Symposium

Inherited Neuronal Ion Channelopathies: New Windows on Complex Neurological Diseases

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Studies of genetic forms of epilepsy, chronic pain, and migraine caused by mutations in ion channels have given crucial insights into molecular mechanisms, pathogenesis, and therapeutic approaches to complex neurological disorders. Gain-of-function missense mutations in the brain type-I sodium channel $Na_V1.1$ are a primary cause of generalized epilepsy with febrile seizures plus. Loss-of-function mutations in $Na_V1.1$ channels cause severe myoclonic epilepsy of infancy, an intractable childhood epilepsy. Studies of a mouse model show that this disease is caused by selective loss of sodium current and excitability of GABAergic inhibitory interneurons, which leads to hyperexcitability, epilepsy, and ataxia. Mutations in the peripheral sodium channel $Na_V1.7$ cause familial pain syndromes. Gain-of-function mutations cause erythromelalgia and paroxysmal extreme pain disorder as a result of hyperexcitability of sensory neurons, whereas loss-of-function mutations cause congenital indifference to pain because of attenuation of action potential firing. These experiments have defined correlations between genotype and phenotype in chronic pain diseases and focused attention on $Na_V1.7$ as a therapeutic target. Familial hemiplegic migraine is caused by mutations in the calcium channel, $Ca_V2.1$, which conducts P/Q-type calcium currents that initiate neurotransmitter release. These mutations increase activation at negative membrane potentials and increase evoked neurotransmitter release at cortical glutamatergic synapses. Studies of a mouse genetic model show that these gain-of-function effects lead to cortical spreading depression, aura, and potentially migraine. Overall, these experiments indicate that imbalance in the activity of excitatory and inhibitory neurons is an important underlying cause of these diseases.

Key words: **sodium channels; calcium channels; epilepsy; migraine; pain; genetic diseases**

Introduction

Voltage-gated sodium and calcium channels are closely related members of the ion channel protein superfamily (Yu and Catterall, 2004). Because of the importance of their functions and the complexity of their structures, these two ion channels are targets for numerous mutations that cause diseases of hyperexcitability, including epilepsy, migraine, chronic pain, periodic paralysis, and cardiac arrhythmia. Voltage-gated Na^+ channels in the brain are complexes of a 260 kDa α subunit in association with auxiliary β subunits ($\beta1$–$\beta4$) of 33–36 kDa (Catterall, 2000). The α subunit contains the voltage sensors and the ion-conducting pore in four internally repeated domains (I–IV), which each consists of six α-helical transmembrane segments (S1–S6) and a pore loop connecting S5 and S6 (Catterall, 2000). The association of β subunits modifies the kinetics and voltage dependence of gating, and these subunits are cell adhesion molecules interacting with extracellular matrix, other cell adhesion molecules, and the cytoskeleton (Isom et al., 1995; Isom, 2002). The voltage-gated ion channels are among the most ancient and conserved gene families, with sequence identity of >50% in the transmembrane domains of human sodium channel α subunits and those of the simplest multicellular eukaryotes. The mammalian genome contains nine functional voltage-gated sodium channel α subunits, which differ in patterns of tissue expression and biophysical properties. The $Na_V1.1$, $Na_V1.2$, $Na_V1.3$, and $Na_V1.6$ sodium channel subtypes, encoded by the *SCN1A*, *SCN2A*, *SCN3A*, and *SCN8A* genes, are the primary sodium channels in the CNS (Catterall, 2000; Goldin et al., 2000; Goldin, 2001; Trimmer and Rhodes, 2004). $Na_V1.1$ and $Na_V1.3$ channels are primarily localized in cell bodies (Westenbroek et al., 1989, 1992), $Na_V1.2$ channels in unmyelinated or premyelinated axons and dendrites (Westenbroek et al., 1989, 1992), and $Na_V1.6$ channels in myelinated axons and in dendrites (Caldwell et al., 2000; Krzemien et al., 2000; Jenkins and Bennett, 2001). These channels participate in generation of both somatodendritic and axonal action potentials (Stuart and Sakmann, 1994; Johnston et al., 1996; Callaway and Ross, 1997; Raman and Bean, 1999; Khaliq and Raman, 2006).

