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Modulation of human dorsal root ganglion neuron excitability by Nav1.7 inhibition

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eLife Assessment

Fujita et al. examine the effects of AM-2099, a Nav1.7 inhibitor, on the excitability of human dorsal root ganglion neurons and compare these results to their prior study of Nav1.8 inhibition by suzetrigine. They show that the Nav1.7 inhibitor primarily alters action potential threshold and initiation, but not repetitive firing, whereas Nav1.8 inhibition elicits much stronger inhibition on repetitive firing. These complementary roles of Nav1.7 and Nav1.8 provide a plausible cellular explanation for the limited clinical success of Nav1.7 inhibitors compared to Nav1.8 inhibitors for chronic pain. While the conclusions are **important** and **solid**, there are some key shortcomings that should be addressed to strengthen the study.

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Abstract

Nav1.7 voltage-gated sodium channels are strongly expressed in human primary painsensing neurons (nociceptors) and selective Nav1.7 inhibitors have been developed as possible therapeutic agents for treating pain, so far with disappointing clinical results. In contrast, a selective Nav1.8 channel inhibitor (suzetrigine) has had successful clinical trials. Because nociceptors express both Nav1.7 and Nav1.8 channels, it is of interest to compare effects of Nav1.7 and Nav1.8 inhibitors on the excitability of human nociceptors. To compare with previous results with suzetrigine, we characterized the effects of a selective Nav.7 inhibitor, AM-2099, on action potential generation and repetitive firing of dissociated human dorsal root ganglion neurons, studied at 37°C. Inhibition of Nav1.7 channels by 600 nM AM-2099 generally produced a substantial depolarizing shift of action potential threshold, an increase in rheobase, a decrease in action potential upstroke velocity, decrease in action potential peak, and prolongation of refractory period. Compared to inhibition of Nav1.8 channels, inhibition of Nav1.7 channels had larger effects on threshold and maximal upstroke velocity, while action potential peak was reduced similarly by both. Nav1.8 inhibition produced much more dramatic reduction of repetitive firing than Nav1.7 inhibition. The results show that although the excitability of human DRG neurons is affected by inhibition of Nav1.7 channels, most notably by an increase in threshold and increase in refractory period, repetitive firing of the neurons in response to strong stimuli is little affected.

Significance statement

Nav1.7 sodium channels are highly expressed in primary pain-sensing neurons and humans with null mutations in Nav1.7 channels have loss of pain sensation. However, unlike the Nav1.8 inhibitor suzetrigine, Nav1.7 inhibitors have so far not reached clinical use. We compared effects

of Nav1.7 on electrical excitability of human dorsal root ganglion neurons with those of suzetrigine and found that while Nav1.7 inhibition affects spike threshold more than suzetrigine, there is little effect on repetitive firing with strong stimuli.

Introduction

The discovery of families with congenital insensitivity to pain traced to loss of function mutations in Nav1.7 voltage-dependent sodium channels (Cox et al., 2006; Goldberg et al., 2007; reviewed by Dib-Hajj et al., 2013; Drissi et al., 2020) suggested that Nav1.7 channels could be an ideal pharmacological target and launched many drug development efforts. However, PF-05089771, the first selective Nav1.7-inhibitor to reach large-scale clinical trials, was much less effective on pain in humans than anticipated (McDonnell et al., 2018; Siebenga et al., 2020; reviewed by Alsaloum et al., 2020) and many though not all Nav1.7-focused drug development efforts have been discontinued (Eagles et al., 2022; Alsaloum et al., 2025; Banh et al., 2025; Yang et al., 2025).

The reasons that Nav1.7 inhibitors have so far failed to advance to successful clinical studies are still unclear. One potential limitation of selectively inhibiting Nav1.7 channels is that primary pain-sensing neurons (nociceptors) express not only Nav1.7 channels but also have prominent expression of Nav1.8 channels (reviewed by Cummins et al., 2007; Bennett et al. 2019; Alles et al., 2021; Goodwin and McMahon, 2021). In the cell bodies of primary nociceptors of rat and mouse, Nav1.8 channels carry most of the sodium current driving the action potential Renganathan et al., 2001; Blair and Bean, 2002; reviewed by Han et al., 2016) and are particularly important for supporting repetitive firing of the neurons (Elliott and Elliott, 1993; Cummins and Waxman, 1997; Rush et al., 1998; Renganathan et al., 2001; Blair and Bean, 2003; Matsutomi et al., 2006; Patrick Harty and Waxman, 2007; Han et al., 2015). Recently, a potent and highly-selective Nav1.8 inhibitor, suzetrigine, was approved by the United States Food and Drug Administration for treatment of acute pain based on results in clinical studies on post-operative pain (Jones et al., 2023; Bertoch et al., 2025). However, although suzetrigine was more effective than placebo in these studies on post-operative pain, it was far from producing complete analgesia.

We lack a good understanding of the different roles of Nav1.7 and Nav1.8 channels in controlling the excitability of human primary nociceptors. Inhibitors of both Nav1.7 and Nav1.8 channels have been shown to reduce excitability of dorsal root ganglion neurons from human donors (Payne et al., 2015; Alexandrou et al., 2016; Osteen et al., 2025; Stewart et al., 2025), but for studies of Nav1.7 inhibitors, data is limited to a handful of cells studied with limited protocols (Alexandrou et al., 2016). Studies with suzetrigine inhibition of Nav1.8 channels showed only modest effects on action potential threshold and upstroke in most human DRG neurons (Stewart et al., 2025), suggesting a possible major role of Nav1.7 channels. Here we have examined effects of a Nav1.7 inhibitor, AM-2099, on excitability of DRG neurons from human neurons. We found that Nav1.7 inhibition has larger effects than Nav1.8 inhibition on action potential threshold and action potential upstroke but much less effect on ability of the neurons to fire repetitively during stimulation by maintained depolarization. Combined with the results of a similar study with suzetrigine, the results show that selective inhibition of either Nav1.7 or Nav1.8 channels alone has limited efficacy for inhibiting firing of human DRG neurons.

