Human Na$_{\alpha}$.8: enhanced persistent and ramp currents contribute to distinct firing properties of human DRG neurons

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VOLTAGE-GATED SODIUM CHANNELS play a critical role in the generation and propagation of action potentials in excitable cells, including cardiomyocytes, muscle cells, and neurons. Nine voltage-gated sodium channels (Na$_{\alpha}$.1–Na$_{\alpha}$.9) have been identified in mammals (Catterall et al. 2005). Different sodium channel subtypes have specific distributions and functions. Sodium channel Na$_{\alpha}$.1.8 is preferentially expressed in peripheral neurons, in particular within small and medium-sized dorsal root ganglion (DRG) neurons (Akopian et al. 1996; Djouhri et al. 2003; Sangameswaran et al. 1996; Shields et al. 2012). Na$_{\alpha}$.1.8 is known to produce a slow-inactivating, tetrodotoxin (TTX)-resistant current and is characterized by significantly depolarized activation and inactivation compared with other sodium channels (Akopian et al. 1996; Sangameswaran et al. 1996). This enables Na$_{\alpha}$.1.8 to act as a major contributor to the action potential upstroke during repetitive firing of DRG neurons evoked by sustained depolarization (Blair and Bean 2002; Renganathan et al. 2001).

Studies in knockout animals have clearly established a role of Na$_{\alpha}$.1.8 in pain (Akopian et al. 1999; Zimmermann et al. 2007). Furthermore, gain-of-function mutations of Na$_{\alpha}$.1.8 have been found in human subjects with painful neuropathies; relatively subtle gain-of-function changes in channel biophysics due to these mutations markedly alter the excitability of DRG neurons (Faber et al. 2012; Han et al. 2014; Huang et al. 2013), underscoring the potential importance of even small differences in human Na$_{\alpha}$.1.8 channel properties, compared with those in rodents, for pain signaling. Na$_{\alpha}$.1.8 mutations linked to small-fiber neuropathy have been functionally profiled after expression in rodent DRG neurons (Faber et al. 2012; Han et al. 2014; Huang et al. 2013). Although rodent DRG neurons provide a tractable heterologous expression system for the functional assays that have been used to assess these mutations (Dib-Hajj et al. 2009), notable differences between rodent and human DRG sodium channels have been reported, including, e.g., an ~10-mV difference in voltage dependence of human Na$_{\alpha}$.1.9 currents compared with rodent Na$_{\alpha}$.1.9 currents (Dib-Hajj et al. 1999). Given the important role of Na$_{\alpha}$.1.8 in pain, there is a need for information about the functional properties of human Na$_{\alpha}$.1.8 channels and their roles in human DRG neuron firing.

Persistent sodium current and ramp current are produced by some sodium channels, and it has been known that persistent and ramp sodium currents play critical functional roles in modulating the excitability of neurons (Cummins et al. 1998; Fleidervish and Gutnick 1996; Kiss 2008; Stafstrom 2007). Persistent sodium currents have been associated with several hyperexcitability disorders, including epilepsy and LQT3 disease (Christe et al. 2008; Saint 2008; Stafstrom 2007; Veeramah et al. 2012), and pain (Fertleman et al. 2006). In the present study, using dynamic clamp as well as voltage clamp and current clamp to study human Na$_{\alpha}$.1.8 both after transfection into rodent DRG neurons and in native human DRG neurons, we investigated the biophysical properties of human Na$_{\alpha}$.1.8. We demonstrate larger persistent current and ramp current as well as slower inactivation of human Na$_{\alpha}$.1.8 channels and demonstrate longer-lasting action potentials in DRG neurons carrying human Na$_{\alpha}$.1.8.

MATERIALS AND METHODS

Plasmid Constructs

The pcDNA5-SCN10A (Na$_{\alpha}$.1.8) plasmid construct that encodes human Na$_{\alpha}$.1.8 protein was purchased from Genionics, and the plasmid pRK-Nav1.8 carrying rat Na$_{\alpha}$.1.8 insert was a gift from Dr. John...
Wood (University College London, London, UK). Both human and rat Na,1.8 constructs were driven by the CMV promoter.

Primary Mouse DRG Neuron Isolation and Transfection

Animal studies were approved by Yale University and Department of Veterans Affairs West Haven Hospital Animal Use Committees. DRG neurons were isolated, as previously reported (Dib-Hajj et al. 2009), from homozygous Nav1.8cre mice (4–8 wk of age, both male and female) that lack endogenous Na,1.8. Briefly, 24 DRGs (from T8 to L4, both sides) were harvested, incubated at 37°C for 20 min in complete saline solution [CSS; in mM: 137 NaCl, 5.3 KCl, 1 MgCl2, 25 sorbitol, 3 CaCl2, and 10 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES), adjusted to pH 7.2 with NaOH] containing 0.5 U/ml Liberase TM (Roche Diagnostics) and 0.6 mM EDTA before 15-min incubation at 37°C in CSS containing 0.5 U/ml Listerase TL (Roche Diagnostics), 0.6 mM EDTA, and 30 U/ml papain (Worthington). Tissue was then centrifuged and triturated in 0.5 ml of DRG medium: Dulbecco’s modified Eagle’s medium-F12 (1:1) with 100 U/ml penicillin, 0.1 mg/ml streptomycin (Invitrogen), and 10% fetal bovine serum (HyClone), containing 1.5 mg/ml bovine serum albumin (BSA) (low endotoxin; Sigma) and 1.5 mg/ml trypsin inhibitor (Sigma). After trituration, Na,1.8 constructs (2 μg of Na,1.8 wild type plus 0.2 μg of EGFP) were transfected into DRG neuron suspension by electroporation using Nucleofector IIS (Lonza) and Amaxa SCN Nucleofector (SVPI-1003). After electroporation, 100 μl of calcium-free Dulbecco’s modified Eagle’s medium (Invitrogen) was added and cells were incubated at 37°C for 5 min in a 95% air-5% CO2 (vol/vol) incubator to allow neurons to recover. The cell mixture was then diluted with DRG medium containing 1.5 mg/ml BSA (low endotoxin; Sigma) and 1.5 mg/ml trypsin inhibitor (Sigma), seeded onto poly-α-lysine-laminin-coated coverslips (BD), and incubated at 37°C to allow DRG neurons to attach to the coverslips. After 40 min, DRG medium was added into each well to a final volume of 1.0 ml [for current-clamp recording culture, medium was supplemented with 50 ng/ml mouse nerve growth factor (mNGF) (Alomone Labs) and 50 ng/ml recombinant human glial cell line-derived neurotrophic factor (hGDNF) (PeproTech)] and the DRG neurons were maintained at 37°C in a 95% air-5% CO2 (vol/vol) incubator for ~40–48 h before recording.