Sodium channels and inherited epilepsy

Despite their amino acid sequence identity of >70%, knock-out of any of the three sodium channel α subunit genes expressed primarily in adult brain (*SCN1A*, *SCN2A*, and *SCN8A*) is lethal, demonstrating that each channel performs some nonredundant

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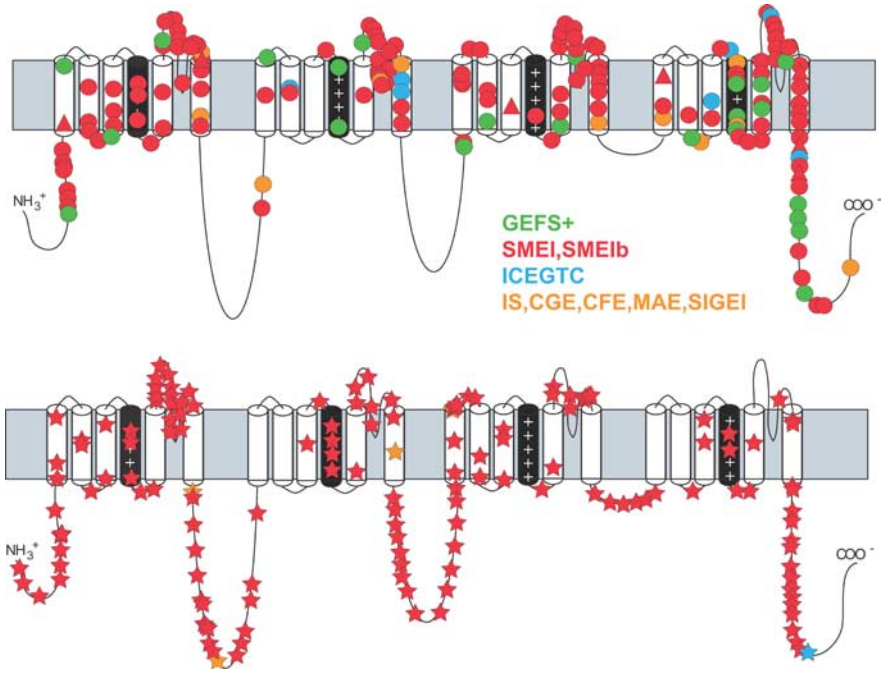

Figure 1. *SCN1A* mutations in patients with epilepsy. Top, Missense mutations (circles) and in-frame deletions (triangles). Bottom, Truncation mutations (stars). Most missense mutations are located in the transmembrane-spanning segments and the proximal half of the C-terminal domains. The clinical type of epilepsy is indicated by color: GEFS+, generalized epilepsy with febrile seizures plus; SMEI, severe myoclonic epilepsy of infancy; SMEIb, borderline SMEI; ICEGTC, idiopathic childhood epilepsy with generalized tonic-clonic seizures; IS, infantile spasms; CGE, cryptogenic generalized epilepsy; CFE, cryptogenic focal epilepsy; MAE, myoclonic astatic epilepsy; SIGEI, severe idiopathic generalized epilepsy of infancy. Modified from Kearney and Meisler (2008).

function. The first channelopathies associated with mutations of neuronal sodium channels were discovered in the mouse: a null mutation of *Scn8a* in the ataxic *med* mouse (Burgess et al., 1995) and a gain-of-function mutation of *Scn2a* in the epileptic *Q54* mouse (Kearney et al., 2001). Screening of human patients with related clinical disorders led to the identification of *SCN1A* mutations in two large families with the autosomal dominant epilepsy disorder generalized epilepsy with febrile seizures plus (GEFS+) [Online Mendelian Inheritance in Men database (OMIM) identification number 604233] (Escayg et al., 2000). More than 20 different mutations were subsequently identified in GEFS+ patients, accounting for ~10% of cases (Fig. 1). GEFS+ is caused by missense mutations that alter multiple biophysical properties of the channel expressed in non-neuronal cells (Meisler and Kearney, 2005). Although increased levels of persistent sodium current is a common observation in heterologous expression systems (George, 2005), loss-of-function mutations are also observed (Barela et al., 2006). Moreover, reduced expression levels of mutant channels in neurons may be more significant than gain-of-function changes (Rusconi et al., 2007) and lead to an overall loss-of-function phenotype at the cellular level in the nervous system. The patient mutation R1648H has been incorporated into the mouse genome, providing an animal model of GEFS+ that will permit more detailed analysis of the effect of mutations on neuronal currents *in vivo* (A. Escayg, personal communication).

