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CRITICAL REVIEW AND INVITED COMMENTARY

Sodium channels and the neurobiology of epilepsy

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Summary

Voltage-gated sodium channels (VGSCs) are integral membrane proteins. They are essential for normal neurologic function and are, currently, the most common recognized cause of genetic epilepsy. This review summarizes the neurobiology of VGSCs, their association with different epilepsy syndromes, and the ways in which we can experimentally interrogate their function. The most important sodium channel subunit of relevance to epilepsy is SCN1A, in which over 650 genetic variants have been discovered. *SCN1A* mutations are associated with a variety of epilepsy syndromes; the more severe syndromes are associated with truncation or complete loss of function of the protein. SCN2A is another important subtype associated with epilepsy syndromes, across a range of severe and less severe epilepsies. This subtype is localized primarily to excitatory neurons, and mutations have a range of functional effects on the channel. SCN8A is the other main adult subtype found in the brain and has recently emerged as an epilepsy gene, with the first human mutation discovered in a severe epilepsy syndrome. Mutations in the accessory β subunits, thought to modulate trafficking and function of the α subunits, have also been associated with epilepsy. Genome sequencing is continuing to become more affordable, and as such, the amount of incoming genetic data is continuing to increase. Current experimental approaches have struggled to keep pace with functional analysis of these mutations, and it has proved difficult to build associations between disease severity and the precise effect on channel function. These mutations have been interrogated with a range of experimental approaches, from in vitro, in vivo, to in silico. In vitro techniques will prove useful to scan mutations on a larger scale, particularly with the advance of high-throughput automated patch-clamp techniques. In vivo models enable investigation of mutation in the context of whole brains with connected networks and more closely model the human condition. In silico models can help us incorporate the impact of multiple genetic factors and investigate epistatic interactions and beyond.

KEY WORDS: Genetics, Mutations, Voltage-gated.

Voltage-gated sodium channels (VGSCs) play essential roles in normal neurologic function, especially in initiation and firing of action potentials (Hu et al., 2009). It is, therefore, not surprising that gene variation can have effects, and even potentially devastating consequences on the nervous system. Indeed, sodium channel mutations are the most important currently recognized cause of genetic epilepsies. The associated phenotypic variation is remarkable. Herein we review the neurobiology of sodium channels, their known mutational spectrum in epilepsies, and challenges in functional characterization to help clarify the complex genotype–phenotype correlations.

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Neurobiology

As the main arbiters of cellular excitability, VGSCs share some key biophysical characteristics. At resting membrane potentials they are in the closed state, and do not pass sodium ions. With mild membrane depolarization, such as that caused by synaptic activity, they open to allow the inward flow of sodium, causing a further rapid depolarization that underlies the rising phase of the action potential (AP). Following this opening, a rapid inactivation (on a millisecond time scale) stops the flow of sodium and the channels enter a closed state and are unavailable for opening. The subsequent opening of voltage-gated potassium channels causes a slow membrane repolarization that causes the sodium channels to recover from inactivation and once again become available for opening. Different sodium channel α subunits have evolved a spectrum of voltage and kinetic properties to support the functional diversity of the organs in which they reside.

Structurally, the pore-forming α subunit of the VGSC consists of four domains (DI–IV), each containing a motif of six transmembrane segments (S1–6), with approximately 2,000 amino acid residues in total (Meisler & Kearney, 2005) (Fig. 1). The fourth segment (S4) of each domain contains voltage-sensing machinery for activation of the channel. The linker between DIII and DIV contains the isoleucine, phenylalanine, and methionine (IFM) motif, which is thought to contain the inactivation machinery for the channel (Meisler & Kearney, 2005).

In the adult brain there are four main subtypes of VGSCs: $Na_V1.1$, $Na_V1.2$, $Na_V1.3$ and $Na_V1.6$, encoded for by the genes *SCN1A*, *SCN2A*, *SCN3A,* and *SCN8A*. For the remainder of this review, however, we will incorporate the Human Genome Organisation (HUGO) gene nomenclature guidelines (Shows et al., 1980) and refer to the gene as the italicized symbol (e.g., *SCN1A*) and the encoded protein using the same symbol but nonitalicized (e.g., SCN1A). This is the official nomenclature of the International Union of Pharmacology (Catterall et al., 2005).

The expression of VGSCs varies with development. *SCN3A* mRNA is present at higher amounts in the neonatal brain, suggesting an important developmental role for this subtype. In humans it is also found in specific areas of the brain in adults suggesting it may also have some important physiologic role in adulthood (Chen et al., 2000). However *SCN1A*, *SCN2A,* and *SCN8A* mRNAs are all present at high amounts in the adult brain (Gazina et al., 2010), and deletion of each of the genes leads to lethality, suggesting they are essential for life and have unique functional roles (Chen et al., 2008). The differing neuronal and cortical distribution of these VGSCs supports this hypothesis.