Primary Human DRG Neuron Isolation

Human DRGs [lumbar (L), L, or L] were obtained from the National Disease Research Interchange (NDRI). Studies with human tissues were approved by human investigation committees at Yale University and Department of Veterans Affairs West Haven Hospital. The DRG neurons were harvested and dissociated within 24 h of clamping the aorta. DRGs were harvested from six Caucasian donors (woman, age 53 yr, L; woman, age 51 yr, L; man, age 46 yr, L; woman, age 53 yr, L; woman, age 51 yr, L; man, age 75 yr, L, and L). The causes of death were anoxia, cardiac arrest, head trauma, and intracranial hemorrhage. None of these patients had been diagnosed with a pain or inflammatory syndrome or with peripheral neuropathy prior to death.

Nerve roots and as much connective tissue as possible were removed, and DRGs were sliced into small fragments in CSS and then incubated on a rotating shaker at 37°C for 40 min in CSS containing 0.5 U/ml Listerase TM (Roche) and 0.6 mM EDTA, followed by a 25-min incubation at 37°C in CSS containing 0.5 U/ml Listerase TL (Roche), 0.6 mM EDTA, and 30 U/ml papain (Worthington). DRGs were then centrifuged (1,000 rpm for 30 s) and triturated in DRG medium containing 1.5 mg/ml BSA (low endotoxin; Sigma) and 1.5 mg/ml trypsin inhibitor (Sigma). After filtering with 100-μm nylon mesh cell strainer (BD), the cell suspension was centrifuged (1,000 rpm for 10 min), the cell pellet was resuspended in 1.2 ml of DRG medium, and 80 μl of cell suspensions were plated on each poly-d-lysine-laminin-coated coverslip (BD). After incubation at 37°C in a 95% air-5% CO2 (vol/vol) incubator for 60 min to allow neurons to adhere, 0.92 ml of DRG medium supplemented with mNGF (50 ng/ml; Alomone Labs) and hGDNF (50 ng/ml; PeproTech) was added into each well. In preliminary experiments to optimize the culture conditions, we determined that supplements of these trophic factors were necessary to obtain viable neurons for recordings. DRG neurons were maintained at 37°C in a 95% air-5% CO2 (vol/vol) incubator and recorded by whole cell patch clamp within 24 h after plating. A total of 86 DRG neurons (size range 25–60 μm) were selected for recording by voltage clamp and current clamp.

Primary Rat DRG Neuron Isolation

DRG neurons were isolated from male Sprague-Dawley rats (4–8 wk of age) as described previously (Rizzo et al. 1994). The tissue was then enzymatically digested at 37°C for 25 min with collagenase A (1 mg/ml; Roche) in CSS and for 25 min with collagenase D (1 mg/ml; Roche) and papain (30 U/ml; Worthington) in CSS at 37°C. Treated tissues were then centrifuged (100 g for 3 min), and the pellets were triturated in DRG medium containing 1.5 mg/ml BSA (low endotoxin; Sigma) and 1.5 mg/ml trypsin inhibitor (Sigma). Cells were then plated on poly-l-ornithine-laminin-coated glass coverslips (BD), flooded with DRG medium after 1 h, and incubated at 37°C in a humidified 95% air-5% CO2 (vol/vol) incubator. Small DRG neurons were recorded by whole cell patch clamp within 24 h after plating.

Electrophysiology

Voltage-clamp recording on transfected mouse DRG neurons. Small transfected mouse DRG neurons (<25-μm diameter) with robust green fluorescence and no apparent neurites were selected for voltage-clamp recording. Fire-polished electrodes (1–2 MΩ) were fabricated from 1.6-mm-inner diameter borosilicate glass micropettes (World Precision Instruments, Sarasota, FL). The pipette potential was adjusted to zero before seal formation, and liquid junction potential was not corrected. Capacitative transients were canceled, and voltage errors were minimized with 80–90% series resistance (1.5–5 MΩ) compensation; cells were excluded from analysis if the predicted voltage error exceeded 3 mV. Currents were acquired with Pulse Software (HEKA Electronics) 5 min after whole cell configuration was established, sampled at a rate of 50 kHz, and filtered at 2.9 kHz. The pipette solution contained the following (in mM): 140 CsCl, 10 NaCl, 0.5 EGTA, 3 MgATP, and 10 HEPES, pH 7.3 with CsOH (adjusted to 315 mosM with dextrose). The extracellular bath solution contained the following (in mM): 140 NaCl, 3 KCl, 1 MgCl2, 1 CaCl2, 10 HEPES, 5 CsCl, and 20 tetraethylammonium chloride (TEA-Cl), pH 7.32 with NaOH (327 mosM). TTX (0.5 μM), CdCl2 (0.1 mM), and 4-aminopyridine (1 mM) were added to the bath solution to block endogenous voltage-gated sodium currents, calcium currents, and potassium currents, respectively.

