Topical Review

Multiple sodium channels and their roles in electrogenesis within dorsal root ganglion neurons

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Dorsal root ganglion neurons express an array of sodium channel isoforms allowing precise control of excitability. An increasing body of literature indicates that regulation of firing behaviour in these cells is linked to their patterns of expression of specific sodium channel isoforms, which have been discovered to possess distinct biophysical characteristics. The pattern of expression of sodium channels differs in different subclasses of DRG neurons and is not fixed but, on the contrary, changes in response to a variety of disease insults. Moreover, modulation of channels by their environment has been found to play an important role in the response of these neurons to stimuli. In this review we illustrate how excitability can be finely tuned to provide contrasting firing templates in different subclasses of DRG neurons by selective deployment of various sodium channel isoforms, by plasticity of expression of these proteins, and by interactions of these sodium channel isoforms with each other and with other modulatory molecules.

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Introduction

Although electrogenesis in neurons has classically been considered to be the product of activity of 'the' sodium channel, we now know that multiple isoforms of voltage-gated sodium channels (Na_v) are present within neurons (Catterall *et al.* 2005). It is becoming increasingly clear that these multiple sodium channel isoforms collaborate in the production of electrical activity by neurons. Particularly fruitful for the study of sodium channels and their roles in electrogenesis are the neurons of the dorsal root ganglia (DRG), a collection of cell bodies of the afferent sensory fibres, which lie between adjacent vertebrae. The soma of the DRG neuron is spherical, and is located on a side branch of the main axon (receiving little synaptic input) and its isolation for techniques such as patch-clamp is relatively easy and can provide excellent recording conditions, making it an especially tractable model neuron. Additionally, changes in excitability of DRG neurons are of importance in a number of pathological conditions. Our understanding of the very different roles particular sodium channels play in influencing excitability of DRG neurons has progressed rapidly. In this review, we examine the roles of sodium channels in the excitability of DRG neurons.

Multiple sodium channel subtypes within DRG neurons

Conduction velocity of the dorsal root fibres has been associated with DRG cell size, classifying these neurons into four main groups: Aα, 30–55 m s^{-1}; Aβ, 14–30 m s^{-1}; Aδ, 2.2–8 m s^{-1}; and C, $<$1.4 m s^{-1} (Harper & Lawson, 1985*a*,*b*). These fibres relay information from muscle and skeletal mechanoreceptors (Aα, Aβ), cutaneous and subcutaneous mechanoreceptors (Aβ, Aδ) and nociceptors (Aδ, C). The slower velocity fibres are generally associated with small cells, and the action potential duration is broader, often displaying an inflection or hump on the falling phase. Many of these characteristics have now been associated with expression of particular sodium channel subtypes in specific subclasses of DRG neurons. Studies of C-fibres (e.g. from sciatic nerve) have shown that they can generate a slow sodium-dependent spike that is resistant to tetrodotoxin

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Table 1. Summary of DRG sodium channels

Na^+ channel Isoform	Type (Pharmacology/ kinetics)	Distribution	Unique biophysical characteristics in DRG	Role in AP generation
$Na_v1.1$	TTX-S, fast	Widespread	Unknown	Unknown
$Na_v1.2$	TTX-S, fast	Embryonic	Depolarized activation/inactivation for a TTX-S channel; can produce resurgent current	May maintain firing when misexpressed in MS damaged neurons
$Na_v1.3$	TTX-S, fast	Embryonic	Rapid repriming, ramp current, persistent current	Ectopic firing when misexpressed in axotomy/SCI.
$Na_v1.4$	TTX-S, fast	Not present	N/A	N/A
$Na_v1.5$	TTX-R, fast	Embryonic	Not fully characterized	Unknown
$Na_v1.6$	TTX-S, fast	Widespread	Rapid repriming; persistent current; can produce resurgent current	Maintains high frequency firing when present
$Na_v1.7$	TTX-S, fast	Widespread	Slow onset of inactivation leading to ramp current, slow repriming	Ramp current amplifies small inputs
$Na_v1.8$	TTX-R, slow	Widespread	Very depolarized activation/ inactivation, rapid repriming	Major contributor to action potential upstroke and repetitive firing in small neurons
$Na_v1.9$	TTX-R, persistent	Most small cells (esp. IB4+)	Hyperpolarized activation, overlapping activation/inactivation curves, ultra-slow inactivation	May be involved in setting RMP, amplification of inputs and/or maintaining activation of $Na_v1.8$.

(TTX), due to the presence of TTX-resistant (TTX-R) sodium channels (Gaumann *et al.* 1992; Kobayashi *et al.* 1993; Jeftinija, 1994; Buchanan *et al.* 1996) and, subsequently, to the specific isoform responsible for the current, $Na_v1.8$ (Akopian *et al.* 1996; Sangameswaran *et al.* 1996). Multiple studies have now shown that there are large variations in sodium current parameters in DRG neurons, linked to expression of a heterogeneous population of sodium channels (as summarized in Table 1; Kostyuk *et al.* 1981; Caffrey *et al.* 1992; Roy & Narahashi, 1992; Elliott & Elliott, 1993; Ogata & Tatebayashi, 1993; Rizzo *et al.* 1994; Rush *et al.* 1998; Cummins *et al.* 1999). Apart from pharmacological intervention using TTX, one can dissect the channels by using parallel methods of immunohistochemistry and electrophysiological recording, giving insight into the expression patterns and possible roles of the various channel isoforms. Using immunocytochemical techniques, large DRG cells have been shown to predominantly express TTX-sensitive (TTX-S) channels, such as $Na_v1.1$, $Na_v1.6$ and $Na_v1.7$, with some TTX-R $Na_v1.8$ expression (Black *et al.* 1996). Small cells, which are likely to be nociceptive in nature, express TTX-S channels (Black *et al.* 1996; Sangameswaran *et al.* 1997; Toledo-Aral *et al.* 1997), in conjunction with TTX-R $Na_v1.8$ and $Na_v1.9$ channels (Amaya *et al.* 2000; Fjell *et al.* 2000). These findings have been further corroborated using intracellular recording, together with immunohistochemistry, to show the distribution of channels in DRG neurons that give rise to particular fibre types (Fang *et al.* 2002, 2006; Djouhri *et al.* 2003*a*,*b*). Although it is not known whether the complement of sodium channels along, or at the terminals of, an axon are the same as at the cell body giving rise to that fibre, it is known that expression of multiple sodium channels, including TTX-R channels, is not limited to the cell body and extends along the fibres (Brock *et al.* 1998; Strassman & Raymond, 1999; Black *et al.* 2002*a*; Black & Waxman, 2002*b*; Rush *et al.* 2005*a*, 2006*b*), demonstrating the likely importance of these channels in conduction and fibre characteristics.