In excitatory neurons, SCN2A and SCN8A are the major sodium channel α subunits. SCN2A is found predominantly at terminals, unmyelinated axons, and at high levels in the axon initial segment (AIS) (Westenbroek et al., 1989; Gong et al., 1999; Whitaker et al., 2001; Hu et al., 2009). SCN8A is found predominantly at the soma and like SCN2A is found at high levels in the AIS, with some distribution at dendrites (Whitaker et al., 2001; Hu et al., 2009; Lorincz & Nusser, 2010). The AIS is the site for AP initiation in neurons with an exquisite molecular architecture. Low-threshold SCN8A and high-threshold SCN2A channels (Rush et al., 2005; Hu et al., 2009) preferentially accumulate at the distal and proximal AIS, respectively. Patch-clamp and modeling studies suggest the distal AIS, the lowest threshold region, promotes AP initiation and the proximal AIS, the highest threshold region, promotes back propagation to the soma and dendrites (Hu et al., 2009; Lorincz & Nusser, 2010). Of interest, the AIS is also emerging as a critical point of convergence for genetic epilepsies.

In contrast, SCN1A is expressed predominantly in γ-aminobutyric acid (GABA)ergic neurons and it may be co-localized with SCN8A as it is in spinal cord neurons (Duflocq et al., 2008). Historically, it was thought that SCN1A was distributed solely in the somatodendritic compartments (Westenbroek et al., 1989); however, recent immunohistochemical evidence has shown it also to be concentrated at the AIS, suggesting its role in the initiation and propagation of APs specifically in GABAergic neurons (Ogiwara et al., 2007; Duflocq et al., 2008). The unique interplay between SCN1A/2A and SCN8A is critical for normal neuronal function and likely to be important in the pathogenesis of genetic epilepsies and a site for interaction of gene function (epistasis) (Martin et al., 2007).

The distribution of SCN1A, SCN2A, and SCN8A varies in the brain. SCN1A has high expression levels in caudal regions, hippocampus, brainstem, cortex, substantia nigra, and caudate (Gong et al., 1999). SCN2A distribution is somewhat complementary to SCN1A, with a rostrocaudal

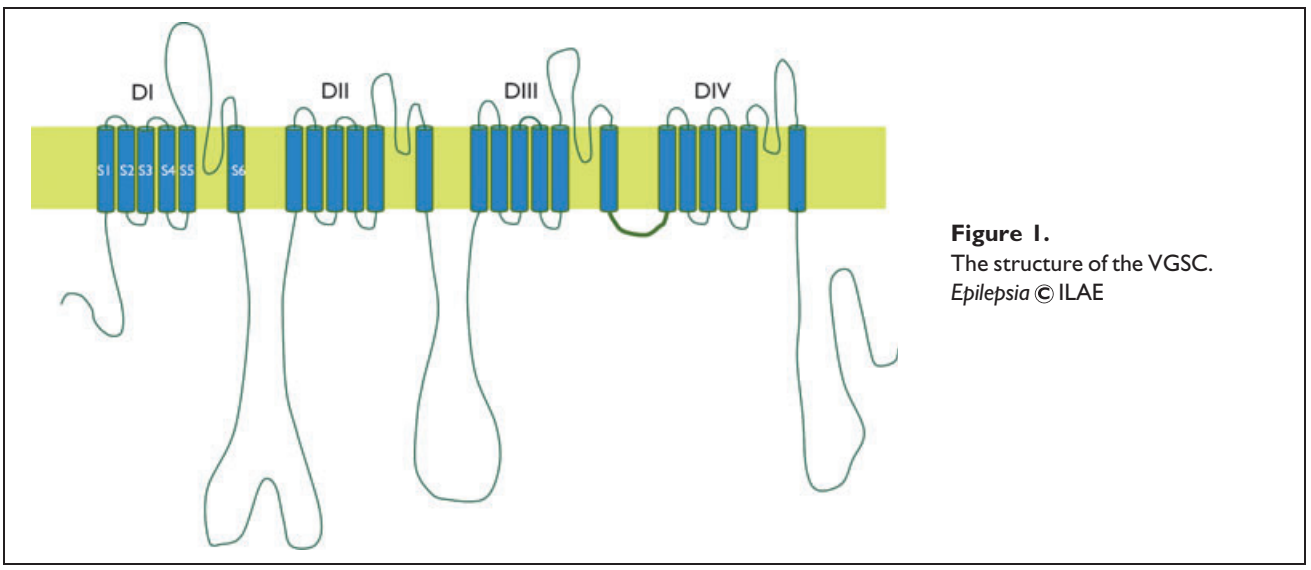

Figure 1.
The structure of the VGSC.
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gradient distribution in the brain, and high expression in the cortex, hippocampus, striatum, and midbrain (Whitaker et al., 2001). SCN8A is more uniformly distributed, with high expression in the hippocampus, cortex, and cerebellum (Whitaker et al., 1999; Kress et al., 2010). In addition there are different excitability thresholds for different neurons, probably determined by differing distribution and expression of sodium channel subunits at the AIS. A recent comparative study showed that in the hippocampus, dentate neurons were less excitable than pyramidal counterparts and that dentate neurons had a lower, yet more proximal expression of SCN8A at the AIS compared to pyramidal cells (Kress et al., 2010).

