

# Precision physiology and rescue of brain ion channel disorders

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Ion channel genes, originally implicated in inherited excitability disorders of muscle and heart, have captured a major role in the molecular diagnosis of central nervous system disease. Their arrival is heralded by neurologists confounded by a broad phenotypic spectrum of early-onset epilepsy, autism, and cognitive impairment with few effective treatments. As detection of rare structural variants in channel subunit proteins becomes routine, it is apparent that primary sequence alone cannot reliably predict clinical severity or pinpoint a therapeutic solution. Future gains in the clinical utility of variants as biomarkers integral to clinical decision making and drug discovery depend on our ability to unravel complex developmental relationships bridging single ion channel structure and human physiology.

## Introduction

The dual premise that inherited monogenic errors in membrane ion currents cause disease, and that drugs designed to restore the biophysics of defective voltage-gated channels hold the key to correcting them is over 50 years old and remains unshaken. Breakthroughs in visualizing, modeling, and optically controlling pore regions of voltage gated-channels, including alternative voltage-sensing (Whicher and MacKinnon, 2016) and temperature sensitive (Arrigoni et al., 2016) domains, combined with the widening search for therapeutic peptide toxins (Verdes et al., 2016) and synthetic allosteric modulators (Changeux and Christopoulos, 2016), all signal continuing grounds for unbridled optimism, even while leaving significant kinetic properties unexplained (Hoshi and Armstrong, 2015). Now a third wave of discovery is unfolding, driven by the steady drumroll of genetic variants within subunits of this molecular superfamily detected by exome sequencing in cases of human epilepsies and cognitive disorders appearing at the earliest stages of life. The challenge to isolate and repair the pathogenic mechanisms of these experiments of nature provides an overarching scientific framework to explore links between the genetic refinement of ion channel biophysics with their cellular biology and to trace the critical steps toward their highest evolutionary achievement, synchronizing neurons in the human neocortex.

Why study channel disorders in epileptic cortical microcircuits in preference to single cell models? Brain tissue is the single largest repository of membrane bound proteins (Uhlén et al., 2015), and enrichment of ion channel gene mutations in epilepsy, a prototypical neuronal synchronization disorder affecting nearly 1% of the world population, comes as little surprise. The first 10 genes linked to epilepsy in humans

and mouse genetic models were all subunits of voltage- and ligand-gated ion channels, which currently constitute nearly one third of nearly 150 known monogenic causes of seizure disorders (Noebels, 2015a). Subsets of these channel genes overlap with those for other disorders such as ataxia, autism, and cognitive development and memory impairment (Spillane et al., 2016), creating a genetic borderland of single channel comorbidities with many different circuits affected by shared molecular errors (Noebels, 2015b). Because individual neurons express up to 300 different channel subunits and develop unique use-dependent cell and isoform-specific profiles, an inherited variant may be tolerated in one neural pathway and damaging in another. The technical simplicity of a transfected heterologous model cell to determine alterations of activation kinetics, an efficient first step, cannot accurately reflect the dynamic and cell-specific compartmental density of the current and can only suggest rather than explain why, where, or when synchronization in a given network will be impaired.

Nevertheless, molecular diagnosis of early-onset ion channel disorders is having an immediate clinical and translational impact in neurology. Causative gene discoveries are widely embraced by clinicians eager to genetically tag and stratify affected individuals, parse their neurological syndromes, and select appropriate pharmacology according to the lost or acquired conductance of the mutated channel. In parallel, they are forging the creation of parent–scientist advocacy groups focused on ion channel research, bioinformatics tools to evaluate variant pathogenicity, relational patient variant database websites that link treating physicians with expert channel physiologists

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(<https://Matchmakerexchange.org>), and molecular pharmacology “pipelines” using cell and network models to screen for existing approved compounds that may restore single variant dysfunction (Griffin et al., 2016; Schutte et al., 2016; Streit et al., 2016). New methods of optically probing (Zhang et al., 2016) and controlling (Rajasethupathy et al., 2016) fast transmembrane voltage changes in excitable cells, coupled with the ability to modify and assay single channel function in neurons induced from patient-derived and unaffected pluripotent stem cells (Liu et al., 2013; Patzke and Südhof, 2016; Sun et al., 2016) are also certain to accelerate personalized evaluation of mutant channels.

**Crystal structures do not predict biological complexity.** If this auspicious trend continues, the next decade should witness a steep increase in our ability to more precisely diagnose human ion channel disorders and challenges the field of channel biophysics to complete a “rough draft” of a large remainder of the nearly 400 known ion channel subunits that are currently understudied. However, even considering those that are best understood, the exact roles of “sticky” or “leaky” mutant activation kinetics versus current density in single transfected cells are unclear. In a developing circuit, the ability of a channel to shape brain network excitability extends beyond simple ion permeation, and important stretches of discontinuity in the biological evidence remain.

Proof of the “missing physiology” in sodium channel variant epilepsy arose when clinicians and biophysicists alike realized that children bearing missense mutations throughout the same sodium channel SCN1A subunit, often at intramembranous loci distant from S4 voltage sensor and S5-6 pore-forming domains, displayed an impressive spectrum of severity in their disorder or different clinical disorders entirely. Most patients bearing gain of function mutations proved resistant to therapy with sodium channel blockers. Likewise, the unexpected hyperexcitability of cortical circuitry resulting from global deletion of a gene for sodium current puzzled cellular electrophysiologists. This anomaly can only be solved by *in situ* analysis of the affected channel as found in the disease circuit rather than recreated in a model cell. We have since learned that the paradoxical brain phenotype is explained by neuronal heterogeneity within the afflicted network combining dissimilar and nonlinear thresholds for action potential electrogenesis at axonal initial segments, along with nonuniform and cell-specific disruption of interaction domains, trafficking defects, or compensatory currents in different cell types. An added genetic explanation invokes epistatic interactions among other variants in each individual’s genetic background. Further exome analysis reveals that in some cases, seemingly identical

SCN1A seizure disorders arise from mutations in other unrelated voltage- or ligand-gated ion channels. These two revelations, allelic and genetic heterogeneity, are accepted integral properties of membrane diseases dictated by voltage control, where the timing and strength of depolarization, neurotransmitter release, and circuit stability all depend on the shared participation of many colocalized conductances, and where the electrical neighborhood of the affected channel plays a key role in sculpting the mutant neuronal phenotype as well as prioritizing targets for therapy. But control of membrane voltage fluctuation is only the beginning of a complex story that must start with an accurate molecular diagnosis.

