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A single sodium channel mutation produces hyper- or hypoexcitability in different types of neurons

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Disease-producing mutations of ion channels are usually characterized as producing hyperexcitability or hypoexcitability. We show here that a single mutation can produce hyperexcitability in one neuronal cell type and hypoexcitability in another neuronal cell type. We studied the functional effects of a mutation of sodium channel $Na_v1.7$ associated with a neuropathic pain syndrome, erythermalgia, within sensory and sympathetic ganglion neurons, two cell types where $Na_v1.7$ is normally expressed. Although this mutation depolarizes resting membrane potential in both types of neurons, it renders sensory neurons hyperexcitable and sympathetic neurons hypoexcitable. The selective presence, in sensory but not sympathetic neurons, of the $Na_v1.8$ channel, which remains available for activation at depolarized membrane potentials, is a major determinant of these opposing effects. These results provide a molecular basis for the sympathetic dysfunction that has been observed in erythermalgia. Moreover, these findings show that a single ion channel mutation can produce opposing phenotypes (hyperexcitability or hypoexcitability) in the different cell types in which the channel is expressed.

Mutations in voltage-gated sodium channels have been associated with a number of neurological disorders including inherited epilepsy, muscle disorders, and primary erythermalgia, an autosomal dominant neuropathy characterized by pain of the extremities in response to mild warmth. Recent studies have demonstrated mutations in primary erythermalgia in $Na_v1.7$ (1), a sodium channel that is preferentially expressed

within primary sensory [such as nociceptive dorsal root ganglion (DRG)] and sympathetic ganglion [e.g., superior cervical ganglion (SCG)] neurons (2-6). The $Na_v1.7$ mutations characterized to date produce changes in channel physiology that include hyperpolarizing shifts in activation, depolarizing shifts in steady-state inactivation, slowing of deactivation, and an increase in the "ramp" current evoked by slow, small depolarizations, all augmenting the response of $Na_v1.7$ channels to small stimuli (3, 6, 7). One of these mutations, F1449V, has been assessed at the level of cell function within DRG neurons, where it produces hyperexcitability (3). However, the effects on cell function of $Na_v1.7$ mutations have not been assessed in sympathetic ganglion neurons, where $Na_v1.7$ is also present.

Because different ensembles of channels are present within DRG and SCG neurons, we hypothesized that the same sodium channel mutation might have different effects on excitability in these two neuronal types. Here we test this hypothesis for one of the first $Na_v1.7$ erythermalgia mutations to be characterized, L858H (2, 7). We show that although the L858H mutation produces a depolarizing shift in resting membrane potential (RMP) in both cell types, it renders DRG neurons hyperexcitable and SCG neurons hypoexcitable. We demonstrate that the opposing functional effects of this mutation are a result of the selective presence of another sodium channel, $Na_v1.8$, in DRG, but not SCG, neurons. These results suggest a contribution of $Na_v1.7$ mutant channels to the sympathetic dysfunction that has been reported in erythermalgia. More generally, these observations show that a single mutation can cause functionally opposing changes in the different types of neurons in which the gene is expressed.

Results

L858H Mutation Produces Depolarization of RMP and Decreases Action Potential Threshold in DRG Neurons. Fig. 1 shows the effect of the $Na_v1.7$/L858H mutation (L858H) on firing threshold in DRG neurons, a cell type that is known to express $Na_v1.7$ (8, 9). DRG neurons expressing wild-type (WT) $Na_v1.7$ produced robust overshooting action potentials in response to stepwise current inputs. The representative cell shown in Fig. 1*A*, with a RMP of approximately -51 mV, produced subthreshold responses to 50- to 130-pA current injections and required a 135-pA input to elicit an all-or-none action potential that arose at a voltage threshold of approximately -15 mV.