Although the AP initiation role of VGSCs at the AIS is critical, VGSCs are also expressed at the soma and dendrites, where they are involved in the integration of synaptic inputs (Gong et al., 1999; Whitaker et al., 2001; Lorincz & Nusser, 2010). In addition, it has also been postulated that VGSCs in this region, as well as axonal compartments, mediate persistent current (Whitaker et al., 2001; Kole, 2011), a steady-state sodium influx that persists orders of magnitude longer than, and is activated at more negative voltages than, the transient current (Crill, 1996). This lifts the resting membrane potential to a more depolarized level, rendering it more likely to initiate APs. The current is also implicated in shaping firing characteristics, generating subthreshold oscillations and sustaining pace making.

Further physiologic variation in function occurs because *SCN1A, SCN2A, SCN3A,* and *SCN8A* are subject to alternative splicing of coding exons 5A and 5N during development. The splicing corresponds to the presence of a neutral amino acid residue in the 5N isoform, and an aspartic acid residue in the 5A isoform, with SCN2A-5N found to be less excitable than SCN2A-5A (Xu et al., 2007a,b). The 5N/5A ratio investigated in mice varies across brain regions, with the higher relative levels of 5N in the cortex, followed by the hippocampus, thalamus, and then cerebellum. For SCN2A the relative amount of 5N decreases markedly during the first few weeks of development, leveling at a ratio of 0.1–0.2 and a similar, albeit less marked, change is seen for SCN3A and SCN8A (Gazina et al., 2010). Alternative splicing is also seen in SCN1A at exon 11 (Lossin, 2009), SCN8A at exon 18, SCN2A, SCN3A, and SCN9A at exon 16, and SCN3A at exon 11 (Plummer et al., 1997; Kerr et al., 2008).

Each α subunit associates with two accessory β subunits. The accessory β subunits are important modifiers of channel function (Brackenbury & Isom, 2011). In the adult brain there are four accessory β subunits: SCN1B, SCN2B, SCN3B and SCN4B (Yu et al., 2003), which generally show a broad distribution in the brain (Whitaker et al., 2000). SCN1B and SCN3B show a complementary distribution in the brain, except for the hippocampus where they are both present at high levels (Shah et al., 2001; Yu & Catterall, 2003; Wimmer et al., 2010). The distribution of SCN2B and SCN4B in the brain often overlaps, except for the hippocampus and cerebral cortex where their distribution is complementary (Whitaker et al., 2000).

SCN1B and SCN3B show sequence homology and both noncovalently link to α subunits. SCN2B and SCN4B are also homologous, and both covalently link to α subunits (Yu et al., 2003). α Subunits are associated with one or more β subunit types (Yu & Catterall, 2003).

The β subunits are thought to affect trafficking and gating of the VGSC as well as establishing contacts with the cytoskeleton and/or extracellular matrix proteins (Moran et al., 2003; Aman et al., 2009), with recent evidence suggesting they may play important roles in cell adhesion independent of α subunits (Patino & Isom, 2010). The SCN4B subunit was also recently identified as a likely candidate for mediating resurgent current (Grieco et al., 2005). Resurgent current is a small transient sodium influx that is activated upon repolarization of the cell. It is thought that a particle blocks the sodium channel shortly after activation, before it inactivates, holding it in this state. Upon repolarization this particle is expelled from the channel and a small sodium influx occurs. The cytoplasmic tail of the SCN4B subunit contains ε-amino groups and an aromatic residue like other known sodium channel blockers, and is present in many brain regions where resurgent current is present (Lewis & Raman, 2011). Mutations in both α and β subunits have been identified in epilepsy.

The heterogeneity of VGSC-subunit composition, the differing macroscopic distribution of particular subunits in the brain, their cellular and subcellular specificity, their developmentally regulated expression, and the occurrence of alternative splicing all underscore the great complexity of physiologic function. Understanding this complexity helps explain the heterogeneous phenotypes observed with various molecular lesions. Moreover, the potential complexity increases further when one considers that different mutations in the same gene may increase or decrease function to varying degrees.

Epilepsies with Sodium Channel Mutations

Among the genetic epilepsies several specific syndromes have been associated with a range of sodium channel mutations, the majority but not all associated with generalized seizure types. The spectrum of epilepsy syndromes associated with sodium channel mutations spans from benign forms of epilepsy such as benign familial neonatal infantile seizures (BFNIS) to the severely disabling and sometimes fatal Dravet syndrome (also known as severe myoclonic epilepsy of infancy, or SMEI).

BFNIS is usually caused by *SCN2A* mutations. Here seizures present around 3 months of life, with a range from the neonatal to the later infantile period. The outcome is excellent in terms of normal development and seizure remission. The condition has largely been recognized in autosomal

dominant pedigrees, and there are associated missense variants in *SCN2A* (Kaplan & Lacey, 1983; Berkovic et al., 2004; Herlenius et al., 2007; Meisler et al., 2010). Age-dependent seizure resolution in BFNIS has been suggested to correlate with the developmental reorganization of the AIS where SCN2A is replaced by SCN8A in the distal AIS diminishing the influence of mutant SCN2A on AIS function (Liao et al., 2010).