Identification of these familial *SCN1A* mutations was followed by the surprising report of mutations in children with the sporadic epilepsy disorder severe myoclonic epilepsy of infancy (SMEI) (OMIM identification number 607208) (Claes et al., 2001). These children carry *de novo* mutations that are not inherited from their parents. More than 300 *SCN1A* mutations have been identified (Fig. 1), accounting for ~70% of cases (Meisler and Kearney, 2005). Mutation hotspots, including several sites of CpG deamination, account for ~25% of new mutations (Kearney et al., 2006a). Genetic screening for *SCN1A* has become the standard of care for children with early-onset seizures. More than half of the SMEI mutations cause loss of function as a result of stop codons or deletions, demonstrating that haploinsufficiency of *SCN1A* is pathogenic. Missense mutations of *SCN1A* in patients with SMEI are concentrated in the transmembrane segments of the protein (Fig. 1). As for the GEFS+ mutations, heterogeneous biophysical abnormalities are associated with these missense mutations when expressed in non-neuronal cells (Meisler and Kearney, 2005), and no clear correlation between biophysical abnormalities of the missense mutations and clinical severity has emerged from this ongoing work. One practical result of these discoveries is the avoidance of treatment with sodium channel blockers, which exacerbate symptoms in patients with reduced expression of *SCN1A*.

There has been considerable controversy regarding claims that routine childhood vaccination may be associated with onset of seizures and mental decline. In 2006, Berkovic et al. studied 14 children with this diagnosis and identified *SCN1A* mutations in 11 of the children (Berkovic et al., 2006). Their observations indicate that vaccination and its associated fever may trigger the first episode of an underlying genetic disorder. *SCN1A* mutations have also been identified in a few families with familial hemiplegic migraine type 3 (FHM3) (OMIM identification number 609634) (Dichgans et al., 2005). Biophysical analysis of three of these mutations demonstrated reduced channel activity in two cases and gain-of-function features, including increased persistent current in a third case (Cestèle et al., 2008; Kahlig et al., 2008). The presence of seizures in addition to migraine in the third family demonstrates the potentially close relationship between these two disorders.

In contrast to *SCN1A*, a small number of pathological mutations have been identified in the other three major neuronal sodium channels. Missense mutations of *SCN2A* were identified in one patient with GEFS+ and nine patients with benign familial neonatal-infantile seizures (BFNIS), a syndrome of mild seizures that remit by the first birthday. Functional analysis of three BFNIS mutations identified a variety of abnormalities with the common feature of reduced channel activity (Misra et al., 2008). A truncation mutation of *SCN2A* was found in a patient with intractable epilepsy and mental decline, similar to SMEI. The first mutation of *SCN3A* was described recently in a child with partial epilepsy resistant to antiepileptic drugs (Holland et al., 2008). This missense mutation SCN3A–K354Q caused an increase in persistent current that is similar in magnitude to epileptogenic mutations of *SCN1A* and *SCN2A*. The relative paucity of mutations in

SCN2A and *SCN3A* may reflect bias of ascertainment, in that fewer patients may have been screened for mutations in these channels.

Ataxic gait and tremor are associated with mutations of *Scn8a* in the mouse (Meisler et al., 2004) and are reproduced by conditional knock-out of *Scn8a* in cerebellar Purkinje and granule cell neurons (Levin et al., 2006). However, no pathogenic variants of *SCN8A* were detected among 100 families with essential tremor (Sharkey et al., 2008). In a screen of 150 families with ataxia, one frame-shift mutation was identified in *SCN8A* that truncates the protein in transmembrane domain 4, removing the C-terminal domain and leading to predicted loss of channel function (Trudeau et al., 2006). The 9-year-old proband who was heterozygous for the null allele also exhibited mental retardation, and three heterozygous family members had cognitive and behavioral deficits, including attention deficit disorder. Behavioral testing of heterozygous *Scn8a* null mice also revealed abnormalities indicative of increased emotionality and anxiety (McKinney et al., 2008). These observations suggest that channelopathies may contribute to cognitive and psychiatric disorders.