Fig. 1. L858H renders DRG neurons hyperexcitable. (*A* and *B*) Action potentials were evoked from small (25 μm in diameter) DRG neurons by using depolarizing current injections from the RMP. V_m, membrane potential. (*A*) Representative traces from a cell expressing WT $Na_v1.7$ show subthreshold responses to 50- to 130-pA

current injections and subsequent all-or-none action potentials evoked by injections of 135 pA (current threshold for this neuron) and 155 pA. (*B*) In contrast, in a cell expressing L858H, action potentials were evoked by a 60-pA current injection. The voltage for take-off of the all-or-none action potential (approximately -14.5 mV, dashed line) was similar for the neurons in *A* and *B*. (*C*) L858H causes a depolarizing shift in the RMP of DRG neurons. DRG neurons expressing WT $Na_v1.7$ had an average RMP of -50.1 ± 0.9 mV (n = 20), whereas those expressing L858H mutant channels had a significantly (*, $P < 0.001$) depolarized RMP of -44.9 ± 1.1 (n = 25). (*D*) The average current threshold for action potential firing of DRG neurons expressing WT $Na_v1.7$ channels was 120.6 ± 23.9 pA (n = 20), whereas that of neurons expressing L858H mutant channels was significantly (*, $P < 0.01$) reduced to 69.2 ± 9.8 pA (n = 25). (*E*) Action potential overshoot in cells expressing WT $Na_v1.7$ channels (67.8 ± 3.0 mV, n = 20) was not significantly different from that in cells expressing L858H mutant channels (64.4 ± 2.6 mV, n = 20; $P > 0.05$). The voltage of action potential take-off was unchanged (WT, -14.5 ± 1.2 mV, n = 20; L858H, -14.5 ± 1.3 mV, n = 25; $P > 0.05$). n.s., not significant.

In contrast, the RMP is approximately -46 mV, i.e., is depolarized by 5 mV, in a representative DRG neuron expressing L858H mutant channels, and this cell required a much lower current input of only 60 pA for firing (Fig. 1*B*). For the entire population of cells studied, the average RMP of DRG cells expressing L858H was significantly more depolarized (-44.9 ± 1.1 mV, n = 25) than that of cells expressing WT $Na_v1.7$ (-50.1 ± 0.9 mV, n = 20) ($P < 0.001$; Fig. 1*C*). The current threshold was significantly decreased, by >40% ($P < 0.01$), in cells expressing L858H channels (69.2 ± 9.8 pA, n = 25) compared with WT $Na_v1.7$ (120.6 ± 23.9 pA, n = 20) (Fig. 1*D*). Despite the depolarized RMP and changes in current threshold, action potential overshoot was not significantly different in cells expressing WT $Na_v1.7$ (67.8 ± 3.0 mV, n = 20) or L858H channels (64.4 ± 2.6 mV, n = 20) ($P > 0.05$; Fig. 1*E*).

L858H Mutation Produces Depolarization of RMP but Increases Action Potential Threshold in SCG Neurons. We next investigated the functional effects of L858H mutant channels in SCG neurons, which are also known to express $Na_v1.7$ (8, 9). Fig. 2*A* shows subthreshold responses evoked by 15- to 20-pA current injections from a representative SCG neuron expressing WT $Na_v1.7$ channels, which required current injections of 25 pA to reach the threshold of approximately -20 mV and fire all-or-none action potentials, from the RMP of -47 mV.

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Fig. 2. L858H renders SCG neurons hypoexcitable. (*A* and *B*) Action potentials were evoked by using depolarizing current injections from resting potential. (*A*) Representative traces from a cell expressing the WT channel show subthreshold responses to 15- to 20-pA current

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injections and subsequent all-or-none action potentials evoked by injections of 25 pA. (*B*) In contrast, in a cell expressing the L858H channel, action potentials required a 70-pA current injection. The voltage for take-off (dashed line) of the all-or-none action potential was unchanged. (*C*) L858H channels caused a depolarizing shift in the RMP of SCG neurons. SCG neurons expressing WT channels had an average RMP of -46.3 ± 0.8 mV (n = 15), whereas those expressing L858H had a significantly ($P < 0.001$) depolarized RMP of -41.6 ± 0.8 (n = 17). (*D*) The average current threshold for action potential firing of SCG neurons expressing WT channels was 22.7 ± 3.6 pA (n = 15), whereas that of neurons expressing L858H channels was significantly (*, $P < 0.01$) increased to 42.9 ± 6.3 pA (n = 17). (*E*) Action potential overshoot in cells expressing WT channels (47.8 ± 3.4 mV, n = 15) was significantly larger (*, $P < 0.001$) than that in cells expressing L858H (23.8 ± 4.7 mV, n = 20). The voltage of action potential take-off was unchanged (WT, -23.1 ± 1.2 mV, n = 15; L858H, -19.8 ± 1.3 mV, n = 17; *, $P > 0.05$).

