurons (grey; n = 2110 total neurons, 1151 *BDKRB1*+ neurons from 3 donors). Data represent mean ± s.e.m.

(<10%) of hDRG neurons (**Fig. 1C, D**), whereas *BDKRB2* was detected in a larger fraction (48%-61%; **Fig. 1C**) of hDRG neurons and at significantly higher levels compared with *BDKRB1* (**Fig. 1D**; η^2=0.268, d = 1.211). More than half of *BDKRB1*-expressing neurons were medium-sized to large-sized neurons with diameter greater than 40 μm (**Fig. 1E**), whereas *BDKRB2* was expressed across sensory neurons of varying sizes (16um-131um) (**Fig. 1F**). The DRG neuron size distribution was consistent between donors (data not shown).

To assess whether *BDKRB2* is specifically expressed by nociceptors in hDRG, we examined whether *BDKRB2* mRNA is localized in cells expressing *SCN10A*. *SCN10A* was chosen as a marker for human nociceptors based on a previously published spatial transcriptomics study, which demonstrated that *SCN10A* is specifically and highly enriched among multiple nociceptor subpopulations in hDRG.[61] *BDKRB2* and *SCN10A* were coexpressed in 1/3 of all sensory neurons analyzed (31.3%, n = 2663 cells; **Figs. 2A, B**). A majority (72.4%) of *BDKRB2*-expressing neurons also expressed *SCN10A*, indicating that *BDKRB2*-expressing cells are predominantly nociceptors (**Fig. 2C**). Among the *SCN10A*-expressing putative nociceptors, around half (51.6%) also expressed *BDKRB2* (**Fig. 2C**). Interestingly, we also observed that *BDKRB2* colocalized with FABP7, a marker of satellite glial cells (**Fig. 2D**). We were unable to quantify the relative proportion of BDKRB2-expressing satellite glial cells, as segmenting individual satellite glial cells that have been imaged using fluorescence or confocal microscopy is challenging due to the tight contact between the multiple satellite glial cells that surround a hDRG neuron.[1] Together, these results suggest that B2 receptor is expressed in both nociceptor and glial subpopulations in the DRG.

4.2. Acute bradykinin sensitizes human dorsal root ganglion neurons

In a previous study, we reported that acute application of BK can induce spontaneous activity and reduce rheobase in hDRG

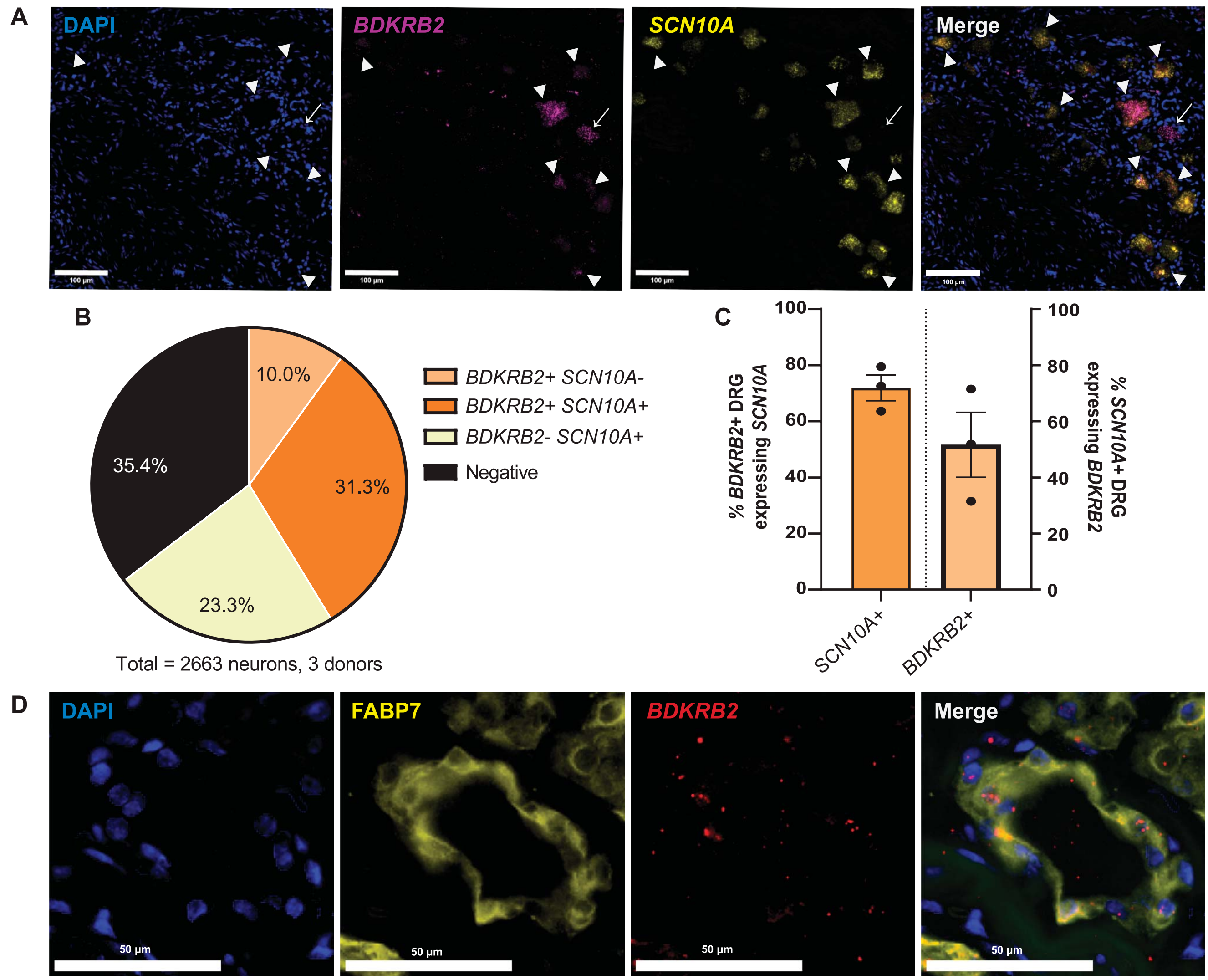

Figure 2. B2 bradykinin receptor is expressed in human nociceptors and satellite glial cells. (A) Representative image of hDRG expressing mRNA for *BDKRB2* (magenta) and *SCN10A* (yellow), the human nociceptor marker. Arrowheads mark cells coexpressing *BDKRB2* and *SCN10A*; arrows mark cell that expresses *BDKRB2*, but not *SCN10A*; scale bar, 100 μm. (B) Distribution of *BDKRB2* and *SCN10A* across human sensory neurons analyzed (2663 neurons from 3 donors). (C) Quantification of BDKRB2 distribution, averaged across donors (N = 3 donors, 2-3 sections and 338-1349 neurons per donor). Left, proportion of *BDKRB2*-expressing neurons that coexpress SCN10A. Right, proportion of *SCN10A*-expressing nociceptors that express *BDKRB2*. (D) Representative image of *BDKRB2* expression in FABP7-expressing satellite glial cells in hDRG; scale bar, 50 μm. Data represent mean ± s.e.m.

neurons.[12] In addition, recent work has suggested that both rodent and human sensory neurons exist in distinct physiological phenotypes that can be distinguished by single- or repetitively firing discharge pattern in response to a stepwise current injection.[3,48,53] Therefore, we tested whether acute BK-induced hyperexcitability is consistent across small-diameter neurons with different physiological phenotypes.

We found that acute (2-3 min) application of 100 nM BK led to spontaneous activity or depolarization in 75% of neurons recorded (**Fig. 3A, B**; 37.01 ± 7.829 μm, mean diameter ± SD). The depolarization in resting membrane potential was seen in both single-spiking and repetitive-spiking hDRG (**Fig. 3C**). In addition, 72% of all neurons displayed either reduced rheobase or increased spike firing (**Fig. 3D, E**), which contributed to a leftward shift in the input–output curve of the number of spikes fired in response to increasing current steps in both pooled neurons and the single-spiker subgroup (**Fig. 3F**). Acute BK additionally led to increased input resistance and reduced rheobase in single spikers, consistent with hyperexcitability (**Table 3**). Although BK treatment led to increased spike firing in many repetitively firing neurons, statistical analysis showed no significant effect of treatment, likely due to the variability in spike frequency between repetitive-firing neurons. Together, these results indicate that acute BK application can increase the intrinsic excitability of human sensory neurons.