A screen of 117 autism families for variants of *SCN1A*, *SCN2A*, and *SCN3A* identified five missense mutations that appeared to cosegregate with the disease (Weiss et al., 2003). One of these, SCN2A–R1902C, reduced binding affinity for calmodulin. A recent study identified a consanguineous family in which individuals affected with autism are homozygous for a 40 kb deletion close to the *SCN7A* gene in the chr 2q24 cluster that includes *SCN1A*, *SCN2A*, and *SCN3A*; this intergenic deletion might alter expression of one of the sodium channel genes in this cluster (Morrow et al., 2008). It seems likely that future screening in patients with a wider variety of neuropsychiatric illness will reveal a broader role for mutations in voltage-gated channels.

The net electrophysiological properties of a neuron are the product of its overall ion channel content, so inheritance of multiple genetic variants may contribute to conditions with polygenic inheritance or variable penetrance among family members. Such genetic interactions can be studied by mating mouse lines having different well defined genetic deficiencies (Fig. 2). In one study, the combination of mild mutations in *Scn2a* and the potassium channel gene *Kcnq2* resulted in a severe seizure disorder, consistent with the roles of these two channels in action potential generation and maintenance of neuronal membrane potential (Kearney et al., 2006b). In another example, the severe epilepsy associated with haploinsufficiency of *Scn1a* was rescued by haploinsufficiency for the sodium channel gene *Scn8a* (Martin et al., 2007). In the third case, combining epileptogenic alleles of *Cacna1a* and *Kcn1a* resulted in correction of disease (Glasscock et al., 2007). These examples suggest that mutations in one channel could be treated by inhibition or activation of a second channel. Large-scale screening projects currently in progress are likely to identify human patients with multiple ion channel variants, and analysis of the underlying mechanisms will be both challenging and informative.

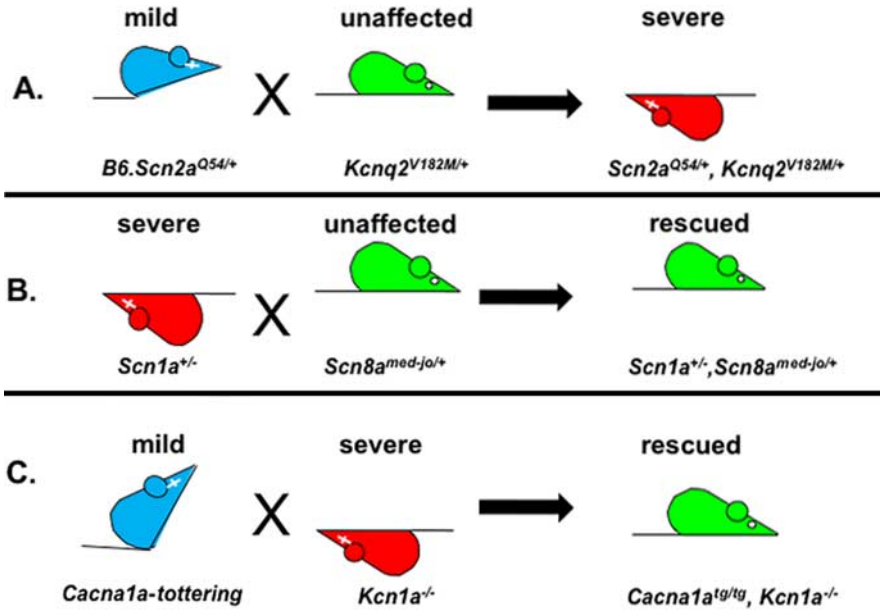

Figure 2. Combining two ion channel mutations can exacerbate or rescue the abnormalities associated with individual mutations. ***A***, Interaction between sodium and potassium channel mutations in the mouse (Kearney et al., 2006b). ***B***, Interaction between mutations in two sodium channel genes, *Scn1a* and *Scn8a* (Martin et al., 2007). ***C***, Interaction between calcium and potassium channel genes (Glasscock et al., 2007).