We cotransfected Na,1.8 channels with EGFP and were able to record the Na,1.8 slow-inactivating TTX-R current in every GFP-positive neuron (48/48 cells transfected with rat or human Na,1.8 channels) in voltage-clamp mode, indicating that in our transfection method all neurons with green fluorescence also produced Na,1.8 sodium currents. For voltage-clamp recording, DRG neurons were held at ~70 mV to inactivate Na,1.9 channels and we recorded both activation and fast inactivation of every cell. We have shown previously (Cummins et al. 1999; Dib-Hajj et al. 1999) that the fast inactivation of Na,1.9 starts at much hyperpolarized voltage (around ~100 mV) but that Na,1.8 starts to inactivate at much depolarized voltage (around ~70 mV). For our analysis, we discarded the cells that displayed TTX-R current with hyperpolarized fast inactivation (less than ~70 mV) to exclude potential contamination of Na,1.9 current.

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within 24 h after plating, with rat DRG neurons (clamp recordings were obtained from native human DRG neurons. 

\[ V_{\text{clamp}} = \frac{1}{2} \left( V_{1/2,\text{act}} - V \right) \] 

where \( V_{1/2,\text{act}} \) is the potential at which activation is half-maximal, \( V \) is the test potential, and \( k \) is the slope factor.

Persistent currents were measured as mean amplitudes of currents recorded between 90 and 95 ms after the onset of depolarization and are presented as a percentage of the maximal transient peak current. Ramp currents were elicited with slow ramp depolarization over a 600-ms period at 0.2 mV/ms. The amplitude of ramp current was presented as a percentage of the maximal peak current.

Voltage-clamp recording on native human DRG neurons. Voltage-clamp recordings were obtained from native human DRG neurons within 24 h after plating, with rat DRG neurons (<30-μm diameter) as a comparator. The pipette solution was the same as for voltage-clamp recording on transfected mouse neurons. For the extracellular bath solution, 140 mM NaCl was substituted with 70 mM NaCl and 3 mM Mg-ATP, pH 7.3 with KOH (adjusted to 315 mosM with dextrose). Sodium current was described by the following rate constant:

\[ I_{\text{Na}} = 5.97 \times 10^{-4} \left( V - 65 \right)^2 \]

Sodium current was described by a double-exponential equation:

\[ I_{\text{Na}} = \frac{\text{membrane potential}}{10.9 - V} + \frac{\text{membrane potential}}{7.86 - V} \]

where \( I_{\text{Na}} \) is maximal conductance and was set to 125 nS to match our voltage-clamp recordings, which show ~6 nA of current, \( V_{\text{m}} \) is membrane voltage, and \( E_{\text{Na}} \) is sodium reversal potential. Currents evoked by voltage clamp were calculated in 10-μs precision with a custom program written in OriginPro 8.5 LabTalk. Human and rat Na1.8 channel steady-state parameters and kinetics were obtained from our voltage-clamp recording on transfected mouse DRG neurons.

Data Analysis

Voltage-clamp and current-clamp data were analyzed with FitMaster (HEKA) and OriginPro 8.5 (OriginLab). Dynamic clamp data were analyzed with pCLAMP 10 (Molecular Devices) and OriginPro 8.5. All data are presented as means ± SE. Statistical significance was examined with two-sample Student’s t-test, Mann-Whitney test, or twoportion z-test, except for dynamic clamp data, which was examined with two-sample paired Student’s t-test.
(Fig. 1A) and human (Fig. 1B) Na\textsubscript{v}1.8 channels. DRG neurons transfected with both rat and human Na\textsubscript{v}1.8 channels generated voltage-dependent, slowly inactivating inward currents. There was no significant difference in the densities of peak sodium channel current between the two groups of cells [rat Na\textsubscript{v}1.8: 356 ± 74 pA/pF (n = 25), human Na\textsubscript{v}1.8: 447 ± 90 pA/pF (n = 23); P > 0.05]. Notably, DRG neurons transfected with human Na\textsubscript{v}1.8 display a threefold larger persistent current compared with DRG neurons transfected with rat Na\textsubscript{v}1.8.

Figure 1, C and D, compare the absolute and normalized amplitudes of persistent current in DRG neurons expressing rat and human Na\textsubscript{v}1.8 channels, respectively. The persistent currents display voltage dependence with a peak within the voltage range −5 to 0 mV for both channels. However, the normalized amplitude of persistent current of DRG neurons expressing human Na\textsubscript{v}1.8 channels (12.3% ± 1.3%, n = 17) is 2.9-fold larger than that of DRG neurons expressing rat DRG neurons (4.2% ± 0.4%, n = 17; P < 0.001) (Table 1).

We also compared the voltage dependence of activation and steady-state fast inactivation between human and rat Na\textsubscript{v}1.8 channels. As Fig. 2A shows, human Na\textsubscript{v}1.8 channels display hyperpolarized activation compared with rat Na\textsubscript{v}1.8 channels. The $V_{1/2}$ of activation for human Na\textsubscript{v}1.8 is $−11.12 ± 1.76$ mV (n = 17), which represents an $−$5-mV hyperpolarizing shift compared with the $V_{1/2}$ of activation for rat Na\textsubscript{v}1.8 ($−6.21 ± 1.62$ mV, n = 17; P < 0.05) (Fig. 2B, Table 1). Human Na\textsubscript{v}1.8 channels display depolarized steady-state fast inactivation compared with rat Na\textsubscript{v}1.8 channels. The $V_{1/2}$ of fast inactivation for human Na\textsubscript{v}1.8 channels ($−31.86 ± 0.58$ mV, n = 15) is significantly different from that for rat Na\textsubscript{v}1.8 channels ($−35.53 ± 1.6$ mV, n = 16; P < 0.05) (Fig. 2C, Table 1). In addition, the offset of fast inactivation for human Na\textsubscript{v}1.8 channels (9.2 ± 1.61%, n = 15) is fourfold bigger compared with rat Na\textsubscript{v}1.8 channels (2.25 ± 0.42%, n = 16; P < 0.001) (Table 1). The hyperpolarized activation combined with depolarized fast inactivation of human Na\textsubscript{v}1.8 produce a large overlap that predicts a large window current (Fig. 2D). The kinetics for open-state inactivation, which reflect the transition from the open to the inactivated state, were significantly slowed for human Na\textsubscript{v}1.8 channel compared with rat Na\textsubscript{v}1.8 channel from 0 to +40 mV (Fig. 2E).