Results

We selected AM-2099 as a suitable Nav1.7 inhibitor, based on its potency and high degree of selectivity, >200-fold for Nav1.7 over Nav1.8 (Marx et al., 2016). Figure 1 shows the effects of 600 nM AM-2099 on action potentials in human DRG neurons studied at 37°C. Action potentials were evoked by short (0.5-ms) current injections. With this protocol, the action potential shape is not affected by the stimulating current, making it easier to interpret changes in action potential shape produced by channel inhibition. Figure 1A-B shows a typical effect of AM-2099: a slowing of the rising phase of the action potential and substantially reduced peak, with little effect on the

falling phase or after-hyperpolarization. Figure 1C-E show collected results for the effect of 600 nM AM-2099 on action potential parameters. In collected results, 600 nM AM-2099 produced a consistent decrease in maximum upstroke velocity to an average of 42 ± 6% of control, from 337 ± 42 to 154 ± 30 mV/mS (mean ± SEM, n=18; p=.0002, two-tailed Wilcoxon test), and a consistent decrease in action potential peak by an average of 16 ± 3 mV, from +30 ± 3 mV to +14 ± 5 mV (n=18; p=.0002, two-tailed test Wilcoxon test). Unlike the substantial decrease in the width of the action potential seen with suzetrigine inhibition of Nav1.8 channels (Stewart et al., 2025), inhibiting Nav1.7 channels by AM-2099 did not have a consistent effect on action potential width, increasing the width in 5 of 13 neurons and decreasing the width in 8 neurons (p =0.2, two-tailed test Wilcoxon test; width measured at 0 mV, with results including only neurons in which the peak was > 0 mV n=13).

AM-2099 consistently produced a depolarizing shift in the action potential threshold, determined by short (0.5-ms) current injections of increasing magnitude (Figure 2). In collected results, the threshold increased by an average of 9.4 ± 1.2 mV from -44.7 ± 2.2 mV to -35.4 ± 3.0 mV (n=18; p=.0002, two-tailed test Wilcoxon test). The 0.5-ms current injection required to reach threshold increased by an average of 20 ± 3% (n=18; p=.0002, two-tailed test Wilcoxon test).

We next explored the effect of inhibiting Nav1.7 channels on repetitive firing evoked by 1-s current injections. Figure 3A-B shows a typical example: the minimal current required to elicit a spike (rheobase) increased substantially but the frequency of firing evoked by large current injections did not change much. In collected results (Figure 3C), rheobase current increased by 69 ± 12%, from 2.4 ± 0.3 nA to 4.1 ± 0.6 nA (mean ± SEM, n=23; p=.0002, two-tailed Wilcoxon test). In 25 neurons tested, 14 fired multiple action potentials in control, and 13 of the 14 still fired multiple action potentials after application of AM-2099 (Figure 3D).

In a previous study, we were surprised to find that inhibiting Nav1.8 channels with suzetrigine had the counter-intuitive effect of decreasing the refractory period (Stewart et al., 2025). In contrast, inhibiting Nav1.7 channels resulted in an increase in the refractory period in 14 of 15 neurons tested (Figure 4), with an average increase to 1.8 ± 0.2 of the value in the control (mean ± SEM, n=15; p=.0013, two-tailed Wilcoxon test).

Discussion

Previous work has shown that, like mouse and rat small-diameter dorsal root ganglion neurons (reviewed by Rush et al., 2007; Bennett et al. 2019; Goodwin and McMahon, 2021), human dorsal root ganglion neurons express both Nav1.7 and Nav1.8 channels (Payne et al., 2015; Alexandrou et al., 2016; Zhang et al., 2017; Osteen et al., 2025). In a previous paper, we used the selective Nav1.8 inhibitor suzetrigine (VX-548) to explore how inhibition of Nav1.8 channels affects action potential generation in the neurons (Stewart et al., 2025). The results presented here using similar protocols show that inhibiting Nav1.7 channels also strongly affects the excitability of human DRG neurons but in different ways than inhibiting Nav1.8 channels. Figure 5 compares the effects of inhibiting Nav1.7 channels on action potential threshold, shape, and ability to fire repetitively with the effects of Nav1.8 inhibition determined in a previous study (Stewart et al., 2025), with recordings in both cases done at 37°C with the same protocols. Inhibiting Nav1.7 channels generally produces a larger effect on action potential threshold (Figure 5A) and maximum upstroke (Figure 5B) than Nav1.8 inhibition, while the effects on action potential peak were similar (Figure 5C). A major difference is that inhibiting Nav1.8 channels was generally much more effective than Nav1.7 inhibition in reducing repetitive firing during 1-sec current injections (Figure 5D).

The different effects of inhibiting Nav1.7 and Nav1.8 channels on action potential shape and repetitive firing fit well with their different voltage-dependence and kinetics as defined from studies on both human and rodent channels (Alexandrou et al., 2016; Köster et al., 2025; Vasylyev et al., 2025a,b) and with the different but overlapping roles of Nav1.7 and Nav1.8 channels in action potentials of mouse and rat DRG neurons (Vasylyev et al., 2025a,b; Xie et al., 2024). Nav1.7 channels are activated more quickly and at more negative voltages than Nav1.8