Previously, we found that acute BK can lead to changes in action potential kinetics in hDRG neurons.[12] Therefore, we examined physiological phenotype-specific changes in action potential kinetics after acute treatment with BK. Intriguingly, we found that changes in AP kinetics were only seen in repetitively-firing neurons (**Fig. 4**, **Table 3**), a phenotype associated with a wider AP half-width and slower decay in hDRG.[66] In repetitive spikers (n = 8), acute BK led to a slower rise time and a faster decay (**Figs. 4A–D**) and reduced the size of the action potential peak (**Fig. 4E**), consistent with our previous findings[12]. This shift in kinetics was not observed in single-spiking neurons (n = 10; **Figs. 4F–J**). Together, this suggests that acute BK may have phenotype-specific effects on AP kinetics.

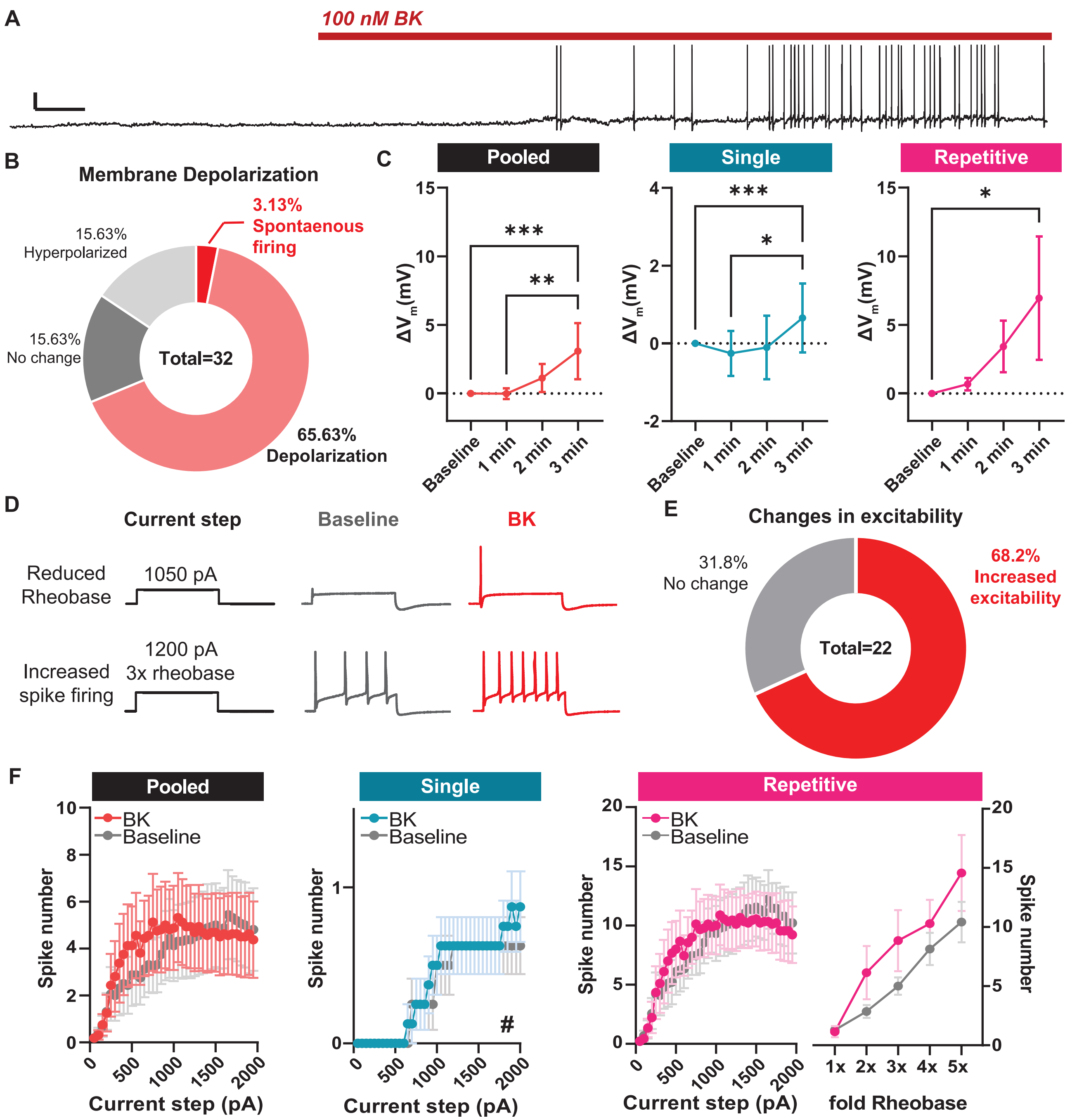

Figure 3. Acute exposure to BK sensitizes small-diameter and medium-diameter hDRG neurons. (A) Representative voltage trace of a sensory neuron with spontaneous action potential firing during bath application of BK (100 nM); scale bars, 20 mV/10 seconds. (B) Distribution of neuronal responses to acute BK based on resting membrane potential depolarization and spontaneous activity during the first 2 minutes of application. (C) Changes in resting membrane potential in hDRG neurons grouped by firing phenotype. Left, pooled analysis of all recorded neurons (n = 19 cells); middle, analysis of single-spiking neurons only (n = 11); right, analysis of repetitively-firing neurons only (n = 8). *$P < 0.05$ **$P < 0.01$, ***$P < 0.001$, Friedman test with Dunn post hoc multiple comparison test. (D) Representative traces reduction in rheobase (top) and increase in the number of spikes fired during suprathreshold current injections (bottom) after treatment with 100 nM BK. (E) Distribution of neuronal response to acute BK based on shifts in excitability, as defined by a leftward shift in the input–output curve. (F) Input–output curve of BK-sensitive hDRG neurons, before and during acute bath application of BK. Left, pooled analysis of all recorded neurons (n = 16 cells); middle, analysis of single-firing BK-sensitive neurons only (n = 8 cells, $^{\#}P < 0.05$, main effect of BK treatment; two-way mixed ANOVA); right, input–output curves of repetitive-firing neurons (n = 7 cells). Data represent mean ± s.e.m.

4.3. Prolonged exposure to bradykinin reduces the excitability of repetitive-firing, but not single-spiking, human neurons

Persistent upregulation in BK levels has been implicated in painful inflammatory disorders in humans, such as arthritis[7,23,46] and endometriosis.[42] Therefore, we examined how prolonged (18-24 hours) exposure to BK modulates the intrinsic excitability of hDRG neurons. Overnight treatment with BK did not significantly change the proportions of intrinsic firing phenotypes (single vs repetitive) of neurons recorded (**Fig. 5A**). Among repetitive neurons, we observed a 50% reduction in the relative proportion of neurons that displayed spike frequency adaptation in BK-treated neurons

Table 3

Action potential kinetics and membrane properties of bradykinin-sensitive hDRG before and after acute bradykinin application.

	Pooled (n = 16)		Repetitive spikers (n = 7)		Single spikers (n = 8)	
	Baseline	BK	Baseline	BK	Baseline	BK
Input resistance, MΩ	158.2 ± 37.87	136.7 ± 27.56	268.7 ± 65.61	211.9 ± 47.57	**74.30 ± 14.17**	**80.68 ± 17.14***
Voltage sag ratio	0.3701 ± 0.04432	0.4116 ± 0.04032	0.2079 ± 0.05263	0.3121± 0.07529	0.4370 ± 0.03242	0.4495 ± 0.03871
Rheobase, pA	793.5 ± 193.6	755.8 ± 180.2	215.3 ± 46.00	222.2 ± 48.76	**1392 ± 236.8**	**1311 ± 221.0†**
Threshold, mV	−21.86 ± 2.435	−19.87 ± 2.707	−21.39 ± 2.277	−17.43 ± 3.973	−21.39 ± 4.539	−22.40 ± 5.289
Peak latency, ms	49.76 ± 26.76	26.81 ± 9.675	89.90 ± 58.90	39.53 ± 19.84	9.369 ± 0.9276	9.568 ± 0.8376
Peak, mV	46.12 ± 2.885	45.64 ± 2.337	**50.50 ± 1.806**	**48.93 ± 2.230***	41.41 ± 5.175	41.78 ± 3.885
Half width, ms	4.684 ± 0.7679	3.975 ± 0.5561	6.150 ± 1.193	5.050 ± 0.7912	3.180 ± 0.7335	2.744 ± 0.3147
Rise time, ms	1.019 ± 0.2497	0.7869 ± 0.07063	**0.7286 ± 0.09075**	**0.7900 ± 0.09307***	1.349 ± 0.4781	0.8288 ± 0.1112
Decay time, ms	7.326 ± 1.562	6.707 ± 1.293	11.26 ± 2.631	9.941± 2.180	4.053 ± 0.5136	4.098 ± 0.4017
fAHP, mV	−37.20 ± 2.619	−39.51 ± 2.510	−37.93± 1.921	−40.97 ± 2.035	−39.19 ± 4.255	−41.17± 3.656

Bolded entries denote comparisons that showed statistical significance.
* $P < 0.05$ compared with baseline, Wilcoxon test.
† $P = 0.0549$ compared with baseline, paired *t* test.

compared with vehicle-treated neurons (**Fig. 5B**; $P = 0.0195$, chi-square test).