We also measured the response to a slow ramp stimulus (−70 to +50 mV over 600 ms). Human Na\textsubscript{v}1.8 channels produce almost twofold larger ramp current (21.9 ± 1.1%, n = 12) compared with rat Na\textsubscript{v}1.8 channels (12.1 ± 2.1%, n = 9; P < 0.01) (Fig. 2F, Table 1).

**Threshold Is Lower in DRG Neurons Transfected with Human Na\textsubscript{v}1.8 Channels**

Our previous work has shown that Na\textsubscript{v}1.8 contributes significantly to the production of sodium-dependent action potentials.

Table 1. **Comparison of biophysical properties between human Na\textsubscript{v}1.8 and rat Na\textsubscript{v}1.8**

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<th>Activation</th>
<th>Fast Inactivation</th>
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<td>$V_{1/2,act}$, mV</td>
<td>$n$</td>
<td>$V_{1/2,fast}$, mV</td>
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<tr>
<td>Rat Na\textsubscript{v}1.8</td>
<td>$−6.21 ± 1.62$</td>
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<tr>
<td>Human Na\textsubscript{v}1.8</td>
<td>$−11.12 ± 1.76$</td>
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Values are means ± SE. $V_{1/2,act}$, $V_{1/2,fast}$, potential at which activation or fast inactivation is half-maximal. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. 

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tials in small DRG neurons (Renganathan et al. 2001). The species-specific differences in gating properties of Na\textsubscript{v}1.8 suggested that there might be a marked difference in the effect of human versus rat Na\textsubscript{v}1.8 on the firing properties of DRG neurons. Here we transfected human Na\textsubscript{v}1.8 channels into Na\textsubscript{v}1.8-null mouse DRG neurons so that we could correlate action potential characteristics in these cells with our transfected Na\textsubscript{v}1.8 channels. As a comparator, rat Na\textsubscript{v}1.8 channels were also transfected into Na\textsubscript{v}1.8-null mouse DRG neurons.

The mean RMP was $-51.1 \pm 1.2$ mV ($n = 26$) in DRG neurons expressing human Na\textsubscript{v}1.8 channels, which was not significantly different from the mean RMP in DRG neurons expressing rat Na\textsubscript{v}1.8 channels ($-51.6 \pm 0.8$ mV, $n = 36$; $P > 0.05$). There was no significant difference for input resistance between DRG neurons expressing human Na\textsubscript{v}1.8 channels ($566 \pm 78$ M\text{\ohm}, $n = 26$) or rat Na\textsubscript{v}1.8 channels ($461 \pm 45$ M\text{\ohm}, $n = 36$; $P > 0.05$).

The current threshold, at which DRG neurons generate the first all-or-none action potential, was smaller by nearly one-half for DRG neurons expressing human Na\textsubscript{v}1.8 channels ($68 \pm 12$ pA, $n = 26$) compared with rat Na\textsubscript{v}1.8 channels ($137 \pm 13$ pA, $n = 36$; $P < 0.001$). The amplitudes of the action potential for DRG neurons transfected with human Na\textsubscript{v}1.8 channels and DRG neurons transfected with rat Na\textsubscript{v}1.8 channels were $109.8 \pm 1.3$ mV ($n = 26$) and $107.8 \pm 1.4$ mV ($n = 36$; $P > 0.05$), respectively.

**DRG Neurons Transfected with Human Na\textsubscript{v}1.8 Channels Display Two Firing Patterns**

Using a 200-ms stimulus at the current threshold for individual neurons, we studied firing of Na\textsubscript{v}1.8-null DRG neurons transfected with rat Na\textsubscript{v}1.8 cDNA (42 neurons) or human Na\textsubscript{v}1.8 cDNA (45 neurons). DRG neurons expressing human Na\textsubscript{v}1.8 channels produced different firing patterns based on the width of the first action potential, which we grouped into type I, with a half-width shorter than 10 ms (Fig. 3A), and type II, with a half-width of longer than 10 ms (Fig. 3B). DRG neurons (36 of 36 cells) expressing rat Na\textsubscript{v}1.8 channels demonstrated only a type I firing pattern with a narrow action potential spike in response to 200-ms stimuli. In contrast, DRG neurons expressing human Na\textsubscript{v}1.8 channels demonstrated two firing patterns, types I (Fig. 3A) and II (Fig. 3B): 77% (20 of 26 cells) were type I, and 23% (6 of 26 cells) were type II. As Fig. 3C shows, some type II neurons transfected with human Na\textsubscript{v}1.8 display extremely prolonged action potentials with a plateau around 0 mV. In addition, we observed that 40% (8 of 20 cells) of type I DRG neurons transfected with human Na\textsubscript{v}1.8 chan-
Fig. 3. DRG neurons transfected with human \( \mathrm{Na}_{1.8} \) channels generate different firing patterns. A: representative type I response: the response to a 200-ms depolarizing stimulus has only 1 single spike, and the action potential half-width is smaller than 10 ms. \( V_m \): membrane voltage. B and C: representative type II response: half-width of the first action potential longer than 10 ms (B) and extremely long action potential (C). Inset in C shows the full length of this extremely broad action potential. D: representative bursting firing response: the first all-or-none response has a burst of multiple spikes.

Channels demonstrated burst-like responses in which the first response to 200-ms stimuli was composed of multiple spikes (Fig. 3D). However, there were no DRG neurons transfected with rat \( \mathrm{Na}_{1.8} \) channels that demonstrated burst firing in type I neurons or a type II firing pattern. In addition, we compared the average half-width of action potentials (using the first spike for both types) between DRG neurons transfected with human \( \mathrm{Na}_{1.8} \) channels and DRG neurons transfected with rat \( \mathrm{Na}_{1.8} \) channels. The half-width of the DRG neurons transfected with human \( \mathrm{Na}_{1.8} \) channels (8.69 ± 1.51 ms, \( n = 26 \)) was significantly larger than that of DRG neurons transfected with rat \( \mathrm{Na}_{1.8} \) channels (5.13 ± 0.26 ms, \( n = 36 \); \( P < 0.01 \)).

