



Role of Sodium Channels in Epilepsy

David I. Kaplan¹, Lori L. Isom², and Steven Petrou^{1,3,4}

¹The Florey Institute of Neuroscience and Mental Health, The University of Melbourne, Parkville, Victoria 3052, Australia

²Department of Pharmacology, The University of Michigan, Ann Arbor, Michigan 48109-0632

³The Centre for Neural Engineering, The Department of Electrical Engineering, The University of Melbourne, Parkville, Victoria 3052, Australia

⁴The Australian Research Council Centre of Excellence for Integrative Brain Function, The University of Melbourne, Parkville, Victoria 3052, Australia

Correspondence: steven.petrou@florey.edu.au

Voltage-gated sodium channels (VGSCs) are fundamentally important for the generation and coordinated transmission of action potentials throughout the nervous system. It is, therefore, unsurprising that they have been shown to play a central role in the genesis and alleviation of epilepsy. Genetic studies on patients with epilepsy have identified more than 700 mutations among the genes that encode for VGSCs attesting to their role in pathogenesis. Further, many common antiepileptic drugs act on VGSCs to suppress seizure activity. Here, we present an account of the role of VGSCs in epilepsy, both through their pathogenic dysfunction and as targets for pharmacotherapy.

SUBTYPES AND STRUCTURE

Voltage-gated sodium channels (VGSCs) are responsible for the generation and propagation of action potentials (APs). Through transiently increasing membrane permeability to sodium ions, these specialized proteins facilitate diffusion down an electrochemical gradient, bringing the membrane toward the sodium equilibrium potential (Hille 2001). This depolarization forms the upstroke of the AP. The existence of ion-permeable channels, responsible for voltage gating of sodium ions across the membrane, was first hypothesized by Hodgkin and Huxley in 1952 (Hodgkin and Huxley 1952). In the time that followed, the existence of VGSCs has been proven beyond refute, and a broad family of channel subtypes have been

identified and characterized (Payandeh et al. 2011; Catterall et al. 2012). There are nine different pore-forming α -subunits ($\text{Na}_v1.1$ – 1.9) encoded for by the genes *SCN1A*–*SCN5A* and *SCN8A*–*SCN11A*). Each α -subunit is comprised of a single \sim 260-kDa protein composed of four repeat domains (I–IV), each containing six transmembrane α -helical segments (S1–S6). The S4 segment acts as a voltage sensor because of a high concentration of positively charged arginine residues that compel the segment to move through the electric field of the membrane (Catterall 1986; Guy and Seetharamulu 1986; Keller et al. 1986). Domains III and IV of the α -subunit are connected by an intracellular loop that forms the channel inactivation gate, acting through a hinged lid mechanism (McPhee et al. 1998). Mutagenesis studies suggest that a phenylalanine

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residue (F1489) on the intracellular loop binds to an inactivation gate receptor, which causes the loop to occlude the channel pore during the inactivation process (Patton et al. 1992; West et al. 1992; Kellenberger et al. 1997; Smith and Goldin 1997). The α -subunit is often coupled to one or two non-pore-forming β -subunits. There are five different β -subunit proteins ($\beta 1$, $\beta 1B$, $\beta 2$, $\beta 3$, and $\beta 4$) encoded by four genes (*SCN1B–SCN4B*; $\beta 1B$ is a splice variant of *SCN1B*) (Brackenbury and Isom 2011). VGSC β -subunits are much smaller than α -subunits (30–50 kDa), each consisting of an extracellular immunoglobulin (Ig) loop, a single transmembrane domain, and a short intracellular domain. The physical interaction between the α - and β -subunits is not well characterized; however, evidence suggests that residues in the A/A' strand of the $\beta 1$ -subunit Ig fold are involved in the α -subunit interaction and residues in the $\beta 1$ carboxyl terminus are critical for association with $\text{Na}_V1.1$ (McCormick et al. 1998; Spanpanato et al. 2004b). In addition, $\beta 2$ - and $\beta 4$ -subunits covalently associate with α -subunits via extracellular disulfide bonds (Chen et al. 2012; Buffington and Rasband 2013). β -subunits modulate the kinetics and voltage dependence of the α -subunits and have also been shown to affect the voltage-gated potassium channels, as well as participate in nonconducting roles, including cell–cell and cell–matrix adhesion, directing neuronal proliferation, migration, and fasciculation, and modulating the effects of pharmacological compounds on VGSCs (Isom et al. 1992, 1995; Patton et al. 1994; Yu et al. 2003; Brackenbury and Isom 2011; Marionneau et al. 2012; Nguyen et al. 2012). Aside from the VGSCs, there is also a specialized sodium channel involved in sensing sodium levels. Na_X is related to the family of VGSCs; however, it differs in its primary structure and is not voltage dependent (Hiyama et al. 2002).

THE ROLE OF SODIUM CHANNELS IN THE NERVOUS SYSTEM

Within the central nervous system (CNS), VGSCs are critically important for regulating neuronal excitability and, therefore, network ac-

tivity. They are expressed ubiquitously throughout central neuron compartments with an increased density at nodes of Ranvier and axon initial segments (AIS). In myelinated neurons, the aggregation of VGSCs at the nodes of Ranvier is necessary for speeding AP propagation by saltatory conduction (Conti et al. 1976; Kaplan et al. 2001). The AIS serves as the site of AP initiation because of its reduced threshold of excitation relative to adjacent regions (Stuart et al. 1997a; Palmer and Stuart 2006). This has been attributed to the high concentration and unique properties of VGSCs at this site (Colbert and Johnston 1996; Kole et al. 2008). Immunohistochemistry studies have been instrumental in determining the distribution of different VGSCs along the AIS. One such study, using adult rat retinal ganglion cells, showed that $\text{Na}_V1.6$ channels are densely expressed at the distal AIS, whereas $\text{Na}_V1.1$ channels are aggregated in a spatially distinct region of the proximal AIS (Van Wart et al. 2007). Work looking at cortical pyramidal neurons in rats showed localization of $\text{Na}_V1.6$ and $\text{Na}_V1.2$ channels at the AIS distributed in a gradient along the length of the segment with increased $\text{Na}_V1.6$ at the distal AIS and $\text{Na}_V1.2$ more proximal (Hu et al. 2009). This gradient has functional consequences as the $\text{Na}_V1.6$ channels have a hyperpolarized voltage dependence of activation relative to the $\text{Na}_V1.2$ channels (Hu et al. 2009). When depolarizing current from synaptic inputs enters the AIS, $\text{Na}_V1.6$ channels in the distal compartment will reach their activation threshold more readily than $\text{Na}_V1.2$ and, consequently, open to generate APs. Transmembrane current generated during these APs will then travel both down the axon and back toward the soma, depolarizing the membrane and activating the $\text{Na}_V1.2$ channels to induce a backpropagating AP. This AP is actively propagated through the dendritic arbor by VGSCs expressed throughout the soma and dendrites (Lorincz and Nusser 2010). The backpropagating AP is important for relating neuronal output to synaptic input, which is necessary for regulating synaptic plasticity (Stuart et al. 1997b). Changes in plasticity can dramatically alter network dynamics by changing the functional topology of a net-