A mouse model of severe myoclonic epilepsy of infancy

SMEI (Dravet syndrome) (Claes et al., 2001) is linked to *de novo* loss-of-function mutations in one allele of the *SCN1A* gene, leading to haploinsufficiency of $Na_V1.1$ channels (Claes et al., 2001; Ohmori et al., 2002; Fujiwara et al., 2003). Because of haploinsufficiency of the loss-of-function genotype, SMEI is genetically dominant (Claes et al., 2001; Sugawara et al., 2002; Fukuma et al., 2004). This rare convulsive disorder begins during the first year of life, with seizures often associated with fever, and progresses to prolonged, clustered or continuous seizures and to status epilepticus (Dravet et al., 1992; Engel, 2001). After the second year of life, patients develop comorbidities, including psychomotor delay, ataxia, and cognitive impairment. They have an unfavorable long-term outcome as a result of ineffectiveness of antiepileptic drug therapy (Dravet et al., 1992; Oguni et al., 2001). It is a surprise that haploinsufficiency of a Na_V channel causes epilepsy, because reduced sodium current should lead to hypoexcitability rather than hyperexcitability. To understand the mechanistic basis for hyperexcitability and comorbidities in SMEI, an animal model was generated by targeted deletion of the *Scn1a* gene in mouse (Yu et al., 2006; Ogiwara et al., 2007).

Homozygous null $Na_V1.1^{-/-}$ mice developed ataxia and died on postnatal day 15 (P15) but could be sustained to P17.5 with manual feeding (Yu et al., 2006). Heterozygous $Na_V1.1^{+/-}$ mice exhibited spontaneous seizures and sporadic deaths beginning after P21, with a striking dependence on genetic background (Yu et al., 2006). Loss of $Na_V1.1$ did not change voltage-dependent activation or inactivation of sodium channels in hippocampal neurons (Yu et al., 2006). However, the sodium current density was substantially reduced in inhibitory interneurons of $Na_V1.1^{+/-}$ and $Na_V1.1^{-/-}$ mice but not in their excitatory pyramidal neurons (Table 1). This reduction in sodium current caused a loss of sustained firing of action potentials in hippocampal and cortical interneurons (Yu et al., 2006; Ogiwara et al., 2007). An immunocytochemical survey also revealed a specific upregulation of

Table 1. Functional impact of deletion of the $Na_V1.1$ channel

Functional effect	Heterozygous knock-out	Homozygous knock-out
Na^+ current in hippocampal pyramidal cells (% WT)	100 ± 5.1	96 ± 6.0
Na^+ current in hippocampal interneurons (% WT)	47.0 ± 7.4	27.5 ± 5.4
Na^+ current in Purkinje neurons (% WT)		
Peak	57.6 ± 0.6	41.6 ± 0.5
Persistent	44.9 ± 4.1	41.0 ± 3.7
Resurgent	49.6 ± 5.5	31.2 ± 3.5
Ataxia	Significant at P21	Severe at P11–P14
Thermally induced seizures	First observed at P20, increasing thereafter	Not tested
Spontaneous seizures	First observed at P21, increasing thereafter	P11–P14
Premature death	Increasing premature death after P21	Death at P15

WT, Wild type. Results are from Yu et al. (2006) and Kalume et al. (2007).