The goal of this review is to highlight supramolecular mechanisms not evident in the study of cultured heterologous cell systems that will help to more accurately describe the relationship between genotype and phenotype in brain ion channel disorders. Examples, descending from the level of the gene to *in silico* genomic network models, are selected from single ion channel–linked cortical synchronization defects that illustrate aspects of subunit biology contributing to genotype–phenotype disparity within brain circuitry. The intervening dynamics have important implications for precision therapy of developmental nervous system disorders and, for the most part, require *in situ* evaluation in developing mammalian brain.

### Channel variants as pathogens

When a class of *de novo* protein-damaging channel variants recurs in similarly affected individuals, it provides convincing evidence in support of disease causality (Claes et al., 2001). However, so called monogenic mutations are accompanied by countless other inherited variants, sometimes within the same channel; characterizing their added roles amid this genetic noise is difficult and largely ignored by contemporary clinical gene testing. Because inherited variants, both common and rare, are found routinely and outnumber by thousands the few *de novo* mutations in a typical individual, understanding their impact is essential to sharpen the candidate disease mechanism.

For these reasons, although the solution to unknown channel variant pathogenicity is first sought by mining functional databases or examining a single model cell selected for ease of study rather than derived from the actual diseased network, it must ultimately incorporate the rich molecular interactomics and neuronal heterogeneity of the brain throughout its development, as can be achieved experimentally by engineering the variant into a “humanized” model. At the molecular genetic level, growing evidence of ion channel biological complexity in neurons provides an extensive source of functional variation to account for clinically diverse allelic syndromes. Alternative splicing and editing of mRNA

transcripts greatly expands the functional spectrum and subcellular localization of Na<sub>v</sub>, Ca<sub>v</sub>, and K<sub>v</sub> channel proteins (Lipscombe and Andrade, 2015; Trimmer, 2015; Onwuli and Beltran-Alvarez, 2016), and an advanced catalogue correlating cell type-specific differences in morphology, physiology, and channel transcripts is being assembled from large-scale single cortical cell transcriptomic projects (Cadwell et al., 2016; Tasic et al., 2016), which in turn offer a molecular anatomical framework for selective spliceform-directed or stop codon read-through therapeutic strategies (Lin et al., 2015). The gene responsible may also encode a protein directly involved in channel localization rather than primary structure that selectively transforms a subset of neurons. An exemplary isoform-specific “secondary” brain channelopathy phenotype that has been traced at this level has been recently reported in mice lacking ANK3 exon 1b isoform transcripts (Lopez et al., 2016). This AnkyrinG protein isoform, previously associated with human bipolar disorder, proportionately tethers Na<sub>v</sub> and KCNQ2/3 channels to the axon initial segment in a distinct subset of fast spiking interneurons, and the cell-specific isoform imbalance provoked by the mutation links a novel monogenic phenotype of epilepsy and bipolar depression distinct from the (lethal) global ANK3 deletion.

The high-resolution portrait of brain ion channel complexity emerging from single cell transcriptomics forces the realization that a ball and stick, Beadle and Tatum view of “one channel–one disease” must be replaced by one that builds on biological mechanisms underlying pleiotropy. This begins with a map of brain channel isoform anatomy and a flexible formulation that first recognizes channel isoform diversity and heteromeric co-assembly among neurons and the ability of a single variant to impact firing properties of entirely novel neuronal subpopulations; second, appreciates its variable contribution to excitability at discrete developmental stages; and third, accounts for the remarkable coordinate regulation of circuit excitability through epitranscriptomic changes that self-adjust (or fail to) to maintain homeostatic firing patterns. Because firing and wiring are mutually reinforcing, membrane excitability itself plays an integral role, in time and space, on the impact of each channel mutation upon neural circuit development, although inevitably, some circuits are more hard-wired than others. The adage, “cells that fire together, wire together,” describes a suprathreshold relationship; however, recent evidence suggests that channel-mediated interactions begin well below that threshold. In immature brain, glutamate and GABA-gated control of sodium, calcium, and chloride currents determine where postsynaptic clusters aggregate to form synapses on dendritic spines (Kwon and Sabatini, 2011; Oh et al., 2016). Even subthreshold channel defects may therefore contribute to the synaptic design

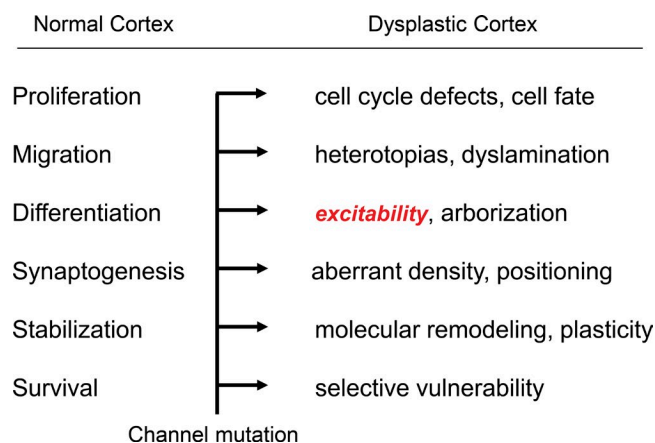


Figure 1. **Pore and non-pore impact of ion channels in developing brain.** Ion channelopathy is cell type and network specific and may produce reversible and irreversible alterations in brain function at multiple stages of development.

of the microcircuits they are destined to control. Recent advances in novel functional cell labeling techniques using targeted recombination of immediate early gene reporters (Guenther et al., 2013) or phosphorylated ribosome capture (Knight et al., 2012) can help isolate and trace circuits activated by channel mutations. Although molecular maturation programs underlying channel plasticity embody forbidding “dark matter” at present, we await large-scale cell type-specific datasets and continue to learn new rules for the developmental excitability blueprint one gene at a time.

#### Separating episodic from static circuit phenotypes

From the vantage point of clinically recognizable phenotypes, the protean network alterations linked to single channel epilepsy variants, from megencephaly (Yang et al., 2012) or brain atrophy (Figueroa et al., 2011) to episodic synchronous discharges and even sudden death (Goldman et al., 2009), emphasize the gaps in our current understanding of channel structure–function relationships, as they arise at multiple levels of organization during brain development (Fig. 1). These intervening mechanisms are essential to unravel, as persistent neurological deficits that appear long after birth may be caused by altered developmental and survival programs rather than ongoing dysfunction of the mutated channel and are thus beyond immediate repair by drugs that restore kinetics of the mutant current.