work. Dendritic VGSCs have also been proposed to contribute to AP generation in the dendrites; however, it still remains unclear as to the relevance of this behavior in vivo (Magee and Johnston 1995; Golding and Spruston 1998). Taken together, these data show the complex relationship between VGSC distribution and network excitability and hint at the vulnerability of brain networks to changes in the function of these channels by mutation or drug action.

EPILEPSY MUTATIONS IN SODIUM CHANNELS

Genetic studies of patients with epilepsy have identified an extensive array of mutations predominantly among the genes that encode for the ion channels, earning epilepsy its classification as a “channelopathy.” A substantial proportion of these mutations are in VGSC genes with the vast majority discovered in *SCN1A*, and fewer in *SCN2A*, *SCN8A*, and *SCN1B* (Wallace et al. 1998, 2002; Mulley et al. 2005; Striano et al. 2006; Herlenius et al. 2007; Patino et al. 2009; Shi et al. 2009; Allen et al. 2013; O’Brien and Meisler 2013; Sawyer et al. 2014).

Mutations in *SCN1A* are occasionally associated with generalized epilepsy with febrile seizures (FSs) plus (GEFS⁺) and much more frequently with the severe epileptic encephalopathy (EE), Dravet syndrome (DS). GEFS⁺ mutations in *SCN1A* are mostly missense mutations, whereas, in DS, 40% are missense mutations, 40% are truncation, and the remainder are splice-variant changes (Marini et al. 2011). Although earlier work suggested that mutations in *SCN2A* are primarily associated with the self-limiting and relatively mild, benign familial neonatal infantile seizures (BFNIS), recent evidence implicates *SCN2A* as an important EE gene (Ogiwara et al. 2009; Liao et al. 2010; Allen et al. 2013; Baasch et al. 2014; Hackenberg et al. 2014). A spate of de novo *SCN8A* mutations among patients with EEs have been identified (Veeramah et al. 2012; Estacion et al. 2014; Larsen et al. 2015). A recent study using high-throughput genetic screening identified 17 mutations in *SCN8A* among patients with EE (Larsen et al. 2015). Seizure onset occurred at

around 5 months of age and all patients had impaired development. Seizure types varied with many patients developing multiple seizure types after initial seizure onset. Mutations in *SCN1B* have been identified in patients with DS, GEFS⁺, and temporal lobe epilepsy (Scheffer et al. 2007; Patino et al. 2009; Ogiwara et al. 2012). Most of the epilepsy-associated mutations discussed in this article are examples of highly penetrant dominant mutations. However, it seems increasingly apparent that, in many cases, epilepsy arises as a consequence of combinatorial effects of multiple mutations that are not individually deleterious. This point is nicely illustrated in a study by Klassen and colleagues (2011) who performed Sanger sequencing on 237 ion-channel genes in 152 patients with idiopathic epilepsy and 139 healthy controls. This study identified thousands of single-nucleotide polymorphisms (SNPs). Interestingly, these were distributed among both epilepsy patients and controls with no clear separation. Further, when the investigation was limited to genes that were known to be associated with epilepsy, the investigators found a significant number of missense mutations among the control group. These findings suggest that common genetic epilepsies are caused by an accumulation of mutant ion channels, rather than highly penetrant rare variants.

FUNCTIONAL ANALYSIS OF SODIUM CHANNEL MUTATIONS: IMPLICATIONS FOR DIFFERENT SPATIAL SCALES

Since the discovery of the first epilepsy-associated genetic mutation, an extensive body of work has focused on understanding the functional consequence of these mutations in model systems. VGSC mutations have been investigated on multiple spatial scales, from the level of single channels heterologously expressed in nonneuronal cell lines, extending to network and whole animal experiments from genetically engineered models. Each of these models offers unique insights into the functional mechanisms of mutations, which can help to build a comprehensive understanding of the mechanisms underlying genetic epilepsies.

CHANGES IN SODIUM CHANNEL BIOPHYSICS: INSIGHTS FROM CELL CULTURES

Mutant channels can be transfected into heterologous expression systems to interrogate changes in channel biophysics. Using quiescent cell lines, currents generated by a heterologously expressed mutant channel can be readily isolated. With this approach, a growing number of VGSC mutations identified in patients with epilepsy have been analyzed. However, these results must be interpreted with caution as a nonneuronal cell line may lack essential components that affect VGSC function and cannot fully recapitulate the internal milieu of a neuron. Moreover, differences in recording conditions, cell line type, and passage history confound comparison of results across different studies.