Among repetitive-spiking neurons, prolonged exposure to BK led to an overall reduction in excitability (**Figs. 5C–E**), including a significant increase in rheobase and reduction in input resistance. We did not observe any difference in action potential kinetics between overnight vehicle-treated and BK-treated groups (**Table 4**). Single spikers from vehicle-treated and overnight BK-treated groups did not display significant differences in measures of excitability or spike kinetics (**Figs. 5F–H**, **Table 4**). Finally, overnight treatment with BK did not significantly reduce the viability of cells compared with vehicle (74.68% vs 77.06%, vehicle vs BK, $P = 0.86$, unpaired *t* test) indicating that observed physiological changes were not likely due to selective cell death of subpopulations of neurons after BK treatment. Together, these results suggest that prolonged BK treatment modulates the excitability of hDRG neurons in a physiological phenotype-dependent manner.

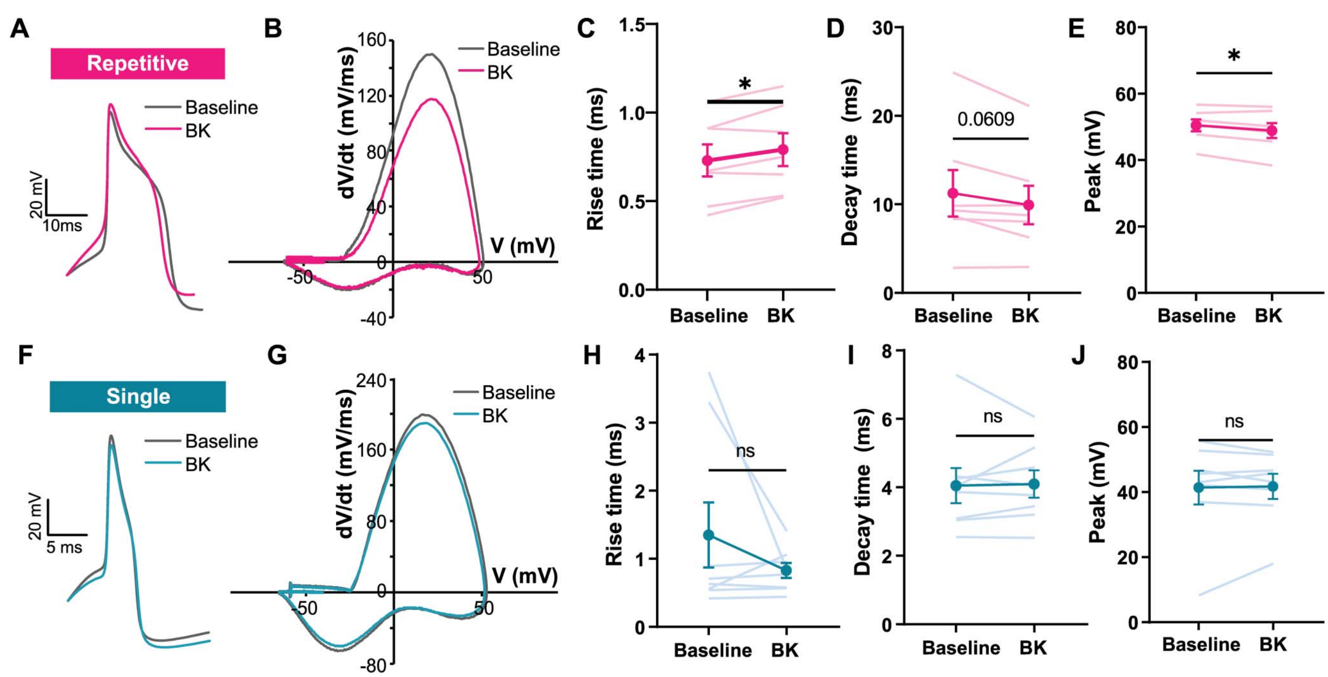

Figure 4. Acute exposure to bradykinin (BK) shifts action potential kinetics of repetitive-firing but not single-spiking hDRG neurons. (A) Example voltage trace of an action potential at rheobase of a BK-sensitive, repetitive-firing neuron, before (gray) and after (pink) acute BK (100 nM); scale bars, 20 mV/10 ms. (B) Representative phase-plane plot of a hDRG neuron in (A) at rheobase, before and during application of BK. (C–E) Changes in rise time, decay time, and peak voltage in action potential spikes of repetitive-firing neurons, before and during acute BK application; n= 7 cells, *$P < 0.05$, paired *t* test. (F) Example voltage trace of an action potential at rheobase of a BK-sensitive, single-spiking neuron, before (gray) and after (blue) acute BK (100 nM); scale bars, 20 mV/10 ms. (G) Representative phase-plane plot of the single-spiking hDRG neuron in (F) at rheobase, before and during application of BK. (H–J) Rise time, decay time, and peak of action potentials from single-spiking neurons before and during acute BK application; n = 8 cells. Data represent mean ± s.e.m.