SCG neurons expressing L858H channels showed a depolarization of the RMP by 5 mV, similarly to DRG neurons, but the effects of L858H on excitability were markedly different. Fig. 2*B* shows a representative SCG neuron where only subthreshold responses were seen with a <70-pA current injection, inputs that produced action potentials with WT $Na_v1.7$ channel expression. When the threshold was reached (approximately -16 mV) with a 70-pA input, the neuron generated an action potential, but it was attenuated, with substantially reduced overshoot. The average RMP of SCG neurons expressing L858H channels was significantly more depolarized (-41.6 ± 0.8 mV, n = 17) than that of cells expressing WT $Na_v1.7$ channels (-46.3 ± 0.8 mV, n = 15) ($P < 0.001$; Fig. 2*C*). For the entire population of SCG cells studied, current threshold was significantly increased by 88% ($P < 0.01$) in cells expressing L858H (42.9 ± 6.3 pA, n = 17) compared with WT $Na_v1.7$ channels (22.7 ± 3.6 pA, n = 15) (Fig. 2*D*). In contrast to DRG neurons where action potential overshoot was maintained after expression of L858H, action potential overshoot was significantly reduced by 50% ($P < 0.001$) in SCG neurons expressing L858H (23.8 ± 4.7 mV, n = 20) compared with WT channels (47.8 ± 3.4 mV, n = 15) (Fig. 2*E*). Thus, in contrast to DRG neurons where L858H mutant channels reduce threshold for single action potentials, expression of L858H in SCG neurons has an opposite effect, increasing threshold.

L858H Mutation Enhances Repetitive Firing in DRG Neurons. Previous studies have shown that 50% of DRG neurons fire repetitively in response to sustained depolarizing stimuli (3, 10-13). In this study, 65% (13 of 20) of DRG neurons expressing WT $Na_v1.7$ fired two or more action potentials in response to prolonged stimulation. Fig. 3*A* shows a representative DRG neuron that fired one action potential in response to a 950-ms input of 100 pA but was capable of firing multiple action potentials with a higher current injection of 250 pA (Fig. 3*A Inset*). With expression of L858H, a higher proportion of

DRG neurons (88%, or 21 of 24) fired two or more action potentials in response to 950-ms current injections. Fig. 3*B* shows a representative DRG neuron expressing L858H, which responded to a 100-pA input with five action potentials, i.e., a higher frequency than for WT $Na_v1.7$ channels. For the entire population of DRG neurons studied, the firing frequency evoked with 50- and 100-pA inputs was increased by 550% ($P < 0.05$) and 280% ($P < 0.05$), respectively, in neurons with L858H compared with WT $Na_v1.7$ channels (Fig. 3*C*).

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Fig. 3. The L858H mutation increases firing frequency in DRG and decreases firing frequency in SCG neurons. (*A*) Representative DRG neuron expressing WT $Na_v1.7$ fires a single action potential in response to a 950-ms input of 100 pA from the RMP of this neuron (approximately -50 mV). (*Inset*) The same neuron fires multiple action potentials in response to a 250-pA stimulus. (*B*) Representative DRG neuron expressing L858H fires five action potentials in response to a 100-pA current injection from the RMP of this neuron (approximately -42 mV). (*C*) For the entire population of DRG neurons studied, the firing frequency evoked by 50-pA current stimuli was 0.32 ± 0.13 Hz after transfection with WT channels ($n = 20$) and 2.06 ± 0.79 Hz after transfection with L858H ($n = 24$; $*$, $P < 0.05$), and the firing frequency evoked by 100-pA stimuli was 0.89 ± 0.28 Hz after transfection with WT and 3.37 ± 1.13 Hz after transfection with L858H ($*$, $P < 0.05$). (*D*) Representative SCG neuron expressing WT $Na_v1.7$ fires six action potentials in response to a 950-ms input of 40 pA from the RMP (approximately -45 mV). (*E*) Representative SCG neuron expressing L858H fires only two action potentials in response to a 100-pA current injection from the RMP (approximately -40 mV). (*Inset*) When the cell was held at -60 mV to overcome the depolarization of the RMP caused by L858H, it produced four action potentials with an identical stimulus. (*F*) For the entire population of SCG neurons studied, the firing frequency evoked by 30-pA stimuli was 5.33 ± 1.5 Hz after transfection with WT channels ($n = 14$) and 0.63 ± 0.01 Hz after transfection with L858H channels ($n = 15$; $P < 0.05$). The firing frequency evoked by 40-pA stimuli was 7.05 ± 1.86 Hz after transfection with WT and 1.96 ± 1.0 Hz after transfection with L858H channels ($*$, $P < 0.05$).