SCN1A MUTATIONS

Of the *SCN1A* mutations, more than one-half are truncation mutations, resulting in haploinsufficiency, whereas the remainder are missense mutations that have effects on the kinetics or voltage dependence of channel gating. Work looking at the effects of such missense mutations from patients with GEFS⁺ showed variable results. Early studies using heterologous expression systems suggest mutations result in a gain of function as a result of impaired channel inactivation (Lossin et al. 2002). This idea was soon challenged by characterization of several loss-of-function GEFS⁺ mutations caused by various different biophysical modifications, such as positive shifts in the voltage dependence of activation or increased slow inactivation (Lossin et al. 2003). To date, no uniform change in Na_v1.1 biophysics has been identified in GEFS⁺ mutations; however, given that these channels are interneuron specific, it is assumed that the disease-causing mechanism will converge at a higher spatial level (Ogiwara et al. 2007; Escayg and Goldin 2010). Under specific circumstances, VGSC gain of function could suppress neuronal excitation. In interneurons, this could lead to disinhibition as a chronically depolarized neuron would reduce channel availability and impair AP firing.

SCN2A MUTATIONS

Missense mutations in *SCN2A* are associated with BFNIS. Characterization of two BFNIS mutations using tsA201 cell lines has shown a depolarizing shift in the voltage dependence of inactivation and an increased persistent current causing an overall gain of function (Kearney et al. 2001; Liao et al. 2010; Lauxmann et al. 2013). This hypothesis is consistent with disease progression with onset at 11 weeks postnatal and remission within 1 year. Na_v1.2 channels are expressed in two different splice forms, neonatal and adult. The adult splice form is more excitable than the neonatal; subsequently, a more excitable mutant channel could induce seizures in a neonate, but be benign in an adult. In a recent study by Gazina and colleagues (2015), a mouse was generated that only expressed the adult isoform of the Na_v1.2 channel, even in infancy. These Na_v1.2^{adult} mice showed an increase in AP firing in cortical neurons and were more susceptible to seizures. In a related study by Xu and colleagues (2007b), the effects of a BFNIS mutation on the adult and neonatal splice forms of the Na_v1.2 channel are compared in HEK293t cells. The mutation results in a gain of function that is more pronounced for the neonatal form of the channel than for the adult. Further, the gain of function caused by the mutation in the neonatal form increases channel excitability to a level similar to that of the adult wild-type channel. This, indeed, suggests that the functional consequence of the mutation is severely diminished in the adult offering an explanation for the self-limiting nature of the disease (Xu et al. 2007b). Biophysical characterizations of *SCN2A* mutations in EEs are currently lacking, and it remains to be seen what, if any, functional changes are correlated with these severe and clinically important disorders.

SCN8A MUTATIONS

De novo mutations in *SCN8A* have been associated with EEs resulting in a broad spectrum of different seizure types, including absence epilepsy. A de novo mutation in the *SCN8A* gene

was identified in a child with EE who died from sudden unexpected death in epilepsy (SUDEP) at age 15 (Veeramah et al. 2012). The patient carried a heterozygous missense mutation, resulting in a N1768D variant Nav1.6 protein. When the mutant protein was expressed in ND7/23 cells, voltage-clamp assays revealed a gain of function with an increase in persistent current, ramp current, a depolarizing shift in steady-state inactivation, and incomplete channel inactivation. Another patient showing developmental delay, intellectual disability, and intractable epilepsy was discovered to carry a heterozygous mutation c.2300C>T in the *SCN8A* gene, resulting in a T767I variant (Estacion et al. 2014). Functional analysis revealed a gain of function with a hyperpolarizing shift of 10 mV in the voltage dependence of activation, as well as an increase in ramp current. Together, these studies would suggest gain of function as a common mechanism for *SCN8A*-related epilepsies; however, an additional study of a de novo missense mutation in a young girl with EE, resulting in a R233G protein variant, showed a loss of function measured as a significant reduction in current amplitude (de Kovel et al. 2014). Mutations in *SCN8A* have also been identified in forms of epilepsy other than EE. Absence seizures have distinct 3-Hz spike-wave discharges and high-frequency bursts associated with behavioral arrest and transient impairment of cognition. Through a chemical mutagenesis screen, mutations in the mouse *Scn8a* gene were shown to cause absence seizures with spike-wave discharges measured through electroencephalography (EEG) (Papale et al. 2009). Voltage-clamp analysis of the mutant channels showed an overall loss of function indicated by a depolarizing shift in the voltage dependence of activation, a hyperpolarizing shift in the voltage dependence of inactivation, and accelerated entry into fast inactivation (Oliva et al. 2014). Severity of the absence phenotype varied depending on the strain of mouse used, and this was shown to be dictated by the variant of other VGSC subtypes expressed in the same neuron. A general trend that can be concluded from this work is that *SCN8A* gain-of-function mutations tend

to result in EE, whereas loss of function seems to induce absence seizures.

SCN1B MUTATIONS

Mutations in *SCN1B*, encoding the $\beta 1$ -/ $\beta 1B$ -subunits, have been associated with a number of different forms of epilepsy. To study these proteins in heterologous expression systems, they must be coexpressed with an α -subunit. All of the *SCN1B* mutations identified in epilepsy patients (except for one identified in the specific carboxy-terminal domain of $\beta 1B$) (Patino et al. 2011) are located in the extracellular Ig fold involved in cell–cell adhesion and α -subunit interaction. Unsurprisingly, characterization of these mutations suggests a loss of function of the β -subunit, resulting in a slowing of α -subunit inactivation, a hyperpolarizing shift in sodium current voltage dependence of inactivation, and increased resilience to use-dependent loss of availability (Meadows et al. 2002; Tammaro et al. 2002; Xu et al. 2007a; Patino et al. 2009). It has also been shown that $\beta 1$ interacts with and modulates certain potassium channel subtypes, best illustrated in a study in which $\beta 1$ was knocked down in mice, resulting in a reduction in A-type potassium current density, an increase in AP half-width, and an increase in repetitive firing (Marionneau et al. 2012). GEFS⁺-related mutations of *SCN1B* can interfere with $\beta 1$ modulation of potassium currents (Nguyen et al. 2012). $\beta 1$ accelerates the activation and reduces cumulative inactivation of the potassium channel K_v1.3; however, these effects are lost with two GEFS⁺ mutations of *SCN1B*, the C121W, and R85C mutations. A mutation in the region of the *SCN1B* gene unique to the $\beta 1B$ splice variant has been suggested to cause aberrant neuronal migration as it causes intracellular retention of the subunit, which must be secreted to act as a cell-adhesion molecule (CAM) (Patino et al. 2011). Determining the effect of these mutations on channel gating is crucially important; however, for a comprehensive picture of how VGSC mutations induce epilepsy, it is necessary to scale out to models that capture the broader aspects of seizure behavior.