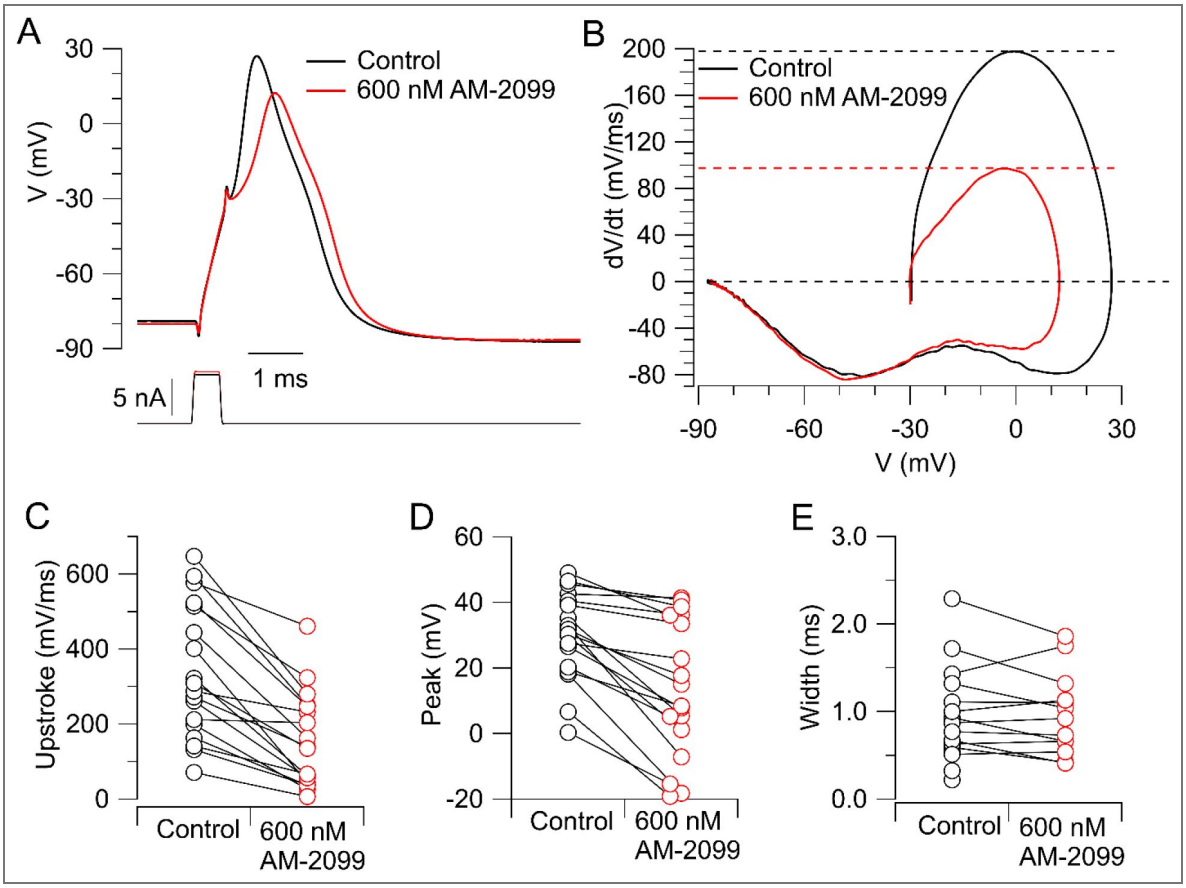

Figure 1. Effect of AM-2099 on action potentials in human DRG neurons.

A, Action potentials evoked by a short (0.5-ms) current injection before and after application of 600 nM AM-2099. Stimulating current was adjusted to be slightly larger in AM-2099 so that the voltage immediately after cessation of the stimulating current was the same as in control (because control has an active component in the last 0.1 ms of the stimulating pulse that is inhibited with AM-2099). B, Phase-plane plot of dV/dt versus V showing reduction of maximum upstroke velocity and peak. Upper dashed lines: maximum upstroke velocity in control (black) and with 600 nM AM-2099 (red). C, Collected results for effect of 600 nM AM-2099 on the maximal upstroke velocity of the action potential in 18 neurons. D, Collected results for effect of 600 nM AM-2099 on peak of the action potential evoked by a short current injection. E, Collected results for effect of 600 nM AM-2099 on the width of the action potential measured at 0 mV. In cells with values only in control, the action potential in AM-2099 had a peak < 0 mV.