L858H Mutation Attenuates Repetitive Firing in SCG Neurons. SCG neurons also fired repetitively in response to prolonged stimuli, but, as with threshold, the effect of the L858H mutation was opposite to that in DRG neurons. Ninety-three percent (13 of 14) of SCG neurons expressing WT $Na_v1.7$ channels produced multiple action potentials in response to prolonged stimulation. Fig. 3*D* shows a representative SCG neuron

expressing WT $Na_v1.7$, where six action potentials were produced in response to a 950-ms current injection of 40 pA. In contrast, an identical stimulus in a SCG neuron expressing L858H evoked only two action potentials, with substantially reduced overshoot (Fig. 3*E*). Interestingly, when this cell was held at -60 mV (a maneuver that reversed the depolarization induced by L858H), multiple overshooting action potentials could be evoked (Fig. 3*E Inset*); thus, the intrinsic ability of this neuron to fire repetitive and full-scale action potentials was not impaired. Compared with SCG neurons expressing WT $Na_v1.7$, a decreased proportion (53%, or 8 of 15) of SCG neurons expressing L858H produced two or more action potentials in response to prolonged stimulation. The firing frequency of SCG neurons with L858H channels in response to 950-ms inputs of 30 and 40 pA was substantially reduced by 88% ($P < 0.02$) and 72% ($P < 0.03$), respectively, compared with SCG neurons with WT $Na_v1.7$ channels (Fig. 3*F*). Attenuated repetitive firing appeared to be caused by the depolarization of RMP by L858H because, in 91% (10 of 11) of the cells tested, multiple action potential firing could be restored by hyperpolarizing to a holding potential of -60 mV.

SCG Neurons Express $Na_v1.7$ but Not $Na_v1.8$. DRG neurons express $Na_v1.8$ as well as $Na_v1.7$ sodium channels (8, 9, 14-16), and action potential generation in these cells involves sequential activation of $Na_v1.7$ and then $Na_v1.8$ (10, 17, 18). SCG neurons normally express $Na_v1.7$ (8, 9), but the full complement of sodium channel isoforms within SCG neurons has not been previously defined. We therefore identified the sodium channel isoforms present in SCG neurons and compared them with DRG neurons using multiplex PCR and restriction enzyme polymorphism analysis (19). Restriction analysis of DRG (Fig. 4*A*, lanes 1-9) demonstrated $Na_v1.1$ (lane 2), $Na_v1.6$ (lane 6), $Na_v1.7$ (lane 7), $Na_v1.8$ (lane 8), and $Na_v1.9$ (lane 9) in the cDNA pool, in agreement with previous results (19). In contrast, profiling of the SCG products (Fig. 4*A*, lanes 10-18) demonstrated $Na_v1.3$ (lane 13), $Na_v1.6$ (lane 15), and $Na_v1.7$ (lane 16). We confirmed the presence of $Na_v1.7$ (Fig. 4*Ba*) and $Na_v1.8$ (Fig. 4*Bb*) protein in adult rat DRG and postnatal day 2 (P2) rat DRG neurons in culture (Fig. 4*B c* and *d*) by immunocytochemistry using isoform-specific antibodies. Consistent with published studies on sodium currents in SCG neurons (20-22), we observed that adult rat SCG neurons express $Na_v1.7$ (Fig. 4*Ca*), but not $Na_v1.8$ (Fig. 4*Cb*), in native tissue and that $Na_v1.8$ expression is not induced in P2 rat SCG neurons under culture conditions (Fig. 4*C c* and *d*).