SUMMARY OF BIOPHYSICAL PATHOMECHANISMS IN EPILEPSY

Functional analyses of epilepsy mutations in VGSCs have revealed a complex picture; however, a specific pattern relating channel mutation to epileptic syndrome has begun to emerge. Changes in channel biophysics tend to be cell-type dependent with VGSCs in interneurons, for example, $\text{Na}_V1.1$ or $\text{Na}_V1.6$, showing loss of function because of haploinsufficiency, depolarizing shifts in the voltage dependence of activation, and hyperpolarizing shifts in voltage dependence of inactivation. Contrastingly, changes in pyramidal cell channels, for instance, $\text{Na}_V1.2$ or $\text{Na}_V1.6$, tend to be gain of function, such as depolarizing shifts in the voltage dependence of inactivation and an increase in persistent current. Broadly, *SCN1A* haploinsufficiency tends to cause DS, as does *SCN1B* loss of function. Gain of function in *SCN2A* relates to BFNIS. Gain of function in *SCN8A* has been associated with EE, whereas loss of function relates to absence. Additional potential pathomechanisms have been proposed, but not yet specifically identified, in patients with epilepsy. Mutations in the VGSC voltage sensor, which substitute the charged arginines with neutral residues, can result in a leak current through the voltage sensor, referred to as the “omega” or “gating pore” current (Sokolov et al. 2005). Although this has not yet been reported in patients with epilepsy, a mutation in $\text{Na}_V1.4$ associated with hypokalaemic periodic paralysis has been shown to have an increased omega current, implicating this mechanism among channelopathies (Sokolov et al. 2007). One other gating phenomenon that has been proposed as a pathomechanism of epilepsy is resurgent sodium current. For certain subtypes of VGSCs, a small transient inward current can be elicited with repolarization of the membrane following a prolonged depolarizing pulse (Raman and Bean 1997). Increased resurgent current amplitude has been associated with an increase in AP firing frequency, implicating it as a potential pathomechanism in epilepsy (Martin et al. 2007; Jarecki et al. 2010; Hargus et al. 2013).

STEM-CELL MODELS

A common criticism of the use of heterologous expression systems to explore mutant channel biophysics is the lack of neuronal context, which may mask pathologically important functional changes. With recent developments in stem-cell- and genome-editing technologies, this limitation can be potentially overcome. In a study by Liu and colleagues (2013), fibroblasts obtained from skin biopsies of patients with DS were used to generate induced pluripotent stem cells (iPSCs), which were then differentiated into forebrain neurons with bipolar and pyramidal morphologies. Electrophysiological assays revealed a marked increase in neuronal excitability in DS-patient-derived excitatory and inhibitory neurons relative to nonepileptic patient control iPSC-derived neurons. Voltage-clamp recordings showed a twofold increase in sodium current density at 3–5 weeks in DS neurons relative to controls, and current clamp experiments showed a reduced threshold of AP generation, increased evoked firing frequency, and spontaneous firing in excitatory and inhibitory DS patient neurons. The observation of similar effects in both pyramidal and bipolar neurons suggested an overall increase in neuronal excitability caused by the mutations. In a related study, glutamatergic neurons were derived from iPSCs from a patient with DS, a patient with FS, and a healthy control (Jiao et al. 2013). In both the DS and FS patients' cell lines, spontaneous APs were recorded, and voltage-clamp analysis showed an increase in sodium current amplitude and incomplete inactivation. These effects were more pronounced in the DS cell line, and treatment with phenytoin appeared to reverse them. Using patient-derived iPSCs preserves the genetic background that contributes to the epilepsy phenotype. Consequently, in spite of similar changes observed in two different DS patients in the Liu et al. (2013) study, differences between the control and patient-derived cells could be caused by a variety of background genetic differences. To constrain the number of variables, new methods using targeted genome editing in stem cells are being developed. A number of single-nucleotide ge-

CRISPR-Cas9 genome-editing methods are now available, including clustered regularly interspersed palindromic repeats (CRISPRs), which can be used to introduce a known epilepsy mutation into iPSCs derived from a healthy control or to repair mutations in patient-derived stem cells (Cong et al. 2013). Comparing the behavior of these cells with control iPSCs from the same patient will reveal the effect of the specific mutation on neuronal biophysics independent of the genetic background. Efforts are underway to extend the iPSC method to generate human brain-like structures to examine synapse formation and function, as well as network properties, allowing for the more detailed and physiologically relevant investigation of epilepsy mechanisms (Lancaster et al. 2013).

SCALING OUT: EPILEPSY AS A NETWORK PHENOMENON

Animal Models of Sodium Channel Mutations

Understanding how mutations affect channel biophysics is crucial to disentangling a complex disease such as epilepsy. As a network scale disorder, it is important to analyze the impact of mutant VGSCs in this context. This is best achieved through the generation of genetically modified animal models harboring epilepsy mutations identified in patients. In one such study, a knockin mouse line was generated with a nonsense mutation R1407X of the *SCN1A* gene that had been independently identified in four patients with DS (Ogiwara et al. 2007). When mice were homozygous for the mutation, they generated spontaneous seizures and died prematurely. Immunohistochemistry suggested that the Nav1.1 channels were localized to the AIS of parvalbumin-positive inhibitory interneurons. Patch-clamp recording of these neurons in the *Scn1a^{RX/+}* mice showed no change in AP threshold, amplitude or half-width; however, the decrement in spike amplitude was significantly increased in the mutant mice. This speaks to a reduction in sodium-channel availability in these neurons, reducing their excitability and, consequently, reducing global inhibition in the mouse brain. This