The goal of separating reversible and irreversible cellular electrical dystrophies is a seminal concern in channelopathies leading to seizures and intellectual impairment, known as epileptic encephalopathies. Whether and when these roles are separable likely hinges upon functions that extend beyond simple voltage control. For example, some dominant missense mutations of *SCN1A*  $\alpha$  subunits are linked to simple childhood febrile seizures without cognitive

loss, whereas others, particularly when arising de novo, create a severe epilepsy phenotype with profound intellectual disability (Brunklau et al., 2014), as also reported for loss of *SCN1B* auxiliary subunits (Wallace et al., 1998; Ogiwara et al., 2012). Both have intracellular domains important for cytoskeletal interactions, but the latter has extracellular immunoglobulin-like motifs involved in cell adhesion (Kruger and Isom, 2016). Could some *SCN1A* deletion mutations interfere with the structural role of co-assembled *SCN1B*'s in brain development, or does the basis for intellectual impairment reside in each subunit?

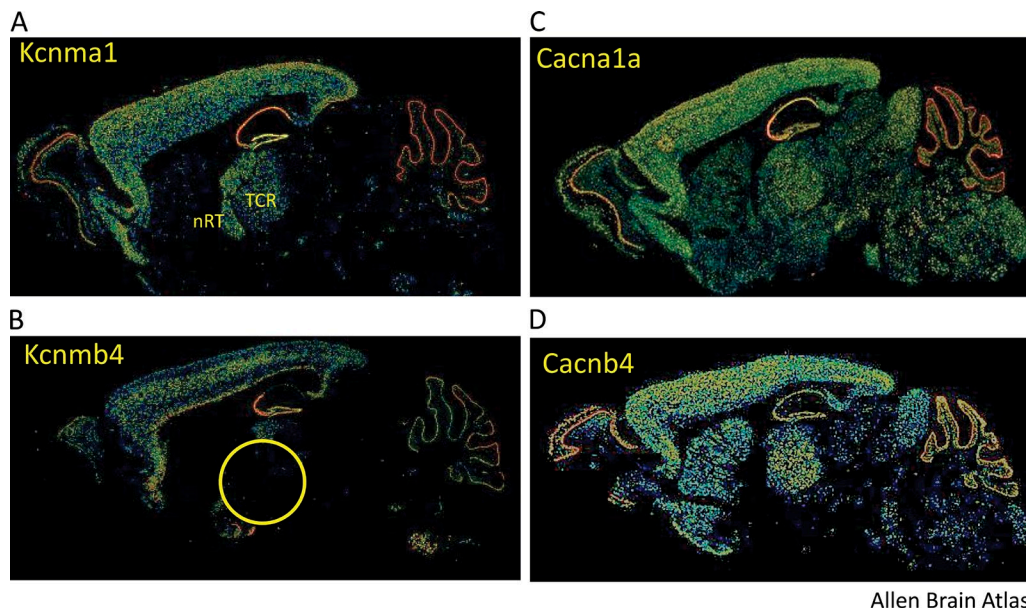
A parallel dichotomy arises among potassium channel mutant phenotypes; however, evidence for non-pore effects is scant. Here, the large diversity of channel subtypes, spatial localization, and coordinate positioning with other channels in membrane subcompartments is so extensive (Trimmer, 2015) that mutation of these channels approaches a near analogue spectral fine tuning adjustment rather than a binary switch for neuronal integration. Nevertheless, distinctive “subunit syndromes” persist. Mutation of *KCNA2*, often co-assembled with *KCNA1* subunits, causes epileptic encephalopathy (Syrbe et al., 2015), as do many other potassium channel  $\alpha$  subunit mutations with seizures (Villa and Combi, 2016), whereas the intellect is inexplicably spared in most human *KCNA1* cases, despite its early and major axonal expression pattern in cortical and hippocampal networks. A variety of point mutations in *KCNA1* show clear phenotype differences based on whether trafficking or kinetics alone are impaired (Rea et al., 2002). Interestingly, a remarkable study of two sets of monogenic twins bearing an F414S and a R307C dominant-negative mutation showed phenotypes that were strikingly disparate in severity (Graves et al., 2010). An early-onset epileptic encephalopathy results from a V378A variant in *KCNB1* subunits that alters both ion selectivity and localization (Thiffault et al., 2015), whereas de novo mutations affect sensor and pore domains and inhibit repetitive firing (Saito et al., 2015). Both loss and gain of function in *KCNQ2* channels, which also localize to axons, correlate with epileptic and cognitive phenotypes (Miceli et al., 2015), and in the latter, clinical mutations, although few in number, offer remarkable insight into the fine structure–function of the voltage sensor. Replacement with either of two alternatively charged amino acids in the same S4 domain produces distinct neurological outcomes, R213W encoding a benign neonatal seizure disorder, and R213Q with severe epileptic encephalopathy; both, however, markedly destabilize the open state, causing a large decrease in voltage sensitivity that could be restored by the  $K_v7$  activator retigabine (Miceli et al., 2013). Missense gain of function mutations in sodium-dependent *KCNT1* potassium channels cause epilepsy with a range of associated phenotypes, including intellectual impairment (Lim

et al., 2016). The mutations appear to affect channel–channel gating cooperativity between multiple channels residing in a single patch (Kim et al., 2014). Quinidine blockade has been variably successful in suppressing *KCNT1*-linked seizures and ameliorating cognitive development (Chong et al., 2016). In animal models, deletion of *Kcnh3* leads to enhanced cognitive performance (Miyake et al., 2009), as well as epilepsy (Zhang et al., 2010). Human BK channel  $\alpha$  subunit (*KCNMA1*) mutation leads to generalized thalamocortical seizures (Du et al., 2005), whereas deletion of its  $\beta4$  (*Kcnmb4*) subunit in mice produced focal hippocampal epilepsy (Fig. 2 A; Brenner et al., 2005). Understanding the prognostic implications of channel gene variants and treating before critical periods of intellectual development will be essential to identify therapeutic opportunities to improve the developmental trajectory of the disorder.

**Mismatches between mutant gene, current, and cell defect.** In rare instances, the pattern of mutant channel expression is remarkably congruent with the malignant circuit; more commonly, the underlying pathogenic circuits are hidden in plain sight by widespread expression of the mutated subunit in cells that do not participate in the clinical phenotype (Fig. 2). In the latter case, these cellular “unaffected carriers” obscure the critical therapeutic targets. A clear lesson emerging from channel disease in the brain is that regardless of overall cellular expression pattern of the pathogenic channel, some cell types are more vulnerable than others to loss of the same subunit, and the emergent neurological disorder arises from very specific afflicted networks which may appear, change, and even disappear during life. Explanations for unequal functional penetrance of the variant at the cellular level at varying stages of life should therefore be incorporated into mechanistic thinking. Acknowledging this key issue at the time of functional variant testing is important because the intrinsic impact of a variant at the single cell level depends on its molecular neighborhood, that is, the remaining nonmutated channel composition. Combined patch clamp–single cell PCR channel expression studies of identified cortical excitatory neurons clearly demonstrate that distinct patterns of evoked cell firing properties are sculpted by the ratios of their channel mRNAs, with some currents exerting far more influence than others (Toledo-Rodriguez et al., 2004). Furthermore, these channel expression profiles, as studied in isolated fast spiking cortical interneurons, undergo a remarkable shift during early postnatal brain development (Okaty et al., 2009). Therefore, designating a clinical channel variant as benign or pathogenic using evidence from a single cell type and age is hazardous because in other cells, the same variant may be functionally silent.