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Fig. 4. DRG neurons express $Na_v1.7$ and $Na_v1.8$; SCG neurons express $Na_v1.7$ but not $Na_v1.8$. (*A*) Restriction analysis of multiplex PCR amplification products from sodium channel domain 1 from adult DRG (lanes 1-9) and SCG (lanes 10-18). M, 100-bp ladder marker (Promega). Lanes 1 and 10 contain amplification products from DRG and SCG, respectively. Lanes 2-9 and 11-18 show results of cutting this DNA with EcoRV, EcoNI, AvaI, AccI, SphI, BamHI, AflII, and EcoRI, which are specific to subunits $Na_v1.1$, $Na_v1.2$, $Na_v1.3$, $Na_v1.5/1.9$,

window] $Na_v1.6$, $Na_v1.7/1.8$, $Na_v1.8$, and $Na_v1.9$ (details can be found in Table 1, which is published as supporting information on the PNAS web site). Restriction products in lanes 2 and 5-9 show the presence of $Na_v1.1$, $Na_v1.6$, $Na_v1.7$, $Na_v1.8$, and $Na_v1.9$ in DRG, in agreement with previous results (19). Restriction products in lanes 13, 15, and 16 show the presence of $Na_v1.3$, $Na_v1.6$, and $Na_v1.7$ in SCG. (*B* and *C*) Immunostaining of $Na_v1.7$ and $Na_v1.8$ channels in DRG and SCG neurons *in vivo* and in cultured neurons. (*B*) $Na_v1.7$ (*a*) and $Na_v1.8$ (*b*) proteins are present in adult DRG neurons *in vivo*; $Na_v1.7$ (*c*) and $Na_v1.8$ (*d*) proteins are present in cultured DRG neurons from postnatal day 2 (P2) rat pups. (*C*) $Na_v1.7$ (*a*), but not $Na_v1.8$ (*b*), protein is present in adult SCG neurons *in vivo*; $Na_v1.7$ (*c*), but not $Na_v1.8$ (*d*), protein is present in cultured SCG neurons from P2 rat pups. (Scale bars, 50 μm.)

Selective Presence of $Na_v1.8$ Within DRG, but Not SCG, Neurons Contributes to Opposing Effects of the L858H Mutation in These Two Cell Types. Having demonstrated that L858H produces hyperexcitability in DRG neurons and hypoexcitability in SCG neurons, we hypothesized that this difference was caused, at least in part, by the presence of $Na_v1.8$ in DRG neurons (14-16) and its absence in SCG neurons. $Na_v1.8$ channels have depolarized voltage-dependence of activation and inactivation (14, 15, 23, 24) and thus allow DRG neurons to fire action potentials even when depolarized (10). We tested this hypothesis by coexpressing $Na_v1.8$ together with L858H in SCG neurons and examining the effects on firing behavior. Fig. 5*A* shows representative suprathreshold action potentials from SCG cells expressing WT $Na_v1.7$ (blue), L858H (red), and L858H plus $Na_v1.8$ (green). As before, expression of L858H channels depolarized the RMP and reduced action potential overshoot. However, when $Na_v1.8$ was coexpressed with L858H, action potential overshoot was restored, even though the depolarization of the RMP induced by L858H persisted. For the population of cells studied, the depolarization of the RMP by 5mV (-41.6 ± 0.76 mV, $n = 17$ with L858H) was maintained with coexpression of $Na_v1.8$ (-40.5 ± 1.01 mV, $n = 17$) ($P > 0.05$; Fig. 5*B*). However, current threshold for firing was reduced when $Na_v1.8$ was coexpressed with L858H ($P < 0.05$; Fig. 5*C*). In addition, action potential overshoot was restored when $Na_v1.8$ was coexpressed with L858H ($P < 0.05$; Fig. 5*D*). These results show that the presence or absence of $Na_v1.8$ is a major determinant of the functional effects of this mutation.