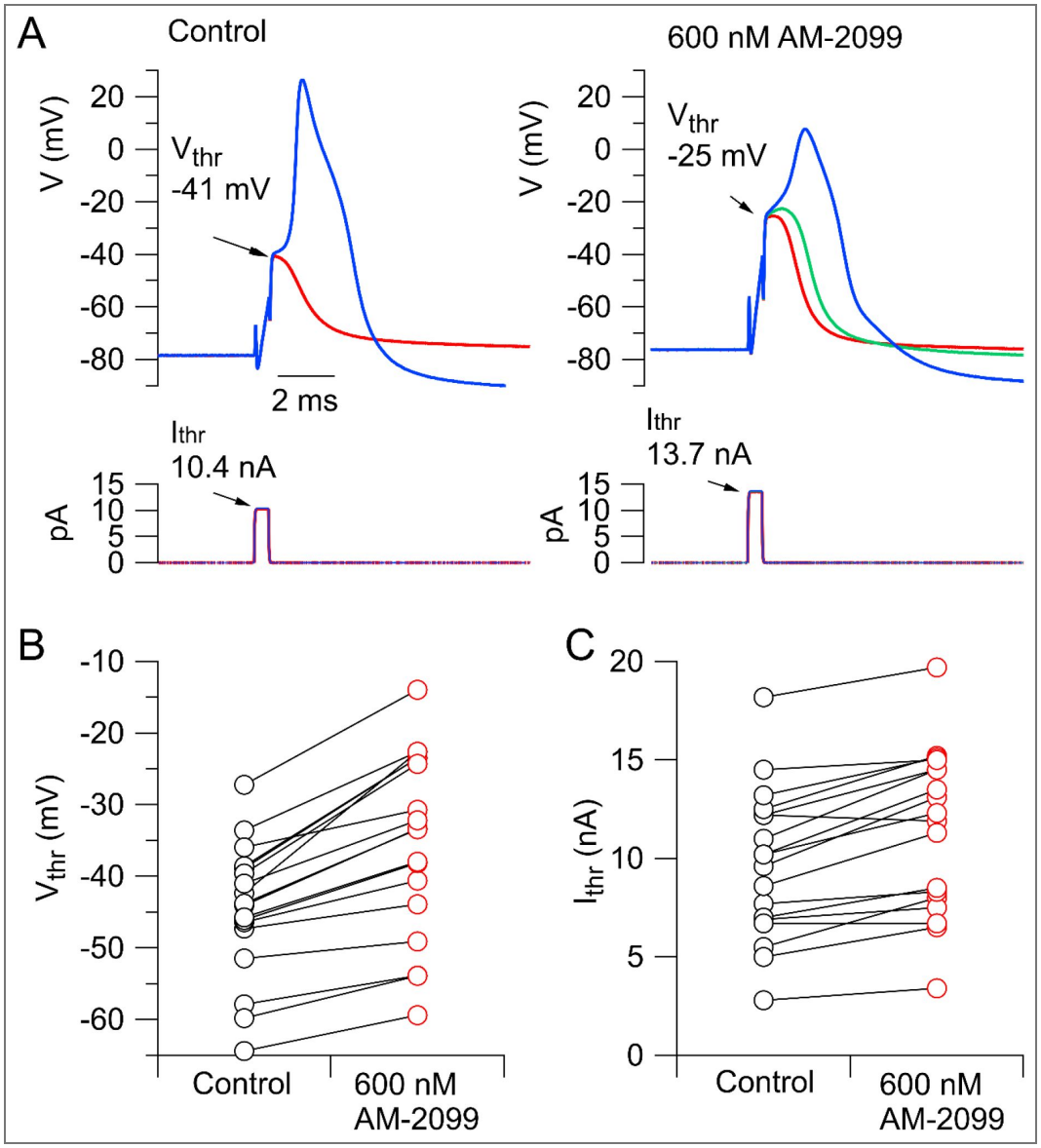

Figure 2. Effect of AM-2099 on action potential threshold.

Short (0.5-ms) current injections of different size were delivered, with gradations to find the level of current injection that first evoked an action potential. The voltage immediately after the smallest current injection that evoked a spike was considered as the threshold voltage. A, Just sub-threshold and supra-threshold current injections in control (left) and after application of 600 nM AM-2099 (right). B, Collected data for threshold voltage before and after 600 AM-2099 (n=18). C, Collected data for minimum current (0.5-ms current injection) that evoked an action potential (n=18).

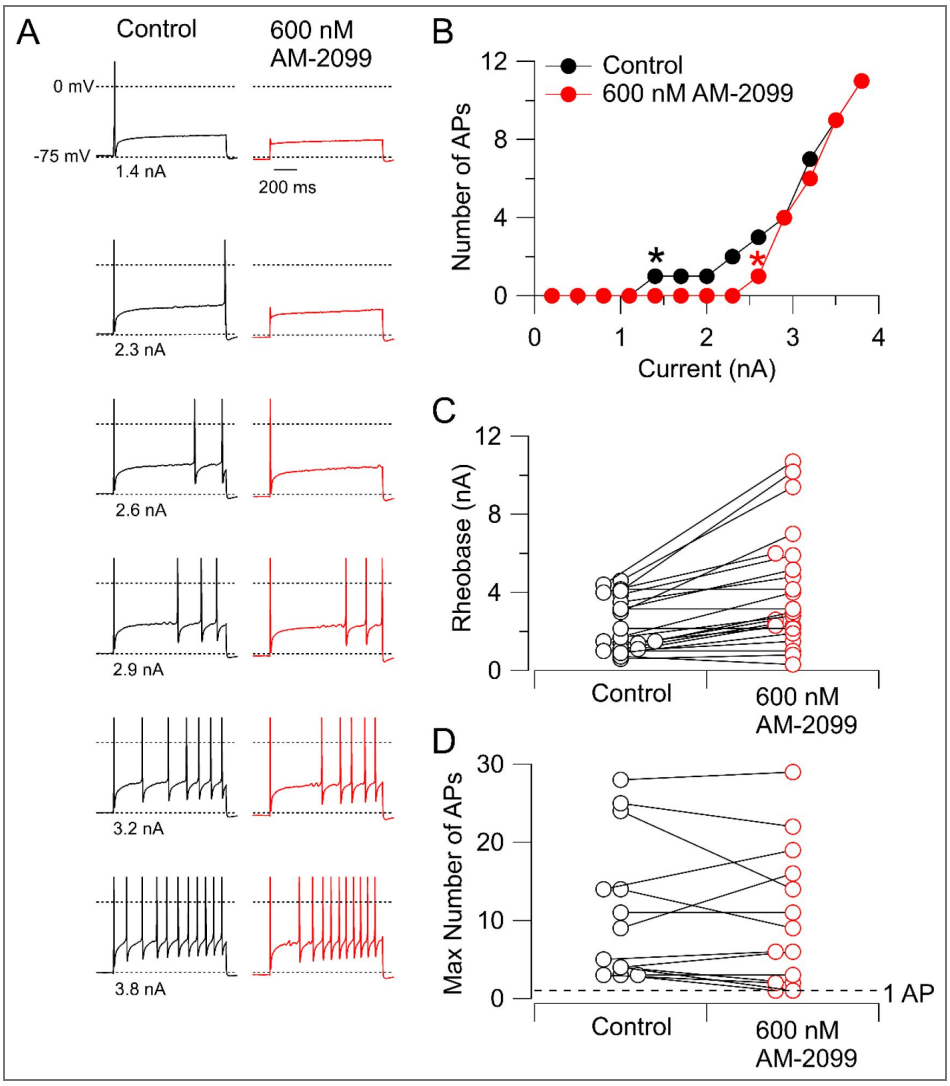

Figure 3. Effect of 600 nM AM-2099 on rheobase and repetitive firing.

A, Firing evoked by 1-s injections of current of increasing magnitude in control and after application of 600 nM AM-2099. B, Number of action potentials as a function of the injected current before and after 600 nM AM-2099 in this neuron. Asterisks indicate rheobase current in control (black) and in AM-2099 (red). C, Collected data for the effect of 600 nM AM-2099 on rheobase current. D, Collected results for the effect of 600 nM AM-2099 on the maximal number of action potentials during 1-s current injections over a range of magnitudes for neurons that fired more than one action potential in control. Dashed line drawn at 1 action potential.