Unexpectedly, inhibitory neurons may be less tolerant of channel defects than excitatory neurons. Epi-





**Figure 2. Match and mismatch of mutant channel expression in channelopathy phenotypes.** (A–D) In situ hybridization of ion channel mRNA transcripts show concordant (A and B) and discordant (C and D) expression in brain circuits mediating mutant channel phenotypes. (A) Expression of *Kcnma1* subunits linked to human thalamocortical absence epilepsy phenotype show strong matching expression in cortical-thalamic (TCR, nRT nuclei) circuit. (B) Mutation of the related  $\beta$  subunit *Kcnmb4* in mouse lacks significant thalamic expression (circle); without it, the mutant mouse shows a hippocampal epilepsy phenotype of temporal lobe epilepsy instead. (C) Diffuse expression of *Cacna1a* transcripts broadly overlaps with pathogenic thalamocortical absence seizure pathway and with cerebellar ataxia, but does not clinically affect most other synaptic pathways. (D) Mutation of the related  $\beta$  subunit *Cacnb4* shows identical seizure/ataxia phenotype as *Cacna1a* mutant mice, but somewhat better defines thalamocortical disease circuitry.

lepsy mutations in sodium channels offer intriguing examples of unequal genetic penetrance at the cellular level, and in these cases, whole cell current in pyramidal cells is not altered despite mutation of the channel. The mismatch was first noted when hemizygous reduction of the *Scn1a* subunit was found to dramatically reduce sodium current in mouse interneurons, while sparing pyramidal neurons, despite apparently equivalent levels of gene expression (Yu et al., 2006). Interestingly, analysis of interneurons in a *Scn1a* mouse model carrying the human Dravet Syndrome  $\text{Na}_v1.1\text{-R1648H}$  mutation confirmed the selective hypoexcitability of interneurons across several brain regions; however, this was not caused by reduced somatic  $\text{Na}_v1$  current, but rather slower inactivation affecting the spike electrogenesis threshold at the axon initial segment (Hedrich et al., 2014). The basis of selectivity for interneurons and resistance in excitatory cells to both deletion and missense *Scn1a* mutations remains unexplained, but the contrasting current measurements suggest that restoring  $\text{Na}_v$  current alone may not rescue all mutations. Reconciling interneuron vulnerability with the paradoxical resistance of excitatory cells may hold a key to understanding network imbalance in these disorders.

In apparent contrast, a gain of function mutation, R192Q, in the widely expressed P/Q type calcium channel *CACNA1A* linked to cortical hyperexcitability, seizures, and premature lethality selectively spares cal-

cium current in interneurons. This variant predictably enhances pyramidal cell  $\text{Ca}_v2.1$  current density, causes left-shift in activation gating, and increases transmitter release at excitatory terminals, but paradoxically has no apparent effect on inward calcium current in cortical interneurons (Vecchia et al., 2014). Allele-specific rescue strategies, including temperature-specific modification of protein folding and trafficking sodium channel variants (Bechi et al., 2015), and quantitative analysis of mRNA transcripts for cell-specific compensatory changes in paralogous calcium channel  $\alpha$  subunits (Etheredge et al., 2007) may also help dissect the issue in both disorders.

Rather than directly altering membrane excitability, loss of function P/Q channel  $\alpha$  subunit mutations demonstrate a more subtle, trans-synaptic mechanism of disinhibition leading to epileptic hypersynchronization. They consistently reduce inward calcium current, but also modify the cooperativity with release machinery rather than reduce total evoked transmitter release at central excitatory synapses, which is sustained by N and R type channel coupling (Qian and Noebels, 2000). Whereas a balanced, genomic loss of P/Q at all excitatory and inhibitory presynaptic terminals ought not produce epilepsy, reduction of P/Q coupled release increases synaptic “jitter” and destabilizes thalamocortical circuit development. This leads to cortical hypersynchronization in the form of generalized spike-