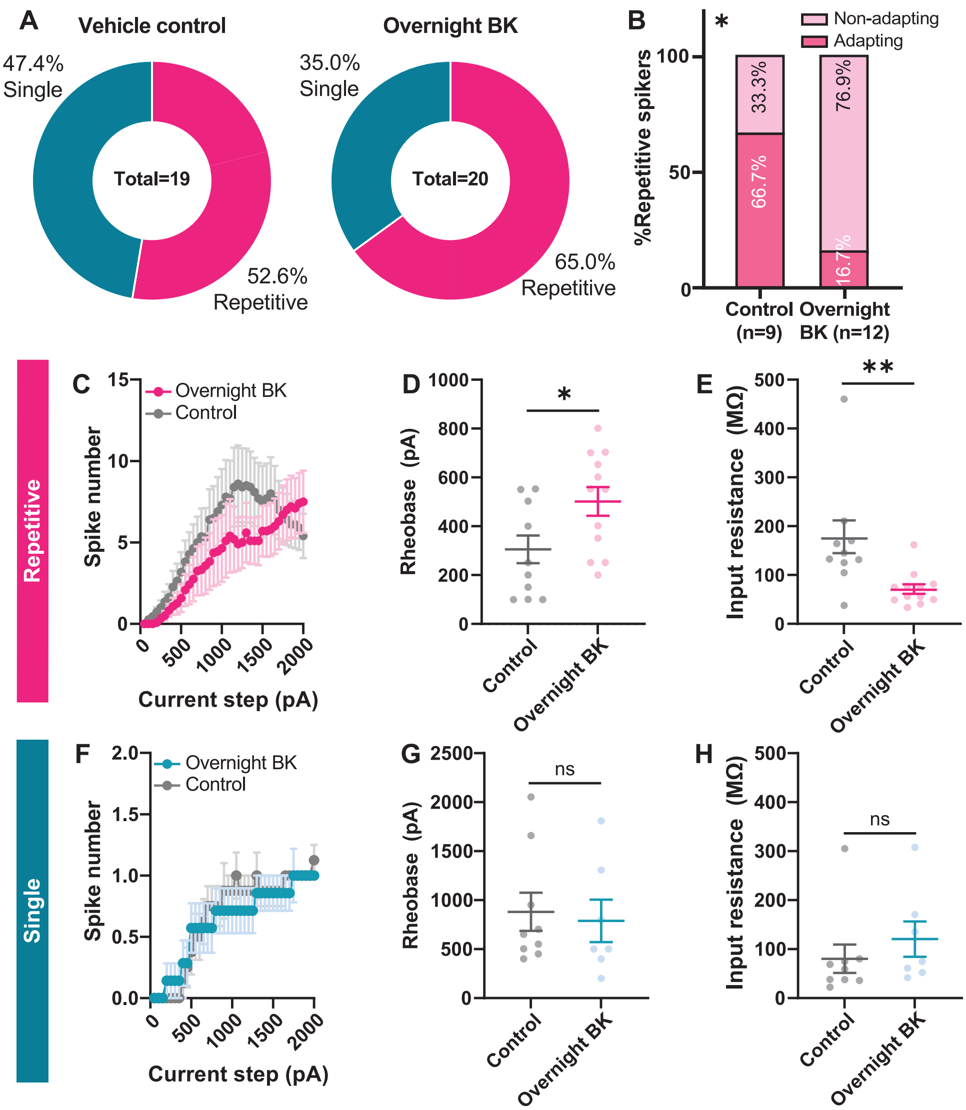

Figure 5. Prolonged exposure to bradykinin (BK) leads to reduced excitability in repetitive, but not single, spiking neurons. (A) Distribution of firing patterns of neurons treated overnight with vehicle or BK (100 nM). (B) Relative frequency of repetitive spikers that have adapting and nonadapting firing patterns; *$P = 0.0195$, chi-square test. (C–E) Input–output curve (C), rheobase (D), and input resistance (E) of repetitive-spiking neurons treated with BK or vehicle overnight; n = 10 to 12 cells, *$P < 0.05$, **$P < 0.01$, unpaired *t* test. (F–H) Input–output curve (F), rheobase (G), and input resistance (H) of single-spiking neurons treated with BK or vehicle overnight; n=7 to 9 cells. Data represent mean ± s.e.m.

4.4. Documented pain history and bradykinin receptor expression or bradykinin-mediated sensitization

Lasting upregulation in B1 and B2 receptor expression and increase in the production of BK have been associated with various rodent models of pain, including myalgia, neuropathic pain, and inflammatory pain.[2,17,28,30,43,50,65] Therefore, we examined whether the history of pain was associated with differences in BK receptor expression in the hDRG. First, we analyzed a previously published bulk RNA-seq data set of hDRG obtained from cancer patients with or without radicular or neuropathic pain.[47] The whole-tissue level of *BDKRB1* was ~1.5 fold higher in hDRG from patients with radicular or neuropathic pain, although this difference was not significant ($P = 0.3539$, Mann–Whitney test) (**Fig. 6A**). *BDKRB2* level in whole-DRG samples was comparable between the 2 groups ($P = 0.6026$, Mann–Whitney test) (**Fig. 6B**).

As whole-DRG RNA-seq does not distinguish between neurons and nonneuronal cells in the tissue, we next assessed whether there are neuron-specific differences in the expression of BK receptors in the hDRG from donors with and without the history of pain. We identified 3 donors with documented pain history in their medical history (**Table 2**) and assessed expression of B1 and B2 receptors in their DRGs using RNAScope in situ hybridization. We observed that the proportion of hDRG neurons that expressed *BDKRB1* was 1.9-fold higher in the "pain history" group compared with the "no pain history" group (**Figs. 6C, D**), although this difference was not statistically significant ($P = 0.4313$, unpaired *t* test). Within *BDKRB1*-expressing cells, the pain history was associated with a significant but small decrease in the average *BDKRB1* RNA expression level per cell ($P < 0.01$, $\eta^2 = 0.018$, d = 0.228; **Fig. 6E**). Proportion of BDKRB2-expressing hDRG neurons was comparable between the 2 groups ($P = 0.5875$; **Figs. 6F, G**). Although the average expression level of *BDKRB2* within each *BDKRB2*+ neuron was significantly lower in the pain history group, the effect size of this difference was trivial ($P < 0.05$, $\eta^2 = 0.002$, d = 0.098;

Table 4

Action potential kinetics and membrane properties of overnight vehicle-treated and bradykinin-treated cells.

	Repetitive spikers		Single spikers	
	BK (n = 12)	Vehicle (n = 10)	BK (n = 7)	Vehicle (n = 9)
RMP, mV	−50.93 ± 2.474	−49.91± 2.505	−50.95 ± 6.153	−54.10 ± 1.888
Input resistance, MΩ	**71.23 ± 9.959***	**168.2 ± 35.44**	120.5 ± 35.93	80.31 ± 28.91
Voltage sag ratio	0.4143 ± 0.0328	0.3898 ± 0.04461	0.4160 ± 0.02921	0.4631 ± 0.03783
Rheobase, pA	**501.6 ± 58.82†**	**290.8 ± 60.73**	788.4 ± 216.6	880.5 ± 195.0
Threshold, mV	−23.50 ± 0.8859	−20.77 ± 3.459	−18.08 ± 2.783	−19.49 ± 3.622
Peak latency, ms	92.87 ± 64.24	112.0 ± 59.59	11.02 ± 1.807	10.64 ± 0.9777
Amplitude, mV	73.22 ± 1.793	71.12 ± 5.403	61.67 ± 5.085	66.43 ± 3.448
Half width, ms	4.296 ± 0.4584	4.798 ± 0.4523	2.871 ± 0.4766	2.444 ± 0.3349
Rise time, ms	0.6500 ± 0.03979	0.7290 ± 0.08034	0.7400 ± 0.08588	0.6833 ± 0.1090
Decay time, ms	6.773 ± 0.5790	8.534 ± 0.7809	4.683 ± 0.8342	3.666 ± 0.5184
fAHP, mV	−36.44 ± 2.002	−38.68 ± 1.930	−39.69 ± 2.976	−40.73 ± 3.277

Bolded entries denote comparisons that showed statistical significance.
† $P < 0.05$, unpaired t-test.
* $P < 0.01$, Mann–Whitney test.

Fig. 6H). Interestingly, we observed a positive linear relationship approaching significance ($P = 0.0721$, $R^2 = 0.5959$) between age and proportion of *BDKRB1*-expressing hDRG neurons (**Fig. 6I**), but not in *BDKRB2*-expressing neurons (**Fig. 6J**).

Next, we tested whether the pain history was associated with any differences in the overall modulatory effects of BK on neuronal physiology. To that end, we reanalyzed the physiology experiments (**Figs. 3–5**) based on donors' pain history (**Table 1**). Acute BK led to depolarization in hDRG neurons from both donors with and without pain history (**Fig. 6K**). The hyperexcitability phenotype of acute BK seemed more robust in repetitively-spiking hDRG neurons obtained from donors with pain history (**Fig. 6L**); effect of acute BK on input–output curves of single-spiking neurons was comparable across both pain groups (**Fig. 6M**). We were unable to assess whether the pain history was associated with difference in the relative magnitude of acute BK-induced hyperexcitability in repetitive spikers due to the small sample size per group resulting from segmentation by both pain history and spiking phenotype (n = 3-5 per spiking phenotype per pain group), Finally, overnight BK treatment led to reduced spike firing and excitability in repetitive neurons from both donors with and without pain history (n = 3-8; **Fig. 6N**, **Table 5**). Together, this suggests that modulatory effect of acute and prolonged BK on neuronal excitability is broadly consistent in sensory neurons obtained from donors with and without pain history.

5. Discussion

In this study, we show that human nociceptors express functional BK receptors and that BK can bidirectionally modulate the membrane excitability of hDRG neurons depending on the duration of exposure and the physiological subtype of the neuron. Our results also suggest that the pain history may be associated with higher B1 receptor expression in hDRG and more robust neuronal response to acute BK. Together, these experiments support the notion that BK signaling is a potential analgesic target for inflammatory pain in humans.