The expression of human \( \mathrm{Na}_{1.8} \) channels produced a significant increase in the proportion of spontaneously firing DRG neurons. Six of 42 (14%) recorded DRG neurons expressing rat \( \mathrm{Na}_{1.8} \) channels displayed spontaneous firing. In contrast, nearly three times more DRG neurons transfected with human \( \mathrm{Na}_{1.8} \) channels were spontaneously active (42%, 19 of 45 neurons; \( P < 0.01 \)). Moreover, as for non-spontaneously active DRG neurons, spontaneously firing DRG neurons expressing human \( \mathrm{Na}_{1.8} \) channels also displayed different firing patterns (Fig. 4). The percentages for type I (Fig. 4, A and B) and II (Fig. 4, C and D) firing patterns were 84% (16 of 19 neurons) and 16% (3 of 19 neurons), respectively. Among 16 type I DRG neurons, 5 cells displayed burst firing (Fig. 4, E and F). In contrast, spontaneously firing DRG neurons expressing rat \( \mathrm{Na}_{1.8} \) channels demonstrated only a type I firing pattern, with no burstlike firing.

We also compared the evoked responses to a series of 500-ms depolarizing stimuli of different magnitudes (50–500 pA) between DRG neurons expressing human \( \mathrm{Na}_{1.8} \) channels and DRG neurons expressing rat \( \mathrm{Na}_{1.8} \) channels. Since DRG neurons expressing rat \( \mathrm{Na}_{1.8} \) channels generate only type I action potentials, we confined this analysis to DRG neurons expressing human \( \mathrm{Na}_{1.8} \) channels that displayed type I firing. Figure 5A shows the responses of representative DRG neurons that expressed human and rat \( \mathrm{Na}_{1.8} \) channels to 500-ms steps at 50-, 100-, 200-, and 400-pA current stimuli. DRG neurons expressing human \( \mathrm{Na}_{1.8} \) channels generated significantly more action potentials over the stimulation range from 50 to 500 pA (Fig. 5B).

Dynamic Clamp Recordings Confirm that Human \( \mathrm{Na}_{1.8} \) Channel Contributes to the Broad Action Potential

Our results indicate that for the population of transfected mouse DRG neurons, human \( \mathrm{Na}_{1.8} \) produces a larger persistent current compared with neurons transfected with rat \( \mathrm{Na}_{1.8} \) channels and suggest that this results in action potentials with longer duration, a more pronounced inflection on the falling phase, and type II action potentials with a plateau-like falling phase. Since these results were obtained in transfected cells where it is not possible to control the expression level, we also examined action potential electrogenesis using dynamic clamp recording where we can precisely titrate the level of human and rat \( \mathrm{Na}_{1.8} \) expression. Dynamic clamp recording allowed us to compare, in the same cell, the effects of addition of a physio-
logical level of rat Na\textsubscript{v}1.8 conductance versus human Na\textsubscript{v}1.8 conductance; the current amplitude (~6 nA) injected by the dynamic clamp was adjusted to be the mean level of conductance observed after transfection of human Na\textsubscript{v}1.8 in Na\textsubscript{v}1.8-null mouse DRG neurons of <25-\textmu m diameter, which is comparable to the average Na\textsubscript{v}1.8 current amplitude that we have previously measured in wild-type mouse DRG neurons (Shields et al. 2012).

For dynamic clamp recording, a single neuron from a Na\textsubscript{v}1.8-null mouse was endowed sequentially with 100% human Na\textsubscript{v}1.8 channel conductance or 100% rat Na\textsubscript{v}1.8 channel conductance. First we performed in silico evaluation of human and rat Na\textsubscript{v}1.8 channels. Figure 6, A and B, show computer simulation of current traces obtained from the rat (Fig. 6A) and human (Fig. 6B) Na\textsubscript{v}1.8 channel model $I_{Na} = g_{max} m^3 h (V_{m} - E_{Na})$ that we developed and used for the dynamic clamp recordings. Compared with modeled rat Na\textsubscript{v}1.8 channels, modeled human Na\textsubscript{v}1.8 channels clearly display larger persistent currents. We performed dynamic clamp recording with 200-ms-long stimulation (the same protocol as we used for our current-clamp recordings on transfected mouse DRG neurons) by injecting either human Na\textsubscript{v}1.8 conductance or rat Na\textsubscript{v}1.8 conductance. Among 39 cells we recorded, 9 cells (23%) displayed spontaneous firing after the injection of human Na\textsubscript{v}1.8 conductance (Fig. 6C); in contrast, no cells showed spontaneous firing after we injected rat Na\textsubscript{v}1.8 conductance.

For the other 30 non-spontaneously active neurons, the RMP was not significantly different between the human Na\textsubscript{v}1.8 channel recordings ($55.3 \pm 1.0 \text{mV, } n = 30$) and rat Na\textsubscript{v}1.8 channel recordings ($55.3 \pm 1.0 \text{mV, } n = 30; P > 0.05$). However, the current threshold required to produce the first all-or-none action potential was significantly reduced after the injection of human Na\textsubscript{v}1.8 conductance ($73 \pm 11 \text{pA, } n = 30$) compared with the injection of rat Na\textsubscript{v}1.8 conductance ($129 \pm 11 \text{pA, } n = 30; P < 0.001$).