In contrast, Dravet syndrome begins at around 6 months of age and has a devastating course with recurrent hemiclonic seizures, development of multiple and usually refractory other seizure types with developmental regression (Dravet et al., 2005). Around 80% of all Dravet syndrome cases are caused by mutations in *SCN1A* (Reid et al., 2009; Heron et al., 2010; Marini et al., 2011), with 95% of these occurring de novo, most often on the paternal allele. Approximately half the mutations are missense and most of the remainder are point mutations predicting truncation of the protein, with a minority being deletions of one or more exons or mutations affecting the promoter region (Marini et al., 2011).

Genetic epilepsy with febrile seizures plus (GEFS+) is a familial syndrome, initially described in autosomal dominant pedigrees, but the majority of families have complex inheritance. The phenotypes are diverse with a spectrum from febrile seizures, febrile seizures plus to more severe phenotypes including epilepsy with myoclonic–atonic seizures and even Dravet syndrome, which may occur with milder phenotypes in families (Scheffer & Berkovic, 1997). The spectrum also includes focal temporal and frontal lobe epilepsies. About 10% of families have missense mutations in *SCN1A* or *SCN1B,* with rare families being described with *SCN2A* mutations or GABA-receptor mutations but the molecular basis of most cases remains uncertain (Scheffer et al., 2009).

The three syndromes described above are numerically the most important thus far described with heterozygous mutations in *SCN1A*, *SCN2A,* and *SCN1B* but the phenotypic spectrum is wider still. *SCN1A* mutations are found in cases previously regarded as ''vaccine encephalopathy''; these cases generally have the phenotype of Dravet syndrome (Berkovic et al., 2006). A related severe infantile epilepsy, which lacks some cardinal features of Dravet syndrome, severe infantile multifocal epilepsy (SIMFE) is usually due to mutations in *SCN1A* (Harkin et al., 2007). Similarly, the severe syndrome of migrating partial seizures of infancy is sometimes due to *SCN1A* mutations (Carranza Rojo et al., 2011), as are less specific epileptic encephalopathies and milder cases with tonic–clonic seizures alone in infancy, some cases with myoclonic–atonic seizures, and cases with predominantly occipital seizures (Harkin et al., 2007; Livingston et al., 2009).

Although BFNIS is due to mutations of *SCN2A*, more severe infantile epileptic encephalopathies, some resembling Dravet syndrome, are also recognized with certain mutations in *SCN2A* (Ogiwara et al., 2009). One case of an epileptic encephalopathy has been described with a homozygous mutation in *SCN1B* (Patino et al., 2009).

Other *VGSC* genes may also be implicated in epilepsies. An *SCN3A* mutation has been reported in a patient with cryptogenic partial epilepsy. This mutation enhanced persistent and ramp currents when the gene was expressed in human embryonic kidney (HEK)-293 cells (Estacion et al., 2010). Of interest, a number of *SCN9A* mutations have also been identified, in patients with febrile seizures and Dravet syndrome. Although *SCN9A* is primarily expressed in the peripheral nervous system (Toledo-Aral et al., 1997), a study using reverse transcription polymerase chain reaction (RT-PCR) suggests its presence in the brainstem, spinal cord, and cortex (Belcher et al., 1995). Nine *SCN9A* missense variants have been identified in patients with Dravet syndrome in the presence of an *SCN1A* mutation, suggesting its role as a modifier gene, whereas six missense mutations have been identified in febrile seizures in the absence of an *SCN1A* mutation, suggesting it can also be disease-causing (Doty, 2010). One of the identified febrile seizure mutations was expressed in mouse, and it was found to exhibit significantly reduced thresholds for electrically induced tonic–clonic seizures, and increased corneal kindling acquisition rates (Singh et al., 2009), supporting this hypothesis. It is worth noting that *SCN1A* and *SCN2A* have an adjacent location on chromosome 2q24 and will cosegregate, regardless of causality (Goldin, 2002). Very recently a case of a de novo heterozygous missense mutation in *SCN8A* was described causing a severe infantile encephalopathy with onset at age 6 months, epileptic spasms at age 4 years, with sudden unexpected death at age 15 years (Veeramah et al., 2012). When the mutation was expressed in ND7 cells it showed increased persistent current, incomplete channel inactivation, and a depolarizing shift in the voltage dependence of inactivation (Veeramah et al., 2012).