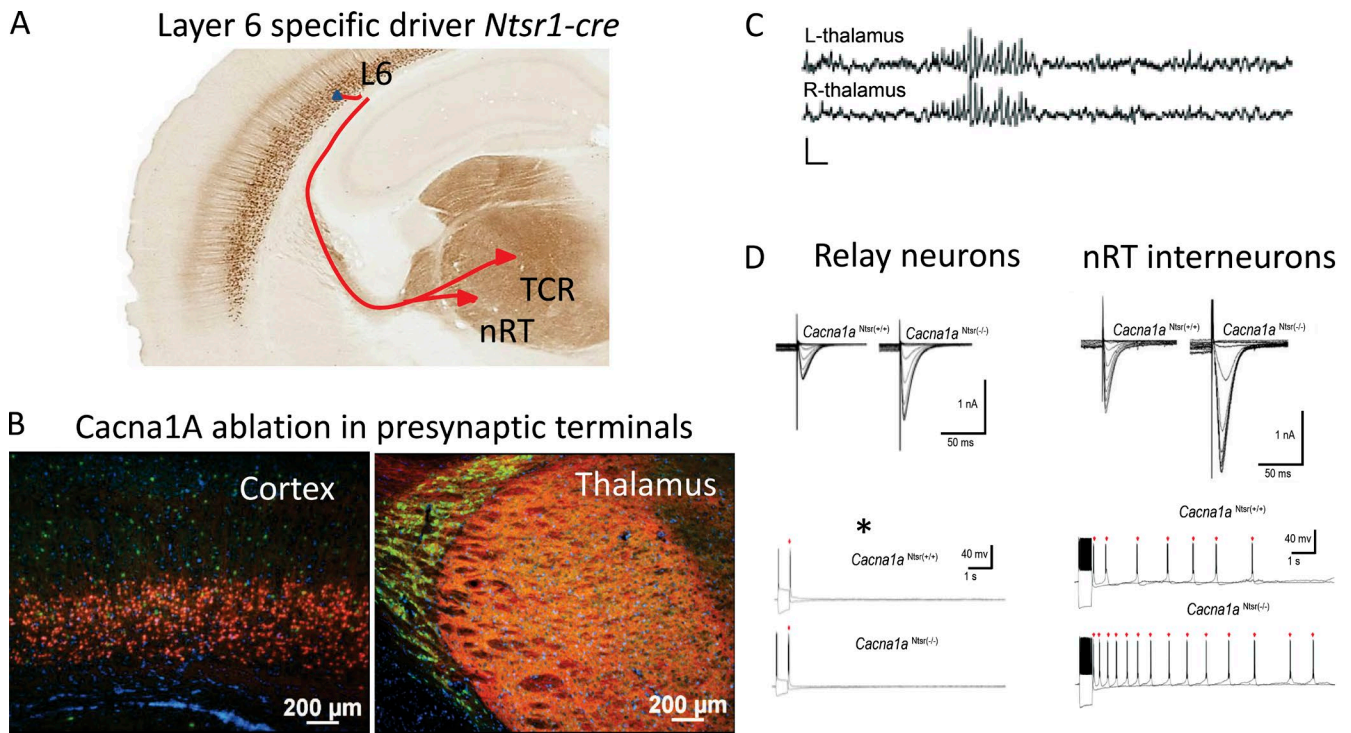


Figure 3. **Genetic isolation of critical epilepsy circuit underlying widespread calcium channelopathy.** (A and B) Cre-driven selective ablation of *Cacna1a* in layer 6 corticothalamic projection neurons only delimits critical synapses sufficient for appearance of thalamocortical seizures, shown in C. (D) Defective glutamate release (N-type, rather than P/Q type) at these thalamic synapses induces postsynaptic enhancement of low threshold T-type calcium current and rebound bursting in downstream thalamic cells. Re-modeling caused by loss of developmental homeostasis at this single class of synapses results in diffusely hypersynchronized cortical networks (reprinted from Bomben et al., 2016).

wave discharges arising from enhanced rebound burst properties and disinhibition in the network (Noebels, 2012). Selective isolation of the critical synapses by conditional *Cacna1a* deletion in layer 6 pyramidal cells has narrowed the generalized P/Q defect to a singular aberrant descending corticothalamic input onto thalamic neurons (Fig. 3; Bomben et al., 2016). Impaired presynaptic P/Q channel-mediated transmission at this single class of excitatory synapses (with no change in layer 6 intrinsic neuronal excitability) up-regulated low threshold T-type (*Cacna1G*) current in postsynaptic thalamic cells. The downstream thalamic inhibitory interneurons in nucleus reticularis displayed enhanced rebound bursting; this firing pattern is a sufficient cause of this seizure type. The mechanism underlying trans-synaptic induction of aberrant burst properties by reduced presynaptic P/Q excitation–release coupling is unknown, but P/Q channels mediate a form of rapid synaptic homeostatic plasticity that might present a novel target for rescue (Frank et al., 2006).

**Subunit reshuffling: Reassembly modifies variant penetrance.** The stoichiometry of  $\alpha$  and  $\beta$  subunits can be altered by mutation, providing another means of sculpting the pathogenic network. The *lethargic* mouse carries a point mutation truncating the *CacnB4* sub-

unit-binding site to the intracellular  $\alpha$  subunit interaction domain of *CACNA1A-F* channels (Burgess et al., 1997).  $\beta 4$  is one of four cytosolic regulatory subunits that bind promiscuously to these  $\alpha$  subunits, each conferring distinctive changes. The  $\beta 1-4$  subunit expression patterns themselves are variably overlapping, creating complex profiles of  $\alpha\beta$  stoichiometry and high voltage-gated calcium current behavior in different cells. Absence of the  $\beta 4$  subunit throughout *lethargic* brain allows replacement at all  $\alpha$  subunits by an alternate family member, resulting in potentially novel patterns of excitation-release coupling (Burgess et al., 1999). In *lethargic* brain, P/Q current is partially rescued in cells expressing  $\beta 1$  or  $\beta 3$  (preferred partners of P/Q subunits) but is impaired in cells lacking these paralogues, so the vulnerable circuit is defined not by the cells where the mutant  $\beta 4$  subunit is lost, but by those where it is not rescued. An additional mechanism invokes alternative gene transcription caused by altered intranuclear activity of a  $\beta 4$  subunit protein fragment (Ronjat et al., 2013).

**Reassignment of subunit function.** Macromolecular reassembly by subunit reshuffling is distinct from functional “subunit switching” (Fig. 4), a shift in transcription that occurs in some neurons during development and