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Fig. 5. Coexpression of L858H and $Na_v1.8$ channels rescues electrogenic properties in SCG neurons. When $Na_v1.8$ was coexpressed with L858H, current threshold and action potential overshoot were restored, although the depolarization of the RMP induced by L858H

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persisted. (*A*) Suprathreshold action potentials recorded from representative SCG neurons transfected with WT (blue), L858H (red), and L858H plus $Na_v1.8$ (green) channels. (*B*) Depolarized RMP in cells with L858H channels (-41.6 ± 0.76 mV, $n = 17$) was maintained with coexpression of $Na_v1.8$ (-40.5 ± 1.01 mV, $n = 17$; $P > 0.05$). n.s., not significant. (*C*) Current threshold for action potential firing was reduced from 42.9 ± 6.3 pA ($n = 17$) for L858H to 26.8 ± 4.3 pA ($n = 17$) for L858H coexpressed with $Na_v1.8$ (*, $P < 0.05$). (*D*) Action potential overshoot in SCG neurons with L858H channel (23.8 ± 4.7 mV, $n = 17$) was increased when $Na_v1.8$ was coexpressed with L858H (41.5 ± 4.6 mV, $n = 17$; *, $P < 0.05$).

Discussion

The L858H erythermalgia mutation results in a single amino acid substitution in the domain II S4-S5 linker within $Na_v1.7$ (2, 7), a sodium channel that is preferentially expressed in DRG and sympathetic ganglia neurons (8, 9, 16, 25). Our experiments show that L858H produces hyperexcitability (decreased threshold and enhanced repetitive firing) within DRG neurons and hypoexcitability (increased threshold and attenuated repetitive firing) within sympathetic ganglion neurons. The latter observation provides a molecular basis for the sympathetic dysfunction that has been reported (26, 27) in erythermalgia. Moreover, this observation suggests the more general hypothesis that other ion channel mutations may have differing physiological effects in different cell types in which the channel is normally expressed. Although opposing functional effects of the same mutation in different types of neurons may at first seem paradoxical, we demonstrate that it is caused by different cell backgrounds, including different repertoires of other ion channels within the two types of neurons.

The L858H mutation produces a hyperpolarizing shift in activation, slows deactivation, and increases the ramp response of $Na_v1.7$ to small stimuli (7). We observed that L858H produces a depolarization, of 5 mV, in the RMP of both DRG and SCG neurons. A similar depolarization has been observed with a mutation of the adjacent residue in the $Na_v1.4$ muscle sodium channel that is associated with muscle weakness (28, 29). The depolarization may be a result of increased window currents predicted from voltage-clamp analysis of L858H to be present between -80 and -35 mV and largest between -60 and -45 mV, close to resting potential (7) because of the hyperpolarizing shift in activation, as for the $Na_v1.4$ mutation (28, 29).

We hypothesized that L858H produces hyperexcitability in DRG neurons and hypoexcitability in SCG neurons because of the selective expression of $Na_v1.8$ sodium

channels in DRG and its absence in SCG neurons. The majority of nociceptive DRG neurons express $Na_v1.8$ (14, 30), which contributes most of the current underlying the action potential upstroke (10, 18). Because it has depolarized voltage-dependence of activation [$V_{1/2}$ = -16 to -21 mV (14, 15, 23, 24)] and inactivation [$V_{1/2}$ = -30 mV (14, 15, 23, 24)] compared with other sodium channels, $Na_v1.8$ permits DRG neurons to generate action potentials and sustain repetitive firing when depolarized (10, 12). This finding led us to predict that $Na_v1.7$ mutations can produce hyperexcitability of DRG neurons because these cells also express $Na_v1.8$ channels that are, because of depolarized inactivation voltage-dependence, still available for activation.

We observed a similar depolarization, of 5 mV, in SCG neurons after the expression of L858H, but in these neurons the mutation produced hypoexcitability, i.e., increased threshold, attenuated repetitive firing, and reduced action potential amplitude. $Na_v1.3$, $Na_v1.6$, and $Na_v1.7$, but not $Na_v1.8$, are present in SCG neurons (Fig. 4). Because of the relatively hyperpolarized steady-state inactivation of $Na_v1.3$, $Na_v1.6$, and $Na_v1.7$ in neurons [$V_{1/2}$ values between -65 and -78 mV (31, 32)], the L858H-induced depolarizing shift in the RMP would be expected to inactivate sodium channels within SCG neurons, with a resultant decrease in excitability and action potential amplitude. Consistently with this, when we held SCG neurons expressing the L858H mutation at potentials hyperpolarized compared with their RMP, excitability and action potential amplitude increased (Fig. 3*E*).