Rodent genetic studies suggest that other sodium channel mutations may also be associated with genetic generalized epilepsy (GGE). A recent N-ethyl-N-nitrosourea (ENU) mutagenesis study provided the first association of *Scn8a* and absence epilepsy-like seizures (Papale et al., 2009). The study authors went on to show a similar phenotype in the null allele $Scn8a^{med}$ and the missense allele $Scn8a^{med\text{-}jo}$ mice. Because three different genetic variants of *Scn8a* all show spike-wave discharges and ''absence'' phenotypes, *SCN8A* might also be an important gene for human absence seizures (Papale et al., 2009). Although these rodent studies are encouraging, full identification of the sodium channels involved in human GGE is only likely to emerge from the initiation and completion of large-scale exome and genome sequencing projects in hundreds or even thousands of patient samples whose epilepsy syndromes have been well characterized.

The phenotypic heterogeneity with known sodium channel mutations between unrelated subjects is remarkable, but

is even more puzzling within families such as large pedigrees with GEFS+. The presence of modifier genes is likely and *SCN9A* variation may be one such mechanism (see above). Other modifiers may be discovered with current large scale sequencing projects. Finally, within families, somatic mosaicism is an additional mechanism for heterogeneity. Here a child is heterozygous for a mutation in all cells and may have a relatively severe syndrome, whereas a parent is mosaic with <50% of the alleles mutated and has a milder syndrome. This occurs due to postzygotic mutation, which can occur any time from the two cell stage onward (Marini et al., 2006; Morimoto et al., 2006; Selmer et al., 2009; Azmanov et al., 2010; Depienne et al., 2010).

Specific Mutations in VGSC

More than 650 *SCN1A* variants have been discovered in patients with Dravet syndrome, >20 in patients with GEFS+, and several others in other epilepsy syndromes (Catterall et al., 2010; Meisler et al., 2010). More than half of these are truncation mutations, the others missense, distributed over the channel (Fig. 2). Due to the large number of mutations identified in this gene, it has been labeled as a ''superculprit'' gene in epilepsy (Lossin, 2009).

There have been approximately 20 genetic variants identified in *SCN2A*, identified in patients with BFNIS, GEFS+, and Dravet-like phenotypes (Fig. 3) (Reid et al., 2009). *SCN2A* is an important candidate gene in epilepsy as it is one of the key subunits at the AIS, and is likely to have important epistatic interactions with *SCN8A*, the other main VGSC at the AIS. It was only recently that *SCN8A* was implicated in epilepsy, when Papale et al. (2009) identified an *Scn8a* mutation in an ENU-induced mouse model of absence epilepsy. The single case of mutation of human SCN8A (see above) is a missense change located at the cytoplasmic end of transmembrane segment 6 in domain 4 (Fig. 4) (Veeramah et al., 2012).

There have been four mutations identified in *SCN1B*, all of which localize to the extracellular domain of the protein, a region critical for gating properties (Fig. 5) (Wallace et al., 1998; Audenaert et al., 2003; Scheffer et al., 2007). These mutations were all identified in GEFS+ patients, with the spectrum of the most commonest mutation (C121W) including temporal lobe epilepsy (TLE) with and without prior FS (Scheffer et al., 2007). Early onset absence epilepsy and febrile seizures was seen with the IVS2-2A>C variant.

Experimental Approaches

As genome sequencing becomes more affordable, the amount of genetic data obtained will continue to grow. Due to the complex nature of the disease and the clinical and genetic heterogeneity, in order to make sense of the genetic