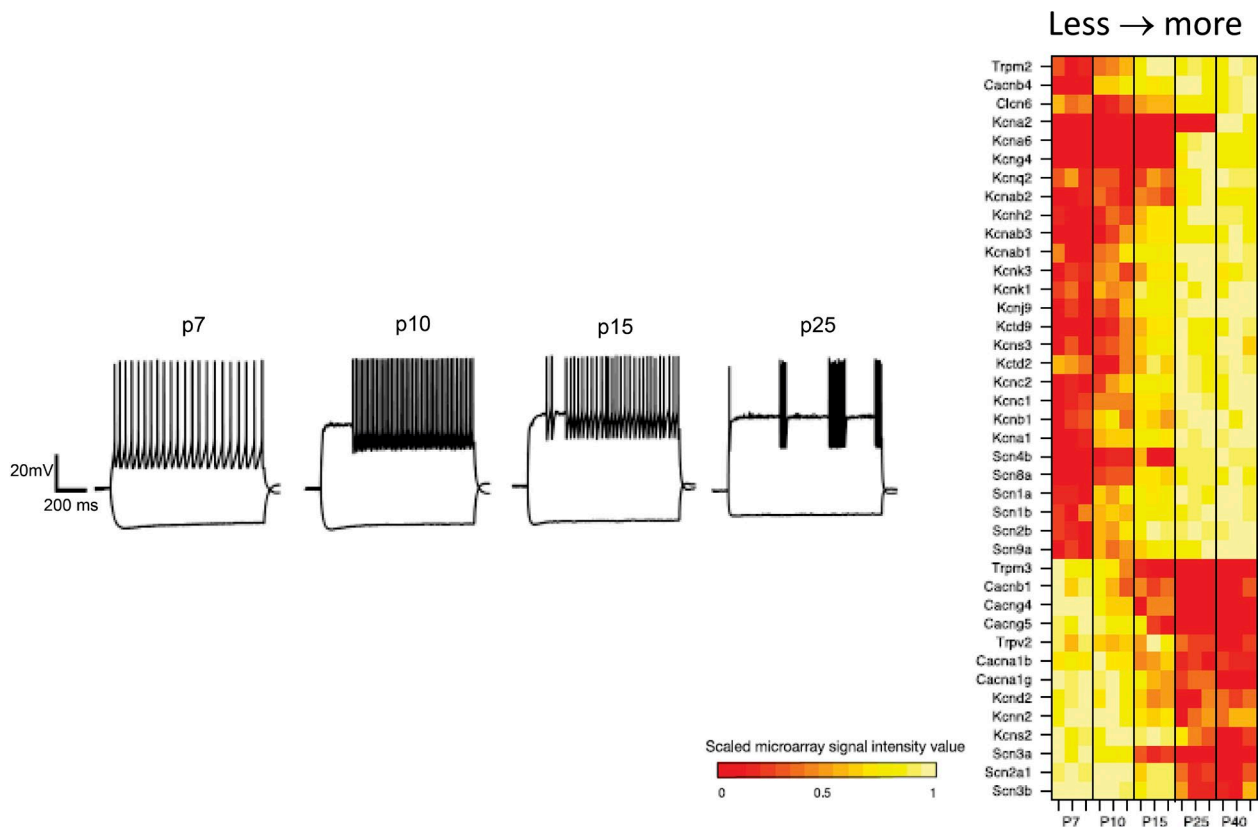


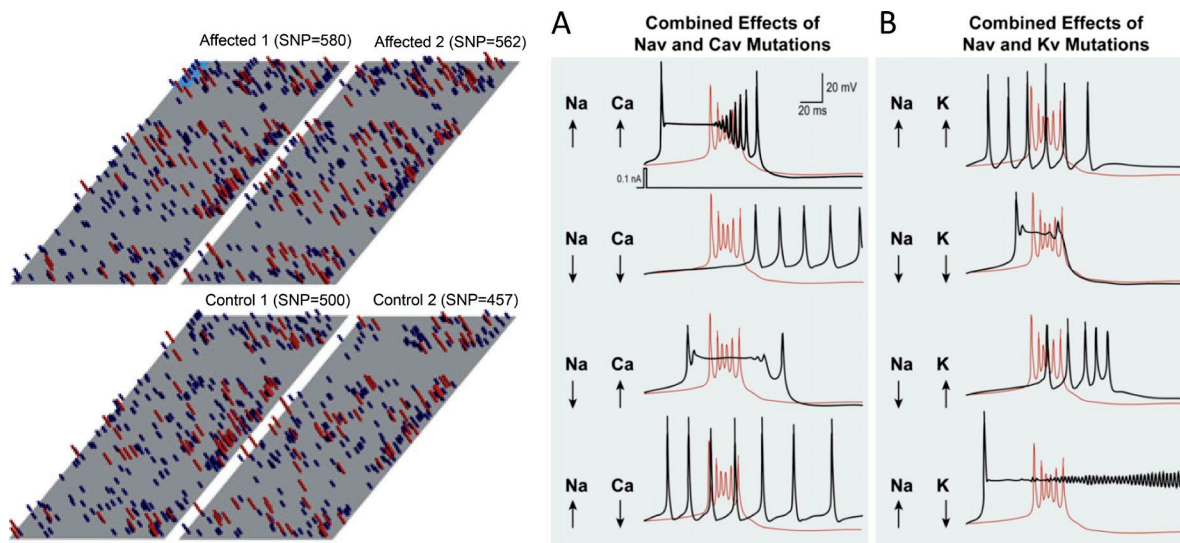
Figure 4. **Maturation of ion channel expression and firing properties in developing interneurons.** Channel subunit switching in a fast spiking neocortical inhibitory interneuron demonstrated by laser capture of parvalbumin + cells at differing ages and analyzed by microarray for sodium, potassium, and calcium channel subunit transcripts. Many subunits show clear reversal of channel transcript pattern in second postnatal week (reprinted from Okaty et al., 2009).

may contribute to the onset of symptoms when the mutant subunit is called into play. In both mouse and human, thalamocortical seizures show a clear onset during early childhood. A second developmental event during the functional maturation of synapses, which is not understood but is possibly caused by altered calcium cooperativity and physical rearrangement, is exemplified by the shared roles of P/Q and N type (*Cacna1B*) channels in exocytosis. In immature brain, excitation-secretion coupling depends primarily on calcium entry through N type channels that is later supplanted at most synapses by P/Q channels (Miki et al., 2013). Functional reallocation from a wild-type N channel release to an ineffective P/Q channel isoform may therefore contribute to the timing of seizure onset in *Cacna1a* mouse mutants (Noebels, 2012).

**Activity-induced channel remodeling.** The potential disease impact of a channel mutation is also driven by its own ability to alter the electrical stability of the circuit. Epitranscriptomic mechanisms underlying channel remodeling and the progressive developmental excitability architecture of cortical circuits point to candidate mechanisms for “acquired channelopathy”

(Nainar et al., 2016). For example, seizures dysregulate  $K_v$  channel transcription (Tsauro et al., 1992), which might result from promoter region methylation (Guo et al., 2011), microRNA effects (Henshall and Kobow, 2015), or A-I RNA editing genes such as ADAR that create *Kcna1* isoforms differing in inactivation, assembly, and surface expression (Bhalla et al., 2004; Streit et al., 2014; Behm and Öhman, 2016), all leading to changes in seizure threshold. Sodium channel processing (Baek et al., 2014) and the stoichiometry of ligand-gated channels are also activity dependent (Scharfman and Brooks-Kayal, 2014). Inherited potassium channelopathy may also arise secondarily from mutations in enzymes that desumoylate potassium channels. *Senp2* deletion leaves *Kcna1* and *Kcnq2* channels hypersumoylated and hypoactive, causing severe epilepsy with premature lethality (Qi et al., 2014). Finally, activity-dependent “neurotransmitter switching” adds another layer of flexibility where remodeling the transmitter phenotype can reverse the identity of a circuit from excitatory to inhibitory (Spitzer, 2015). Overall, this level of supramolecular plasticity means that once established, seizures themselves alter the landscape and hence the evolving im-





**Figure 5. Human ion channel variant complexity requires massive simulation to solve complex personal excitability profiles.** (Left) Unexpected complexity of novel nonsynonymous single nucleotide variants detected among 237 channel subunits sequenced in two individuals with epilepsy (upper plots, affected 1 and 2) and without (lower, control 1 and 2). Overall, the numerical burden of channel variants did not significantly differ between these groups (epilepsy adults  $n = 152$ , neurological normal adults  $n = 139$ ), indicating that pattern rather than load is a major contributor to phenotype. (Right) Computer simulation of a single hippocampal neuron firing pattern when current amplitudes are varied in a simple “two hit” model of a digenic mutation interactions between  $\text{Na}_v/\text{Ca}_v$  (A) and  $\text{Na}_v/\text{K}_v$  (B). In the future, personalized models of complex masking and degenerate current/firing pattern outcomes can be systematically tested in large networks incorporating an individual’s full compound variant profile (“channotype”) and correlated with real-life sensitivity to ion channel-based therapies (reprinted from Klassen et al., 2011).

pect of a channel variant, including its role in pharmacoresistance (Kobow et al., 2013).

