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TOPICAL REVIEW

Resurgent current of voltage-gated Na^+ channels

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Abstract Resurgent Na^+ current results from a distinctive form of Na^+ channel gating, originally identified in cerebellar Purkinje neurons. In these neurons, the tetrodotoxin-sensitive voltage-gated Na^+ channels responsible for action potential firing have specialized mechanisms that reduce the likelihood that they accumulate in fast inactivated states, thereby shortening refractory periods and permitting rapid, repetitive, and/or burst firing. Under voltage clamp, step depolarizations evoke transient Na^+ currents that rapidly activate and quickly decay, and step repolarizations elicit slower channel reopening, or a 'resurgent' current. The generation of resurgent current depends on a factor in the Na^+ channel complex, probably a subunit such as $Na_V\beta4$ (*Scn4b*), which blocks open Na^+ channels at positive voltages, competing with the fast inactivation gate, and unblocks at negative voltages, permitting recovery from an open channel block along with a flow of current. Following its initial discovery, resurgent Na^+ current has been found in nearly 20 types of neurons. Emerging research suggests that resurgent current is preferentially increased in a variety of clinical conditions associated with altered cellular excitability. Here we review the biophysical, molecular and structural mechanisms of resurgent current and their relation to the normal functions of excitable cells as well as pathophysiology.

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Introduction

In all excitable cells, voltage-gated Na^+ channels are closed (deactivated) at negative potentials, open (activate) upon depolarization, and then rapidly become non-conducting (inactivated, but see below), decreasing current flow by about 99% within a few milliseconds (Hodgkin & Huxley, 1952*a*). In voltage clamp, the brief macroscopic Na^+ current evoked by a step depolarization is therefore often referred to as a 'transient Na^+ current', while the proportionately tiny, residual Na^+ current that lasts throughout the step is called 'persistent Na^+ current'. In many cells, relief of inactivation occurs only at strongly negative potentials, without additional current flow (Hodgkin & Huxley, 1952*c*; Kuo & Bean, 1994). In cells such as cerebellar Purkinje neurons, however, non-conducting Na^+ channels have the distinctive characteristic of reopening in response to repolarization after steps to positive potentials. The current that flows through channels that reopen in response to negative voltage changes, after the decay of macroscopic transient current, is called 'resurgent Na^+ current' (Fig. 1*A*; Raman & Bean, 1997).

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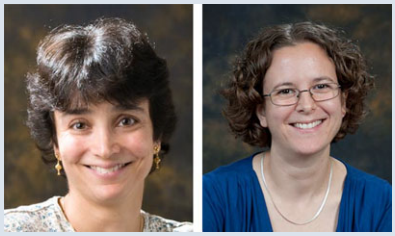

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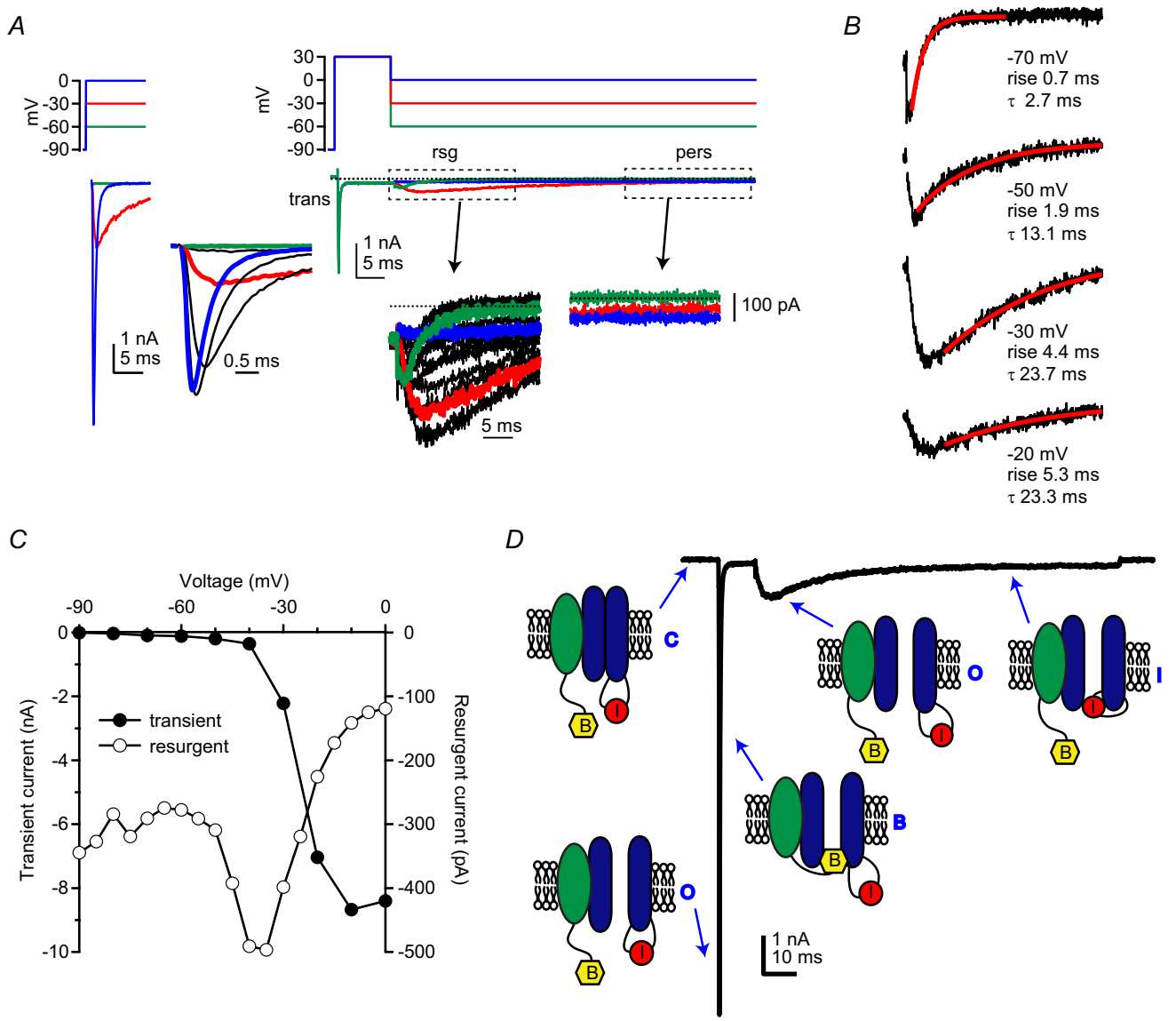