In rodents, BK's hyperalgesic effect on naïve animals is mediated by the B2 receptors expressed on peptidergic nociceptors.[10,13,38,41,60] Consistent with these findings in rodents, we found that B2 receptors are particularly enriched among putative *SCN10a*+ nociceptors in the hDRG; we did not distinguish between peptidergic and nonpeptidergic populations of human nociceptors in our study, as hDRG, unlike rodent DRG, has a high overlap between these markers.[57]

The present findings support and extend our previous reports that hDRG neurons can be activated or sensitized by acute BK.[12] The hyperexcitability-inducing effects of acute BK may partly account for the increases in pain rating seen in healthy human volunteers that receive intradermal BK injection and in other inflammatory disorders such as arthritis that are associated with elevated plasma and synovial kinin levels.[7,23,37] One possible mechanism underlying acute BK-induced hyperexcitability is G_q-mediated inhibition of M-type K^+ currents and activation of the Ca^{2+}-gated chloride channel, ANO1, both of which contribute to BK-induced membrane depolarization and spike firing in rat DRG.[32,64] The fact that multiple subpopulations of hDRG neurons express the genes for both Kv7 channels and ANO1[61] further bolsters this idea, although additional functional studies are needed. Together, these results indicate that peripheral mechanism underlying acute BK-induced pain may be conserved from rodents to humans.

Overnight incubation with BK and other inflammatory reagents has been previously used to mimic chronic inflammatory disorders such as arthritis in vitro.[24,60] Surprisingly, prolonged BK led to a reduction in the excitability in a subset of hDRG neurons. The reduction in excitability in neurons with a repetitive-spiking phenotype was also accompanied by a partial increase in the incidence of repetitive spikers without spike frequency adaptation, a cluster associated with smaller number of action potentials discharged at lower current steps.[66] Similar shift towards a hypoexcitable firing phenotype has been seen previously in hDRG neurons following prolonged depolarization[53] and in DRG from rats with spinal cord injury that were exposed in vitro to high concentrations of the cytokine macrophage migration inhibitory factor.[3] In conjunction with these studies, our findings provide additional evidence that human sensory neurons can engage in a homeostatic shift towards a more hypoexcitable state in the context of prolonged inflammation. There are several potential mechanisms that may play a role in the

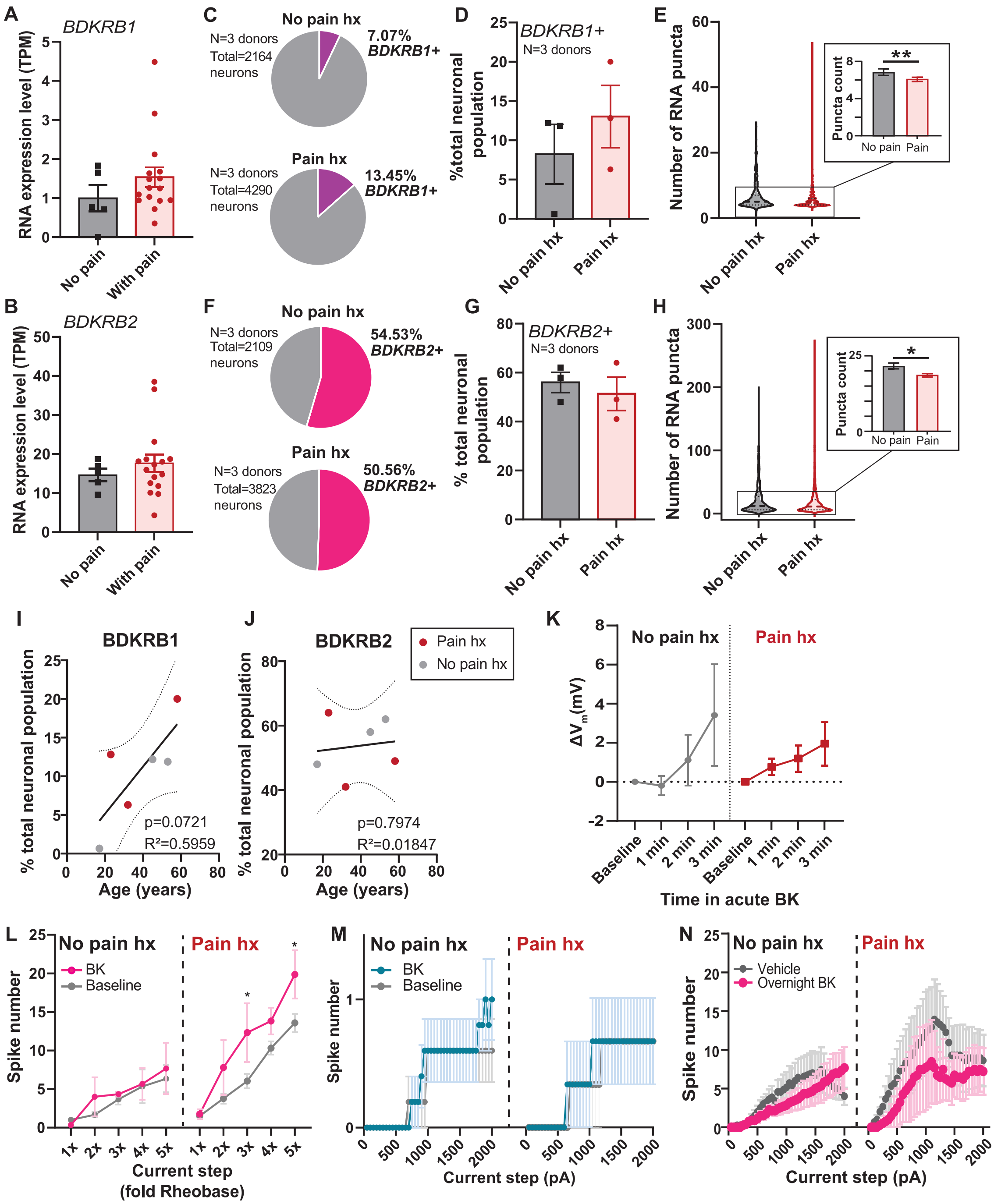

Figure 6. Bradykinin (BK) receptor expression and BK-mediated sensitization in donors with documented pain history. Whole-tissue *BDKRB1* (A) and *BDKRB2* (B) expression level, in TPM, in hDRG from cancer patients with or without neuropathic pain (data reanalyzed from North et al., 2019[43]). (C) Pie chart showing relative proportion of *BDKRB1*-positive hDRG neurons in a pooled sample of hDRG neurons (n= 2164-4290 from 3 donors per group; 1207-1632 neurons per each donor in the "pain hx" group, 300-950 neurons per each donor in the "no pain hx" group). (D) Percentage of *BDKRB1*-expressing DRG neurons per individual donor (N = 3 donors, 2-3 sections and 323-1632 cells per donor). (E) Distribution of *BDKRB1* RNA expression within each *BDKRB1*-positive cell. Dotted line on violin plot indicates median number of *BDKRB1* puncta per neuron. Inset shows a bar graph of the mean puncta count per cell for *BDKRB1* (n = 152-429 *BDKRB1*+ neurons from 3 donors for each condition; **$P < 0.01$, Mann–Whitney test). (F) Pie chart displaying relative proportion of *BDKRB2*-positive hDRG in a pooled sample of hDRG neurons (n = 2109-3823 neurons pooled from 3 donors per group; 963-1890 neurons per donor in the "pain hx" group, 340-1044 neurons per donor in the "No pain hx" group). (G) Percentage of *BDKRB2*-expressing DRG neurons per individual donor (N = 3 donors, 338-1890 cells per donor). (I) Distribution of *BDKRB2* RNA expression level within each *BDKRB2*-positive cell. Dotted line on violin plot indicated median number of *BDKRB2* puncta per neuron. Inset shows a bar graph of the mean puncta count per cell for *BDKRB2* (n = 655-1542 *BDKRB2*+ neurons from 3 donors for each condition; *$P < 0.05$, Mann–Whitney test). (I) Percentage of *BDKRB1*-expressing hDRG neurons per donor plotted against donor's age. Line shows line of best fit predicted by simple linear regression analysis (y = 0.3050*x - 0.9456), ± standard error of the slope (dotted line). (J) Percentage of *BDKRB2*-expressing hDRG neurons per donor plotted against donor's age. Solid line shows line of best fit predicted by simple linear regression analysis (y = 0.07413 * x + 50.85), ± standard error of the slope (dotted line). (K) Change in resting membrane potential over time during acute BK application in hDRG neurons from donors with (right; n = 5) and without (left; n = 15) history of pain. (L) Input–output curves of BK-sensitive repetitive-spiking hDRG neurons from donors with (right; n = 4) and without (left; n = 3) history of pain, before and during acute BK treatment. *$P < 0.05$, two-way repeated measures ANOVA with Šídák post hoc test. (M) Input–output curves of BK-sensitive single-spiking hDRG neurons from donors with (right; n = 3) and without (left; n = 5) history of pain, before and during acute BK treatment. (N) Effect of overnight BK or vehicle on input–output curves of hDRG neurons from donors with and without documented pain history (n=3-8 per group). Data represent mean ± s.e.m.