work supports the hypothesis that *SCN1A*-based DS results from an imbalance of the excitation:inhibition (E/I) ratio caused by the selective loss of VGSCs in inhibitory neurons (Fig. 1) (Yu et al. 2006; Ogiwara et al. 2007). To explore mechanisms in GEFS⁺, a missense *SCN1A* mutation, *R1648H*, identified in GEFS⁺ patients, was knocked into mice with mixed genetic backgrounds, and electrophysiological assays were used to compare the biophysical properties of inhibitory and excitatory neurons in this model (Martin et al. 2010). Voltage-clamp analysis showed that in wild-type animals sodium current density was substantially greater in inhibitory bipolar cells than in excitatory pyramidal neurons with a ratio of 2.7, whereas in the *Scn1a^{RH/RH}* and *Scn1a^{RH/+}* mice, this ratio was closer to one as a result of a reduced sodium current in bipolar cells. Consistent with this result, bipolar cells in *Scn1a^{RH/RH}* mice showed significantly reduced excitability compared with those in the wild-type mice. This result suggests that GEFS⁺ may share a similar imbalance of E/I as in DS, albeit with differences that account for the different clinical presentations. Beyond *SCN1A*, mutations in other VGSC genes have also been investigated using rodent models. In a recent study, a de novo missense mutation in *SCN8A* that had been identified in a patient with EE and SUDEP was knocked into a mouse line and shown to be sufficient to recapitulate the epileptic phenotype (Wagnon et al. 2015). This gain-of-function mutation resulted in increased persistent current in transfected neurons and was proposed to account for neuronal hyperexcitability in the mice. Mice that were heterozygous for the mutation showed no symptoms for the first 2–3 months of age, at which point seizure onset ensued and death occurred within a month. Generalized tonic–clonic seizures were observed that would last no more than 1 minute and were accompanied by distinct EEG signatures. Taken together, these studies show the power of animal models of genetic epilepsies for exploring pathogenesis.

Using mice to understand the implications of epilepsy mutations has obvious benefits; they are mammalian, their genome has been thoroughly analyzed, and a wealth of tools and pro-

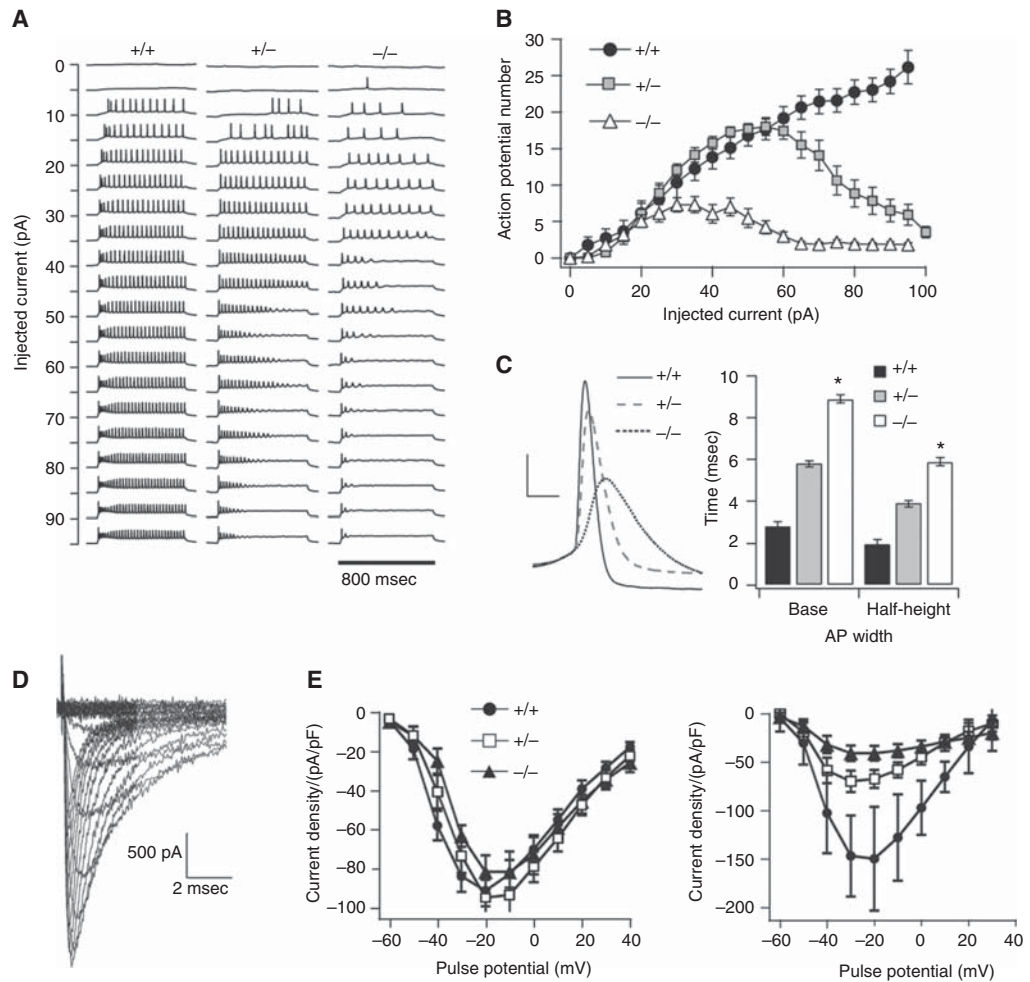


Figure 1. *Scn1a* knockout and heterozygous mice show a reduction of sodium currents localized to inhibitory interneurons (Yu et al. 2006). (A) Sample voltage traces from current clamp recordings of mouse γ -aminobutyric acid (GABA)ergic interneurons in the wild-type (WT) (left), heterozygous (middle), and null (right) *Scn1a* mice, showing a marked reduction in cell excitability in the heterozygous and null mice, summarized in B. (C) The size of action potentials (APs) was also reduced with the mutation, whereas the width of APs increased. (D) Voltage-clamp recordings of isolated sodium currents. (E) I–V curves showing that the mutation does not affect current density in pyramidal neurons (left), whereas it does cause a reduction in current in GABAergic neurons (right) (figure created from data in Yu et al. 2003, 2006).