Figure 1. Transient, resurgent and persistent Na^+ currents
A, TTX-sensitive Na^+ currents in a voltage-clamped Purkinje neuron. Left, a family of transient currents evoked by step depolarizations from −90 mV to −60 (green), −30 (red), 0 (blue) mV. Right, in a different cell, transient current evoked by a 10 ms step to +30 mV followed by resurgent and persistent currents elicited by repolarization to the same voltages. Recordings were made at room temperature, in normal Na^+ gradients (150 mM external, 10 mM internal). Recordings were repeated in 900 nM TTX to block all current and subtractions gave the illustrated TTX-sensitive current. Insets, currents at higher gain or faster sweep speed, with currents at more voltages shown for transient (Δ10 mV) and resurgent (Δ5 mV) components. In physiological Na^+ concentration gradients, resurgent current can be maximized by step depolarizations to +30 mV for 5–50 ms (depending on the decay time of transient current; slower decay requires a longer step) followed by repolarization to −30 to −40 mV; thus this voltage protocol probably maximizes the resurgent current amplitude. *B*, resurgent current kinetics and amplitude vary with repolarization potential. Currents from (*A*) are shown from the repolarization step only. The time of peak relative to the time of the repolarization step (rise) is prolonged at less negative repolarizations, reflecting less efficient displacement of the blocker. The single exponential decay time constant (τ) also increases with less negative steps, owing to less concerted transitions into non-conducting states as well as the slower transition of open channels into inactivated, rather than closed states. *C*, current–voltage relation of transient and resurgent Na^+ currents from (*A*). Maximal Na^+ conductance was 120 nS in the cell from which transient currents are shown and 45 nS in the cell from which resurgent currents are shown. Resurgent current scale is 20× the transient current scale. *D*, schematic of the primary channel states occupied during different phases of transient and resurgent current. The α subunit is blue with a red inactivation gate 'I', and the blocking subunit (possibly $Na_V\beta 4$) is green with a yellow blocking domain 'B'. Upon depolarization, channels make transitions from closed

Properties and distribution of resurgent current

Although resurgent current flows through the same channels as transient and persistent current, it exhibits different voltage dependence and kinetics. Resurgent current is distinct from persistent current because it is dynamically gating; at any given potential, it has a rising phase, a peak and a falling phase. With a prolonged repolarizing step, the resurgent current decays to a persistent (steady-state) current. It can be useful to subtract this persistent component when measuring resurgent current amplitudes. Besides being activated by negative rather than positive voltage steps, resurgent current activates and decays more slowly than transient current. For instance, in Purkinje neurons at room temperature, transient current evoked by a step depolarization to −30 mV activates within 500 μs and decays in about 1 ms, while resurgent current evoked by a step repolarization to −30 mV rises in 3–5 ms and has a decay constant near 20 ms (Fig. 1*B*). The slow rise of resurgent current distinguishes it from tail current, which rises instantaneously, flowing through the few channels that remain open throughout the depolarizing step. Tail currents also scale directly with driving force, increasing in amplitude at more negative potentials (Hodgkin & Huxley, 1952*b*), whereas resurgent currents have a non-monotonic voltage dependence on repolarization. Resurgent current is generally not detectable until the membrane potential is repolarized below 0 mV. With progressively more negative steps, the peak current increases until about −30 mV, and then decreases, remaining measurable to at least −90 mV (Fig. 1*C*). At its maximum near −30 mV, peak resurgent current is about 5–10% of the amplitude of transient current measured at 0 mV (Aman & Raman, 2007; Lewis & Raman, 2011). The mechanistic bases for these properties are further discussed below.

Historically, resurgent current was considered a quirk of Purkinje neurons. Since its identification, however, resurgent current has been found in 19 other classes of cells throughout the nervous system (Table 1). The cerebellum and brainstem contain several cell types in which every neuron has resurgent current; in the globus pallidus, parts of the hippocampus and the dorsal root ganglia (DRG), only subsets of certain neuronal classes express the current. Conversely, resurgent current is consistently absent from CA3 pyramidal neurons of the hippocampus, some spinal neurons and neurons of the superior cervical ganglion (Raman & Bean, 1997; Pan & Beam, 1999; Han *et al.* 2012).

Open channel block as a mechanism for resurgent Na^+ current

The observation of Na^+ currents apparently gated by both positive and negative voltage steps raises questions of how such currents arise. Analysing the currents can reveal specific channel states, probably corresponding to one or more physical conformations of ion channel proteins (Fig. 1*D*). Non-conducting channels that are ready or 'available' to open in response to depolarization are defined as being in 'closed' states, 'C', and conducting channels are in 'open' states, 'O'. Non-conducting channels that are unavailable to open – usually because of ongoing or previous depolarization – are in 'inactivated' states, 'I'. Most voltage-gated Na^+ channels recover from inactivation directly (I→C), without reopening and passing measurable current (Kuo & Bean, 1994). In cells with resurgent current, however, the non-conducting states favoured at positive voltages evidently make transitions directly to open states even at moderately negative potentials (just below –10 mV). These non-conducting states must therefore be distinct from classical inactivated states.

Early studies of Na^+ currents in squid axons demonstrated that open channels can be reversibly blocked or plugged by exogenous compounds applied to the intracellular face of the membrane. Such compounds, including *N*-methyl-strychnine, pancuronium ion and thiazine dyes, obstruct channels opened by depolarization, but cannot pass through the pore (Yeh & Narahashi, 1977; Cahalan & Almers, 1979; Armstrong & Croop, 1982); the pentapeptide KIFMK works similarly in expressed Na^+ channels (Tang *et al.* 1996). Repolarization of channels blocked by these agents generates currents with a slow rising phase, termed 'hooked tail currents' (Yeh & Narahashi, 1977; see also Armstrong, 1971). The 'hook' arises as the blocking agent slowly dissociates from its binding site at negative potentials, after which open channels again pass current before changing conformation into more stable non-conducting states. Binding of the blocking agent therefore appears voltage-dependent. Moreover, the voltage-sensitivity arises because unbinding is facilitated by inwardly permeating ions that repel and displace the blocker (Armstrong, 1971; Tang *et al.* 1996). The similarity of hooked tails to resurgent current suggests that cells with resurgent current contain an endogenous factor that functions as an open channel blocking particle, which can drive open channels into a 'blocked' state, 'B'. Thus, most voltage-gated Na^+

('C') to open ('O') states, eliciting a large transient current. This current decays as channels become blocked ('B'). Upon repolarization, channels briefly revisit open states before making transitions into fast inactivated states ('I'). Traces from Aman & Raman (2010). pers, persistent (current); rsg, resurgent (current); trans, transient (current); TTX, tetrodotoxin.