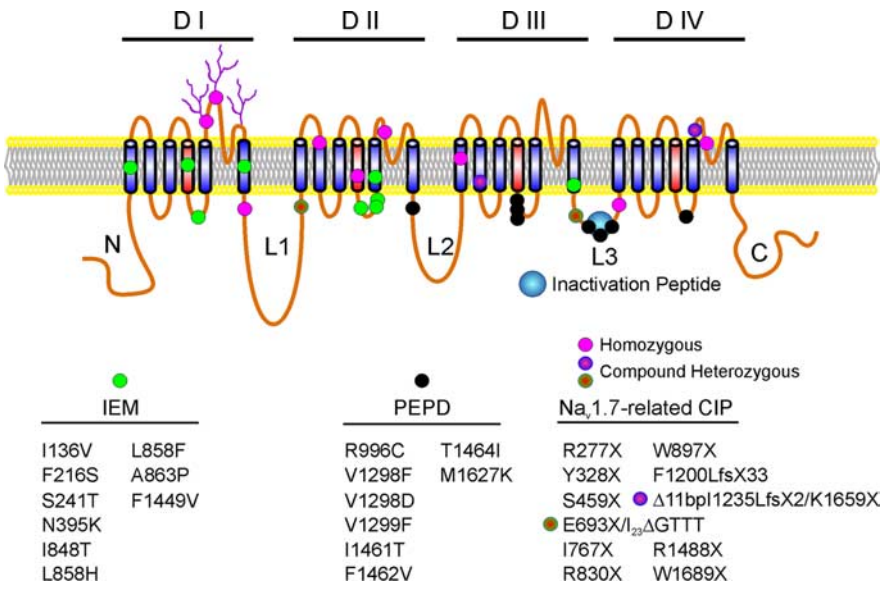

Figure 3. Schematic of voltage-gated sodium channel showing locations of the known mutations in $Na_v1.7$-related inherited pain disorders. Inherited erythromelalgia (green symbols) and PEPD (black symbols) mutations are gain-of-function and inherited as a dominant trait. $Na_v1.7$-related CIP is caused by loss-of-function mutations that are inherited as a recessive trait. Homozygous $Na_v1.7$-related CIP mutations carry the same nonsense mutation on both alleles of SCN9A (solid magenta), whereas two pairs of compound heterozygous mutations (blue–magenta and red–green) carry different mutations, which produce nonfunctional channels on the two alleles. Used with permission from Dib-Hajj et al. (2007).

$Na_V1.3$ channels in a subset of hippocampal interneurons (Yu et al., 2006) (supplemental data, available at www.jneurosci.org as supplemental material), but this upregulation was insufficient to compensate for the loss of the sodium current of $Na_V1.1$ channels (Yu et al., 2006). These results suggest that reduced sodium currents in GABAergic inhibitory interneurons in $Na_V1.1^{+/-}$ heterozygotes may cause the hyperexcitability that leads to epilepsy in patients with SMEI. Loss of excitability of GABAergic inhibitory interneurons would allow hyperexcitability of dentate granule and pyramidal neurons, and this gain-of-function effect may cause epilepsy that is genetically dominant.

Ataxia, spasticity, and failure of motor coordination contribute substantially to the developmental delay and functional impairments of SMEI patients and are major determinants of their poor quality of life, burden of care, and premature deaths (Dravet, 2003; Dravet et al., 2005). However, reduced sodium current in hippocampal interneurons would be unlikely to cause these deficits. How might loss of $Na_V1.1$ channels cause ataxia, spasticity, and failure of motor coordination? Purkinje cells are GABAergic inhibitory neurons that serve as the output pathway for information on movement, coordination, and balance from the cerebellar cortex. Degeneration of Purkinje neurons and abnormal expression of voltage-gated ion channels in them are associated with ataxia (Fletcher et al., 1996; Raman and Bean, 1997; Grüsser-Cornehls and Bäurle, 2001; Sausbier et al., 2004), and conditional deletion of $Na_V1.6$ channels in Purkinje neurons is sufficient to cause ataxia (Levin et al., 2006). The mouse genetic model of SMEI was used to investigate the relationship between loss of $Na_V1.1$ channels and ataxia based on the hypothesis that deletion of the $Na_V1.1$ channel reduces the excitability of Purkinje neurons (Kalume et al., 2007).

Behavioral assessment indicated severe motor deficits in homozygous mutant mice, including irregularity of stride length during locomotion, impaired motor reflexes in grasping, and mild tremor in limbs when immobile, consistent with cerebellar dysfunction (Yu et al., 2006; Kalume et al., 2007). A milder impairment of normal gait was observed in the heterozygotes after P21 (Kalume et al., 2007). Immunohistochemical studies showed that $Na_V1.1$ and $Na_V1.6$ channels are the primary sodium channel isoforms expressed in cerebellar Purkinje neurons (Kalume et al., 2007). The amplitudes of whole-cell peak, persistent, and resurgent sodium currents in Purkinje neurons were reduced by 58–69%, without detectable change in the kinetics or voltage dependence of channel activation or inactivation (Table 1). Nonlinear loss of sodium current in Purkinje neurons from heterozygous and homozygous mutant animals suggested partial compensatory upregulation of $Na_V1.6$ channel activity (Table 1). Current-clamp recordings revealed that the firing rates of Purkinje neurons from mutant mice were substantially reduced, with no effect on threshold for action potential generation (Kalume et al., 2007). The results show that $Na_V1.1$ channels play a crucial role in the excitability of cerebellar Purkinje neurons, with major contributions to peak, persistent, and resurgent forms of sodium current and to sustained action potential firing. Loss of these channels in Purkinje neurons of mutant mice and SMEI patients may be sufficient to cause their ataxia and related functional deficits. These findings suggest the hypothesis that loss of sodium

currents in different classes of GABAergic neurons may underlie the multiple comorbidities in SMEI, including light hypersensitivity, altered circadian rhythms, and cognitive impairment.