protocols are available. However, there are additional animal models that can often be more appropriate to answer certain questions about genetic epilepsies. Zebrafish have become a popular animal model in the neurosciences and, more recently, within epilepsy research (Hortopan et al. 2010). A line of zebrafish carrying a mutation in the *scn1Lab* gene (the zebrafish gene most equivalent to human *SCN1A*) have been shown

to recapitulate the epilepsy phenotype both in terms of behavior and electrographic activity (Baraban et al. 2013). Using these fish, a semi-automated high-throughput drug screen was developed to identify effective antiepileptic drugs (AEDs). *Drosophila melanogaster* has also been used as an epilepsy model and has the advantage that it can breed rapidly and has long served as a tool for genetic studies, with a host of



methods for genome editing. It has recently been used to investigate a number of epilepsy mutations in *SCN1A* (Sun et al. 2012; Schutte et al. 2014). In one such study, a GEFS⁺ mutation, K1270T, was knocked into the *para* VGSC gene of *Drosophila* (Sun et al. 2012). These flies developed thermogenic seizures and electrophysiological analysis revealed temperature-dependent changes in VGSC biophysics, implicating a possible mechanism. Despite the simplicity of working with these nonmammalian animal models, using species so phylogenetically distant from humans introduces distinct obstacles for translation into clinical outcomes.

Downstream Effects on Cellular Physiology

VGSC mutations can lead to a host of downstream effects that may not be intuitively apparent from electrophysiological analysis, highlighting the value of using genetically modified animal models to understand the pathomechanisms of VGSC mutations. Mutations in *SCN1B* have been identified in patients with GEFS⁺. β 1-subunits, encoded by *SCN1B*, are both VGSC modulators and CAMs necessary for neurite growth, cell migration, and normal cell morphology (Brackenbury and Isom 2011).

The generation of an *Scn1b* null mouse line, *Scn1b*^{-/-}, has offered great insight into the role of this protein and its involvement in epilepsy (Chen et al. 2004). *Scn1b*^{-/-} mice appear ataxic and show spontaneous seizures, lasting from seconds to minutes, which have been correlated with high-voltage, synchronous discharges on the EEG (Chen et al. 2004). Further characterization of this mouse line has shown significant cytoarchitectural defects, including a reduction in the number of nodes of Ranvier in neurons of the optic nerve, aberrant pathfinding in the corticospinal tract and cerebellum, reduced density of neurons in the dentate gyrus, and increased cell proliferation in the hilus (Chen et al. 2004; Brackenbury et al. 2013). Importantly, pathfinding differences occur in *Scn1b* null brain before seizure onset, suggesting that hyperexcitability may result from these defects. This work offers a broad picture of the importance of the β 1-subunit, and shows how its absence leads to

an epileptic phenotype, consistent with the finding that inheritance of two mutant *SCN1B* alleles in human patients results in DS (Patino et al. 2009; Ogiwara et al. 2012). In light of this, it is not surprising that a specific *SCN1B* mutation identified in humans with epilepsy could induce epilepsy in a mouse. Mice carrying the GEFS⁺ missense mutation β 1-C121W have been well characterized, revealing a host of downstream effects. These mice show a FS phenotype in which seizures are induced by an increase in core temperature with a reduced threshold relative to wild-type littermates (Fig. 2) (Wimmer et al. 2010). Electrophysiological analysis of subicular pyramidal neurons showed an increase in excitability in heterozygous CW mice relative to wild-type CC littermates. In response to 400 msec suprathreshold current injections, cells fired initial bursts followed by tonic AP firing. In the CW mice, bursts were more protracted and tonic-firing frequency was increased. Further, the threshold for AP initiation was, on average, 2.7 mV more hyperpolarized in CW mice relative to CC mice. Using fluorescent labeling of β 1-subunits in combination with immunofluorescent staining against the AIS protein ankyrin-G, they showed that the native β 1(C121) protein is aggregated at the AIS, whereas the β 1(W121) mutant is excluded from this compartment. Changes in VGSC functioning at the AIS can have significant implications for neuronal excitability, as this is the site of AP initiation. Scaling out even further, changes in neuronal excitability can have unpredictable effects on network behavior. This notion is explored in a related study in which the same mouse line was used to investigate network properties of the C121W mutation (Fig. 2) (Hatch et al. 2014). Tetanic stimulation of the CA1 in a slice preparation resulted in a brief oscillation as recorded by an extracellular recording electrode. In CW mice, the duration of oscillations was extended and the latency to oscillation following the stimulus was reduced. This effect was also shown to be exacerbated with increased bath temperature. These studies begin to paint a picture of the elaborate consequences of small changes in channel structure.