Table 1. Distribution of resurgent Na^+ current, $Na_V\beta4$ expression and firing properties

Cell type with resurgent current	$Na_V\beta4$	Spike pattern	References
Cerebellar Purkinje cells	Yes	Spontaneous; repetitive; bursts*	Raman & Bean, 1997; Yu *et al.* 2003, Buffington & Rasband, 2013
Subthalamic nuclei	Yes	Spontaneous; repetitive; bursts	Do & Bean, 2003; Buffington & Rasband, 2013
Cerebellar nuclei	Yes	Spontaneous; repetitive; bursts	Raman *et al.* 2000; Yu *et al.* 2003; Afshari *et al.* 2004
Cerebellar granule cells	Yes	Repetitive; bursts	D'Angelo *et al.* 2001; Chadderton *et al.* 2004; Afshari *et al.* 2004; Magistretti *et al.* 2006; Bant & Raman, 2010
Cerebellar unipolar brush cells	Probably†	Spontaneous; repetitive; bursts	Yu *et al.* 2003; Afshari *et al.* 2004; Russo *et al.* 2007
Large dorsal root ganglion cells	Yes	Repetitive	Abdulla & Smith, 2001; Yu *et al.* 2003; Cummins *et al.* 2005
Mesencephalic trigeminal neurons	Yes	Repetitive; bursts	Yu *et al.* 2003; Enomoto *et al.* 2006; Buffington & Rasband, 2013
Medial nucleus of the trapezoid body	Yes	Repetitive	Wang *et al.* 1998; Leão *et al.* 2006; Buffington & Rasband, 2013
Medial vestibular nuclei: GABAergic	Yes	Spontaneous; repetitive	Gittis & du Lac, 2008; Kodama *et al.* 2012
Medial vestibular nuclei: Non-GABA	No	Spontaneous; repetitive	Gittis & du Lac, 2008; Kodama *et al.* 2012
Cartwheel cells (cochlear nucleus)	Probably†	Spontaneous; repetitive; bursts	Raman & Trussell unpublished; Yu *et al.* 2003; Kim & Trussell, 2007
Globus pallidus (*some cells*)	Some	Spontaneous; repetitive	Yu *et al.* 2003; Mercer *et al.* 2007
Layer II pyramidal cells of perirhinal cortex, area 35 (*most cells*)	Probably not‡	Repetitive (most cells)	Yu *et al.* 2003; Castelli *et al.* 2007*a*
Hippocampal dentate gyrus (*60%*)	No	Other	Yu *et al.* 2003; Castelli *et al.* 2007*b*
Ventral CA1 pyramidal cells (*35%*)	Low	Other	Yu *et al.* 2003; Castelli *et al.* 2007*b*; Buffington & Rasband, 2013
Calyx of Held (bushy cells of ventral cochlear nucleus)	Probably†	Repetitive	Yu *et al.* 2003; Kim *et al.* 2010
10% small dorsal root ganglia cells	Yes	Repetitive	Abdulla & Smith, 2001; Yu *et al.* 2003; Jarecki *et al.* 2010
Substantia nigra pars reticulata (GABAergic)	Yes	Spontaneous; repetitive	Yu *et al.* 2003; Ding *et al.* 2011
Substantia nigra pars compacta (dopaminergic) – small currents	Some	Spontaneous; repetitive	Yu *et al.* 2003; Ding *et al.* 2011
Medial entorhinal cortex	Possibly	Repetitive with accommodation	Allen Brain Atlas; Hargus *et al.* 2011; Nigro *et al.* 2012

*Spontaneous, fires regularly without synaptic input; repetitive, fires with little accommodation with repeated or sustained depolarizing input; bursts, fire bursts of action potentials ± depolarizing input; other, none of the above; †based on high expression in brainstem and cerebellum; ‡based on low expression in this layer of cortex.

channels of Purkinje cells open with depolarization (C→O) but then, rather than inactivating, they become blocked by an open channel blocking particle (O→B). Upon repolarization, blocked channels reopen as the blocker unbinds (B→O), producing resurgent current. Open channels either inactivate (>∼–50 mV, O→I) or deactivate into closed states (<∼-50 mV, O→C). The various channel conformations and state transitions have been quantitatively elaborated in a 13-state kinetic scheme, comprising five closed states, six inactivated states, one open state and one blocked state, which describes Purkinje Na^+ currents recorded in 50 mM extracellular Na^+ (Raman & Bean, 2001). Here, we qualitatively summarize the constraints on the model, which future simulations that improve on the original must retain.

Most importantly, a channel cannot be both blocked and inactivated. Despite being mutually exclusive, these states differ in both onset and stability. Inactivation – specifically, fast inactivation owing to binding of the fast inactivation gate (see below) – unlike block, does not require open channels, and inactivation is an absorbing state, whereas blocker binding is rendered unstable by permeating Na^+. In fact, O→B is more rapid than O→I at all potentials (Raman & Bean, 2001), such that a depolarization to *any* voltage, even below 0 mV, initially favours open channel block. The higher the driving force on Na^+, the more rapidly the blocker will be displaced – within a few milliseconds at −30 mV but over hundreds of milliseconds at +60 mV – producing an apparent voltage dependence of open channel block (Afshari *et al.* 2004; Aman & Raman, 2007). Indeed, in reverse concentration gradients, when extracellular Na^+ is low, the blocker remains bound at negative potentials and resurgent current does not flow (Aman & Raman, 2010). In a fixed Na^+ gradient, the rate of the key transition B→O depends on the affinity of the blocker for the channel; a higher affinity will yield slower dissociation, prolonging the rise of resurgent current. It will likewise lengthen the decay time by desynchronizing the O→I transition. At moderately negative potentials, channels may re-open and re-block repeatedly, delaying inactivation and yielding burst-like single channel openings that increase the amplitude and duration of resurgent current (Raman & Bean, 1997; Aman & Raman, 2010). The O→I transition rate will also contribute to current amplitude and decay time, with slower transitions producing larger and longer resurgent currents. At more negative voltages, the O→C rate begins to dominate, leading to a more rapid curtailment of resurgent current at progressively more negative potentials (Raman & Bean, 2001; Lewis & Raman, 2011).

Transient, resurgent and persistent current thus represent different components of voltage-clamped Na^+ current produced by a single voltage-gated Na^+ channel. Strictly speaking, because all current flows through a common open state, current can only be identified as belonging to one of the three categories in a voltage-clamped cell, in which channels have been pre-equilibrated into primarily closed or blocked states. In a non-voltage-clamped cell, in which voltage is continually changing and channels make transitions among all states, reference to transient, persistent or resurgent current is rendered ambiguous. For convenience, however, resurgent current can be defined as the current arising as channels unblock (B→O), as distinguished from transient current that arises as channels open from available states (C→O).