Trafficking and expression of sodium channels

Trafficking and expression of sodium channels can be regulated by association of channels with cofactors, in many cases in an isoform-specific manner. In DRG neurons, one such protein is annexin II/p11, which binds to the N-terminus of $Na_v1.8$ and facilitates insertion of functional $Na_v1.8$ channels into the cell membrane (Okuse *et al.* 2002). CAP-1A, a linker protein that binds clathrin and $Na_v1.8$, plays a complementary role, removing $Na_v1.8$ channels from the cell membrane (Liu *et al.* 2005). Contactin, a glycosyl-phosphatidylinositol (GPI)-anchored neuronal surface glycoprotein (Ranscht, 1988; Brummendorf *et al.* 1989; Gennarini *et al.* 1989), has been found to interact with $Na_v1.2$ via the β1-subunit and increases channel density at the plasma membrane in heterologous cells (Kazarinova-Noyes *et al.* 2001; Chen *et al.* 2004) and produces similar effects with $Na_v1.3$ and $Na_v1.9$ (Liu *et al.* 2001; Shah *et al.* 2004). In DRG neurons, contactin has been found to regulate current density of TTX-R sodium channels $Na_v1.8$ and $Na_v1.9$ in the subset

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of largely nociceptive, α-D-galactosyl lectin-binding IB4+ neurons, although TTX-S channels ($Na_v1.6$ and $Na_v1.7$) were unaffected (Rush *et al.* 2005*a*). TTX-S channels may instead be regulated by β2 subunits and this interaction has been shown to modulate the response to pain (Pertin *et al.* 2005; Lopez-Santiago *et al.* 2006). However, contactin may also play a role in the pathological re-emergence of $Na_v1.3$ in adult DRG neurons and accumulation of the channel in the neuroma of transected sciatic nerve (Shah *et al.* 2004). In addition to affecting plasma membrane channel density, the activation and inactivation kinetics of TTX-S currents are accelerated by β2, and other β-subunits are involved in modulation of $Na_v1.8$ (Shah *et al.* 2000; Vijayaragavan *et al.* 2004). Although some proteins may function primarily or solely as channel chaperones, such as annexin II/p11 with $Na_v1.8$ (Okuse *et al.* 2002), alteration of biophysical parameters can also occur with other cofactors. Fibroblast growth factor homologous factor (FHF) 2A and 2B have been demonstrated to be present in DRG neurons, and associate with $Na_v1.6$ to increase current density but also modulate the channel's biophysical properties, for instance, depolarizing steady-state inactivation (Wittmack *et al.* 2004; Rush *et al.* 2006*b*). Calmodulin can bind to the C-terminus of sodium channels (Herzog *et al.* 2003*b*) and can also modulate both the current density and biophysical properties of sodium currents generated by $Na_v1.6$ and $Na_v1.8$ in DRG neurons (Herzog *et al.* 2003*b*; Choi *et al.* 2006). Several of these proteins have been demonstrated to be coexpressed with their target interacting sodium channel, not only in the cell body but also along the nerve fibre (Wittmack *et al.* 2004; Liu *et al.* 2005; Rush *et al.* 2005*a*, 2006*b*). Thus, there are multiple sodium channel associated proteins in DRG neurons and associated fibres that control not only the density of different channel isoforms at the membrane but also some of the biophysical properties of the channels themselves.

Activation and inactivation characteristics

The slowly TTX-R inactivating sodium channel, now known to be $Na_v1.8$, which is specifically expressed within DRG and trigeminal neurons (Akopian *et al.* 1996; Sangameswaran *et al.* 1996), has more depolarized steady-state activation and inactivation (Fig. 1) characteristics than the TTX-S channels (Kostyuk, 1981; Caffrey *et al.* 1992; Roy & Narahashi, 1992; Elliott & Elliott, 1993; Ogata & Tatebayashi, 1993; Rizzo *et al.* 1994; Cummins & Waxman, 1997; Rush *et al.* 1998). DRG neurons are thus rather unique in having a mix of channels with an unusually wide range of varied characteristics. As we will see throughout this review, it has been possible over the last few years to examine the specific roles of different sodium channel isoforms in the firing behaviour of DRG neurons. Using knock-out mice, $Na_v1.8$ channels were shown to be essential for generation of single action potentials in most small DRG neurons and in fact generate about 80% of the inward current underlying the action potential upstroke in these cells (Renganathan *et al.* 2001). When $Na_v1.8$ was absent, the more hyperpolarized voltage-dependent properties of the TTX-S channels were revealed by inhibition of firing with even minor depolarization (Fig. 2). Although TTX-R channels are the major contributor to the action potential upstroke, TTX-S channels also play a significant part, especially around threshold, with a relatively minor involvement of high threshold calcium channels (Blair & Bean, 2002). The depolarized voltage dependence of inactivation of $Na_v1.8$, together with rapid recovery from inactivation (see below), also allows repetitive firing with sustained depolarization (Fig. 3) (Renganathan *et al.* 2001; Blair & Bean, 2003).

$Na_v1.9$, the second TTX-R sodium channel discovered within DRG neurons (Dib-Hajj *et al.* 1998), displays very slow activation and inactivation, with a large overlap between activation and steady-state inactivation that is centred near resting potential (Cummins *et al.* 1999). The kinetics of $Na_v1.9$ are so slow that it does not contribute to the action potential upstroke. In cells where it is present, $Na_v1.9$ enhances and prolongs the response to depolarizations that are subthreshold for action potential electrogenesis (Herzog *et al.* 2001) and lowers threshold for single action potentials and for repetitive firing (Baker *et al.* 2003). Because of its large predicted window current (a current produced by a significant crossover of activation and inactivation properties; Attwell *et al.* 1979) around RMP, it might be expected that $Na_v1.9$ might depolarize membrane potential (Herzog *et al.* 2001; Baker *et al.* 2003). Yet resting potential has been reported to be shifted in a depolarizing direction in $Na_v1.9-/-$ knockout DRG neurons (Morisset *et al.* 2005; but see Priest *et al.* 2005), and in IB4– DRG neurons (which tend not to express $Na_v1.9$) compared to IB4+ neurons (which express high levels of $Na_v1.9$; Fang *et al.* 2006). A possible explanation of this apparent paradox is that $Na_v1.9$ might provide a return pathway for Na^+ which is necessary for operation of Na^+/K^+-ATPase. Consistent with this speculation, block of persistent sodium conductance in optic nerve axons (Stys *et al.* 1993) and astrocytes (Sontheimer *et al.* 1994) produces a transient hyperpolarization as the depolarizing influence of the sodium conductance is blocked, followed by progressive depolarization due to failure of the Na^+/K^+-ATPase. Up-regulation of $Na_v1.9$ within DRG neurons with GTP is accompanied by a fall in threshold, repetitive firing in response to stimulation, and spontaneous activity in some cells even at relatively hyperpolarized levels, indicating that the effect of $Na_v1.9$ on threshold is not entirely due to the change in resting potential (Baker *et al.* 2003).