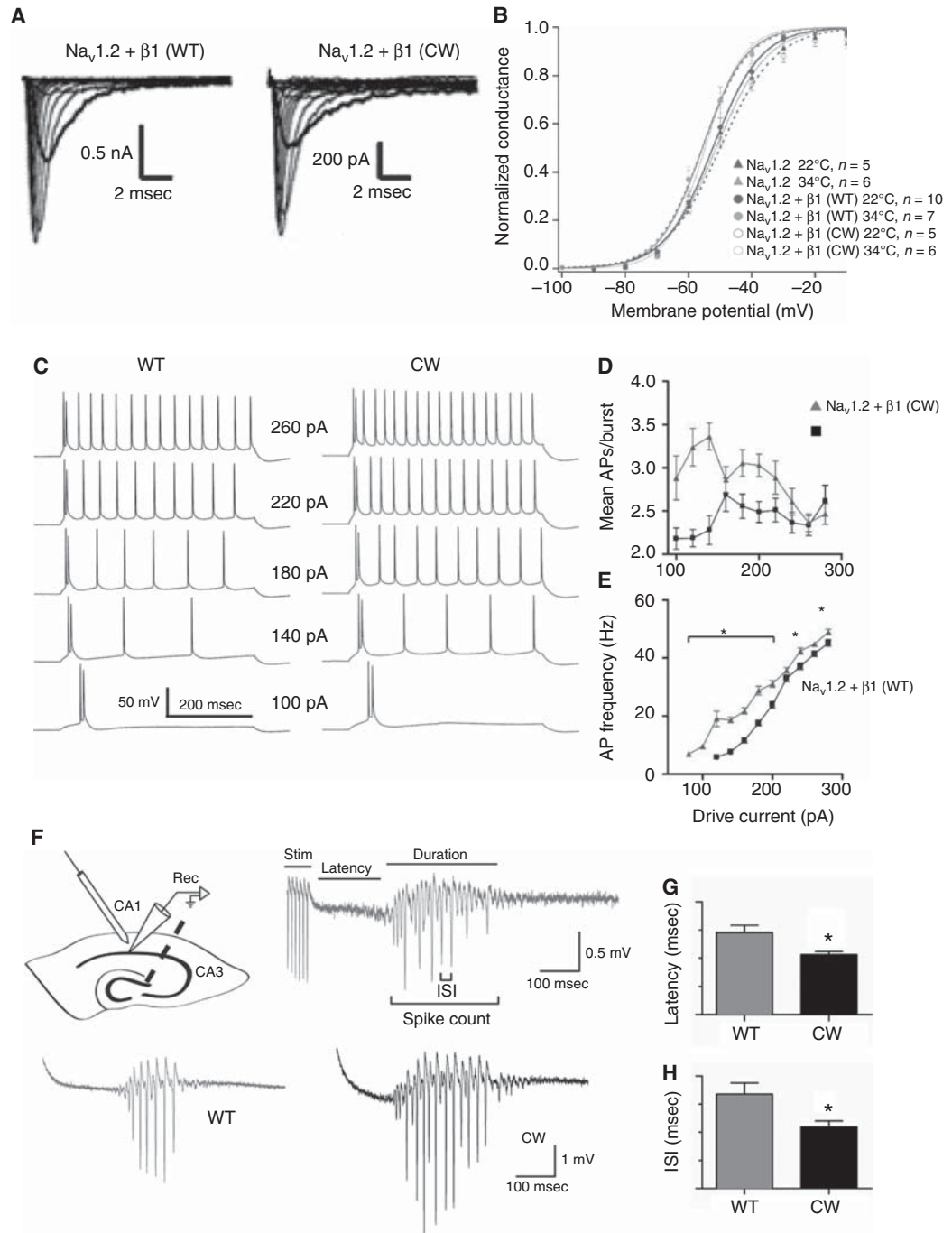


Figure 2. The functional consequences of channel mutations can be analyzed on different spatial scales (Wimmer et al. 2010; Egri et al. 2012; Hatch et al. 2014). (A) Current traces from voltage-clamp analysis of $\text{Na}_v1.2$ VGSC α -subunits coexpressed with either the wild-type (WT) $\beta 1$ -subunit (left) or the GEFS⁺ mutation C121W (right) in Chinese hamster ovary (CHO) cells. (B) Voltage dependence of activation for the $\text{Na}_v1.2$, $\text{Na}_v1.2 + \beta 1$ (WT), and $\text{Na}_v1.2 + \beta 1$ (CW) at 22°C and 34°C. (Legend continues on following page.)

BRIDGING THEORY AND EXPERIMENT: COMPUTATIONAL MODELING OF SODIUM CHANNEL MUTATIONS

Computational Models Can Reveal a Pathological Mechanism

Changes in channel biophysics induced by mutations can be quantified through voltage-clamp analysis. Through the use of animal models, the effects of channel mutations on both neuronal and network excitability can be measured. A challenge arises when trying to causally relate these changes, measured on different spatial scales. Computational modeling of neuronal biophysics has emerged as a useful means of overcoming this challenge. Biophysical properties of channels, neurons, and networks can be measured through common electrophysiological techniques. These properties can then be described mathematically and simulated on a computer (Hodgkin and Huxley 1952; Lytton 2008). A prime example of computational modeling is shown in a previously discussed study looking at the C121W heterozygous mouse (Wimmer et al. 2010). The AIS is a small compartment, notoriously difficult to access with a patch-clamp electrode. To circumvent this limitation, the investigators produced a biophysically realistic computational model of a pyramidal neuron using NEURON simulation software, and systematically altered the behavior of AIS VGSCs in an attempt to recreate the effects of the mutation on AP dynamics measured at the soma (Hines and Carnevale 1997). Negatively shifting the voltage dependence of activation for VGSCs in the AIS, they were able to mirror the increase in excitability measured in the CW mouse. This illustrates the importance of AIS VGSCs to neuronal and network excit-

ability and offers an explanation of how loss of $\beta 1$ modulation at the AIS could result in aberrant AP firing. Computational models can also be used to investigate the implications to neuronal activity of different changes in channel biophysics induced by epilepsy mutations. Spampanato and colleagues (2001, 2003, 2004a) used previously quantified changes in $\text{Na}_v1.1$ biophysics caused by three different GEFS⁺ mutations to generate a NEURON simulation of the somatic compartment to explore the consequences on AP firing. Despite having distinct effects on channel biophysics, all three mutations converged at the scale of the neuron and resulted in an increase in AP firing, indicating how different mutations can lead to a single convergent pathology. Modeling can also be used to predict the effects of mutations at the network scale. In one study, a physiologically realistic model of the dentate gyrus was used to study the effects of functional changes in different ion channels (Santhakumar et al. 2005). Previous studies had shown that the most common changes in channel biophysics caused by epilepsy mutations were small shifts in the voltage dependence of activation and inactivation. In one such study, these properties were systematically varied in all voltage-dependent channels both independently and in combination with one another. Network excitability was most sensitive to changes in VGSC activation with even small hyperpolarizing shifts having a profound effect. Again, this study presents the power of VGSCs in regulating network excitability, and the potential for pathological behavior when they malfunction. Conversely, it alludes to the potential of exploiting VGSCs to reestablish physiological network activity (Thomas et al. 2009).

Figure 2. (Continued) Increasing temperature results in a shift of the voltage of half activation, which is most significant in the $\text{Na}_v1.2 + \beta 1$ (CW). (C) Current clamp recordings showing the voltage response of mouse subicular neurons to current injections in WT (left) and C121W heterozygous (right) mice. (D) For most current injections, the C121W mouse showed an increase in action potentials (APs) frequency (E) and the average number of APs per burst. (F) Through tetanic stimulation, network oscillations could be induced in the mouse hippocampus. (G) The latency and (H) interspike interval of these oscillations was reduced in the C121W mouse (figure created from data in Wimmer et al. 2010, Egri et al. 2012, and Hatch et al. 2014). ISI, Interspike interval.