Physiological roles of resurgent current

Classical Hodgkin–Huxley kinetics (Hodgkin & Huxley, 1952*d*) cannot effectively model Na^+ channels with resurgent current, because the activation and inactivation gates are not independent. Instead, the onset of block is contingent on channels being open and not inactivated; conversely, a blocked channel can neither inactivate nor deactivate (see Armstrong, 1971). Nevertheless, the physiological effects of resurgent current can be predicted largely by analysing channel gating. The central point is that, upon repolarization, displacement of the blocker is much faster than recovery from fast inactivation (Raman & Bean, 2001; Aman & Raman, 2007, 2010). The time course of restoration of Na^+ channel availability determines the duration of refractory periods (Hodgkin & Huxley, 1952*c*), as well as the number of action potentials that can be fired repetitively before cumulative inactivation precludes further spiking (Colbert *et al.* 1997; Engel & Jonas, 2005). Thus, by preventing fast inactivation at positive potentials but dissociating rapidly at negative potentials, the blocking particle creates a cycle of opening, block and unblock that is permissive for rapid, repetitive action potential firing. Simulations, as well as experimental work modulating resurgent current (see below), confirm that repetitive firing in Purkinje cells depends upon restoration of Na^+ channel availability, primarily through recovery from an open blocked state (Khaliq *et al.* 2003).

Indeed, many neurons with resurgent current share a capacity for repetitive spiking: rapid spontaneous firing, continuous regular firing during steady depolarization or burst firing (Table 1). The spiking pattern of any given cell, however, depends on its full complement of channels (Fig. 2); resurgent current facilitates but does not guarantee rapid, spontaneous, or repetitive firing, much as hyperpolarization-activated cation current (I_h) produces pacemaking in some cells but not others (McCormick & Pape, 1990; Magee, 1998). In Purkinje neurons, which fire action potentials 50–100 spikes s^{-1} spontaneously and up to ∼250 spikes s^{-1} with depolarization (Khaliq & Raman, 2005; Monsivais *et al.* 2005), the effect of resurgent current is shaped by extremely large, high-threshold,

rapidly deactivating K^+ currents (Raman & Bean, 1999; Khaliq *et al.* 2003), carried mostly by K_V3 channels (Akemann & Knöpfel, 2006) and BK channels (Benton *et al.* 2013). These K^+ channels are closed at inter-spike potentials, permitting a high input resistance against which even small Na^+ currents can depolarize effectively. The K^+ currents activate powerfully with depolarization, however, truncating Purkinje action potentials so that they are among the briefest in the nervous system (Carter & Bean, 2009). Na^+ current flows on the downstroke of action potentials, from unblock of blocked channels as well as from tail currents through open channels (Raman & Bean, 1997; Khaliq *et al.* 2003; Carter & Bean, 2009). Importantly, the K^+ currents are big enough to counteract this depolarization and terminate the action potential, but deactivate quickly enough to prevent a deep afterhyperpolarization that could preclude another action potential from initiating spontaneously (Raman & Bean, 1999; Khaliq *et al.* 2003). Shifting the balance of channel availability to favour Na^+ channels, e.g. via hyperpolarization, can change simple spikes to bursts resembling complex spikes (Raman & Bean, 1997). Firing rates, however, are set mostly by K^+ currents; in the medial vestibular nucleus, for example, a strong but short-lasting repolarization is provided by K_V3 currents in fast-spiking neurons and by BK currents in more slowly spiking GABAergic neurons (Gittis *et al.* 2010). In fact, introducing an open Na^+ channel blocker into neurons with small K^+ currents that lack a native blocker can actually impede rather than favour repetitive firing (Bant *et al.* 2013).

Molecular basis for resurgent Na^+ current

The first hint toward the molecular basis of resurgent current came from studies of mice lacking the neuronal voltage-gated Na^+ channel α subunit $Na_V1.6$ (*Scn8a*) (Burgess *et al.* 1995). In $Na_V1.6$-null Purkinje cells, transient current amplitudes fall by only ~30%, consistent with expression of $Na_V1.1$ (*Scn1a*) and $Na_V1.2$ (*Scn2a*). In contrast, resurgent current decreases by ~90% (50 mM external Na^+; Raman *et al.* 1997). A similarly drastic preferential reduction of resurgent current is seen in $Na_V1.6$-null neurons from the DRG (large sensory neurons), mesencephalic trigeminal ganglia and hippocampal CA1 region (Cummins *et al.* 2005; Enomoto *et al.* 2007; Royeck *et al.* 2008), demonstrating the necessity of $Na_V1.6$ for normal resurgent current in these cell types.