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Repriming: recovery from inactivation

Several recent studies have shed light on the distinct patterns of recovery from inactivation (repriming) of the different sodium channel isoforms in DRG neurons. The TTX-S currents of intact large DRG neurons tend to have relatively fast repriming characteristics (Cummins & Waxman, 1997; Everill *et al.* 2001), which correlates with expression in these neurons of $Na_v1.6$ (Black *et al.* 1996), an isoform that displays this characteristic when expressed and studied in isolation in DRG (Herzog *et al.* 2003*a*; Rush *et al.* 2005*b*). In contrast, TTX-S currents in uninjured small DRG neurons, which include nociceptors, tend to have slow repriming (Elliott & Elliott, 1993; Cummins & Waxman, 1997; Rush *et al.* 1998; Black *et al.* 1999), similar to the behaviour of $Na_v1.7$ when expressed in cell lines or in a DRG background (Cummins *et al.* 1998; Herzog *et al.* 2003*a*). From this evidence, differential expression of $Na_v1.6$ or $Na_v1.7$ appears to endow these neurons with distinct TTX-S repriming properties. However, $Na_v1.6$ is expressed in all DRG cell sizes, including small cells giving rise to unmyelinated fibres (Black *et al.* 2002*a*; Rush *et al.* 2005*a*) and $Na_v1.7$ is also expressed in most DRG cells (Sangameswaran *et al.* 1997; Toledo-Aral *et al.* 1997). One possible explanation for this apparent discrepancy is that there are differences in the amount of non-functional channel protein for $Na_v1.6$ and $Na_v1.7$ channels in small and large DRG neurons. An alternative explanation is that functional properties of $Na_v1.6$ and/or $Na_v1.7$ are modified by other factors, such as associated proteins or kinases, that are differentially expressed in specific populations of DRG neurons. One likely candidate is fibroblast growth factor homologous factor 2A (FHF2A). This protein associates with $Na_v1.6$ via the C-terminus of the channel, is present in DRG neurons and strongly inhibits repriming of $Na_v1.6$, leading to a large accumulation of inactivation during repetitive stimulation (Rush *et al.* 2006*b*). Expression of FHF2A in small DRG neurons may help explain the

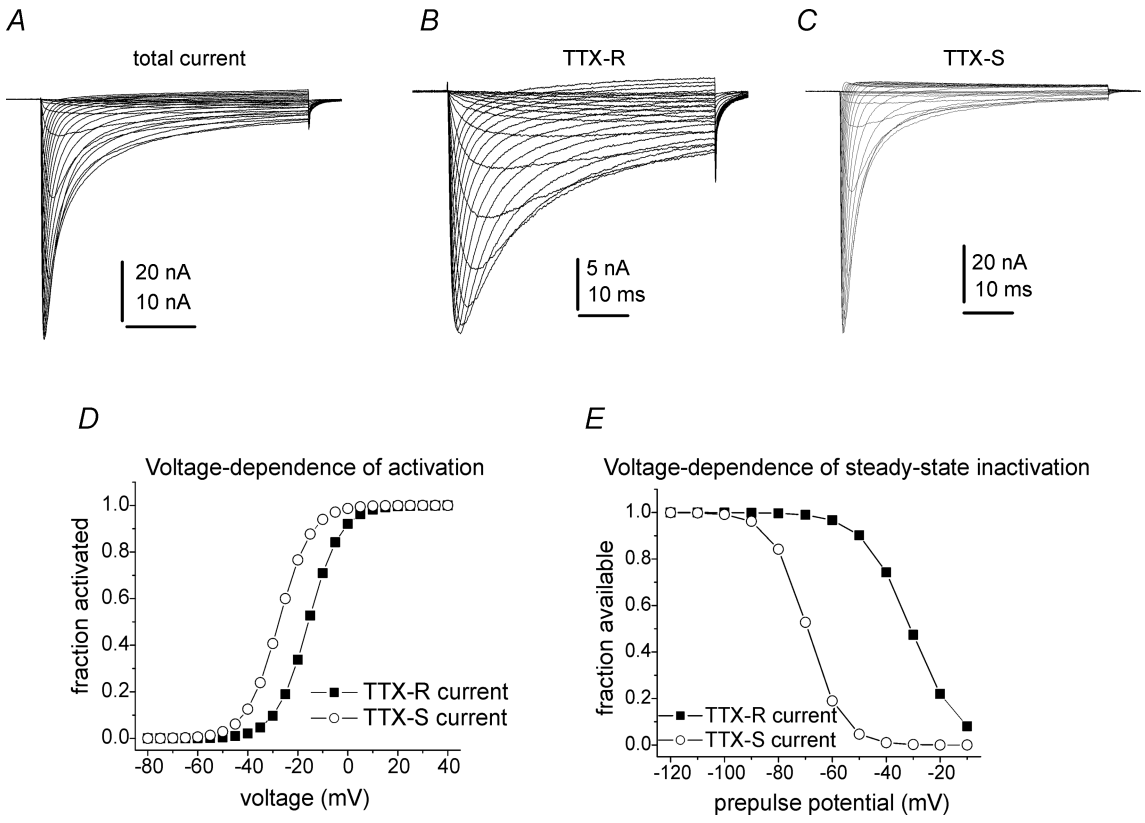

Figure 1. TTX-R currents carried by $Na_v1.8$ have more depolarized voltage dependence than TTX-S currents in DRG neurons

Total sodium current in this DRG cell elicited using a holding potential of −100 mV and 25 ms step depolarizations to voltages ranging from −80 to +40 mV (*A*) can be pharmacologically separated on the basis of sensitivity to TTX (100 nM), allowing isolation of TTX-R currents (*B*) and by subtracting *B* from *A*, TTX-S currents (*C*). *D*, the voltage dependence of activation for TTX-R currents (■) is depolarized compared to that of TTX-S currents (○) in DRG neurons. *E*, the voltage dependence of steady-state inactivation is more depolarized for the TTX-R currents (■) than for TTX-S currents (○). The voltage dependence of inactivation was estimated by measuring currents elicited by 20 ms test pulses to −10 mV after 500 ms inactivating prepulses ranging from −130 to −10 mV. Adapted from data in Cummins & Waxman (1997) with permission; ©1997 by the Society for Neuroscience.