Chronic pain

In the peripheral nervous system, $Na_V1.7$ channels are expressed in sympathetic neurons, sensory neurons, and their axons, whereas $Na_V1.8$ and $Na_V1.9$ are exclusively expressed in sensory neurons, including peripheral terminals, axons, and cell bodies. Recent human studies have directly linked $Na_v1.7$ to three pain disorders (Fig. 3): inherited erythromelalgia (IEM) (OMIM identification numbers 133020 and 603415), paroxysmal extreme pain disorder (PEPD) (OMIM identification number 167400), and $Na_v1.7$-associated congenital insensitivity to pain (CIP) (OMIM identification number 243000 (Dib-Hajj et al., 2007)). Dominantly inherited gain-of-function mutations in *SCN9A*, the gene encoding $Na_v1.7$, cause the painful neuropathy IEM, characterized by episodes of burning pain, erythema, and mild swelling in the hands and feet, which are triggered by mild warmth or exercise (Dib-Hajj et al., 2007; Drenth and Waxman, 2007). A different set of gain-of-function mutations has been identified in $Na_v1.7$ in patients with PEPD, previously referred to as familial rectal pain (Fertleman et al., 2006). Severe pain in PEPD patients along with flushing are induced by bowel movement or probing of the perianal areas and are sometimes accompanied by tonic nonepileptic seizures and cardiac deficits (Fertleman et al., 2007). In contrast, recessively inherited loss-of-function mutations in $Na_v1.7$ have been identified in individuals with complete inability to experience pain coupled with impaired sense of smell (Cox et al., 2006; Goldberg et al., 2007). These studies provide complementary and compelling evidence for a central role of this channel in pain signaling.

$Na_v1.7$ is mainly a peripheral sodium channel with robust expression levels in dorsal root ganglion (DRG) neurons and sympathetic ganglion neurons (Black et al., 1996; Sangameswaran et al., 1997; Toledo-Aral et al., 1997). Within DRG neurons, $Na_v1.7$ is present in 85% of functionally identified nociceptors (Djouhri et al., 2003). $Na_v1.7$ produces a fast activating and inactivating current that is sensitive to nanomolar concentrations of tetrodotoxin (Klugbauer et al., 1995; Sangameswaran et al., 1997) but recovers (reprimes) slowly from fast in-