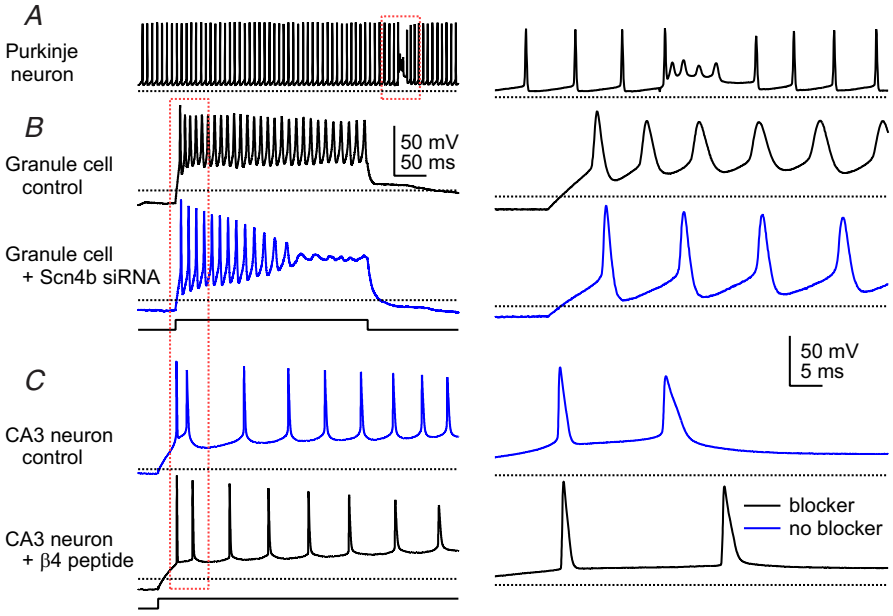

Figure 2. Firing patterns of different neurons, with and without an open channel Na^+ blocker
A, spontaneous firing in a Purkinje cell in a cerebellar slice, with an evoked complex spike (within box). This cell has an endogenous blocking protein. The brevity of spikes results largely from repolarization by high-voltage-activated K^+ currents. *B*, action potentials evoked by current injection in two different cultured granule cells, transfected with non-targeted siRNA (control) or siRNA targeted against Scn4b to reduce native $Na_V\beta4$ expression. The primary difference is in the ability of the cells to fire throughout the step. *C*, action potentials evoked by current injection in two different CA3 pyramidal cells in a hippocampal slice, either without a blocker (control) or with the $\beta4$ peptide added intracellularly. With peptide, the firing rate does not always increase but the spike waveform consistently narrows and threshold is reduced. Left scale bars apply to all left records, right scale bars to right records. Black dotted line, –60 mV. Boxes on left indicate regions expanded on right. All recordings are from mouse. Traces from Khaliq and Raman (2005, Purkinje), Bant and Raman (2010, granule) and Bant *et al.* (2013, CA3).

Surprisingly, in $Na_V1.1$-null mice, transient and resurgent current in Purkinje cells decrease *in parallel* (Kalume *et al.* 2007), raising the possibility that $Na_V1.1$ somehow regulates $Na_V1.6$ expression in these cells.

Considerable evidence suggests, however, that $Na_V1.6$ is neither solely responsible, nor even an obligate factor, for resurgent current. First, in CA3 pyramidal neurons and large spinal motoneurons, as well as in heterologous expression systems, expressing $Na_V1.6$ does not generate resurgent current, suggesting that $Na_V1.6$ lacks an intrinsic blocking domain (Raman & Bean, 1997; Smith *et al.* 1998; Pan & Beam, 1999). Second, even in Purkinje neurons of $Na_V1.6$-null mice, resurgent current can be restored by peptide toxins that slow the rate of fast inactivation (Grieco & Raman, 2004). Third, heterologously expressing α subunits of skeletal muscle ($Na_V1.4$), cardiac muscle ($Na_V1.5$) or peripheral neurons ($Na_V1.7$) in DRG neurons that contain an endogenous blocking protein can also produce resurgent currents (Jarecki *et al.* 2010). Fourth, in the subthalamic nuclei, cerebellar nuclei, globus pallidus and cerebellar granule layer, loss of $Na_V1.6$ only mildly to moderately reduces resurgent current (Do & Bean, 2004; Aman & Raman, 2007; Mercer *et al.* 2007; Osorio *et al.* 2010), suggesting that $Na_V1.1$ and $Na_V1.2$ normally carry resurgent current in these cells. Interestingly, $Na_V1.6$ independent, tetrodotoxin-sensitive resurgent current in cerebellar granule cells as well as tetrodotoxin-resistant $Na_V1.8$-mediated resurgent currents in DRG cells both have remarkably slow decay times of ~50 ms and ~800 ms respectively, hinting at effects of the α subunit on resurgent current kinetics (Bant & Raman, 2010; Tan *et al.* 2014). Thus, the native blocking particle, unlike the fast inactivation gate, is not part of $Na_V1.6$ or any other α subunit. Because it is retained in excised patches and appears subject to proteolysis and dephosphorylation (see below), the blocker is probably a protein within the Na^+ channel complex (Grieco *et al.* 2002). It can interact with several α subunits, although blocking efficiency apparently varies across cells.

At present, the only identified endogenous open channel blocker is $Na_V\beta4$ (*Scn4b*) (Grieco *et al.* 2005; Bant & Raman, 2010). Like other known Na^+ channel β subunits (*Scn1b, Scn2b, Scn3b*), this ~200 amino acid subunit has one transmembrane domain, an extracellular domain with an immunoglobulin-like fold, and a short cytoplasmic C-terminus (Yu *et al.* 2003, Gilchrist *et al.* 2013). Like $Na_V\beta2$, $Na_V\beta4$ associates covalently with α subunits (Yu *et al.* 2003; Buffington & Rasband, 2013; Fig. 3*A*). A distinguishing feature of $Na_V\beta4$, however, is that it contains a nine-amino-acid insert in the C-terminus (residues 158–166). In mice, the amino acids immediately after the transmembrane segment, which include the insert, have the sequence KKLITFILKKTREK (residues 154–167). Based on its loose resemblance to the KIFMK peptide, Grieco *et al.* (2005) tested whether a peptide with this sequence (the '$\beta4$ peptide') could block Na^+ channels. In inside–out patches from Purkinje neurons, the native blocking particle was first destroyed by brief exposure of inside–out patches to trypsin or alkaline phosphatase, which enlarged and slowed transient currents and removed resurgent currents. Next, the $\beta4$ peptide was applied to the intracellular face of the patches. It blocked the channel upon depolarization and unblocked upon repolarization, shortening transient currents and restoring resurgent-like currents. Importantly, the kinetics of peptide-dependent and native transient and resurgent currents were indistinguishable. The $\beta4$ peptide and native blocking particle therefore must have similar affinities for Purkinje Na^+ channels. Thus, $Na_V\beta4$ emerged as a good candidate for an endogenous open channel blocker.

Indeed, when expression of $Na_V\beta4$ is reduced by siRNA in cultured cerebellar granule cells, resurgent current is reduced proportionately to the degree of knockdown, and repetitive firing is compromised. (Note that these cells do not fire spontaneously.) The $\beta4$ peptide restored both resurgent current and sustained repetitive firing, supporting the idea that both siRNA-induced changes result from the loss of an open channel blocking action by the subunit. Strikingly, $Na_V\beta4$ knockdown also negatively shifted steady-state inactivation curves of transient currents, and the $\beta4$ peptide restored the curves to normal, more depolarized values, illustrating the intimate relation between block – or the blocking sequence itself – and fast inactivation (Bant & Raman, 2010). The $\beta4$ peptide can also induce a resurgent-like current in neurons lacking endogenous blocking proteins, including CA3 pyramidal cells (Grieco *et al.* 2005) and HEK cells expressing $Na_V1.1$, 1.4, 1.5, 1.6 and 1.7 (Wang *et al.* 2006; Aman *et al.* 2009; Theile *et al.* 2011), demonstrating generalizability of an open channel block by this peptide and its utility for studying resurgent current.