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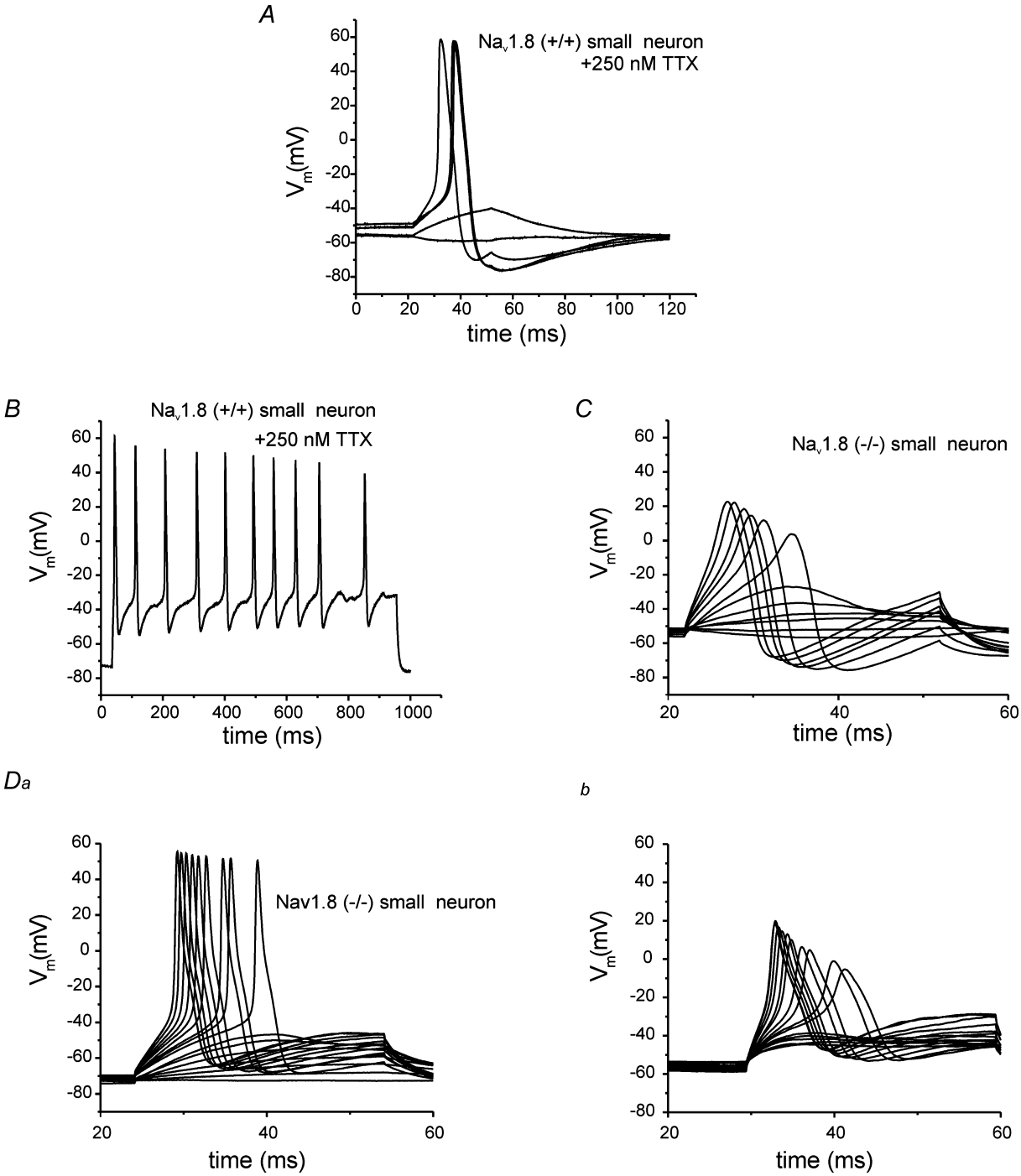

Figure 2. The TTX-R Na$_v$1.8 channel is a major contributor to the action potential upstroke in small DRG neurons

A, TTX-R single action potentials recorded from small DRG neurons from a Na$_v$1.8+/+ mouse, in the presence of 250 nM TTX. *B*, a longer injection of current for 1 s generates a train of TTX-R action potentials in the same neuron. *C*, recordings under similar conditions as in (*A*), but in the absence of TTX, demonstrate that only graded potentials can be elicited from ~80% of knockout Na$_v$1.8−/− mouse small DRG neurons. Full-sized action potentials cannot be recorded in these neurons, which lack Na$_v$1.8 expression. Around 20% of small Na$_v$1.8−/− DRG neurons have a more hyperpolarized RMP that allows overshooting action potentials (*Da*). Depolarizing the RMP in these neurons (*Db*, same cell as *Da*) inactivates the TTX-S channels and demonstrates the importance of Na$_v$1.8 in supporting action potential firing from a depolarized RMP. Adapted from Renganathan *et al.* (2001) with permission; © 2001 The American Physiology Society.

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anomalous repriming and channel expression observation outlined above. In addition, there may be as yet unknown cofactors that accelerate repriming of $Na_v1.7$ in larger DRG neurons. An emerging trend (see Cummins *et al.* 2001) is that the cellular background in which particular channels are expressed is an important determinant of the behaviour of sodium channel isoforms.

Recovery from inactivation is a critical channel characteristic that also plays a role in nerve injury. Chronic constriction injury (CCI) and axotomy of the sciatic nerve both trigger a down-regulation of $Na_v1.8$ and $Na_v1.9$ and an up-regulation of $Na_v1.3$ expression in nociceptive DRG neurons (Waxman *et al.* 1994; Dib-Hajj *et al.* 1996; Cummins & Waxman, 1997; Dib-Hajj *et al.* 1998; Cummins *et al.* 2000; Kim *et al.* 2002) that is accompanied by the emergence of a rapidly repriming TTX-S current in these cells (Fig. 4) (Cummins & Waxman, 1997). $Na_v1.3$ is normally only expressed during early stages of development and is practically undetectable in the adult rat nervous system (Beckh *et al.* 1989; Waxman *et al.* 1994; Felts *et al.* 1997). The change in channel expression is likely to be due to reduced access of axotomized DRG neurons to peripheral pools of neurotrophic factors such as nerve growth factor (NGF) and glial derived neurotrophic factor (GDNF), as the effects can be reversed by increasing their levels after nerve injury (Dib-Hajj *et al.* 1998; Fjell *et al.* 1999; Boucher *et al.* 2000; Cummins *et al.* 2000; Leffler *et al.* 2002).