Table 5

Rheobase and input resistance of overnight vehicle-treated and BK-treated repetitive spiking neurons, segmented by donors' pain history.

	No pain history		Pain history	
	BK (n = 8)	Vehicle (n = 8)	BK (n = 4)	Vehicle (n = 3)
Rheobase (pA)	483.2 ± 68.33	369.7 ± 63.60	538.5 ± 124.9*	133.5 ± 33.45
Input resistance (MΩ)	76.82 ± 14.09†	190.0 ± 46.26	60.06 ± 10.10‡	147.5 ± 9.114

* $P = 0.0571$, Mann–Whitney test compared with vehicle.
† $P < 0.05$, Mann–Whitney test compared with vehicle.
‡ $P < 0.01$, unpaired t test compared with vehicle.

shift we observed in our study. Overnight exposure to BK has been associated increased trafficking of low voltage-activated calcium channels in rat DRG, which may contribute to changes in neuronal excitability and firing patterns we observed in our study.[24] In addition, BK receptor internalization and desensitization after prolonged BK exposure may further contribute to the hypoexcitability observed in our experiments. Internalized, endosomal GPCRs have been shown to engage in further signaling that modulates neuronal excitability in mouse DRGs.[25,26] As BK receptor desensitization is dependent on both duration and concentration,[4,14,39,52] it may be interesting to test whether prolonged exposure to low (eg, 1 nM) concentrations of BK also leads to hypoexcitability.

A large number of preclinical studies have shown that pain-inducing injury and inflammation can upregulate B2 receptor levels and induce B1 receptor synthesis in the rodent DRG.[13,15,28,30,34,43,49,63,65] In this study, we attempted to determine whether donor's history of pain similarly correlates with differential levels of BK receptor expression. Usage of tissue from human organ donors presents several limitations, including (a) variation in pain phenotype, (b) inability to test which neurons within the DRG innervate the site of pain, (c) large variability in demographic and medical histories within the donor pool, and (d) limited availability of samples. Despite these sources of variability, our analyses suggest that donor's pain history may be associated with greater number of B1 receptor-expressing neurons in the hDRG; this is also supported by our functional data that suggests that in repetitive-spiking neurons from donors with pain history, acute BK has a more robust effect on neuronal excitability. If pain-inducing injury or inflammatory insult leads to de novo synthesis of B1 receptors at low levels in hDRG neurons, then this would account for the seemingly opposite trends of greater percentage of *BDKRB1*+ neurons but lower average within-cell expression level of *BDKRB1* in hDRG from pain donors. These trends may also explain why the relative increase in B1 receptor expression in the pain history group is much smaller in the whole-DRG transcriptomics data set from cancer patients,[47] as whole-tissue transcriptomics cannot distinguish between differing expression levels in multiple neurons. In addition, the difference in the pain pathology of the donors in the 2 data sets (radicular, neuropathic, or cancer vs predominantly musculoskeletal), as well as the inclusion of nonneuronal cells in the whole transcriptomics analysis, may additionally contribute to the differences in the results of the 2 analyses. In line with several preclinical rodent studies that have implicated B1 receptor as an integral player in various pain models,[13,30,43,63,65] our findings—that B1 receptor expression in hDRG may be dynamically regulated by pain history—point to B1 receptor blockade as a potential strategy for achieving analgesia for some pain conditions in humans. Additional studies using hDRG as translational platform may shed light on the relative efficacy of B1 and B2 receptor antagonists in reducing neuronal hyperexcitability and inform which of the 2 BK receptors should be targeted to most effectively reduce pain in humans.

We were surprised to see a positive linear trend between age and proportion of B1 receptor-expressing neurons in the hDRG, which suggests that age alone may also be an important factor associated with BK receptor expression. Age-related increases in B1 or B2 receptor levels have been previously reported in rat and human cardiac tissue.[27,31] A larger data set and sample pool would allow us to more effectively conclude whether pain history, pathology, and age are significant predictors of B1 receptor expression among hDRG neurons at a population level.

Previous studies have shown that satellite glial cells in rodent DRG and trigeminal ganglia express functional BK receptors and appear to play a role in BK-induced inward current in rat sensory neurons.[8,22] Our results demonstrate that B2 receptors are also expressed on human satellite glial cells. This finding raises the possibility that satellite glial cells may be involved in BK-mediated neuronal hyperexcitability in hDRG. Recent studies have shown that satellite glial cells in mouse and rat DRG can release inflammatory interleukins, PGE2, and TNF-α, which can, in turn, induce hyperexcitability in DRG neurons.[35,59] Whether activation of B2 receptors on satellite glial cells can trigger similar inflammatory glia–neuronal signaling is an interesting avenue for further research.

Although many studies in rodents have previously shown that BK antagonists can be analgesic across multiple different models of pain,[11,15,18,20,43,45,65] there have been few published clinical studies assessing the efficacy of BK receptor antagonists in human pain patients. Icatibant (HOE-140) is a B2 receptor antagonist that has been FDA-approved for the treatment of hereditary angioedema (HAE), a rare and painful inflammatory disorder. Although BK signaling has been implicated in multiple other painful disorders (notably, arthritis) in human studies,[7,23,42] the progress towards repurposing this orphan drug for other inflammatory or generalized pain conditions has been slow. For example, according to the NIH's database on clinicaltrials.gov, only one clinical study has been conducted since 2000 to study icatibant's efficacy against pain in non-HAE patients. A study published in 2008[58] found that arthritis patients who received intra-articular injections of icatibant experienced a significant improvement in pain at rest and during activity compared with the placebo group. Despite the small sample size, results of this study provide promising evidence of analgesic efficacy of BK receptor antagonists in a wider range of painful inflammatory disorders.[58]

Altogether, our findings suggest the mechanism underlying BK-mediated pain and sensitization are generally conserved from rodents to human. The consistency in BK receptor expression patterns and its acute effects across species makes BK signaling an ideal target for further study and drug development that could be probed using both preclinical (eg, rodent) and translational (eg, primary human neuronal culture) approaches. In the context of previous studies in rodents and the clinical work outlined above, our study further supports the notion that BK signaling in the peripheral nervous system is a viable target for pain relief in multiple disorders in humans.

Conflict of interest statement

The authors have no conflict of interest to declare.

Acknowledgements

This work was supported by the American Heart Association through Predoctoral Fellowship #828671 (J.Y.) and by the National Institutes of Health through the NIH HEAL Initiative under award number U19NS130607 (R.G.). The authors thank the donors, their families, and Mid-America Transplant, without whom this research would not have been possible; J. Lemen for his support during surgical extractions; and the past and present members of the Gereau lab for their helpful comments and critiques. The authors declare no conflicts of interest.

Data availability statement: The data that support the findings of this study are available from the corresponding author, R. W. Gereau, upon reasonable request.

Supplemental digital content

Supplemental digital content associated with this article can be found online at http://links.lww.com/PAIN/B904.