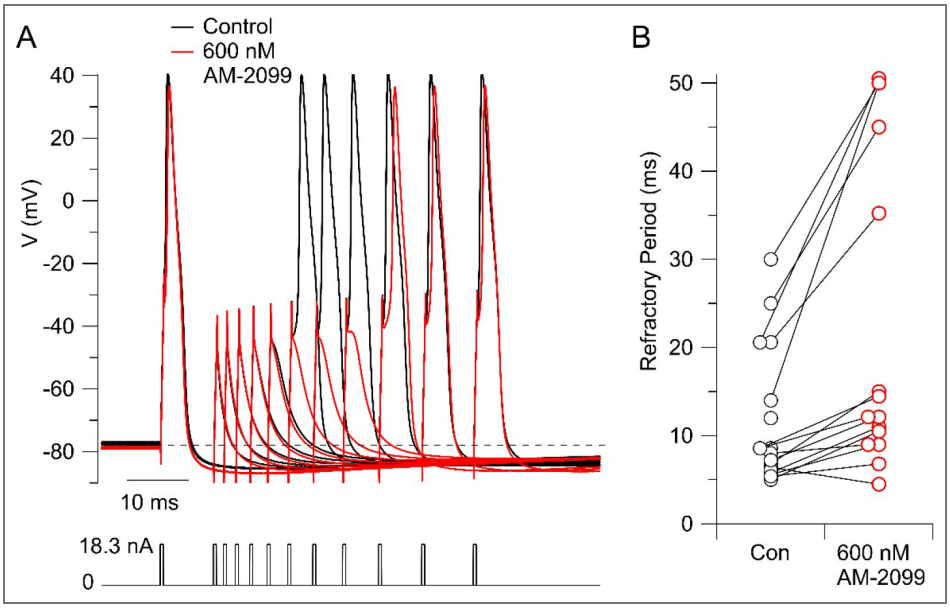

Figure 4. Prolongation of refractory period by AM-2099.

A, Action potentials were evoked by a pair of 0.5-ms current injections with a variable time between them, with magnitude of both set at 1.5-times the threshold current determined in control. The time between the two current injections was varied from longer to shorter to determine the refractory period with this stimulus. The figure shows superimposed sweeps from 11 different sets of times in control (black) and with 600 nm AM-2099 (red). In control, the second stimulus evoked an action potential with a spacing (start to start) of 20.6 ms, while after AM-2099, a spacing of 35.2 ms was required to evoke an action potential using the same stimuli. B, Collected results in 15 neurons.

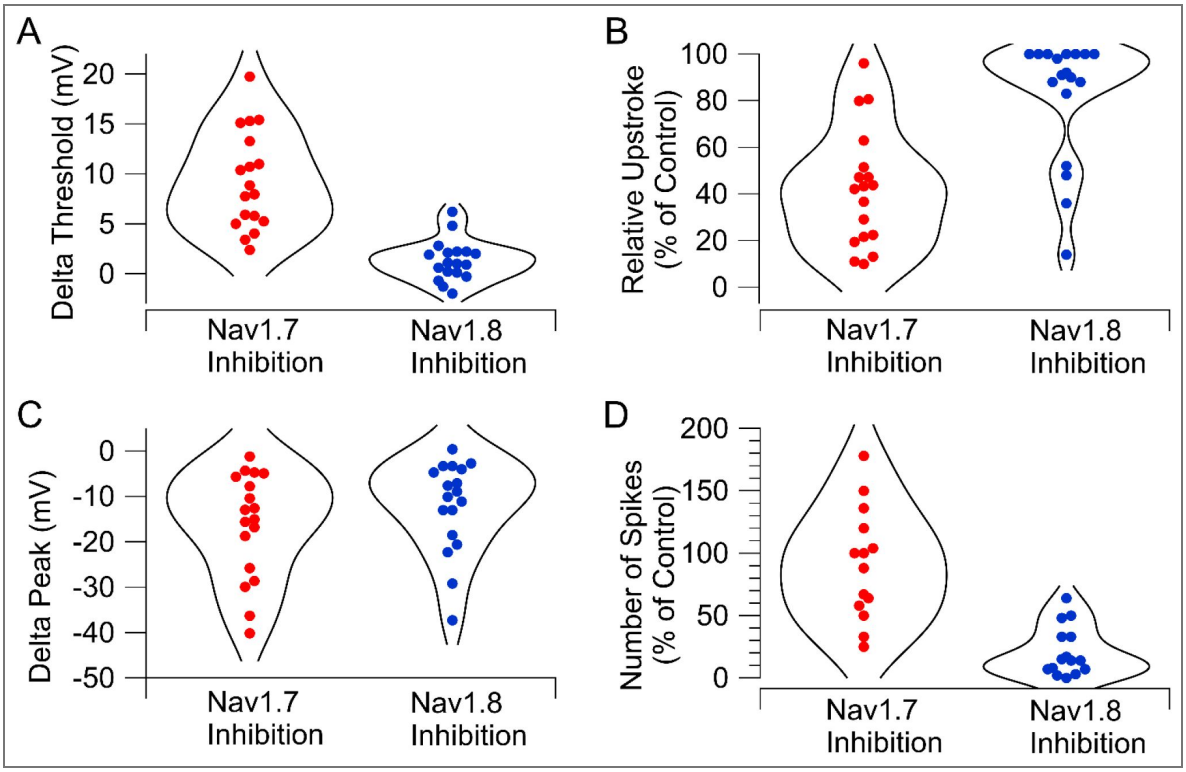

Figure 5. Comparison of Nav1.7 and Nav1.8 inhibition on action potential firing in human DRG neurons.

A, Change in action potential threshold by inhibiting Nav1.7 channels (600 nM AM-2099) or Nav1.8 channels (10 nM VX-548). B, Inhibition of maximum upstroke of action potential by inhibiting Nav1.7 channels (600 nM AM-2099) or Nav1.8 channels (10 nM VX-548). C, Reduction of action potential peak by inhibiting Nav1.7 channels (600 nM AM-2099) or Nav1.8 channels (10 nM VX-548). D, Effect of inhibiting Nav1.7 channels (600 nM AM-2099) or Nav1.8 channels (100 nM VX-548) on maximum number of action potentials evoked by 1-s depolarizations of different magnitudes. Data for effects of 100 nM VX-548 from Stewart et al., 2025.

channels, consistent with the dominant effect of Nav1.7 channels on threshold and maximum upstroke velocity. Nav1.8 channels inactivate more slowly than Nav1.7 channels and a substantial fraction of Nav1.8 channels remain open during the shoulder of the action potential (Stewart et al., 2025). An apparent quantitative difference between human and rodent DRG neurons is the dominance of Nav1.7 channels in the upstroke of the action potential in human neurons, while TTX-resistent current from Nav1.8 channels appears to dominate in the upstroke of action potentials in small-diameter rat and mouse neurons (Renganathan et al., 2001; Blair and Bean, 2002; Matsutomi et al., 2006; Rush et al., 2007; Han et al., 2015a). Interestingly, this quantitative difference between mouse and rat DRG neurons was mirrored in a PCR analysis of seven sodium channel subtypes in DRG neurons of human and mouse, showing higher relative expression of Nav1.7 compared to Nav1.8 in human neurons compared to mouse neurons (Chang et al., 2018).