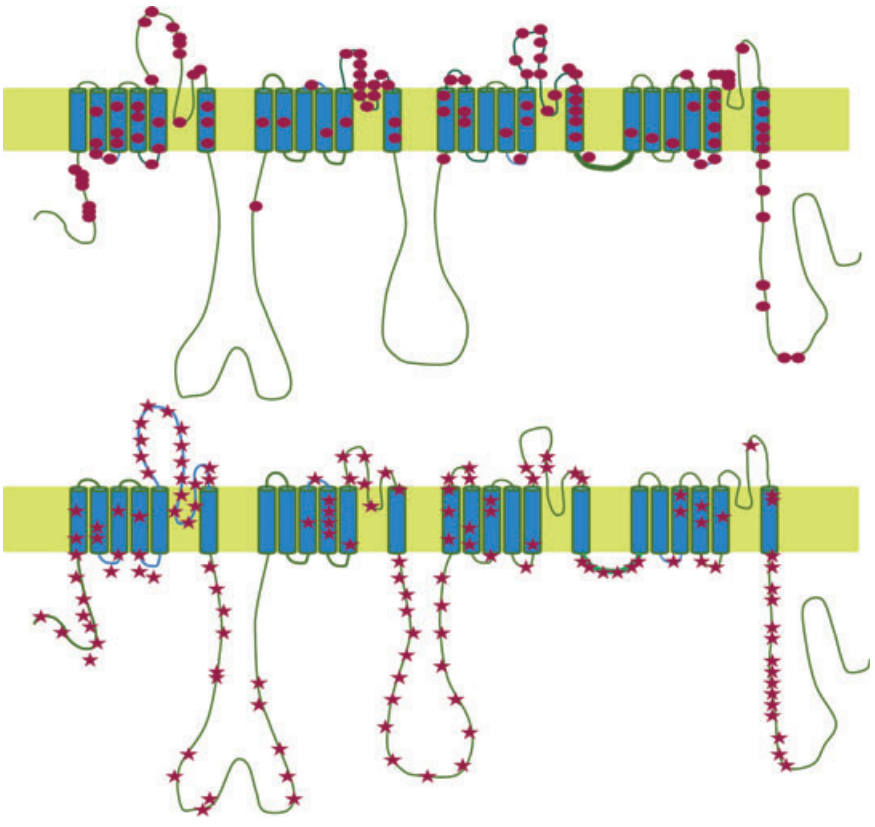

Figure 2.
Mutations associated with SMEI in the SCN1A channel. Missense mutations are circles; truncation mutations are stars. Adapted from (Catterall et al., 2008).
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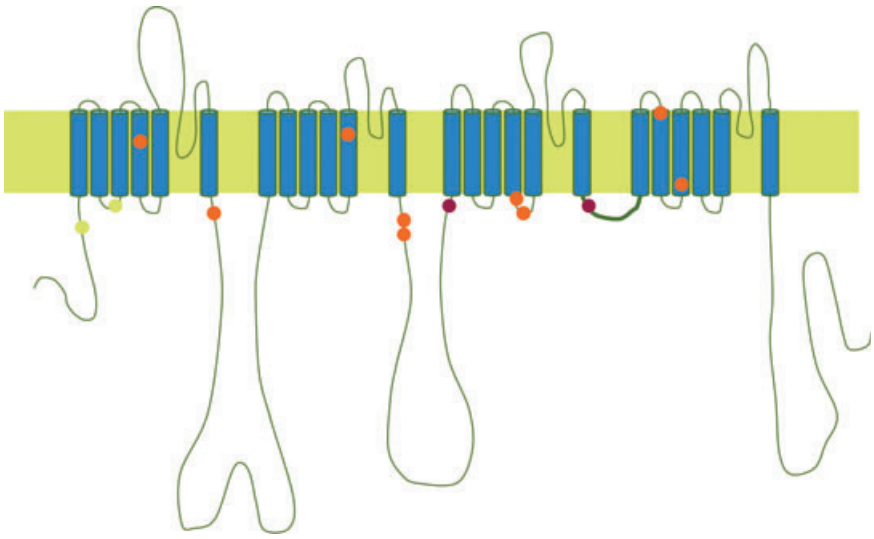

Figure 3.
Mutations associated with epilepsy in the SCN2A channel. SMEI variants are in red, GEFS+ are in yellow, and BFNIS are orange.
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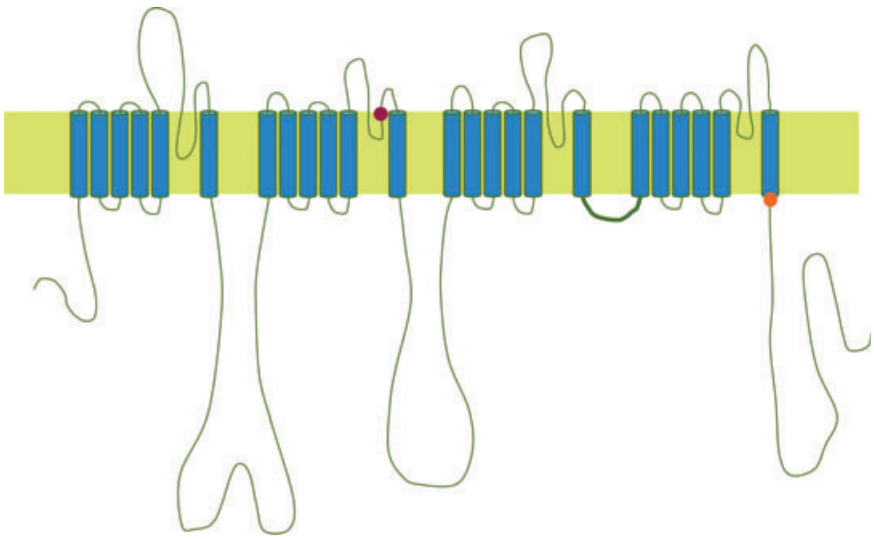

Figure 4.
Mutations associated with epilepsy in the SCN8A channel. Rodent variant in red; human variant in green.
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data and elucidate the mechanisms of the disease, functional models on different physiologic levels are required to investigate this.

In vitro

Heterologous expression systems are a relatively effective and efficient starting point for analyzing genetic variation data from patients (Faisal, 2007; Mantegazza et al., 2010). Some commonly used cells are the HEK cell line, the Chinese Hamster Ovary (CHO) cell line, and *Xenopus oocytes*, which have been chosen because, for the most part, they have low levels of expression of native ion channels that could confound interpretation of experiments. These models provide an effective method for detecting functional changes of mutations at the molecular level, as the gene of interest can be transfected into the cells and a clean profile of the gating properties of the channel established through electrophysiologic studies.