As described above, mouse DRG neurons transfected with rat Na\textsubscript{v}1.8 channels displayed only a type I firing pattern. Here, in the dynamic clamp recording, we also observed that DRG neurons displayed only type I firing behavior after the injection of rat Na\textsubscript{v}1.8 conductance. In contrast, after injection of human Na\textsubscript{v}1.8 channel conductance 80% (24 of 30 neurons) of DRG neurons displayed a type I firing pattern, while 20% (6 of 30 neurons) of DRG neurons produced type II broad action potentials. No burst firing behavior was observed. For each DRG neuron that displayed type I firing pattern, we compared the action potential width with input of human...
Na\textsubscript{1.8} conductance and with rat Na\textsubscript{1.8} conductance in the same cell. As Fig. 6D shows, the half-width of the action potential was significantly wider after inputting human Na\textsubscript{1.8} conductance (6.31 ± 0.31 ms, n = 24) compared with inputting rat Na\textsubscript{1.8} conductance (4.44 ± 0.25 ms, n = 24; \(P < 0.001\)). Figure 6, E and F, show representative type I action potential traces for the same DRG neuron after inputting rat (Fig. 6E) or human (Fig. 6F) Na\textsubscript{1.8} conductance. For the six DRG neurons that demonstrated type I action potentials after inputting rat Na\textsubscript{1.8} but type II broad action potentials after inputting human Na\textsubscript{1.8} conductance, five of six cells produced extremely prolonged action potentials that did not repolarize to RMP even after the 200-ms stimulation. The plateaus of responses generated by these five cells were around 0 mV. Figure 6G shows representative action potential traces recorded from one of these five cells. Figure 6H shows the action potential traces recorded from the sixth type II firing cell. The action potential configuration of this cell is similar to the action potential shape shown in Fig. 4D from a spontaneously active DRG neuron transfected with human Na\textsubscript{1.8}, with one major spike and two minor spikes.

**Human Na\textsubscript{1.8} Displays Larger Persistent Current and Ramp Current in Native Human DRG Neurons**

Our data above show that the human Na\textsubscript{1.8} channel displays larger persistent current and ramp current when assessed in transfected mouse DRG neurons. To determine whether Na\textsubscript{1.8} channels also produces a large persistent current or ramp current within native human DRG neurons, we performed voltage-clamp recording on acutely isolated human DRG neurons. As in the experiments described above, rat DRG neurons were used as a comparator. Figure 7, A and B, show representative Na\textsubscript{1.8} current traces recorded from rat and human DRG neurons, respectively. As Fig. 7C shows, compared with Na\textsubscript{1.8} channels of rat DRG neurons, Na\textsubscript{1.8} channels of human DRG neurons produce significantly larger persistent current between \(-40\) mV and 0 mV. The normalized peak persistent current for Na\textsubscript{1.8} channels of human DRG neurons was 6.1 ± 0.7\% (\(n = 9\)), which was \(-1.8\)-fold larger than that of Na\textsubscript{1.8} channels of rat DRG neurons (3.4 ± 0.2\%, \(n = 19\); \(P < 0.01\)) (Fig. 7C). We also measured the response to a slow ramp stimulus and show that Na\textsubscript{1.8} channels in human DRG neurons produce almost twofold larger ramp current (18.6 ± 1.7\%, \(n = 8\)) compared with Na\textsubscript{1.8} channels in rat DRG neurons (9.8 ± 0.7\%, \(n = 15\); \(P < 0.001\)) (Fig. 7D). The rate of fast inactivation as a function of stimulus pulse potential is shown in Fig. 7E, which shows that the kinetics of Na\textsubscript{1.8} channel in human DRG neurons is significantly slower over the voltage range between \(-5\) and \(+30\) mV compared with Na\textsubscript{1.8} channel in rat DRG neurons.
Human DRG Neurons Generate Broader Action Potentials

Our observation that human DRG neurons produce a larger Na\textsubscript{1.8} persistent current and ramp current, and slower kinetics of inactivation, suggested that the properties of action potentials generated by human DRG neurons might be different from those of rat DRG neurons. To test this hypothesis, we performed current-clamp recording from both rat and human DRG neurons.

Human DRG neurons manifested distinct properties compared with rat DRG neurons. The RMP for human DRG neurons was \(-55.0 \pm 0.8\) mV \((n = 59)\), which was depolarized by 6 mV compared with the RMP of rat DRG neurons \((-61.0 \pm 1.9\) mV, \(n = 30); P < 0.01\). The input resistance of human DRG neurons \((153 \pm 20\) M\(\Omega, n = 55)\) was significantly smaller than that of rat DRG neurons \((659 \pm 51\) M\(\Omega, n = 30); P < 0.001\). The action potential amplitude was different between human \((111.7 \pm 1.6\) mV, \(n = 59)\) and rat \((118.5 \pm 1.8\) mV, \(n = 30); P < 0.01\) DRG neurons, consistent with the more depolarized RMP. The current threshold of human DRG neurons \(251 \pm 29\) pA, \(n = 30); P < 0.001\), consistent with the smaller input resistance of human DRG neurons.

Figure 8A shows representative action potentials recorded from a rat DRG neuron. All of the rat DRG neurons that we recorded \((30 of 30 cells)\) displayed only type I firing pattern. In contrast, 90\% \((53 of 59 cells)\) of human DRG neurons demonstrated a type I firing pattern (Fig. 8B), while 10\% \((6 of 59 cells)\) of human DRG neurons demonstrated a type II firing pattern with prolonged action potentials. Figure 8C shows the prolonged action potential recorded from one of these human DRG neurons. We also measured the duration of the action

Fig. 6. Dynamic clamp recording confirms the effect of human Na\textsubscript{1.8} on DRG neuron behavior. A and B: current traces obtained from rat (A) and human (B) Na\textsubscript{1.8} model \(I_{Na} = g_{max}m^3h(V_m - E_{Na})\). C: representative spontaneously firing action potential trace after input of human Na\textsubscript{1.8} channel conductance. D: comparison of action potential half-width between human Na\textsubscript{1.8} group and rat Na\textsubscript{1.8} group. ***P < 0.001. Action potential width in each neuron was measured after injection of human Na\textsubscript{1.8} conductance and subsequently in the same cell with injection of rat Na\textsubscript{1.8} conductance (open and filled symbols connected for each neuron). Two larger symbols indicate means \(\pm\) SE. E and F: type I representative response to rat (E) or human (F) Na\textsubscript{1.8} conductance injection. G: type II representative response displaying extremely long action potential to human Na\textsubscript{1.8} conductance injection. H: type II response displaying early afterdepolarization-like action potential to human Na\textsubscript{1.8} conductance injection.
neurons (2 of 32 cells, 6.3%; 23.4%) displayed spontaneous firing compared with rat DRG proportion of native human DRG neurons (18 of 77 cells, threefold longer compared with rat DRG neurons. Nav1.8-null mouse DRG neurons, with an average half-width that is nearly threefold larger persistent current and ramp current and slower kinetics of fast inactivation in human Nav1.8 channels, both in native human DRG and in transfected mouse DRG neurons. We demonstrate significantly broader action potentials in human Nav1.8 channels in human DRG neurons produce a ramp current that is almost double that produced by Nav1.8 channels in rat DRG neurons. E: kinetics of open-state inactivation measured as a function of voltage for Nav1.8 channel in rat DRG neurons (n = 19) and in human DRG neurons (n = 9). Time constants were obtained by fitting currents elicited as described in A and B with a single-exponential function. *P < 0.05.