Despite strong evidence that $Na_V\beta4$ is a functional blocking protein in some cells, however, it has been impossible to reconstitute resurgent current in heterologous systems of non-excitable cells. Expressing full-length $Na_V\beta4$ with $Na_V1.6$, $Na_V1.1$ or $Na_V1.7$ in HEK cells produces channels without resurgent current (Chen *et al.* 2008; Aman *et al.* 2009; Theile *et al.* 2011), revealing that the subunit does not function as a blocking protein under all conditions. Possibly, other proteins or modifications to $Na_V\beta4$ are required for the subunit to act as a blocker or for the α subunit to be receptive to block by the protein. The lack of reconstitution may also indicate peculiarities of the expression system, however. Na^+ channel gating properties in HEK cells are greatly altered from those in excitable cells, with activation curves of transient currents shifted 20–30 mV depolarized (Aman & Raman, 2007; Aman *et al.* 2009; Fu *et al.* 2011). Such changes may influence the efficacy of an open channel block; even $\beta4$ peptide-induced block is unstable in HEK

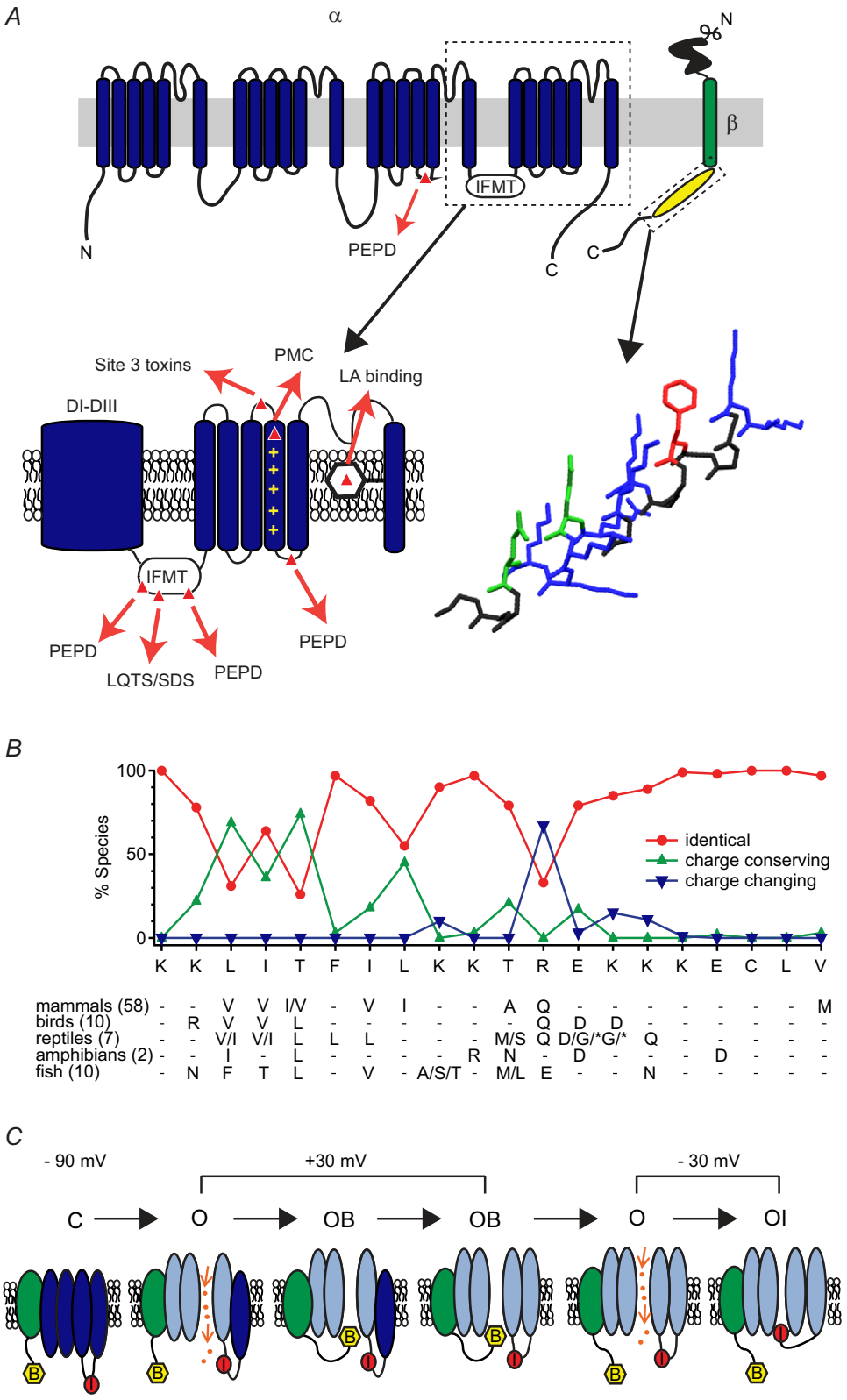

Figure 3.
A, schematic of the topology of Na^+ channel α (upper left) and β (upper right) subunits. Boxed regions are shown in more detail in lower panels. Mutations in the α subunit that affect resurgent current are highlighted in red, and correspond to the human diseases paroxysmal extreme pain disorder (V1299F, T1464I, M1627K in $Na_V1.7$), LQTS/SDS (F1468L in $Na_V1.5$) and PMC (R1448P in $Na_V1.4$) (Jarecki *et al.* 2010; Theile *et al.* 2011).

cells, requiring extremely large depolarizations (+60 mV) for binding and only weak repolarization (–10 mV) for unbinding (Aman *et al.* 2009).

Reconstitution notwithstanding, many neurons with resurgent current indeed express $Na_V\beta4$, and some cells without resurgent current (CA3 pyramidal neurons) lack $Na_V\beta4$ (Table 1). Moreover, much like $Na_V1.6$ (Caldwell *et al.* 2000; Royeck *et al.* 2008), $Na_V\beta4$ expression is enriched in axons and nodes of Ranvier (Buffington & Rasband, 2013), positioning it to participate in spike initiation in several cell types. In fact, in neurons from the perirhinal cortex, resurgent current appears restricted to the initial segment (Castelli *et al.* 2007*a*). In many spontaneously firing neurons, which usually have high densities of somatic Na^+ channels, cell bodies have resurgent current, however (Raman & Bean, 1997; Do & Bean, 2003; Afshari *et al.* 2004). Even pharmacologically silencing Na^+ channels in Purkinje initial segments or first nodes of Ranvier does not disrupt action potential generation (Khaliq & Raman, 2006), suggesting a broader subcellular distribution of $Na_V\beta4$ in some neurons. Moreover, the roles of $Na_V\beta4$ may be quite diverse; it appears involved in neurite outgrowth and is specifically downregulated in mouse models of Huntington's disease (Ohyama *et al.* 2006).