More than one half of *SCN1A* mutations in epilepsy are truncation mutations, leading to channels with complete loss of function (LoF). This intuitively follows that this decreases the inhibition in the system, and therefore, results in overall hyperexcitability. The other half of the mutations are missense, with a small number of them studied functionally (Ragsdale, 2008; Catterall et al., 2010; Meisler et al., 2010). These studies have revealed some of these mutations to be gain of function (GoF) and some LoF; however, conflicting results are reported in different studies, detailed in Table S1.

Conflicting results are also reported in heterologous system studies of SCN2A (Table S1), with both GoF and LoF gating properties reported. This discrepancy is likely due to

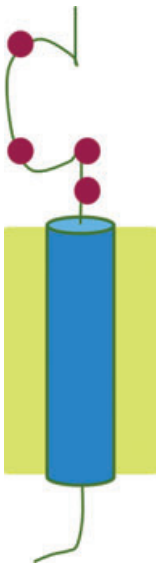

Figure 5.
Mutations associated with epilepsy in the SCN1B subunit.
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different expression systems being used, coexpression with different β subunits, the inherent variability between batches of cell lines and passage number, and differences in methodology involving passage techniques, culture media, and original cell source (Thomas & Smart, 2005; Kurejova et al., 2007). Apparent differences in the summary effects of variants (GoF or Lof) can also be due to differences in the particular channel properties interrogated by different studies. These discrepancies raise the question of how accurately these systems model neuronal conditions in higher order systems, and if we can usefully interpret results from these studies.

SCN1B mutations expressed in both HEK293T cells (Xu et al., 2007a) and *Xenopus* oocytes (Wallace et al., 1998) have shown to be all LoF mutations, appearing to interfere with the ability of the SCN1B to modulate the gating properties. Although there are some conflicting results in the literature, in general, SCN1B expression has been found to decrease the excitability of the partner α subunit (Qu et al., 2001; Yu et al., 2003; Ferrera & Moran, 2006; Aman et al., 2009). Therefore a mutation that interferes with this mechanism of action will increase the excitability of the α subunit to which it is bound. In the case of SCN2A, localized to excitatory neurons, this follows intuitively that this would increase the excitability of the system. In the case of SCN1A, however, localized to GABAergic neurons, it would intuitively follow that an *SCN1B* mutation would increase the inhibition in the system and therefore result in hypoexcitability. This does not allow an intuitive link between the mutation and the mechanism by which it is causing seizures. It is possible that the SCN1B differentially modulates different VGSC α subunits; a dedicated study examining this hypothesis would be valuable.

The heterologous expression system is not designed to model a neuron, but simply offers a way in which to detect functional changes between channels in an effective and efficient manner. Due to the large discrepancies between studies it is more useful to compare results from different mutants only if they have been characterized in the same study. Furthermore, the expression system chosen may affect the functional repertoire of a given channel and for this reason changes in biophysical properties, although useful markers that the mutation is altering function may not always be predictive of in vivo mechanisms in patients. With this in mind, the expression system chosen to analyze this may not be as crucial as the way in which it is interpreted. Both oocytes and mammalian cell lines are used as model systems, and like most models they have their strengths and weaknesses. For example, the nonmammalian nature of the *Xenopus* oocytes can induce changes in channels' properties, and care should be taken when interpreting results from this system. Experimental reproducibility of the functional tests used to analyze mutations is critical for achieving the necessary statistical power to determine pathogenecity. Implementation of large-scale functional studies, which are now more feasible because of recent advances in automated patch-clamping, can provide this power for a range of mutations to establish functional "biomarkers" that are common to pathogenic variants. Although this may not immediately lead to establishment of pathomechanisms, it will provide a framework on which to create diagnostic and prognostic assessment.

In vivo

Animal models can be used to model genetic disease. In human disease studies the most common models incorporate variant data originally sourced from humans. In this way controlled experiments can be carried out to investigate the effects of environment, drugs, and gene-to-gene interactions on the variant of interest. For VGSCs in epilepsy, eight mouse models have been developed in this way (Kearney et al., 2001; Yu et al., 2006; Martin et al., 2007; Ogiwara et al., 2007; Papale et al., 2009; Singh et al., 2009; Tang et al., 2009; Martin et al., 2010).

Two *SCN1A* R1648H mouse models have been created and the sodium currents analyzed in separate studies. The first study used a bacterial artificial chromosome (BAC) transgenic approach and the second used a gene targeted knock-in approach. Both studies recorded from excitatory and GABAergic dissociated cortical neurons. In the GABAergic neurons, both studies found a slowed recovery from inactivation and greater use-dependant inactivation (Tang et al., 2009; Martin et al., 2010). In excitatory neurons, however, the BAC model of Tang et al. (2009) showed a hyperpolarizing shift in the voltage dependence of inactivation, whereas the knock-in mouse model of Martin et al.

(2010) showed essentially no change in the function of the excitatory neurons. The differences in the excitatory phenotype were attributed to differences between transgenic and knock-in approaches and it was concluded that knock-in approaches generate more accurate disease models. In this case the most parsimonious explanation is that the transgenic *Scn1a* gene is expressed in excitatory pyramidal neurons where it is normally absent (Duflocq et al., 2008; Lorincz & Nusser, 2010; Wimmer et al., 2010).