The emergence of $Na_v1.3$ in injured DRG neurons provides a clear example of the maladaptive effects that changes of sodium channel expression can have on a

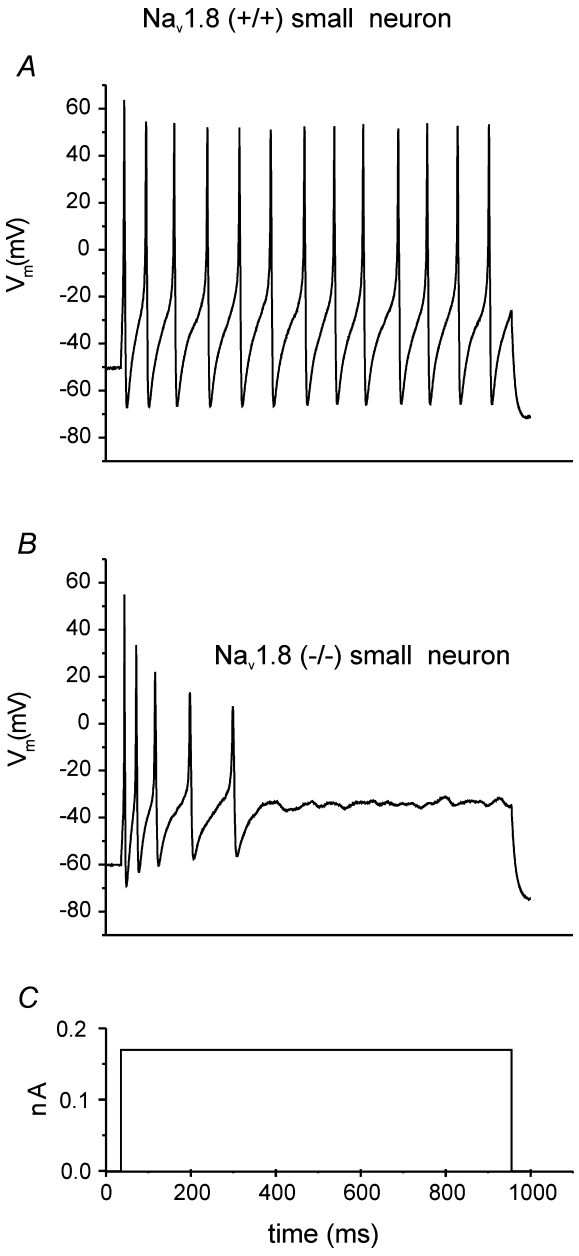

Figure 3. $Na_v1.8$ supports repetitive firing in small DRG neurons in response to sustained depolarization
Long injections of current for 1 s (*C*) generate a train of action potentials in $Na_v1.8+/+$ small DRG neurons (*A*). The depolarized steady-state properties and rapid recovery from inactivation of the $Na_v1.8$ channel permit this firing despite a relatively depolarized RMP. In contrast, $Na_v1.8-/-$ neurons are incapable of sustaining high frequency firing in response to identical stimuli (*B*). Adapted from Renganathan *et al.* (2001) with permission; © 2001 The American Physiology Society.

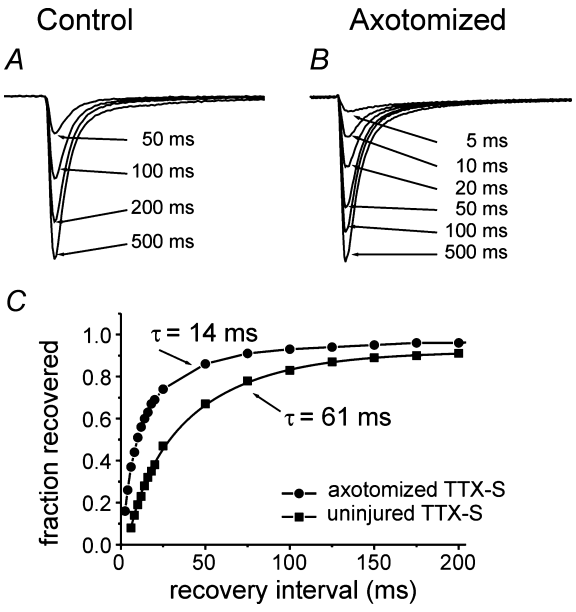

Figure 4. A rapidly repriming TTX-S sodium current, largely attributable to $Na_v1.3$ channels, emerges in DRG neurons following transection of their peripheral axons
A, family of TTX-S sodium current recordings from intact DRG neuron, showing recovery from inactivation at −80 mV. *B*, family of TTX-S sodium currents from a similar neuron 7 days after peripheral axotomy, demonstrating accelerated recovery from inactivation. *C*, single exponential fits showing accelerated repriming following peripheral axotomy. Modified from Black *et al.* (1999) with permission; © 2001 The American Physiology Society.

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neuron. The TTX-S current of intact small, nociceptive DRG neurons normally recovers from inactivation slowly (Elliott & Elliott, 1993; Cummins & Waxman, 1997; Rush *et al.* 1998; Black *et al.* 1999). In contrast, the TTX-S current recorded from axotomized small DRG neurons reprimes rapidly (Cummins & Waxman, 1997), similar to the current produced when $Na_v1.3$ is expressed in a cell line or in DRG neurons (Cummins *et al.* 2001). Thus, high frequency firing may be boosted in axotomized DRG neurons by $Na_v1.3$. In addition, $Na_v1.3$ has slow onset of inactivation, allowing it to produce robust ramp current around resting membrane potential (RMP) (Cummins *et al.* 2001), and produces a persistent, as well as a transient current (Lampert *et al.* 2006). Therefore, previously subthreshold inputs may generate action potentials in the presence of $Na_v1.3$ (see Fig. 5). These changes are likely to contribute to the hyperexcitability and ectopic firing that have been observed (Wall & Gutnick, 1974; Lisney & Devor, 1987; Matzner & Devor, 1994) in spinal sensory neurons after injury.

Slow inactivation

In addition to fast inactivation, DRG sodium channels can also undergo slow inactivation (Ogata & Tatebayashi, 1992; Rush *et al.* 1998), a process that occurs over a longer time course. The mechanism is thought to be separate from the III–IV linker associated with fast inactivation, as the phenomenon is maintained in channels of squid axon, despite removal of fast inactivation with pronase (Rudy, 1978). The exact structural rearrangements are still being elucidated, and appear to involve many different regions of the channel (for reviews see Goldin, 2003; Ulbricht, 2005). Interestingly, different sodium channel isoforms undergo different levels of slow inactivation (Vilin *et al.* 2001) and thus the mixed populations of channels in DRG neurons are likely to be affected differently by RMP and periods of depolarization. In comparisons of small DRG TTX-S and TTX-R currents, despite the more depolarized fast inactivation characteristics, TTX-R channels had quicker and much more complete slow inactivation at depolarized voltages (Rush *et al.* 1998; Blair & Bean, 2003) and this perhaps explains the differential block by agents sometimes used in the treatment of neuropathic pain, such as phenytoin and carbamazapine (Rush & Elliott, 1997; Cardenas *et al.* 2006). The midpoint of TTX-R slow inactivation is actually more hyperpolarized than that for fast inactivation (Blair & Bean, 2003), in marked contrast to the reverse situation commonly found for TTX-S currents (Kuo & Bean, 1994; Fleidervish *et al.* 1996; Fazan *et al.* 2001). Build-up of slow inactivation is likely to be responsible for the use-dependent inhibition of TTX-R currents on repetitive stimulation (Rush *et al.* 1998; Blair & Bean, 2003; Tripathi *et al.* 2006) and the cell-to-cell variations in TTX-R current properties seen in these studies might reflect regulation of $Na_v1.8$ by calmodulin