DISCUSSION

In this study, we demonstrate differences in gating of human Na\(_{\text{1.8}}\) compared with rat Na\(_{\text{1.8}}\) channels, paralleled by different action potential properties of human DRG neurons compared with rat DRG neurons. We demonstrate two- to threefold larger persistent current and ramp current and slower kinetics of fast inactivation in human Na\(_{\text{1.8}}\) channels, both in native human DRG and in transfected mouse DRG neurons. We demonstrate significantly broader action potentials in human DRG neurons, with an average half-width that is nearly threefold longer compared with rat DRG neurons. Na\(_{\text{1.8}}\)-null DRG neurons that express human Na\(_{\text{1.8}}\) channels, or are endowed with the same conductance level of human Na\(_{\text{1.8}}\) current by dynamic clamp, fire action potentials that are unexpectedly long-lasting, similar to those produced by native human DRG neurons.

The basic biophysical properties of native human and rodent DRG neurons show large and statistically significant differences that are predicted to alter excitability of these neurons. The mean input resistance (153 ± 20 MΩ) and action potential threshold (964 ± 85 pA) of human DRG neurons are different from the input resistance (659 ± 51 MΩ) and threshold for action potential (251 ± 29 pA) for native rat DRG neurons but are comparable to those reported by Davidson et al. (2014) from a different set of human donors (input resistance: 97.51 ± 10.09 MΩ; threshold: 1,430 ± 110 pA), confirming that these properties are intrinsic to human DRG neurons. The action potential width in human DRG neurons (6.83 ± 0.78 ms) was threefold greater than that of rat DRG neurons (2.33 ± 0.12 ms), and human DRG neurons manifested increased firing frequencies compared with rat DRG neurons. Despite higher threshold for the initial spike in human DRG neurons, which is attributable at least in part to the lower input resistance in these neurons, they fire more action potentials once they reach that threshold. These data demonstrate clear species-specific differences in firing properties of DRG neurons, consistent with species-specific differences in ion channel conductances that underlie electrogenesis in these neurons.

Although multiple ion conductances contribute to electrogenesis in human and rodent DRG neurons, Na\(_{\text{1.8}}\) has been shown to be a major contributor to the action potential upstroke.
in these neurons and is essential for repetitive firing (Blair and Bean 2002; Renganathan et al. 2001). The slower kinetics of inactivation and the twofold increase in ramp and persistent currents of human Naᵥ1.8 channels compared with rat Naᵥ1.8 channels suggest that this channel is a major contributor to regulating action potential width and the increase in firing frequency in native human DRG neurons. Several factors may contribute to this species-specific difference in Naᵥ1.8 properties, including the substantial divergence of the primary sequence of the two channels (83% identical) and a possible difference in the distribution and affinity of interaction with channel partners, including β-subunits. It is important to note that the main differences between human and rodent Naᵥ1.8 were recapitulated when the channels were expressed in the Naᵥ1.8-null DRG neurons, suggesting a more prominent effect of divergent sequence that may be reflected in altered channel folding or in altering the affinity of interaction with auxiliary proteins. Although we cannot exclude a contribution of β-subunits or other channel partners to the different properties of human and rat Naᵥ1.8 channels, our data are more consistent with a major role of intrinsic Naᵥ1.8 gating properties in conferring species-specific differences in excitability of DRG neurons.

The Naᵥ1.8 persistent current in native human DRG neurons (6.1%) is smaller than the human Naᵥ1.8 persistent current recorded in transfected rodent DRG neurons (12.3%), but the molecular basis for the smaller persistent current in native human DRG neurons is not clear. The persistent current in native rat DRG neurons (3.4% of peak current) is comparable to the rat Naᵥ1.8 persistent current recorded in transfected rodent DRG neurons (4.3% of peak current), suggesting that overexpression of Naᵥ1.8 channels per se would not cause the large persistent human Naᵥ1.8 current in these neurons. One possibility is that the modulation of Naᵥ1.8 channels differs in different cell backgrounds. Other possibilities include interindividual variability in the expression levels of Naᵥ1.8 among the different donors or donor-specific differences in Naᵥ1.8, for example, single-nucleotide polymorphisms that may impact channel function.