Not all cells with resurgent current express detectable $Na_V\beta4$, however; two salient examples are GABAergic neurons of the medial vestibular nucleus (Gittis & du Lac, 2008; Kodama *et al.* 2012) and hippocampal dentate granule neurons, in which just over half the cells have resurgent current (Yu *et al.* 2003; Castelli *et al.* 2007*b*). Such observations raise the possibility that additional blocking proteins remain to be discovered. If they exist, these blocking proteins may share structural features with $Na_V\beta4$ that permit them to interact with a binding site (or sites) in the permeation pathway, and these features are probably evolutionarily conserved. To date, resurgent current has been recorded in Purkinje neurons from rat, mouse, electric fish and chick (Raman & Bean, 1997; Raman *et al.* 1997; de Ruiter *et al.* 2006; Lewis & Raman, 2011). Across species, the putative blocking sequence of $Na_V\beta4$ is highly conserved at some sites and diverges at others; in 50 vertebrates, the sequence is K+ΦΦxFΦΦK+xxxKK–CLV, where Φ, + and – indicate neutral, positive and negative residues, and x is a non-conservative substitution. Four other species have either an F6L or a K10S substitution (Fig. 3*B*). Despite the variability, $\beta4$ peptides with mouse, human, chick, frog and cow sequences all generate resurgent current when introduced into mouse CA3 neurons, suggesting that the most relevant residues for the open channel block include the aromatic phenylalanine and the positively charged residues (Lewis & Raman, 2011). Indeed, altering the properties or position of F6 or the charge or side chain branching pattern of K10/K11 greatly changes the affinity of the blocker. Such mutational studies also suggest that the channel-bound peptide may assume a helical conformation, with the phenylalanine stabilizing the open channel block at all potentials and the unbranched alkylammonium lysine side chains favouring binding at positive voltages while allowing unbinding with repolarization (Grieco *et al.* 2005; Lewis & Raman, 2011). Thus, the FΦΦK+ motif may be a guide toward finding additional open channel blocking subunits.

Clues about the blocker binding site come from studies of local anaesthetics. The $\beta4$ peptide cannot block $Na_V1.5$ channels in which a phenylalanine residue in the local anaesthetic binding site has been mutated (Ragsdale *et al.* 1994; Wang *et al.* 2006). Likewise, briefly exposing inside–out Purkinje cell patches to chymotrypsin, which preferentially cleaves aromatic residues, not only removes native open channel block, but also makes channels insensitive to blockade by the local anaesthetic derivative QX-314 (Grieco *et al.* 2005). Additionally, the native Purkinje open channel blocker and the $\beta4$ peptide each protect Na^+ channels from inhibition by lidocaine (Bant *et al.* 2013). Together, these data support the idea that native open channel blockers, and probably $Na_V\beta4$, may

The site 3 toxin binding site and a residue implicated in both LA and $\beta4$ peptide binding (F1760K in $Na_V1.5$) are also highlighted (Wang *et al.* 2006; Hanck & Sheets, 2007). Lower right, the $\beta4$ peptide rendered as an idealized α-helix in DeepView. Charged residues are blue (positive) and green (negative); the phenylalanine that contributes to stable block is red. *B*, evolutionary conservation and divergence among species of the region of $Na_V\beta4$ corresponding to the $\beta4$ peptide. Sequences from 86 vertebrate species were compared to the mouse $\beta4$ sequence with each residue assigned as identical, charge conserving, or charge changing. Charged residues and a phenylalanine residue are particularly highly conserved. Below, list of substitutions at each residue for each class. *C*, schematic illustrating how voltage sensor movement influences the susceptibility of a channel to block. At rest, all four domains (blue ellipses) are in an inward position and the channel is non-conducting ('C'). Upon depolarization, the voltage sensors of DI–DIII deploy, opening the channel and allowing current to flow ('O'). Deployment of the voltage sensor of DIV is delayed relative to the other domains. The blocker can bind ('OB') before the DIV sensor deploys. Subsequent outward movement of DIV, which usually favours fast inactivation, stabilizes the bound blocker. Upon repolarization, inwardly permeating Na^+ ions knock off the blocker, returning the channel to a conducting state. With prolonged time at a moderately negative potential (e.g. −30 mV), where block is unstable, channels eventually make transitions into fast inactivated states ('OI'). IFMT, isoleucine, phenylalanine, methionine, and threonine (motif); LA, local anaesthetic; LQTS, long QT syndrome; PEPD, paroxysmal extreme pain disorder; PMC, paramyotonia congenita.

be endogenous ligands for the local anaesthetic binding site. As in the case of local anaesthetics (Ragsdale *et al.* 1994), binding may involve interactions of the conserved aromatic rings of the α subunit and the blocking protein, in a manner that permits the charged lysine side chains to obstruct the permeation pathway.

Structural determinants of the open channel block

Ultimately, a full mechanistic description of resurgent current requires a structural understanding of the Na^+ channel complex and its gating properties. Much is known about the physical counterparts of the four main states, C, O, I and B. Voltage-gated Na^+ channels comprise four domains (DI, DII, DIII, DIV), which form a central pore that opens and closes in response to movements of the voltage-sensing regions (which include the fourth transmembrane segment, S4) of each domain (Stühmer *et al.* 1989). At strongly negative voltages, the voltage sensors of all domains are in the resting (inward) position, holding the channel closed. With depolarization, the sensors move, each with distinct voltage dependence and kinetics, and the voltage sensor of DIV deploys less readily than those of DI–III. The open state is favoured only after the voltage sensors of DI, DII and DIII all have moved to the activated (outward) position (Cha *et al.* 1999; Chanda & Bezanilla, 2002). The DIV voltage sensor need not activate for channel opening, but its deployment is required for stable binding of the fast inactivation gate, thought to be the intracellular linker between DIII and DIV (Chahine *et al.* 1994; Eaholtz *et al.* 1994; Sheets *et al.* 1999; Capes *et al.* 2013).

A central question is how an open channel blocker from a separate subunit binds the Na^+ channel more rapidly than a highly localized intrinsic inactivation gate. As mentioned above, when toxins or mutations restrict DIV voltage sensor movement, binding of the fast inactivation gate is slowed (Hanck & Sheets, 2007). Even with DIVS4 in the resting position, however, native or exogenous blocking proteins can readily bind the open channel. Thus, the longer the gap between DI–DIII voltage sensor movements (opening) and DIVS4 movement (inactivation), the more effective an open channel blocker may be at binding during the interval (Grieco & Raman, 2004; Lewis & Raman, 2013). Notably, DIVS4 can deploy after activated channels have been blocked and stabilizes binding of the blocking protein, preventing inactivation even when its onset would otherwise be likely (Lewis & Raman, 2013). Upon repolarization, resurgent current flows at voltages that favour the maintained deployment of DI–DIII (i.e., an open pore) as well as blocker unbinding (Fig. 3*C*).