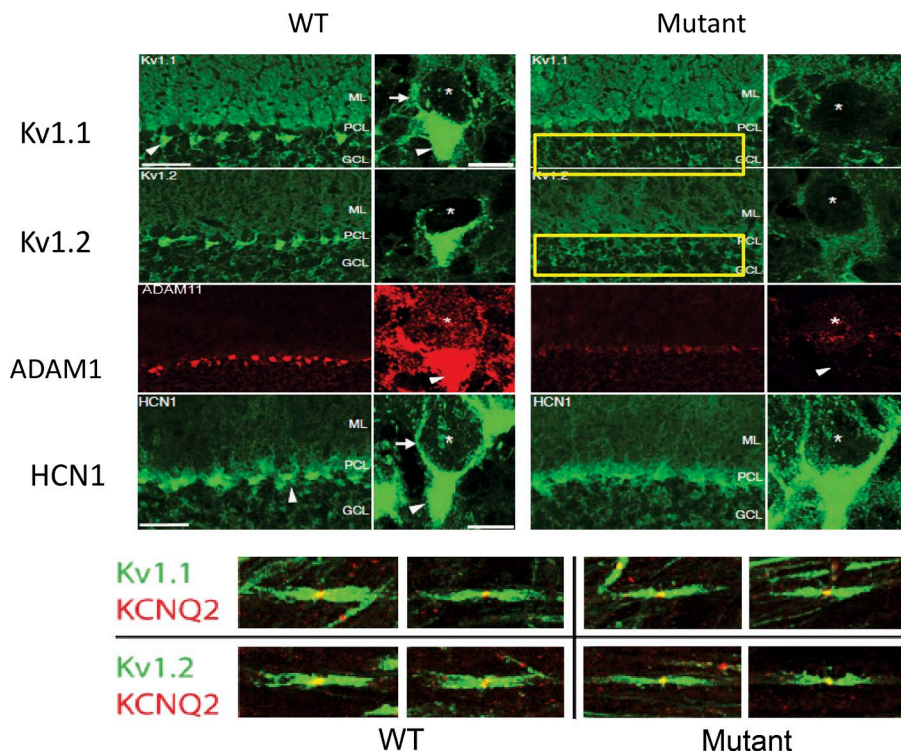
#### Genomic channel variant architecture and cellular epistasis

Extensive personal ion channel gene variation was first uncovered in a large-scale parallel Sanger sequencing study of 237 ion channel subunits in several hundred individuals with epilepsy and neurologically unaffected controls to assess the impact of their global “channotype” (Fig. 5 A; Klassen et al., 2011). A major finding was the unexpectedly large number of variants, including pathogenic rare channel variants, present across all channel genes in both groups, implying extensive epistatic interaction and therefore no simple numerical threshold for epilepsy risk. Thus although epilepsy may arise from only one (or many more) channel variant(s), it is the specific identity and pattern, rather than the numerical sum, of channel variants in an individual that determines the final clinical phenotype. This makes sense if one considers the wide range of their conductances, valences, and uneven distribution across brain circuits. A second finding with major therapeutic implications for pharmacoresistance was the detection of individuals bearing a complex variant profile with multiple channel variants known to cause epilepsy (sometimes as many as eight). Computational simulations of multiplex current variation in a single model neuron demonstrated their enhancing or suppressant interaction.

Experimental genetic validation of inter-channel epistasis by combining pairs of known variants supports this model. Early studies in *Drosophila* mutants demonstrated simple compound interaction of two axon excitability loci, later shown to encode ion channels (Ganetzky and Wu, 1982). In mouse brain, similar digenic suppression of seizure phenotypes has been demonstrated, surprisingly even when both are individually epileptogenic. Crossing *Kcna1*-null mice amplified transmitter release in P/Q loss of function mutants and masked the seizure disorder, a result that could be phenocopied by pharmacological potassium channel blockade (Glasscock et al., 2007). Phenotypic suppression occurred only in pathways where both channels were coexpressed, supporting an anatomical basis for predicting candidate ion channel modifiers. Epistatic modulation may also be bidirectional, and crossing epileptogenic *Scn2a* and *Kcnq2* variants increased seizure severity (Kearney et al., 2006). Co-mutation of *Scn8a* can dramatically influence phenotype severity of mice carrying the Dravet syndrome *Scn1a*-R1648H mutation (Hawkins et al., 2011). Alternatively, channel variants influencing sodium channels can be isolated from the background by classical genetic techniques (Calhoun et al., 2016). These studies point to the importance of full channel profiling rather than single gene testing in the epilepsy clinic.

**Large-scale simulation of personal variant profiles.** Ultimately, computational models must be extended by in-





**Figure 6. Secondary channelopathy resulting from mutation of nonsubunit interacting gene.** ADAM11 is critical for retention of  $K_v1.1/K_v1.2/K_v\beta2$  subunits at the presynaptic terminal (arrows) and "pinneaux" (arrowheads) of basket cells onto Purkinje cells (asterisks). Tethered channel subunit proteins such as ADAM11, a member of the large membrane disintegrin and metalloproteinase family, play a key role in selective targeting and retention of  $K_v1.x$  channels to wild-type presynaptic terminals, as shown by their absence at cerebellar inhibitory basket cell terminals (yellow boxes) in the epileptic ADAM11 $\Delta^{12-18}$  (a truncation removing the domain containing the integrin-binding site) mutant mouse. HCN1, another channel located at the basket cell presynaptic terminal, is not altered. Bars: (left) 50  $\mu\text{m}$ ; (insets) 10  $\mu\text{m}$ . (Bottom) The same heteromeric  $K1.x$  channel  $\alpha$  subunits are spared at mutant peripheral nodes of Ranvier, which depend on ADAM22 (modified with permission from Kole et al., 2015).

incorporating evermore complex pattern of conductances to assess a complete personal channel variant profile, first cell by cell in single defined neuron types, and then combined in circuits (Fig. 5 B). The extent of multiplex variation also has important therapeutic implications for precision rescue, namely whether a polygenic profile of epileptogenic variants can better predict pharmacoresistance (as debated in cancer therapy; Bredberg, 2011), because drugs acting on a single class of channels may not suppress epileptogenic defects in others.