Article history:
Received 21 April 2023
Received in revised form 8 June 2023
Accepted 26 June 2023
Available online 13 September 2023

References

[1] Avraham O, Chamessian A, Feng R, Yang L, Halevi AE, Moore AM, Gereau RW, Cavalli V. Profiling the molecular signature of satellite glial cells at the single cell level reveals high similarities between rodents and humans. PAIN 2022;163:2348–64.
[2] Banik RK, Kozaki Y, Sato J, Gera L, Mizumura K. B2 receptor-mediated enhanced bradykinin sensitivity of rat cutaneous C-fiber nociceptors during persistent inflammation. J Neurophysiol 2001;86: 2727–35.
[3] Bavencoffe A, Spence EA, Zhu MY, Garza-Carbajal A, Chu KE, Bloom OE, Dessauer CW, Walters ET. Macrophage migration inhibitory factor (MIF) makes complex contributions to pain-related hyperactivity of nociceptors after spinal cord injury. J Neurosci 2022;42:5463–80.
[4] Blaukat A, Pizard A, Breit A, Wernstedt C, Alhenc-Gelas F, Müller-Esterl W, Dikic I. Determination of bradykinin B2 receptor in vivo phosphorylation sites and their role in receptor function. J Biol Chem 2001;276:40431–40.
[5] Boix F, Røe C, Rosenborg L, Knardahl S. Kinin peptides in human trapezius muscle during sustained isometric contraction and their relation to pain. J Appl Physiol 2005;98:534–40.
[6] Burgess GM, Mullaney I, McNeill M, Dunn PM, Rang HP. Second messengers involved in the mechanism of action of bradykinin in sensory neurons in culture. J Neurosci 1989;9:3314–25.
[7] Cassim B, Shaw OM, Mazur M, Misso NL, Naran A, Langlands DR, Thompson PJ, Bhoola KD. Kallikreins, kininogens and kinin receptors on circulating and synovial fluid neutrophils: role in kinin generation in rheumatoid arthritis. Rheumatology 2009;48:490–6.
[8] Ceruti S, Fumagalli M, Villa G, Verderio C, Abbracchio MP. Purinoceptor-mediated calcium signaling in primary neuron-glia trigeminal cultures. Cell Calcium 2008;43:576–90.
[9] Cesare P, Dekker LV, Sardini A, Parker PJ, McNaughton PA. Specific involvement of PKC-ε in sensitization of the neuronal response to painful heat. Neuron 1999;23:617–24.
[10] Cesare P, Mcnaughton P. A novel heat-activated current in nociceptive neurons and its sensitization by bradykinin. Proc Natl Acad Sci U S A 1996;93:15435–9.
[11] Chen Q, Vera-Portocarrero LP, Ossipov MH, Vardanyan M, Lai J, Porreca F. Attenuation of persistent experimental pancreatitis pain by a bradykinin b2 receptor antagonist. Pancreas 2010;39:1220–5.
[12] Davidson S, Copits BA, Zhang J, Page G, Ghetti A, Gereau RW. Human sensory neurons: membrane properties and sensitization by inflammatory mediators. PAIN 2014;155:1861–70.
[13] Dray A, Patel IAA, Perkins MNN, Rueff A. Bradykinin-induced activation of nociceptors: receptor and mechanistic studies on the neonatal rat spinal cord-tail preparation in vitro. Br J Pharmacol 1992; 107:1129–34.
[14] Fathy DB, Leeb T, Mathis SA, Leeb-Lundberg LMF. Spontaneous human B2 bradykinin receptor activity determines the action of partial agonists as agonists or inverse agonists. Effect of basal desensitization. J Biol Chem 1999;274:29603–6.
[15] Ferreira J, Beirith A, Mori MAS, Araújo RC, Bader M, Pesquero JB, Calixto JB. Reduced nerve injury-induced neuropathic pain in kinin B1 receptor knock-out mice. J Neurosci 2005;25:2405–12.
[16] Ford ZK, Reker AN, Chen S, Kadakia F, Bunk A, Davidson S. Cannabinoid receptor 1 expression in human dorsal root ganglia and CB13-induced bidirectional modulation of sensory neuron activity. Front Pain Res 2021; 2:1–9.
[17] Fujita M, Andoh T, Ohashi K, Akira A, Saiki I, Kuraishi Y. Roles of kinin B1 and B2 receptors in skin cancer pain produced by orthotopic melanoma inoculation in mice. Eur J Pain 2010;14:588–94.
[18] George J, Pulickal SJ, Singh A, Gautam M, Prasoon P, Kumar R, Ray SB. Locally mediated analgesic effect of bradykinin type 2 receptor antagonist HOE 140 during acute inflammatory pain in rats. J Burn Care Res 2014; 35:e391–e398.
[19] Gerdle B, Hilgenfeldt U, Larsson B, Kristiansen J, Søgaard K, Rosendal L. Bradykinin and kallidin levels in the trapezius muscle in patients with work-related trapezius myalgia, in patients with whiplash associated pain, and in healthy controls—a microdialysis study of women. PAIN 2008;139: 578–87.
[20] Gonçalves ECD, Vieira G, Gonçalves TR, Simões RR, Brusco I, Oliveira SM, Calixto JB, Cola M, Santos ARS, Dutra RC. Bradykinin receptors play a critical role in the chronic post-ischaemia pain model. Cell Mol Neurobiol 2021;41:63–78.
[21] Hamza M, Wang XM, Adam A, Brahim JS, Rowan JS, Carmona GN, Dionne RA. Kinin B1 receptors contributes to acute pain following minor surgery in humans. Mol Pain 2010;6:12.
[22] Heblich F, England S, Docherty RJ. Indirect actions of bradykinin on neonatal rat dorsal root ganglion neurones: a role for non-neuronal cells as nociceptors. J Physiol 2001;536:111–21.
[23] Hernández CCC, Donadi EA, Reis ML. Kallikreins and kininogens in saliva and plasma of patients presenting with rheumatoid arthritis. Scand J Rheumatol 2002;31:38–40.
[24] Huang D, Liang C, Zhang F, Men H, Du X, Gamper N, Zhang H. Inflammatory mediator bradykinin increases population of sensory neurons expressing functional T-type $Ca2+$ channels. Biochem Biophys Res Commun 2016;473:396–402.
[25] Jimenez-Vargas NN, Gong J, Wisdom MJ, Jensen DD, Latorre R, Hegron A, Teng S, DiCello JJ, Rajasekhar P, Veldhuis NA, Carbone SE, Yu Y, Lopez-Lopez C, Jaramillo-Polanco J, Canals M, Reed DE, Lomax AE, Schmidt BL, Leong KW, Vanner SJ, Halls ML, Bunnett NW, Poole DP. Endosomal signaling of delta opioid receptors is an endogenous mechanism and therapeutic target for relief from inflammatory pain. Proc Natl Acad Sci U S A 2020;117:15281–92.
[26] Jimenez-Vargas NN, Pattison LA, Zhao P, Lieu TM, Latorre R, Jensen DD, Castro J, Aurelio L, Le GT, Flynn B, Herenbrink CK, Yeatman HR, Edgington-Mitchell L, Porter CJH, Halls ML, Canals M, Veldhuis NA, Poole DP, McLean P, Hicks GA, Scheff N, Chen E, Bhattacharya A, Schmidt BL, Brierley SM, Vanner SJ, Bunnett NW. Protease-activated receptor-2 in endosomes signals persistent pain of irritable bowel syndrome. Proc Natl Acad Sci U S A 2018;115:E7438–E7447.
[27] Kintsurashvili E, Duka A, Ignjacev I, Pattakos G, Gavras I, Gavras H. Age-related changes of bradykinin B1 and B2 receptors in rat heart. Am J Physiol Heart Circ Physiol 2005;289:H202-5.