Our results are generally consistent with those of Alexandrou et al. (2016), who found that the Nav1.7 inhibitor PF-05089771 blocked action potential generation in most but not all (8 of 15) human DRG neurons tested when action potentials were evoked by current injections that were just-suprathreshold in control. We saw more consistent effects of inhibiting Nav1.7 channels, with an increase in voltage threshold in 18 of 18 neurons. The difference could reflect in part the different recording conditions (37°C in our experiments versus room temperature in the Alexandrou et al. recordings) but could also reflect intrinsic heterogeneity in cell populations.

The strong effect of inhibiting Nav1.7 current on action potential threshold is reflected in the increase in rheobase current necessary to evoke spiking in the 1-s current injection protocols (Figure 3). The effect of inhibiting Nav1.7 channels with AM-2099 is concordant with an increase in rheobase in iPSC-derived nociceptors from human patients with Nav1.7 loss-of-function mutations (McDermott et al., 2019) and with an increase in rheobase with the Nav1.7 inhibitor PF-05089771 in iPSC neurMcDermott et al., 2019ons from healthy controls but not patients with Nav1.7 loss-of-function mutations (McDermott et al., 2019).

In contrast to the counter-intuitive decrease in refractory period seen with Nav1.8 inhibition (Stewart et al., 2025), Nav1.7 inhibition almost always (14 of 15 neurons) increased the refractory period. The overall effects on refractory period of inhibiting either channel reflect a complex combination of the actual reduction in sodium channel availability along with secondary effects resulting from the change in action potential shape. The increase in refractory period with Nav1.7 inhibition is easily understood from the fact that Nav1.7 channels strongly control action potential threshold. The reduction in action potential peak (seen with both Nav1.7 and Nav1.8 inhibition) and a reduction in action potential width (which was dramatic with Nav1.8 inhibition but generally not with Nav1.7 inhibition) would both be expected to have a secondary effect of tending to reduce the refractory period by producing less activation of voltage-activated potassium channels and therefore reducing the post-spike potassium conductance that contributes to the refractory period. Because inhibiting Nav1.8 channels has much less effect on action potential threshold compared to inhibiting Nav1.7 channels, the effects on potassium conductance to decrease refractory period – resulting from the changes in action potential shape - apparently dominate with Nav1.8 inhibition but not Nav1.7 inhibition.

A major limitation in our current knowledge of nociceptor excitability is that while we have relatively detailed information on ion channel composition and electrophysiological properties of cell bodies, we have only limited information about the electrophysiology of the key functional elements of the neurons: the peripheral terminals where action potentials are first generated, the axons where the action potentials are propagated, and the fine branches and synaptic boutons of the central terminals where action potentials trigger synaptic transmission (Bennett et al., 2019; Goodwin and McMahon, 2021). Nav1.7 channels are clearly involved in generation and propagation of action potentials, because selective Nav1.7 inhibitors applied to the nerve can inhibit firing of C-fibers evoked by cutaneous stimulation, although often incompletely (Goodwin et al., 2022; Deng et al., 2023). A recent study making *in vivo* pig single nerve fiber recordings of C-fiber nociceptors excited by transcutaneous stimulation found that inhibiting Nav1.7 channels by protoxin II reduced firing more dramatically at low stimulus intensities than high intensities

(Soares et al., 2025), which could be consistent with Nav1.7 channels regulating firing near threshold stimulation but Nav1.8 channels being more important for repetitive firing with strong stimulation. In general, although studies on the cell body probably have major limitations for predicting clinical effects, they can help define the pharmacological properties of individual channels and perhaps elucidate some ways that overall firing patterns can be regulated by particular channels. For example, the different effects on refractory period and on repetitive firing of inhibiting Nav1.8 versus Nav1.7 channels seems likely to apply wherever both are expressed.

Despite the clear differences between Nav1.7 and Nav1.8 channel inhibition across the entire population of cells tested, there is striking cell-to-cell variability in the effects of inhibiting either Nav1.7 or Nav1.8 channels. For example, although AM-2099 generally had small effects on maximal repetitive firing, it reduced repetitive firing to half or less of control values in 3 of the 14 neurons that had repetitive firing in control (Figures 3, 5D). The relatively large effects of Nav1.7 inhibition on repetitive firing in a subset of neurons is consistent with a previous study quantifying components of sodium current using action potential clamp, in which 2 of 10 neurons tested had a large contribution of Nav1.7 current in the second action potential during repetitive spiking (Stewart et al., 2025). Similarly, although VX-548 inhibition of Nav1.8 channels had only a small effect on maximal upstroke velocity in most cells, in 4 of 18 cells upstroke velocity was reduced to less than 50% of the control value, similar to the typical effect of Nav1.7 inhibition. In the future, larger data sets in combination with single cell measurements of mRNA levels may help interpret the large cell-to-cell variability in terms of defined subpopulations of C-fiber nociceptors (cf. Shiers et al., 2020; Middleton et al., 2021; Tavares-Ferreira et al., 2022; Körner et al., 2022; 2026).

Clinical studies with the first selective Nav1.7 inhibitors to reach studies on human pain showed relatively poor efficacy compared to the Nav1.8 inhibitor suzetrigine (reviewed by Eagles et al., 2022; Alsaloum et al., 2025; Yang et al., 2025). The reasons for the low efficacy of the Nav1.7 inhibitors so far tested on human pain are unclear, and may include poor target engagement as a result of high plasma protein binding (Deng et al., 2023). Recognizing the limitations of studies on cell bodies, our results raise the possibility that another factor distinguishing the two targets could be the greater effect of Nav1.8 inhibition to reduce repetitive firing of the neurons compared to Nav1.7 inhibition. It seems plausible that this difference may extend to initial generation of neuronal firing in the peripheral terminals, where computer modeling suggests that Nav1.8 channels play a major role in regulating repetitive firing (Barkai et al., 2020). The perception of pain is correlated with higher firing frequencies of nociceptors (Yarnitsky et al., 1992; Djouhri et al., 2006; reviewed by Namer and Lampert, 2025), so it is possible that the greater efficacy of Nav1.8 inhibition compared to Nav1.7 inhibition to reduce repetitive firing of human nociceptive neurons contributes to differences in clinical efficacy.