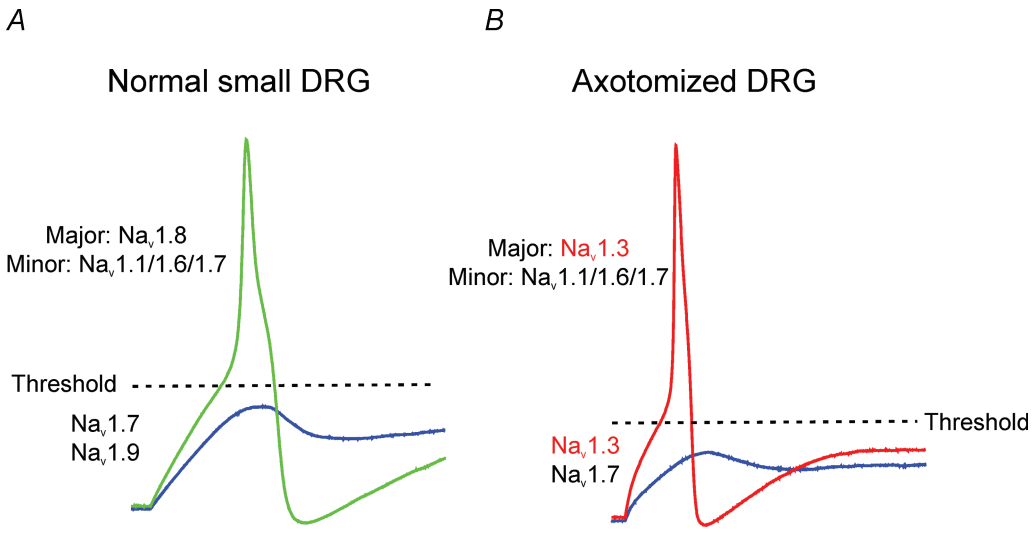

Figure 5. Putative roles of different sodium channel subtypes in electrogenesis in small DRG neurons
Representation of subthreshold responses and overshooting action potentials are shown from small intact and axotomized DRG neurons. Under normal conditions, $Na_v1.7$ (and $Na_v1.9$ in some cells) is likely to bring the neuron towards threshold, and $Na_v1.8$ is largely responsible for the overshooting action potential. Note the large depolarization necessary for action potential generation, due to the depolarized voltage dependence of activation of $Na_v1.8$. In contrast, axotomized DRG neurons are likely to have a more hyperpolarized threshold for overshooting action potential, in line with the predominance of TTX-S ($Na_v1.1$, $Na_v1.3$ and $Na_v1.6$) channels requiring less depolarization for activation and the boosted ramp currents with the presence of $Na_v1.3$. Note that $Na_v1.8$ and $Na_v1.9$ are no longer involved due to down-regulation of expression after axotomy.

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(Choi *et al.* 2006). Interestingly, use-dependent slow inactivation of $Na_v1.8$ current is stronger and develops more rapidly in IB4+ DRG neurons, compared to IB4– DRG neurons, and recovery from slow inactivation is slower in IB4+ neurons (Choi *et al.* 2007), adding to evidence (Stucky & Lewin, 1999; Braz *et al.* 2005; Fang *et al.* 2006) that these subclasses of DRG neurons are functionally distinct.

Resurgent current

Current that occurs upon repolarization is termed resurgent current and was first recorded from cerebellar Purkinje neurons (Raman & Bean, 1997*a*). It is a transient current that displays slow re-activation and inactivation upon rapid repolarization. Initially, resurgent current was linked with the presence of $Na_v1.6$ in Purkinje neurons (Raman *et al.* 1997*b*). Evidence to date suggests that at least some subpopulations of DRG neurons can produce resurgent current (Cummins *et al.* 2005; Rush *et al.* 2005*b*). About 60% of DRG neurons transfected with $Na_v1.6$ produce resurgent currents attributable to this channel (Cummins *et al.* 2005). Other studies have led to the suggestion that other sodium channel isoforms may also be able to produce a resurgent current as it can be recorded from neurons that do not express $Na_v1.6$ (Afshari *et al.* 2004; Do & Bean, 2004). In line with this is the observation that approximately 8% of DRG neurons transfected with $Na_v1.2$ can produce resurgent current attributable to this channel isoform (Rush *et al.* 2005*b*).

With normal channel isoform expression in the adult, the resurgent current may be limited to large DRG neurons (Cummins *et al.* 2005). The cofactor(s) that permit $Na_v1.6$ and $Na_v1.2$ to produce resurgent current in some cell types, but not in others, are still under study. Phosphorylation of the sodium channels may play a role (Grieco *et al.* 2002) and open channel block by the sodium channel $\beta 4$ subunit appears to be involved, for the $Na_v1.6$ channel at least (Grieco *et al.* 2005). The specific role of the resurgent current in DRG neurons has still to be elucidated but in other neurons it has been associated with rapid burst firing in response to large depolarizations (Raman & Bean, 1997*a*; Khaliq *et al.* 2003; Swensen & Bean, 2003; Magistretti *et al.* 2006).

Ramp currents

As we have discussed, $Na_v1.8$ carries a current that has very depolarized voltage dependence of activation and inactivation and is extremely resistant to depolarization. However, TTX-S currents do still play a role in the action potential, especially around threshold (Blair & Bean, 2002). $Na_v1.7$ is one of the major candidates that has been suggested to amplify subthreshold generator potentials by producing a prominent ramp current (response to small, slow depolarizing stimuli), largely due to its slow onset of closed-state inactivation (Cummins *et al.* 1998). It is thought that under conditions where there is slow depolarization of a neuron, this particular channel would tend to remain available for activation, in comparison with other channels that are able to activate only at relatively hyperpolarized potentials. This property would be predicted to make $Na_v1.7$ a significant source of ramp currents and this isoform has indeed been shown to produce a robust ramp current in response to slow depolarizations (Cummins *et al.* 1998; Herzog *et al.* 2003*a*) (see Fig. 6). More recently, mutations in $Na_v1.7$, associated with the neuropathic pain syndrome erythromelalgia (Cummins *et al.* 2004; Yang *et al.* 2004; Dib-Hajj *et al.* 2005; Drenth *et al.* 2005; Han *et al.* 2005), have helped to demonstrate its role as a threshold channel that can interact with $Na_v1.8$ to produce hyperexcitability and repetitive firing behaviour of nociceptive neurons (Dib-Hajj *et al.* 2005; Rush *et al.* 2006*a*). $Na_v1.7$ appears to bring the neuron closer to the potential needed for activation of $Na_v1.8$, thereby increasing excitability. In marked contrast, when the effects of an erythromelalgia mutation were examined in a cell type that lacks $Na_v1.8$ (sympathetic ganglion neurons), a reduction in excitability was seen (Rush *et al.* 2006*a*), presumably due to inactivation of the TTX-S sodium channels essential for the action potential upstroke in those neurons. Subsequent addition of $Na_v1.8$ into sympathetic neurons allowed these cells to fire action potentials despite depolarization of resting membrane potential induced by the mutant $Na_v1.7$ channels, demonstrating how sodium channel isoforms with different biophysical characteristics can interact with one another (Fig. 7). These data may help to explain both the pain caused by mild stimulation (due to hyperexcitability of nociceptive DRG neurons) and also the sympathetic dysfunction associated with this disorder (flushing of the extremities due to loss of vasoconstrictive tone) which is due to hypoexcitability of sympathetic ganglion neurons (Rush *et al.* 2006*a*).