[28] Lee YJ, Zachrisson O, Tonge DA, McNaughton PA. Upregulation of bradykinin B2 receptor expression by neurotrophic factors and nerve injury in mouse sensory neurons. Mol Cel Neurosci 2002;19:186–200.
[29] Lenhard W, Lenhard Z. Computation of effect sizes. 2016. Available at: https://www.psychometrica.de/effect_size.html.
[30] Levy D, Zochodne DW. Increased mRNA expression of the B1 and B2 bradykinin receptors and antinociceptive effects of their antagonists in an animal model of neuropathic pain. PAIN 2000;86:265–71.
[31] Liesmaa I, Shiota N, Kokkonen JO, Kovanen PT, Lindstedt KA. Bradykinin type-2 receptor expression correlates with age and is subjected to transcriptional regulation. Int J Vasc Med 2012;2012:159646.
[32] Liu B, Linley JE, Du X, Zhang X, Ooi L, Zhang H, Gamper N. The acute nociceptive signals induced by bradykinin in rat sensory neurons are mediated by inhibition of M-type K+ channels and activation of Ca2+-activated Cl- channels. J Clin Invest 2010;120:1240–52.
[33] Longhurst H, Cicardi M. Hereditary angio-oedema. Lancet 2012;379: 474–81.
[34] Lu J, Xing J, Li J. Bradykinin B2 receptor contributes to the exaggerated muscle mechanoreflex in rats with femoral artery occlusion. Am J Physiol 2013;304:H1166–74.
[35] Luo H, Liu HZ, Zhang WW, Matsuda M, Lv N, Chen G, Xu ZZ, Zhang YQ. Interleukin-17 regulates neuron-glial communications, synaptic transmission, and neuropathic pain after chemotherapy. Cell Rep 2019; 29:2384–2397.e5.
[36] Hargreaves K, Troullos ES, Dionne RA, Schmidt EA, Schafer SC, Joris JL. Bradylkinin is increased during acute and chronic inflammation Therapeutic implications. Clin Pharmacol Ther 1988;44:613–21.
[37] Manning DC, Raja SN, Meyer RA, Campbell JN. Pain and hyperalgesia after intradermal injection of bradykinin in humans. Clin Pharmacol Ther 1991;50:721–9.
[38] Mathivanan S, Devesa I, Changeux J-PP, Ferrer-Montiel A. Bradykinin induces TRPV1 exocytotic recruitment in peptidergic nociceptors. Front Pharmacol 2016;7:1–12.
[39] McGehee DS, Goy MF, Oxford GS. Involvement of the nitric oxide-cyclic GMP pathway in the desensitization of bradykinin responses of cultured rat sensory neurons. Neuron 1992;9:315–24.
[40] McGuirk SM, Dolphin AC. G-protein mediation in nociceptive signal transduction: an investigation into the excitatory action of bradykinin in a subpopulation of cultured rat sensory neurons. Neuroscience 1992;49: 117–28.
[41] Meng J, Wang J, Lawrence G, Dolly JO. Synaptobrevin I mediates exocytosis of CGRP from sensory neurons and inhibition by botulinum toxins reflects their anti-nociceptive potential. J Cel Sci 2007;120: 2864–74.
[42] Meng X, Li Y, Li Q, Yang J, An M, Fu X, Zhang S, Chen J. Involvement of bradykinin and bradykinin B1 receptor in patients with endometriosis. Exp Ther Med 2021;22:1240.
[43] Meotti F, Campos R, da Silva K, Paszcuk A, Costa R, Calixto J. Inflammatory muscle pain is dependent on the activation of kinin B1 and B2 receptors and intracellular kinase pathways. Br J Pharmacol 2012; 166:1127–39.
[44] Moy JK, Hartung JE, Duque MG, Friedman R, Nagarajan V, Loeza-Alcocer E, Koerber HR, Christoph T, Schröder W, Gold MS. Distribution of functional opioid receptors in human dorsal root ganglion neurons. PAIN. 2020;161:1636–49.
[45] Murase S, Terazawa E, Queme F, Ota H, Matsuda T, Hirate K, Kozaki Y, Katanosaka K, Taguchi T, Urai H, Mizumura K. Bradykinin and nerve growth factor play pivotal roles in muscular mechanical hyperalgesia after exercise (Delayed-onset muscle soreness). J Neurosci 2010;30: 3752–61.
[46] Nishimura M, Segami N, Kaneyama K, Suzuki T, Miyamaru M. Relationships between pain-related mediators and both synovitis and joint pain in patients with internal derangements and osteoarthritis of the temporomandibular joint. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2002;94:328–32.
[47] North RY, Li Y, Ray P, Rhines LD, Tatsui CE, Rao G, Johansson CA, Zhang H, Kim YH, Zhang B, Dussor G, Kim TH, Price TJ, Dougherty PM. Electrophysiological and transcriptomic correlates of neuropathic pain in human dorsal root ganglion neurons. Brain 2019;142:1215–26.
[48] Odem MA, Bavencoffe AG, Cassidy RM, Lopez ER, Tian J, Dessauer CW, Walters ET. Isolated nociceptors reveal multiple specializations for generating irregular ongoing activity associated with ongoing pain. Pain 2018;159:2347–62.
[49] Perkins MN, Campbell E, Dray A. Antinociceptive activity of the bradykinin B1 and B2 receptor antagonists, des-Arg9, [Leu8]-BK and HOE 140, in two models of persistent hyperalgesia in the rat. PAIN 1993;53:191–7.
[50] Petersen M, Eckert AS, Second Von Banchet G, Heppelmann B, Klusch A, Kniffki KD. Plasticity in the expression of bradykinin binding sites in sensory neurons after mechanical nerve injury. Neuroscience 1998;83: 949–59.
[51] Ray P, Torck A, Quigley L, Wangzhou A, Neiman M, Rao C, Lam T, Kim JY, Kim TH, Zhang MQ, Dussor G, Price TJ. Comparative transcriptome profiling of the human and mouse dorsal root ganglia: an RNA-seq-based resource for pain and sensory neuroscience research. Pain 2018;159: 1325–45.
[52] Roberts RA, Gullick WJ. Bradykinin receptors undergo ligand-induced desensitization. Biochemistry 1990;29:1975–9.
[53] del Rosario J, McIlvried LA, Pullen MY, Wangzhou A, Sheahan TD, Slivicki RA, Price TJ, Copits BA, Bertels Z, Chamessian A, Li J, Widman A, Yi J, Gereau RW. Sustained depolarization induces homeostatic plasticity in mouse and human sensory neurons. 2022 Neuroscience Meeting Planner. San Diego, CA: Society for Neuroscience, 2022. p. Program No. 318.19.
[54] Rostock C, Schrenk-Siemens K, Pohle J, Siemens J. Human vs. Mouse nociceptors—similarities and differences. Neuroscience 2018;387: 13–27.
[55] Schuligoi R, Donnerer J, Amann R. Bradykinin-induced sensitization of afferent neurons in the rat paw. Neuroscience 1994;59:211–5.
[56] Sheahan TD, Valtcheva MV, McIlvried LA, Pullen MY, Baranger DAA, Gereau RW. Metabotropic glutamate receptor 2/3 (mGluR2/3) activation suppresses TRPV1 sensitization in mouse, but not human, sensory neurons. eNeuro 2018;5:412–29.
[57] Shiers S, Klein RM, Price TJ. Quantitative differences in neuronal subpopulations between mouse and human dorsal root ganglia demonstrated with RNAscope in situ hybridization. PAIN 2020;161: 2410–24.
[58] Song IH, Althoff CE, Hermann KG, Scheel AK, Knetsch T, Burmester GR, Backhaus M. Contrast-enhanced ultrasound in monitoring the efficacy of a bradykinin receptor 2 antagonist in painful knee osteoarthritis compared with MRI. Ann Rheum Dis 2009;68:75–83.
[59] Souza GR, Talbot J, Lotufo CM, Cunha FQ, Cunha TM, Ferreira SH. Fractalkine mediates inflammatory pain through activation of satellite glial cells. Proc Natl Acad Sci U S A 2013;110:11193–8.
[60] Supowit SC, Zhao H, Katki KA, Gupta P, DiPette DJ. Bradykinin and prostaglandin E1 regulate calcitonin gene-related peptide expression in cultured rat sensory neurons. Regul Pept 2011;167:105–11.
[61] Tavares-Ferreira D, Shiers S, Ray PR, Wangzhou A, Jeevakumar V, Sankaranarayanan I, Cervantes AM, Reese JC, Chamessian A, Copits BA, Dougherty PM, Gereau IVRW, Burton MD, Dussor G, Price TJ. Spatial transcriptomics of dorsal root ganglia identifies molecular signatures of human nociceptors. Sci Transl Med 2022;14:8186.
[62] Valtcheva MV, Copits BA, Davidson S, Sheahan TD, Pullen MY, McCall JG, Dikranian K, Gereau RW. Surgical extraction of human dorsal root ganglia from organ donors and preparation of primary sensory neuron cultures. Nat Protoc 2016;11:1877–88.
[63] Vellani V, Zachrisson O, McNaughton PA. Functional bradykinin B1 receptors are expressed in nociceptive neurones and are upregulated by the neurotrophin GDNF. J Physiol 2004;560:391–401.
[64] Wright AB, Sukhanova KY, Elmslie KS. Kv7 channels are potential regulators of the exercise pressor reflex. J Neurophysiol 2021;560:126.
[65] Yamaguchi-Sase S, Hayashi I, Okamoto H, Nara Y, Matsuzaki S, Hoka S, Majima M. Amelioration of hyperalgesia by kinin receptor antagonists or kininogen deficiency in chronic constriction nerve injury in rats. Inflamm Res 2003;52:164–9.
[66] Yi J, Bertels Z, Rosario JDel, Slivicki RA, Widman AJ, Gereau RW. Bradykinin-mediated sensitization and receptor expression in human sensory neurons. J Pain 2022;23:14.