SODIUM CHANNELS AS A TARGET FOR AEDs

The Modulated Receptor Model

Given the central role of VGSCs in regulating neuronal excitability, it is intuitively satisfying that many common AEDs exert their action on the CNS by targeting VGSC function. The principal mode of action of these AEDs appears to be use-dependent block, in which inhibition of sodium currents is stronger when the membrane potential experiences repeated excursions to depolarized levels more frequently, exposing new drug-binding sites and allowing for selective block of channels only when they are in active neurons (Adler et al. 1986; McLean and Macdonald 1986; Ragsdale et al. 1991). Such activity occurs during seizures, in which neurons fire bursts of high-frequency APs, and AEDs may prevent seizures by impeding these high-frequency bursts through gradual inhibition of VGSCs (McNamara 1994). This manner of drug–channel interaction has been described by the modulated receptor hypothesis, which posits that for channels that transition through multiple states, a drug will bind to the channel with varying affinities depending on the state (Hondeghe 1987). Modulated receptor behavior is characterized through voltage-clamp assays in which complex protocols are designed to tease apart the precise kinetics of drug–channel interaction. In a seminal study by Kuo and Bean (1994), the investigators showed that the AED phenytoin binds preferentially to VGSCs in the fast inactivated state, but with a slow binding rate. Using acutely dissected rat hippocampal neurons, they first showed that the degree of sodium current inhibition by phenytoin is greater at depolarized potentials. This suggests that the drug binds the channel in the inactivated state, as this is the state most significantly occupied when the membrane is depolarized. As previously mentioned, VGSC inactivation is usually described as having fast and slow components. The fast component occurs within 5–10 msec, whereas the slow component can take hundreds of milliseconds to initiate. Given the delayed onset of phenytoin block, it would seem that the drug binds pref-

erentially to the slow inactivated state; however, Kuo and Bean show that the drug, in fact, binds to the fast inactivated state, but with slow kinetics, so that the binding appears to be linked to slow inactivation. To do this, they held the membrane at voltages at which fast inactivation is almost complete but there is very little slow inactivation, and showed that phenytoin exposure still slows recovery from inactivation. This implied that phenytoin is acting on the channel despite minimal slow inactivation. Further studies have shown that other related AEDs act on VGSCs with similar kinetics (Kuo and Bean 1994; Yang and Kuo 2002).

Mechanisms Beyond Use-Dependent Block

Use-dependent block of VGSCs is clearly an effective way to control seizures; however, evidence from the clinic suggests that some forms of epilepsy are not responsive to these drugs and can even be exacerbated by them (Snead and Hosey 1985; Genton et al. 2000; Thomas and Petrou 2013). In some of these cases, the most effective drugs have broad pharmacological effects including, but not exclusively, modulation of VGSC biophysics. Riluzole is currently used for the treatment of amyotrophic lateral sclerosis; however, it was originally developed as an AED. It has been shown to induce a hyperpolarizing shift in the voltage dependence of inactivation in tetrodotoxin (TTX)-sensitive VGSCs, as well as reduce resurgent sodium current and partially block P/Q-type calcium channels in presynaptic terminals reducing glutamate release (Song et al. 1997; Wang et al. 2004; Theile and Cummins 2011). In addition, an indirect mechanism involving the inactivation of a pertussis-sensitive guanosine triphosphate (GTP)-binding protein has been implicated (Doble 1996; Song et al. 1997; Wang et al. 2004). The AED lacosamide (LCM) is used to treat partial seizures and has been shown to enhance VGSC slow inactivation (Errington et al. 2008). Electrophysiological studies have shown that LCM enhances slow inactivation; however, evidence suggests this is not its only mechanism of action. Radioligand-binding studies and molecular dynamics simulations have implicated the protein



CRMP-2, which plays a role in axonal sprouting, suggesting a plasticity-related effect, however, this is disputed (Wang et al. 2010; Wolff et al. 2012). Blocking VGSCs during hyperexcitation appears to be a logical means of suppressing seizures, yet, as discussed earlier, some epilepsies are thought to result from specific loss of VGSCs in inhibitory neurons, causing an imbalance of E/I. Under these circumstances, a VGSC activator could conceivably rescue the loss of channel function in inhibitory neurons. Compounds that selectively activate $\text{Na}_v1.1$ are novel candidates for achieving this targeted activation (Jensen et al. 2014). Given their predominant expression among inhibitory interneurons, activation of $\text{Na}_v1.1$ is postulated to increase global inhibition and potentially prevent seizures. In genetic disorders of *SCN1A*, such as DS, these selective openers would specifically target any cell in which *SCN1A* expressed and, presumably, would restore function at the neuronal level to provide a whole brain therapeutic effect. Further research into this method is needed, yet promising results are beginning to emerge.

CONCLUSION

VGSCs are fundamentally important for the generation and propagation of APs and play a pivotal role in the regulation of network excitability. To date, the vast majority of identified epilepsy-associated mutations lie in the genes that encode VGSCs. There have been extensive efforts to understand the functional consequences of these mutations using a range of experimental models on varying spatial and temporal scales. Analysis of changes in VGSCs' biophysics using heterologous expression systems have been instrumental in characterizing mutant channels, offering some insight into the relationship between channel mutations and epileptic phenotypes. The development of animal models has helped to facilitate this process by exposing some of the higher organizational scale consequences of channel mutations. Some common epilepsies may be attributable to the combined effects of multiple mutations that cannot be readily modeled in heterologous

expression studies and would, thus, rely on a combination of biological and computational models for investigation of pathomechanisms. Despite this complexity, the efficacy of AEDs that target VGSCs emphasizes their disproportionate control over the electrical stability of the brain. Epilepsy is a spectrum of disorders in which many factors converge to induce a complex phenotype. Changes in VGSC biophysics contribute to the factors that determine seizure susceptibility as they are exquisitely sensitive sites for determining neuronal excitability.

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