The biophysical properties of mutant channels are in some cases dependent upon the cells in which they are expressed or the source species of the cDNA encoding the gene, and both these experimental choices can confound interpretation. One example is for the mutation studied above in the mouse models. Spampanato et al. (2001) examined the rat Scn1a (R1648H) mutation in *Xenopus* oocytes and found less use-dependant inactivation and accelerated recovery from inactivation, exactly the opposite of what was found in the in vivo studies. Lossin et al. (2002) studied the human SCN1A (R1648H) mutation in tsA201 cells and found concordance with the in vivo studies for recovery from inactivation but discordance for increases in persistent sodium current, which were only seen in the in vitro study. However, recording persistent current is difficult in neurons, as acknowledged by the authors (Tang et al., 2009), and may depend on space clamp issues and the contamination of the total current by other sodium channels that would reduce the relative contribution of persistent current. In this case the tsA201 cells appear to provide a model system that more closely approximates the biophysical changes in seen in mouse neurons that are expressing mutant sodium channels. The issues of cell type or cDNA species of origin are frequent confounds, and it would be highly beneficial to the field if all in vitro studies were performed using human cDNAs and expressed in the HEK293 cells which, thus far, have provided good concordance with mouse model data.

Studies in the SCN1B (C121W) mouse model found that wild-type Scn1b subunits were localized to the membrane of the AIS of excitatory pyramids and that mutant Scn1b subunits were excluded (Wimmer et al., 2010). This supports the electrophysiologic results from in vitro studies, which found that mutant Scn1b subunits did not modulate gating properties (Wallace et al., 1998; Xu et al., 2007a,b) and suggest that haploinsufficiency is the prime cause of the disease phenotype. This was further demonstrated by electrophysiologic studies performed on neurons from the mutant mice, which demonstrated increased AIS excitability, specifically at higher temperatures (Wimmer et al., 2010). The increased temperature sensitivity reflects the phenotype of the human GEFS+ syndrome in which the mutant was originally discovered, increasing confidence in translating mouse model findings to humans.

A second way of using mouse models is ENU-induced mutagenesis as used by Papale et al. (2009), which can identify the impact of different genes that may contribute to human pathology and suggests avenues of research that may not have been thought about otherwise.

In silico

In silico modeling can be used to interpret changes in the biophysical properties of VGSCs in the context of whole neurons and networks. But potentially more importantly, in silico analysis can incorporate the impact of multiple genetic and other factors on seizure susceptibility to capture the suspected polygenic nature of human epilepsy or to investigate epistatic interactions. In diseases such as cancer it has been hypothesized that the severity of the disease is a result of an accumulation of genetic variants or ''mutation load,'' which eventually crosses a threshold that separates affected and unaffected individuals (Fraser, 1976; Wray & Goddard, 2010). Recent evidence suggests that this may also hold true for neurologic disorders, and this has been demonstrated in the case of ion channel variants in epilepsy (Klassen et al., 2011). Thomas et al. (2009) revealed that very small changes in two or three ion channels could result in large changes in network excitability when modeled in combination. Earlier work by Thomas et al. (2007) identified the importance of activation kinetics in regulating excitability, whereas inactivation kinetics had a much smaller effect in single neuron models. Spampanato et al. (2004) used a model of spiking neurons to look at three GEFS+ mutations in *SCN1A*. Functional profiles of each of the channels were determined from *Xenopus* oocyte expression system recordings. Applying these profiles to the model they found that all mutations increased the propensity of the model neurons to fire action potentials, and each mutation was capable of driving repetitive firing in a mixed population of mutant and wild-type channels demonstrating the dominant nature of these mutations. This highlights the potential power in this type of modeling, especially when used in conjunction with high content automated in vitro patch-clamp techniques. Because biophysical profiles of the genetic variants are identified, these data can be incorporated into higher order in silico models to identify the effect of different genetic variant combinations at different neurobiologic scales.

Many antiepileptic drugs have sodium channel blocking properties. The question is often asked: why these drugs have unpredictable efficacy, and may even worsen seizure control, in subjects with epilepsies due to sodium channel mutations. Clearly better understanding of the heterogeneous mechanisms underlying these epilepsies is needed to better tailor therapies.

Conclusion

VGSCs are integral proteins required for normal neurologic function, and have been identified as culprits in neurologic disease. There have been a large number of mutations discovered in these genes, and the rate of discovery is

continuing to increase. The level of genetic heterogeneity is high, likely contributable to cellular and age-dependant expression of subunits, modifier gene effects, and mosaicism. A better understanding of how these mutations are causing functional changes is needed to better elucidate the mechanisms of the disease. Models of the disease at different molecular scales are needed to examine this.

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None of the authors has any conflict of interest to disclose.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. In vitro studies of epilepsy missense variants of SCN1A and SCN2A channels that do not result in complete loss of function.

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