The expression of Nav1.7 channels can be modified in various pathological conditions associated with pain, including as chemotherapy-induced peripheral neuropathy (Li et al., 2018; Akin et al., 2021; Braden et al., 2022) and early-life stress (Alvarez et al., 2021). The efficacy of various Nav1.7 inhibitors in a variety of animal models of pain suggests that there may be particular forms of human pain that would respond to Nav1.7 inhibition more robustly than the acute pain from initially tested. Further studies of roles of Nav1.7 channels in excitability of human nociceptors in conditions such as chemotherapy-induced peripheral neuropathy and other forms of human neuropathic pain (Raja et al., 2020; Stucky and Mikesell, 2021) would be illuminating and may be feasible as it becomes possible to test neurons from human donors with known pain conditions.

Materials and Methods

Preparation of human DRG neurons

Neurons were obtained from the dorsal root ganglia (DRGs) of human donors. The procurement network of AnaBios Corporation includes only US-based Organ Procurement Organizations and Hospitals. Policies for donor screening and consent are those established by the United Network for Organ Sharing (UNOS). Organizations supplying human tissues to AnaBios follow the standards

and procedures established by the US Centers for Disease Control (CDC) and are inspected biannually by the Department of Health and Human Services (DHHS). The distribution of donor medical information is in compliance with HIPAA regulations to protect donor privacy. All transfers of donor tissue to AnaBios are fully traceable and periodically reviewed by US Federal authorities.

Neurons were dissociated from dorsal root ganglia and suspended in medium (Davidson et al., 2014) and shipped in suspension in a container with temperature maintained at 4°C (Lesnak et al., 2025). The neurons were then plated on round 12 mm poly-D-lysine-treated coverslips placed in 24- or 48-well plates. Coverslips (Fisherbrand, Cat#12-545-80) were prepared by exposure UV for approximately 15-30 minutes and then incubated overnight at 4°C with 0.01 mg/mL poly-D-lysine diluted in sterile water. After trituration of the tissue, 60 µL of the sample was plated on each coverslip and incubated at 37°C (5% CO_2) for 1-2 hours to allow the cells to settle and attach to the coverslip. Each well was then gently flooded with 1 mL of culture media consisting of BrainPhys media (Stemcell technologies, Cat. 05709), 1% penicillin/streptomycin, 1% GlutaMAX, 2% NeuroCult SM1 (Stemcell technologies, Cat 05711), 1% N-2 Supplement (Thermo Scientific, Cat # 17502048) and 2% Fetal Bovine Serum. Plates were housed in a 5% CO_2 incubator set at 37°C for up to 6 days.

Electrophysiology with human DRG neurons

For recording, coverslips were placed into the recording chamber containing about 2 mL of Tyrode's solution at room temperature. We generally selected smaller cells for recording and selected cells in which glia had partially peeled off, which facilitated recording. Prior to recording, if the cell was strongly adhered to the coverslip (as was usual 2-3 days after plating), an electrode with a blunt tip was used to scrape the surrounding area of the cell and gently maneuvered to ensure detachment from the coverslip. Both the "scraping" electrode and recording electrode were pulled from borosilicate capillaries (VWR International, Cat #53432-921) on a Sutter P-97 puller (Sutter Instruments).

Whole-cell patch-clamp recordings were made with either an Axon Instruments Multiclamp 700B amplifier (Molecular Devices) controlled by pClamp9.2 software (Axon Instruments), filtered at 10 kHz with a low-pass Bessel filter, and digitized at 100 kHz by a Digidata 1322A data acquisition interface or a Sutter Instruments dPatch integrated amplifier/data acquisition system, with filtering at 10 kHz and digitization at 100 kHz. Whole-cell recordings were obtained using patch pipettes with resistances of 1.2-2.5 MΩ. The shank of the electrode was wrapped with Parafilm (American National Can Company) in order to reduce capacitance and allow optimal series resistance compensation (bridge balance) of 60-80% without oscillation. Seals were obtained and the whole-cell configuration established with cells in room-temperature Tyrode's solution consisting of 155 mM NaCl, 3.5 mM KCl, 1.5 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, 10 mM glucose, pH adjusted to 7.4 with NaOH. After establishing whole-cell recording, cells were lifted off the bottom of the recording chamber and placed in front of an array of quartz flow pipes (250 µm internal diameter, 350 µm external diameter, Polymicro Technologies) attached with styrene butadiene glue (Amazing Goop, Eclectic Products) to an aluminum rod (1×1 cm) whose temperature was controlled by resistive heating elements and a feedback-controlled temperature controller (TC-344B; Warner Instruments). Solution changes were made (in < 1 second) by moving the cell between adjacent pipes. Experiments were done with temperature controlled at 37°C. Whole-cell recordings were obtained using patch pipettes filled with a K-gluconate-based internal solution containing (in mM) 139.5 K-Gluconate, 1.6 $MgCl_2$, 1 EGTA, 0.09 $CaCl_2$, 9 HEPES,14 creatine phosphate (Tris salt), 4 MgATP, 0.3 GTP (Tris salt), pH adjusted to 7.2 with KOH. Membrane potentials are corrected for a liquid junction potential of -13 mV between the internal solution and the Tyrode's solution in which the current was zeroed before recording.

Cells were recorded at their natural resting membrane potential without any injection of steady holding current. For the data in Figures 1, 2, and 4, action potentials were evoked by short (0.5-ms) injections of current so that the action potential occurred after the current injection. For the data in Figure 3, 1-s current steps were applied with the increments of the steps adjusted

depending on the input resistance of each cell. Action potentials were defined using a criteria of action potential peak >-20 mV and height >40 mV. Experiments were included in combined data sets only if the resting potential varied by less than 5 mV during the application of AM-2099. Capsaicin sensitivity was tested at the end of the experiment by holding the cell at -70 mV in voltage clamp and briefly applying 1 μM capsaicin.