$Na_v1.3$ also produces a prominent ramp current and this also amplifies small depolarizing stimuli. This factor may contribute to hyperexcitability of axotomized DRG neurons in which expression of this channel isoform is up-regulated (Cummins & Waxman, 1997).

Sodium channel combinations and interactions

Thus far, we have discussed many of the specific biophysical characteristics that define particular sodium channel isoforms, how they behave in the DRG neuron background and what their individual influence may be on the neurons and neuronal processes where they are expressed. However, in some conditions, there are not only long-term chronic changes in expression of multiple sodium channels, but also shorter term acute alterations in behaviour of existing channels, in response to changes in their environment.

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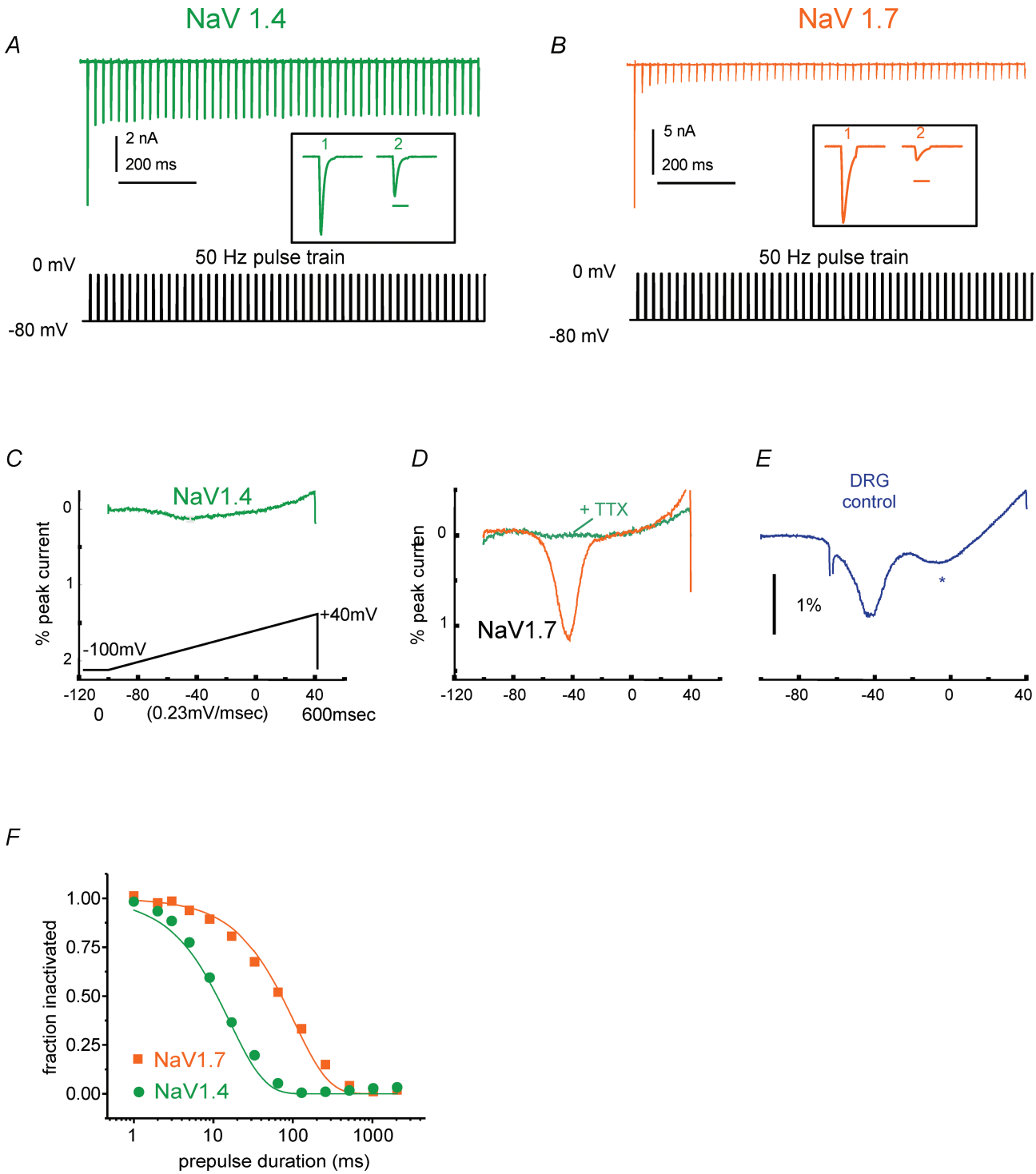

Figure 6. Distinct biophysical characteristics of the $Na_v1.7$ sodium channel shape its role in DRG neurons
Trains of 50 Hz stimulation show that sodium channel $Na_v1.7$ is unable to follow and sustain high frequency firing (*B*), when compared to, for example, $Na_v1.4$ (*A*). Thus $Na_v1.7$ is unlikely to play a major role in the action potential upstroke during repetitive firing. Using ramp stimulation to mimic small graded subthreshold potentials, $Na_v1.4$ produces only a small response (*C*), in marked contrast to the large ramp current evoked with $Na_v1.7$ (*D*), similar to those that can be recorded from small DRG neurons (*E*) (the asterisk marks a second component, blocked by cadmium and therefore likely to be due to calcium influx). *F*, $Na_v1.7$ has much slower onset of inactivation than $Na_v1.4$. Because of this, small changes in membrane potential, such as generator potentials, produce less closed-state inactivation in $Na_v1.7$. Resistance to this form of inactivation enables $Na_v1.7$ to respond to small, slow depolarizations close to RMP, boosting these depolarizations and bringing the cell closer to action potential threshold. Adapted from Cummins *et al.* (1998) with permission; ©1998 by the Society for Neuroscience.