To test the hypothesis that the selective presence within DRG neurons of $Na_v1.8$, with its depolarized $V_{1/2}$ of activation and inactivation (14, 15, 23, 24), contributes to the opposing functional effects of L858H in DRG and SCG neurons, we expressed $Na_v1.8$ within SCG neurons, where it is not normally present. In agreement with this hypothesis, the coexpression of $Na_v1.8$ with L858H tended to restore action potential threshold and amplitude toward the values seen with WT $Na_v1.7$, protecting against the hypoexcitability conferred by L858H in the absence of $Na_v1.8$, even though L858H produced a depolarizing shift of 5 mV in the RMP. Although we cannot exclude an additional contribution of differential expression of other molecules, such as potassium channels, to the opposing changes in excitability in DRG and SCG neurons expressing L858H, our results show that $Na_v1.8$, which is selectively expressed within DRG neurons, is a major contributor to this effect.

Our results show that the same sodium channel mutation can produce hyperexcitability in one type of neuron in which the channel gene is normally expressed, while producing hypoexcitability in another type of neuron where the gene is also expressed. Thus, the effects of sodium channel mutations on neuronal function should not be considered as unidirectional or predictable on the basis of the changes in channel function *per se*: they also depend on the cell background in which the mutation is expressed. More generally, we suggest the possibility that mutations of other ion channels, e.g., other sodium channel isoforms or calcium or potassium channels, may have different functional effects in different types of cells.

Materials and Methods

SCG and DRG Cultures. As described in ref. 33, we isolated SCG from deeply anesthetized (ketamine/xylazine, 80:10 mg/kg, i.p.) 1- to 5-day-old Sprague-Dawley rats, washed them with cold Hanks' balanced salt solution (HBSS) (Ca^{2+}- and Mg^{2+}-free), incubated them for 40 min at 37°C in HBSS (Ca^{2+}- and Mg^{2+}-free) containing 0.2% trypsin (Worthington), washed them twice in warm Leibovitz's L-15 medium (Invitrogen), and triturated them with a fire-polished Pasteur pipette in Leibovitz's L-15 medium containing 0.75 mg/ml BSA/trypsin inhibitor. SCG cells were pelleted by low-speed centrifugation, resuspended in modified Leibovitz's L-15 medium supplemented with 1 μg/ml nerve growth factor (Alomone, Jerusalem), 5% rat serum, 38 mM glucose, 24 mM sodium bicarbonate, and penicillin/streptomycin (each 50 units/ml), plated on 12-mm circular coverslips precoated with poly(D-lysine)/laminin (BD Biosciences, Franklin Lakes, NJ) and incubated at 37°C in 5% CO_2. DRG neurons from age-matched animals were cultured as described in ref. 34.

Cell Culture Immunocytochemistry. We incubated cultured DRG and SCG neurons sequentially in complete saline solution, 4% paraformaldehyde in 0.14 M Sorensen's buffer (pH 7.4) for 10 min, PBS, blocking solution (PBS containing 5% normal goat serum, 2% BSA, and 0.1% Triton X-100) for 30 min, and primary Ab [rabbit anti-$Na_v1.7$, 6 μg/ml, and rabbit anti-$Na_v1.8$, 3.2 μg/ml (Alomone)] in blocking solution overnight at 4°C. The next day, we incubated the coverslips sequentially in PBS, goat anti-rabbit IgG-Cy3 secondary Ab (0.5 μg/ml, Amersham Pharmacia), and PBS. Coverslips were mounted on slides with Aqua-Poly/Mount and examined with a Nikon E800 microscope equipped with epifluorescent optics by using METAVUE software (Universal Imaging, Downington, PA).