Drugs

AM-2099 (Marx et al., 2016) was purchased from MedChemExpress (Catalog HY-100727). A stock solution was prepared at 10 mM in DMSO, aliquoted, and frozen at - 20°C. Capsaicin stock was prepared at 1 mM from powder in DMSO and stored at room temperature. External control solutions contained concentrations of DMSO to match those in the drug-containing solutions. All external solutions contained 1 mg/mL Pluronic PF-68 (Sigma).

Data analysis and statistics

Data were analyzed using programs written in Igor Pro 6, 8 or 9 (Wavemetrics, Lake Oswego, OR), using DataAccess (Bruxton Software) to read pClamp files into Igor Pro.

Data availability

All data is contained in figures.

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Additional information

Author contributions

AF, SJ, RGS, and TO designed and executed experiments, analyzed data, and contributed to writing the manuscript; AF and KC designed procedures for and supervised dissection and preparation of tissue; BPB helped design experiments, analyze data, and write the manuscript.

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Peer reviews

Reviewer #1 (Public review):

Summary:

Fujita and colleagues investigated two selective peripheral nerve voltage-gated sodium channel inhibitors targeting either Nav1.7 or Nav1.8 on the excitability of human dorsal root ganglion neurons. The authors discovered that Nav1.8 inhibition is more effective at suppressing repetitive firing of DRG neurons, and this may explain the greater clinical efficacy observed for suzetrigine.

Strengths:

The study is interesting, and the findings are conceptually satisfying in that they may explain one aspect of Nav1.7 vs Nav1.8 targeting success.

Weaknesses:

(1) The use of postmortem human DRG neurons provides translational relevance, but the use of these cells is also a liability, given their high degree of variability. Of note are the 10 to 20-fold differences in baseline properties among cells, which dwarf the effects of the test compounds. The experiments may suffer from undersampling.

(2) A potential confounder when using post-mortem human DRG neurons is heterogeneity of cell types. The methods clearly state that the cells selected for recording were of 'generally' small size, but specific criteria for what constitutes 'small' or other unstated selection criteria

were not provided. A table of individual cell capacitance and input resistance values, along with information about individual donors (age, sex, ethnicity), is important to include. Additionally, some discussion of how DRG neuron heterogeneity impacts the findings. This relates to concern #1 about sample size determination and how cell heterogeneity factored into this calculation.

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Reviewer #2 (Public review):

Summary:

The authors examine the functional role of Nav1.7 voltage-gated sodium channels in human sensory neuron electrogenesis using a Nav1.7 selective inhibitor and human dorsal root ganglion neurons obtained from organ donors. Patch-clamp electrophysiology is used at physiological temperature to measure the impact of Nav1.7 inhibition on sensory neurons' action potential firing. This is an important topic as Nav1.7 and Nav1.8 have been identified as therapeutic targets for the treatment of pain, but there has been mixed success with isoform-specific inhibitors in clinical trials. The data suggest that Nav1.7 and Nav1.8 have overlapping yet complementary functions in nociceptor neurons and that targeting both may be most effective for reducing nociception.

Strengths:

The data are of high quality. Action potential properties are measured at 37 degrees Celsius. Threshold is measured using brief pulses. The Nav1.7 inhibitor has been reported to be highly selective for Nav1.7 over Nav1.8 and moderately selective for Nav1.7 over Nav1.1 and Nav1.6. Data are collected using identical conditions and protocols to a previous study on the role of Nav1.8 in similar neurons.

Weaknesses:

The study relies on a single Nav1.7 inhibitor that has not been extensively characterized. One prior study indicates that the IC50 is around 140 nM, thus the 600 nM concentration used in this study could be predicted to reduce Nav1.7 currents by 80%. However, there is no voltage-clamp data in the current study to confirm this, and therefore, it is unclear if the batch of AM-2099 is as potent as reported in the paper that initially described its selectivity. The impact of Nav1.7 inhibition is compared to data from a previous study by this lab, and this is a minor concern. It would have been interesting to see if the combined inhibition of Nav1.7 and Nav1.8 completely blocked action potential generation in the human DRG neurons.

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Reviewer #3 (Public review):

Summary:

In this manuscript, Fujita/Jo/Stewart/Osorno et al. investigate the contribution of Nav1.7 in regulating the excitability and firing properties of human dorsal root ganglion (hDRG) neurons in vitro. The authors characterize the effects of a previously reported Nav1.7-selective blocker AM-2099 in cultured hDRG neurons from postmortem organ donors. The authors observed modest changes in many of the properties expected by inhibiting Nav channels, including decreased action potential upstroke rate and amplitude, while increasing the voltage and current thresholds for spike generation. However, AM-2099 did not change the maximum number of APs in response to suprathreshold stimulation, leading the authors to conclude that Nav1.7 inhibition alone has limited efficacy in reducing the firing properties

of hDRG neurons and that Nav1.7 blockers may have limited efficacy as analgesics. This is surprising, given that patients with loss-of-function mutations in Nav1.7 suffer from congenital insensitivity to pain. While it may indeed be true that pharmacological inhibition of Nav1.7 is unlikely to produce analgesia, the present study was limited to a single concentration of AM-2099. The manuscript would be significantly strengthened by a more careful and thorough pharmacological characterization of this compound, which has not been widely used or validated in native human DRG neurons.

Strengths:

Experiments are well-designed and executed, and the results presented are convincing. The focus on voltage-gated sodium channels in native human DRG neurons is highly relevant to recent efforts to develop safer analgesic options for chronic pain in people.

Weaknesses:

Only a single concentration of AM-2099 was used for all experiments. This compound was reported to be selective for cloned human Nav1.7 channels in heterologous systems, but has not been validated in other studies after the original publication in 2016. Since the original study reported a substantial state-dependent block of recombinant Nav1.7 channels, more detailed pharmacological characterization of AM-2099 is needed in human DRG neurons to fully support these claims. This study would be significantly strengthened by the inclusion of dose-response curves to assess how much of the sodium current is inhibited at this concentration, confirming selectivity in hDRG, and whether maximal inhibition of Nav1.7 still has limited efficacy in reducing the firing of native human sensory neurons.

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