Persistent and ramp sodium currents have been shown to control the excitability of many different cell types (Bennett et al. 2000; Brumberg et al. 2000; Crill et al. 1996; Cummins et al. 1998; Enomoto et al. 2006; Fleidervish and Gutnick 1996; Golomb et al. 2006; Kuo et al. 2006; Lamas et al. 2009; Vervaeke et al. 2006; Wu et al. 2005). Our observation that the persistent and ramp currents are severalfold larger for human Naᵥ1.8 compared with rat Naᵥ1.8 suggests that these aspects of Naᵥ1.8 play an important role in controlling the excitability of DRG neurons. First, our observations indicate that enhanced persistent and ramp currents of human Naᵥ1.8 channels are associated with reduced current threshold and increased firing frequency in transfected DRG neurons. It is interesting in this regard that mutations of human Naᵥ1.8 that increase the ramp current reduce threshold in DRG neurons (Faber et al. 2012; Huang et al. 2013). Second, we found that DRG neurons display different firing patterns after expression of human Naᵥ1.8 channels compared with rat Naᵥ1.8 channels. Previously, Theiss et al. (2007) reported that persistent sodium currents also play a major role in determining firing patterns in rat ventral horn spinal interneurons. In that study, cells with repetitive-firing patterns had significantly larger sodium persistent currents, and treatment with riluzole, which is known to reduce the persistent currents, changed repetitive or burst firing into a single-spiking pattern. Third, we found that human...
native human DRG neurons, Na\textsubscript{1.8}-transfected DRG neurons, and DRG neurons after input of human Na\textsubscript{1.8} conductance via dynamic clamp all display prolonged action potentials. This result is in agreement with previous reports that persistent sodium currents maintain prolonged action potentials in other types of neurons (Fleidervish and Gutnick 1996; Maltsev et al. 2007; Song et al. 2006). It is also supported by the observation (Mantegazza et al. 1998) that during perfusion with ATXII, a toxin that is known to increase sodium persistent currents, neocortical pyramidal neurons display a metastable condition characterized by very long-lasting plateau action potentials. We in fact observed these “long-lasting” plateau action potentials within DRG neurons endowed with human Na\textsubscript{1.8} currents (Fig. 3C and Fig. 6G). The plateaus of these prolonged action potentials occur close to 0 mV, a potential that coincides with the peak of the Na\textsubscript{1.8} persistent sodium current, around 0 mV, as observed in voltage-clamp recordings. Fourth, our observations suggest that the enhanced human Na\textsubscript{1.8} persistent current may also contribute to the spontaneous activity of DRG neurons. We found that native human DRG neurons and DRG neurons transfected with human Na\textsubscript{1.8} channels or after input of human Na\textsubscript{1.8} conductance all demonstrate enhanced levels of spontaneous firing activity. In this regard, it is notable that spontaneous activity in injured DRG neurons is inhibited by riluzole (Xie et al. 2011). These observations provide evidence that intrinsic biophysical properties of Na\textsubscript{1.8} channels contribute to regulation of firing in human native DRG neurons, and to the marked difference in the firing of human and rodent DRG neurons.

NGF and GDNF have been known to play important roles in the regulation of sodium channel expression in DRG neurons. In rat DRG neurons, NGF has been shown to increase Na\textsubscript{1.8} expression and affect the electrophysiological properties of DRG neurons (Fang et al. 2005; Fjell et al. 1999). GDNF has also been shown to significantly increase Na\textsubscript{1.8} currents in rat DRG neurons (Cummins et al. 2000; Fjell et al. 1999). However, these effects are generally seen in DRG neurons in culture for several days. As described in MATERIALS AND METHODS, our preliminary experiments indicated that supplements of these trophic factors were necessary to obtain viable human DRG neurons for recordings, in agreement with recently published data (Davidson et al. 2014); thus both NGF and GDNF were added into the primary human DRG culture. Therefore it is possible that differences in exposure to NGF and/or GDNF could have contributed to the different firing behavior that we observed between human and rat DRG neurons. We observed, in Na\textsubscript{1.8}-null neurons treated with NGF and GDNF and transfected with rat versus human Na\textsubscript{1.8}, a pattern of differences in firing properties that paralleled the differences between native human and rodent DRG neurons, suggesting a minimal effect of the acute application of these factors in our culture system. Our data, however, do not formally preclude an effect of NGF and/or GDNF on Na\textsubscript{1.8} expression or modulation that may have contributed to the different firing behavior that we observed between human and rat DRG neurons. Although it is possible that production of bigger current by transfected human Na\textsubscript{1.8} channels compared with rat Na\textsubscript{1.8} channels could contribute to the different firing patterns of DRG neurons expressing these channels, our voltage-clamp data show comparable levels of human Na\textsubscript{1.8} and rat Na\textsubscript{1.8} current densities. Since persistent current levels were normal-ized to peak currents, the increased persistent current of human Na\textsubscript{1.8} reflects a difference in the gating properties of the channel, and the effect on neuronal firing cannot be attributed to higher levels of expression of human Na\textsubscript{1.8} channels. Our dynamic clamp recording (Kemenes et al. 2011; Samu et al. 2012; Sharp et al. 1993; Vasylyev et al. 2014) permitted us to more directly examine the effect on the same DRG neuron of precisely calibrated levels of human or rat Na\textsubscript{1.8} channel currents. Our dynamic clamp recordings in DRG neurons recapitulate the differential effects of human Na\textsubscript{1.8} channels compared with rat Na\textsubscript{1.8} channels on firing properties of DRG neurons, further supporting the conclusion that the unique properties of human Na\textsubscript{1.8} channels shape the pattern of DRG neuron firing.

A role of Na\textsubscript{1.8} in human pain has been demonstrated by the link of Na\textsubscript{1.8} mutations to human pain syndromes (Faber et al. 2012), validating this channel as a target for pain treatment. Our results show that human Na\textsubscript{1.8} channels produce a large persistent current that contributes to determining the firing patterns of DRG neurons. Na\textsubscript{1.8} is known to be present in the central portion of primary afferent axons in the dorsal horn (Amaya et al. 2000), and functional Na\textsubscript{1.8} channels are known to be present along the neurites of DRG neurons in vitro (Vasylyev and Waxman 2012). It has been demonstrated that in some presynaptic terminals persistent currents can modulate transmitter release (Engel and Jonas 2005; Huang and Trussell 2008). Thus it is reasonable to suggest that the presence of Na\textsubscript{1.8} in or close to the central terminals of DRG neurons may have an effect on transmitter release in the dorsal horn, with enhanced synaptic transmission at the terminals of C-type cells. Whether this occurs within the human dorsal horn remains to be determined. Notwithstanding this uncertainty, the distinct properties of human Na\textsubscript{1.8} and the distinct properties of action potentials in human DRG neurons compared with rodent DRG neurons should be taken into account when extrapolating from rodent studies of pain to humans and testing novel blockers for treatment of pain.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

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