Tissue Immunocytochemistry. Adult Sprague-Dawley rats were deeply anesthetized (80 mg/kg ketamine/10 mg/kg xylazine, i.p.) and perfused with PBS and then 4% paraformaldehyde in 0.14 M Sorensen's buffer. SCG and DRG were excised, rinsed with PBS, and cryoprotected in 30% sucrose in PBS overnight at 4°C (35). Cryosections (10 μm) of DRG and SCG were incubated sequentially in (*i*) PBS containing 5% normal goat serum, 2% BSA, and 0.1% Triton X-100 for 30 min; (*ii*) primary Ab [rabbit anti-$Na_v1.7$, 6 μg/ml (Alomone), and rabbit anti-$Na_v1.8$, 0.3 μg/ml (35)] overnight at 4°C; (*iii*) PBS,

six times for 5 min each time; (*iv*) goat anti-rabbit IgG-biotin (1:250); (*v*) PBS, six times for 5 min each time; (*vi*) avidin-horseradish peroxidase (1:250); (*vii*) PBS, six times for 5 min each time; (*viii*) 0.4% diaminobenzidine and 0.003% hydrogen peroxide in PBS for 7 min; and (*ix*) PBS containing 0.02% sodium azide and coverslipped with Aqua-Poly/Mount.

Reverse Transcription-Multiplex PCR. We synthesized first-strand cDNA from total cellular RNA isolated from L4-L5 DRG and SCG dissected from adult Sprague-Dawley rats, using the RNeasy Mini Kit (Qiagen, Valencia, CA) (19). We amplified fragments of sodium channel templates in the cDNA pool by multiplex PCR using four forward and three reverse primers (F1-F4 and R1-R3) designed against highly conserved sequences in domain 1 of -subunits (19). We investigated the presence of specific sodium channel templates by digesting 1/20th of the volume of the multiplex amplicons in a 10-μl final volume with restriction enzymes that produce distinct restriction products, as described in ref. 19.

Transfection of DRG and SCG Neurons and Current-Clamp Recordings. We transfected DRG and SCG neurons as described in ref. 3, using Rat Neuron Nucleofector Solution (Amaxa, Gaithersburg, MD) and a channel/GFP ratio of 5:1, with WT tetrodotoxin-resistant $Na_v1.7$ (36) or L858H mutant derivative (7), and, in a cotransfection assay, we combined the L858H mutant channel and WT $Na_v1.8$ channel to transfect SCG neurons. Transfected DRG and SCG neurons were incubated in DMEM (Ca^{2+}- and Mg^{2+}-free) plus 10% FCS (for 5 min at 37°C) to allow recovery, diluted in regular culture medium (for DRG, DMEM/FCS; for SCG, modified Leibowitz's L-15) supplemented with nerve growth factor and glial cell-derived neurotrophic factor (50 ng/ml), plated on precoated 12-mm circular coverslips, and incubated at 37°C in 5% CO_2.

- Whole-cell current-clamp recordings were made from transfected small-diameter (25 μm) DRG or SCG neurons with robust GFP fluorescence, within 24-60 h, at room temperature (21-25°C) by using an Axopatch 200B amplifier (Axon Instruments, Union City, CA). The pipette solution contained 140 mM KCl, 0.5 mM EGTA, 5 mM Hepes, and 3 mM MgATP (pH 7.3), adjusted to 315 mosM/liter with glucose. External solution contained 140 mM NaCl, 3 mM KCl, 2 mM $MgCl_2$, 2 mM $CaCl_2$, and 10 mM Hepes (pH 7.3), adjusted to 310 mosM/liter with glucose. Pipette potential was set to zero before seal formation without correction for liquid junction potentials. We cancelled capacity transients before switching to current-clamp mode and compensated for series resistance (3-6 M) by 70%. Traces were acquired from cells with stable RMP, excluding cells where RMP changed by >10%, by using CLAMPEX 8.1 software (Axon Instruments), filtered at 5 kHz, at a sampling rate of 20 kHz. Input resistances, measured by recording voltage changes evoked by injection of hyperpolarizing current, were not significantly different between groups. We measured action potential threshold at the beginning of the sharp upward rise of the action potential and determined current threshold by a series of depolarizing currents from 0 to 200 pA in 5-pA increments. We assessed repetitive firing by recording responses to sustained (950 ms) injection of depolarizing current.

Statistical Analysis. Data are presented as mean ± SEM. Data were analyzed by using CLAMPFIT 8.2 (Axon Instruments) and ORIGIN 6.1 (Microcal Software, Northampton, MA) software. Statistical significance was determined by using Student's *t* test, where we assumed that the apparent Gaussian nature of the data sets would be extended to the population.

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Footnotes

Abbreviations: DRG, dorsal root ganglion/ganglia; SCG, superior cervical ganglion/ganglia; RMP, resting membrane potential.

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