Ultimately, therefore, the amplitude of resurgent current depends on several factors (Raman & Bean, 2001; Aman & Raman, 2007, 2010; Lewis & Raman, 2011, 2013). First, *the number of channels that previously have undergone open channel block*. The greater the proportion of channels that bind a blocker upon opening by depolarization, the larger the ratio of resurgent to transient current will be. Second, *the binding affinity of the blocker*, which can have opposing effects; tighter binding increases the proportion of blocked channels at positive potentials, which can increase resurgent current amplitudes upon repolarization. A higher affinity also slows the current rise time, however, which desynchronizes reopening, reducing peaks, while prolonging current flow. Third, *the duration of the step preceding repolarization*. With long depolarizations, permeating Na^+ eventually unblocks channels, permitting channels to inactivate stably; thus, longer depolarizations elicit smaller resurgent currents upon repolarization. Fourth, *the repolarization potential*; more negative voltages generate higher driving forces on Na^+, increasing both the ease of displacement of bound blocking proteins as well as the synchrony of reopening, and thereby raising resurgent current amplitudes. The effect of hyperpolarization is counterbalanced, however, by the fact that less negative voltages permit repeated channel reopening before inactivation, enlarging as well as prolonging resurgent currents. Consequently, resurgent current tends to be maximal near -30 mV, but this value may change with alterations of Na^+ gradients or voltage sensing by any domain. Fifth, *the transition rates of the voltage sensors and fast inactivation gate*. Upon depolarization, faster DIVS4 deployment limits the open channel block by favouring fast inactivation, setting the stage for small resurgent currents. Upon repolarization, faster DI–DIII deactivation rates or faster binding of the inactivation gate will curtail peak resurgent current as well as speed its decay.

It is worth noting that recovery from *any* form of inactivation while channels remain activated will generate currents resembling resurgent current. Such effects have been produced pharmacologically by toxins that either shift the voltage dependence of voltage-sensing domains so that DIS4, DIIS4 and/or DIIIS4 activate more negatively or destabilize DIVS4 deployment so that channels recover at more depolarized voltages (Schiavon *et al.* 2006, 2012). Under these conditions, after depolarization-evoked opening and fast inactivation, subsequent repolarization permits DIVS4 to deactivate while other domains remain temporarily activated, leading to a repolarization-dependent flow of current. Such current is mechanistically distinct from open channel block-dependent resurgent current. It demonstrates the generalizable principle, however, that recovery through open states can be simply mimicked by non-conducting states, entered at positive voltages, becoming unstable at negative potentials at which some channels either remain activated or deactivate more slowly than the inactivation

gate unbinds. It may also provide a means by which some cells can make a resurgent-like current without a blocking protein, although no such instances have yet been described. Such cells would be typified by large window currents and slow channel closure. In these cases, the observed resurgent-like current might be relatively insensitive to Na^+ gradients.

Resurgent current and disease

These biophysical considerations may be relevant to research suggesting that the resurgent current is altered in several disease states (Fig. 3*A*), including paroxysmal extreme pain disorder, long QT syndrome, paramyotonia congenita (Jarecki *et al.* 2010), epilepsy (Hargus *et al.* 2011, 2013) and chemotherapy-induced neuropathy (Sittl *et al.* 2012). Increases in inflammatory mediators can also increase resurgent currents through $Na_V1.8$ (Tan *et al.* 2014). Some of these conditions result from mutations in α subunits that slow the rate of fast inactivation: In paroxysmal extreme pain disorder and long QT, the fast inactivation gate of $Na_V1.7$ or $Na_V1.5$ is directly disrupted; in paramyotonia congenita, DIVS4 of $Na_V1.4$ is mutated. When channels with these mutations are transfected into neurons that contain a native blocking particle, they generate resurgent currents that are much larger than in wild-type channels; in fact, transfection of wild-type $Na_V1.4$ makes no resurgent current (Jarecki *et al.* 2010).

In rat models of status epilepticus, resurgent current is increased in layer II stellate neurons of the medial entorhinal cortex, correlating with increased $Na_V1.6$ expression (Hargus *et al.* 2013). The transient current activation curve shifts slightly negative and inactivation curve slightly positive, increasing the window in which resurgent current can flow. $Na_V1.6$ also appears implicated in cooling-induced neuropathy induced by the anti-cancer drug oxaliplatin; treatment slows inactivation of $Na_V1.6$ in pain-sensing DRG neurons (Sittl *et al.* 2012). The slowing of fast inactivation associated with nearly all these conditions is expected to alter firing patterns by prolonging transient currents, increasing persistent current and enlarging resurgent current, thus reducing accumulation of channels in non-conducting states. In non-voltage-clamped cells, such changes will increase sub-threshold Na^+ current, probably generating the increases in action potential firing rate or duration that underlie the disorders (Jarecki *et al.* 2010). If so, amelioration of the conditions might be achieved by reducing Na^+ currents, most simply by stabilizing inactivation. Indeed, $\beta4$ peptide-induced resurgent current in expression systems can be decreased by carbamazepine, riluzole or anandamide (Theile & Cummins, 2011). Such drugs are expected to be effective in reducing rapid or excessive firing in cells with or without a native blocking particle. Another approach, to target only those cells with resurgent current, might be to stabilize native open channel blockers with compounds that increase blocker channel affinity, making them less easily displaced.

Conclusion

Voltage-gated Na^+ channels that underlie action potentials in all excitable cells – in central and peripheral neurons and in cardiac and skeletal muscle – have the capacity to produce resurgent as well as transient and persistent current. Whether resurgent current is normally present depends on two main factors: first, the expression of an open channel blocking protein within the Na^+ channel complex, a role that can be assumed by $Na_V\beta4$, though not necessarily uniquely, and, second, the gating properties of the specific Na^+ channel complex, particularly the movement of the DIV voltage sensor relative to channel opening. The cycle of open channel block and unblock prevents channels from entering absorbing inactivated states, thereby keeping Na^+ channel availability high enough for repetitive and/or burst firing. Modulating either Na^+ channel gating or the blocking particle itself can alter this cycle, as well as the firing properties that it facilitates, which may have significant consequences for both normal physiology and disease.

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Additional information

Author contributions

AHL and IMR wrote the manuscript and made figures together. Both authors approved the final version for publication.

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