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Thus, in order to understand the overall likely effects on firing behaviour, it is important to consider how the rise in expression of one channel or change in biophysical characteristics of another might relate to each other. For example, in models of inflammatory pain such as carrageenan injection into the hindpaw there is an up-regulation of TTX-S channels $Na_v1.3$ and $Na_v1.7$ in DRG

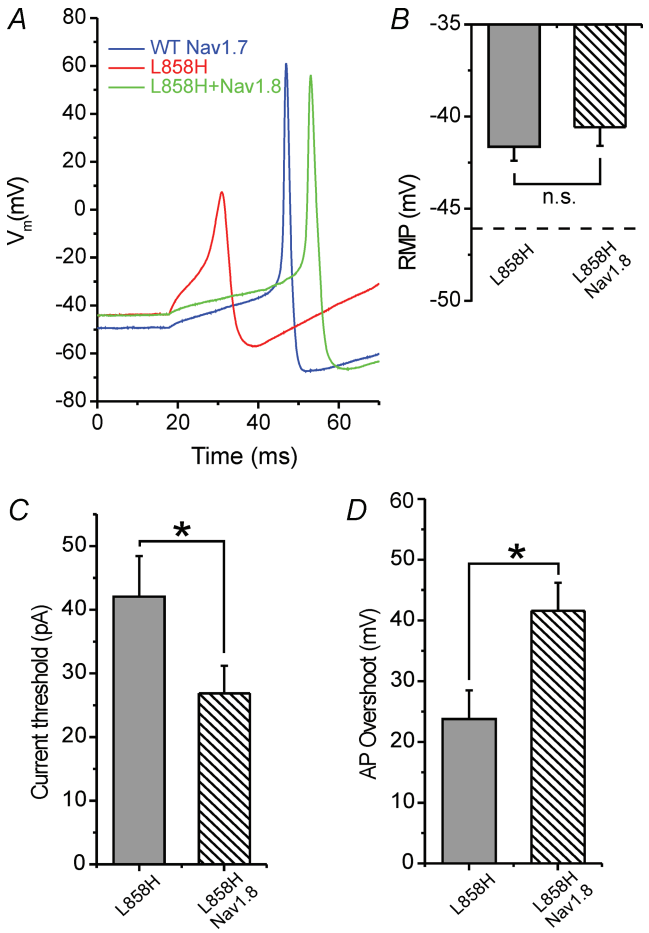

Figure 7. The erythromelalgia $Na_v1.7$ mutation L858H inhibits firing in SCG neurons, which can be rescued by coexpression of $Na_v1.8$ channels
When $Na_v1.8$ is coexpressed with the $Na_v1.7$ mutant L858H, current threshold and action potential overshoot are restored, even though the depolarization of RMP induced by L858H persists. *A*, suprathreshold action potentials recorded from representative superior cervical ganglion (SCG) neurons transfected with WT (blue), L858H (red) and L858H plus $Na_v1.8$ (green) channels. *B*, depolarized RMP in cells with L858H channels is maintained with coexpression of $Na_v1.8$ ($P > 0.05$). Dashed line indicates RMP in cells expressing WT $Na_v1.7$ alone. *C*, current threshold for action potential firing is reduced by L858H coexpression with $Na_v1.8$, when compared to L858H alone ($P < 0.05$). *D*, action potential overshoot in SCG neurons with L858H channel is increased when $Na_v1.8$ is coexpressed with L858H (41.5 ± 4.6 mV, $n = 17$; $P < 0.05$). Thus, the introduction of $Na_v1.8$, with depolarized activation and inactivation characteristics, allows SCG neurons to fire full overshooting action potentials, despite the depolarization induced by the L858H erythromelalgia mutation in $Na_v1.7$. Adapted from Rush *et al.* (2006*a*) with permission; © 2006 National Academy of Sciences, USA.

after a few days, together with a parallel increase in the TTX-S current (Black *et al.* 2004). This is in addition to an increased expression of $Na_v1.8$ and a boosted TTX-R current (Tanaka *et al.* 1998; Black *et al.* 2004). In contrast, expression of $Na_v1.6$ and $Na_v1.9$ are relatively unaffected in the inflammatory response. These changes might amplify previously subthreshold inputs (due to up-regulation of $Na_v1.3$ and $Na_v1.7$) and help in sustaining repetitive firing under prolonged depolarization ($Na_v1.3$ and $Na_v1.8$).

$Na_v1.8$ is also likely to be involved in acute inflammatory pain as administration of agents such as prostaglandin E2 (PGE2) and 5-HT produces an increase in current amplitude and a hyperpolarizing shift in activation, both likely to increase excitability (England *et al.* 1996; Gold *et al.* 1996; Cardenas *et al.* 1997, 2001). Recent evidence also points towards the involvement of $Na_v1.9$ in the inflammatory response. Acute administration of inflammatory mediator PGE2 causes a large increase in the amplitude of $Na_v1.9$ (Rush & Waxman, 2004), a channel which, when knocked out in the mouse, produces deficits in the inflammatory response but not in neuropathic pain models (Priest *et al.* 2005). These data suggest that $Na_v1.9$ may play a significant role in the increased excitability of nociceptive fibres during inflammation, either by effects on resting potential, by sustaining depolarization, or by lowering threshold (Herzog *et al.* 2001; Baker *et al.* 2003). However, in another inflammatory model, induced jejunitis was linked to changes in $Na_v1.8$ but knocking out $Na_v1.9$ had no effect (Hillsley *et al.* 2006). There is still much to be done to fully understand the intricate roles of sodium channel isoforms in the variety of pain models and to translate these results to the clinic.

Overview: does complexity of sodium channel expression represent a therapeutic opportunity?

Although the expression of an especially large repertoire of sodium channel isoforms makes DRG neurons complex, the accessibility and spherical shape of the these cells, and their involvement in a number of readily modelled disease states involving sodium channels, have made them a very tractable model for the study of the roles of sodium channels in electrogenesis. Excitability of DRG neurons can be affected by changes during development and a variety of pathologies and injuries. Sodium channels, which were once thought to be uniform in terms of biophysical properties and simply responsible for the rising phase of an action potential, have now been shown to be able to play multiple roles in electrogenesis, and thus can finely tune the behaviour of excitable cells. The presence of multiple sodium channel isoforms within DRG neurons suggests the possibility of pharmacologically targeting specific channel subtypes, thus possibly achieving therapeutic activity whilst limiting undesirable side-effects. Changes in expression levels or

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physiological properties of specific sodium channel isoforms are likely to contribute to altered function of DRG neurons in a variety of pathologies and these isoforms may therefore be tractable targets for subtype specific modulators.

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