An optimized model to simulate variant interactions in single cell behavior would incorporate not only their kinetics in light of recent past behavior, but their localization. Defining the channel microenvironment can help predict the efficiency of gene–gene interactions in distinct subcompartments, both directly among physical channel dyads as well as those within effective electrotonic distance. For example,  $K_v4.2$  is a major dendritic  $K^+$  conductance; monogenic deletion in mice does not cause seizures, but may modulate their threshold in dendritic trees. In contrast, deletion of the nine different  $K_v$  channels localized in axons all lead to epilepsy, suggesting that tight coupling to impulse electrogenesis and release rather than dendritic integrative properties is more pathogenic. Evidence for the importance of channel proximity to transmitter release sites extends to distal preterminal regions, where mutation of ADAM11, a nonenzymatic disintegrin metalloproteinase, eliminates retention of  $K_v1.1$  and  $K_v1.2$  channels at presynaptic terminals while sparing nodal  $K_v$  channels and leads to epilepsy in mice (Fig. 6; Kole et al., 2015).

Similarly, membrane currents in the vicinity of mutated channels pinpoint an effective target for pharmacological rescue, essential when a mutation has eliminated the protein entirely. For example, deletion of  $K_v1$  channels in juxtaparanodal regions at nodes of Ranvier leads to ectopic burst firing, yet there is no physical channel left to target. However,  $Kcnq2$  channels reside in the adjacent paranode, and opening these channels is sufficient to allow transcompartmental rescue of axon hyperexcitability (Fig. 7; Glasscock et al., 2012). The direct interaction of BK channels with calcium current (Kim and Oh, 2016) and their growing pharmacology (Hoshi and Heinemann, 2016) suggest an opportunity to co-regulate these neighboring epileptogenic currents. Their role in circadian excitability patterns (Whitt et al., 2016) is a novel link between channel function and epilepsies with strong diurnal clinical phenotypes.

At the circuit level, large multineuronal *in silico* networks are being explored in a model motor pattern neuronal generator to understand the general problem of homeostatic control of network excitability (O’Leary et al., 2014). This pursuit initially revealed the seminal principle of degenerate solutions among multiplex currents contributing to rhythmicity. A translational corollary of this rhythmic network current “redundancy” as applied to synchronous cortical activity is that it multiplies potential therapeutic options for seizure control. Although substantial biological nonlinearities stand in the way of building realistic models (Gjorgjieva et al., 2016), the quest for ever larger *in silico* network mod-

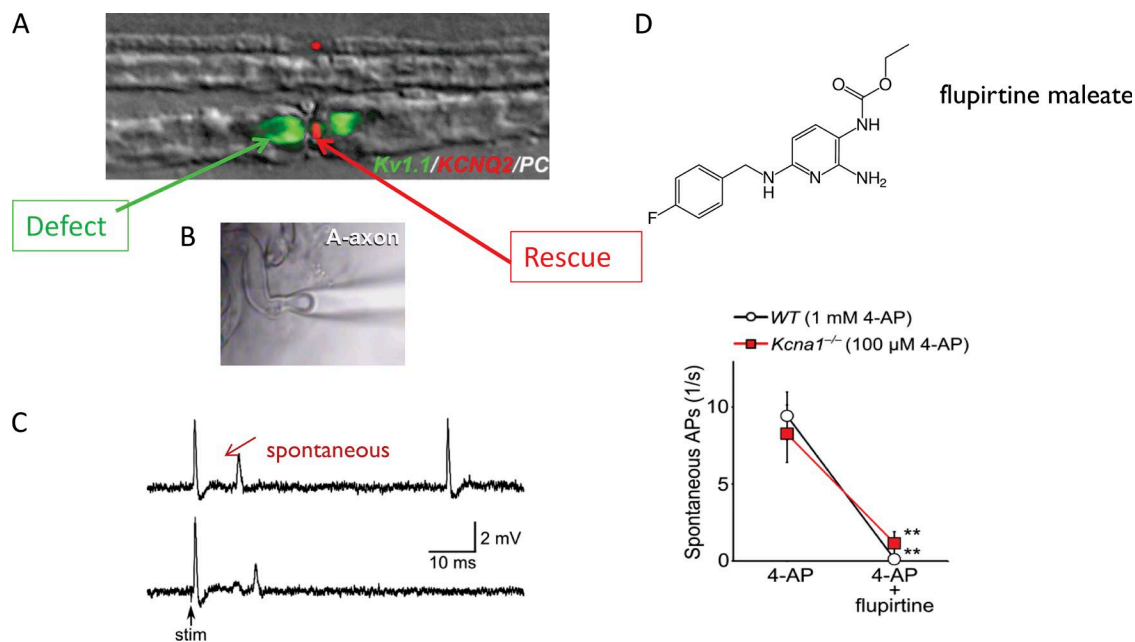


Figure 7. **Transcompartmental rescue of  $K_v1.1$  potassium channel null mutation by opening neighboring KCNQ2 current.** (A) Genetic deletion of repolarizing  $K_v1.1$  channel normally residing in flanking paranodal regions provokes generation of spontaneous and stimulated ectopic impulses recorded in isolated mouse vagal axons (B and C). (D) Hyperexcitability can be restored to wild type level by opening KCNQ2 channels in nodal membrane with flupirtine. The identification of currents that reside in the vicinity of a mutated channel may predict useful therapeutic targets (modified with permission from Glasscock et al., 2012).

els to simulate combinatorial channel gene variant patterns may one day speed our ability to analyze complex personal ion channel profiles for optimal treatment strategies.

### Conclusion

Ion channel subunits comprise the single largest gene family underlying disorders of heart, muscle, and brain and the most frequently tested for precision clinical diagnosis of a broad phenotypic spectrum of central nervous system disease. These disorders collectively constitute an enormous public health burden, with a greater number of life years diminished than cancer. The significance of each variant, which may spell the difference between lifelong disability or sudden death, requires the most accurate functional interpretation to assign causality, stimulate drug discovery, and guide the use of gene variant-specific therapies.

Despite rapid technological innovation, we face a profound lack of functional information regarding the majority of ion channel genes and their myriad splice forms, interactomes, and the unexpected complexity of their coordinate regulation within cells. Clinical exomes point to recurring mysteries, including bioinformatically “benign” variants with seemingly slight or no change in gating kinetics that lead to devastating disease, or functionally damaging yet clinically silent mutations, implicating the involvement of unknown non-pore functions and epistatic compensation.

Genetic testing has permanently expanded the ion channel basic research mandate, and focused studies of the functional biology of human channel mutations are now essential to the success of precision medicine. New collaborative and computational approaches are required to uncover, validate, and simulate variant pathogens in complex combinations. Fortunately, rather than distracting from the fundamental goal of refining canonical protein structure–function relationships, genetic testing will accelerate discovery of unsuspected aspects of channel biophysics and biology in developing brain circuitry and optimize the selection of therapeutic targets. Their profound clinical impact and translational potential illustrate why ion channel mutations represent some of the most intriguing and medically essential molecular lesions to understand and treat.

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