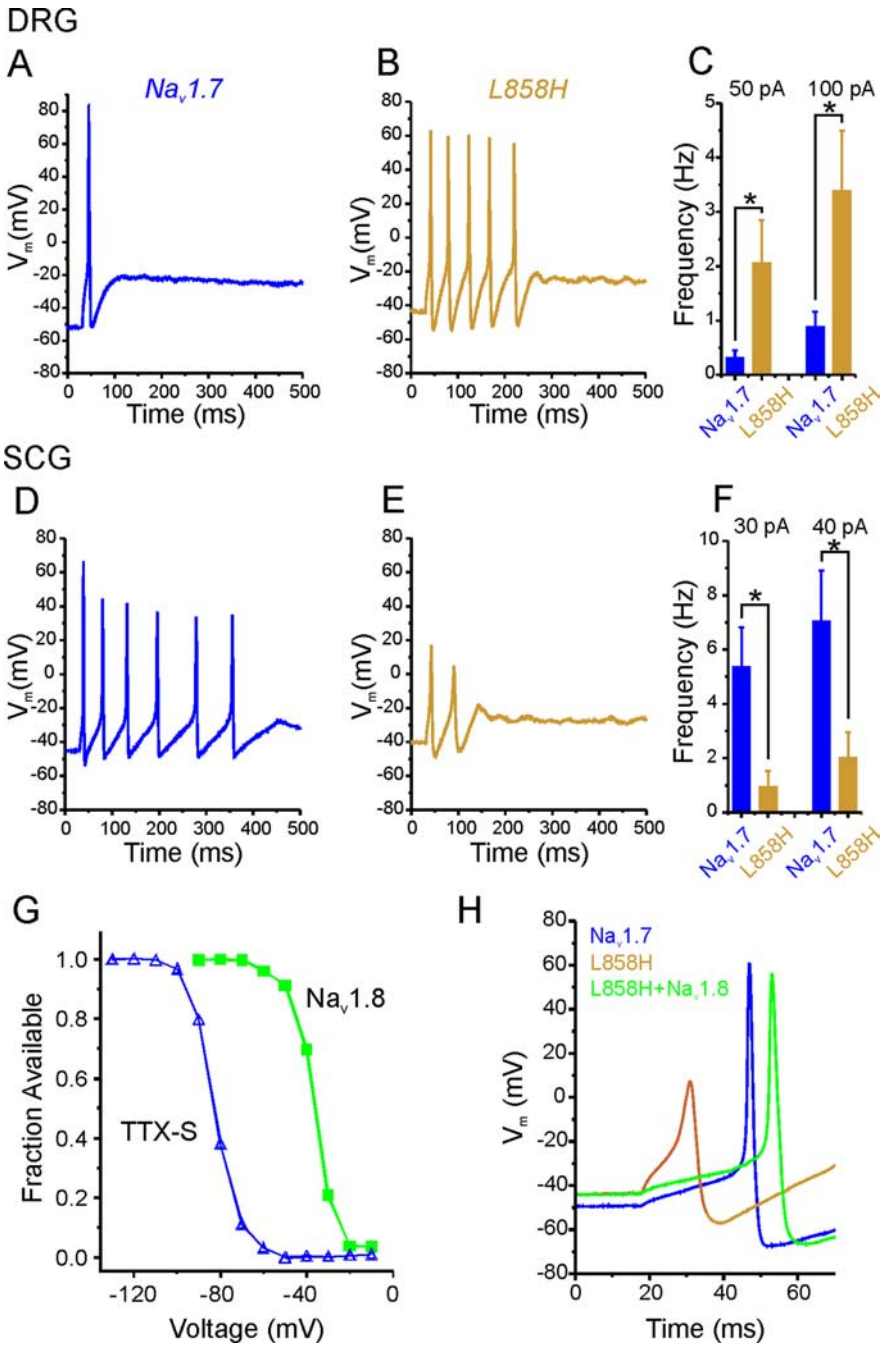

Figure 4. L858H IEM mutation increases firing frequency in DRG and decreases firing frequency in SCG neurons. ***A***, Representative DRG neuron expressing wild-type $Na_v1.7$ fires a single action potential in response to a 950 ms input of 100 pA from the resting membrane potential (RMP) of this neuron (approximately −50 mV). ***B***, Representative DRG neuron expressing L858H fires five action potentials in response to a 100 pA current injection from RMP of this neuron (approximately −42 mV). ***C***, For the entire population of DRG neurons studied, the firing frequency evoked by 50 pA current stimuli was 0.32 ± 0.13 Hz after transfection with wild-type channels ($n = 20$) and 2.06 ± 0.79 Hz after transfection with L858H ($n = 24$; $p < 0.05$), and the firing frequency evoked by 100 pA stimuli was 0.89 ± 0.28 Hz after transfection with wild-type and 3.37 ± 1.13 Hz after transfection with L858H ($p < 0.05$). ***D***, Representative SCG neuron expressing wild-type $Na_v1.7$ fires six action potentials in response to a 950 ms input of 40 pA from RMP (approximately −45 mV). ***E***, Representative SCG neuron expressing L858H fires only two action potentials in response to a 100 pA current injection from RMP (approximately −40 mV). ***F***, For the entire population of SCG neurons studied, the firing frequency evoked by 30 pA stimuli was 5.33 ± 1.5 Hz after transfection with wild-type channels ($n = 14$) and 0.63 ± 0.01 Hz after transfection with L858H channels ($n = 15$; $p < 0.05$); firing frequency evoked by 40 pA stimuli was 7.05 ± 1.86 Hz after transfection with wild-type and 1.96 ± 1.0 Hz after transfection with L858H channels ($p < 0.05$). ***G***, $Na_v1.8$ sodium channel (green) has markedly depolarized voltage dependence compared with TTX-sensitive (TTX-S) sodium channels, which include $Na_v1.1$, $Na_v1.3$, $Na_v1.6$, and $Na_v1.7$ (blue). ***H***, Action potential overshoot in SCG neurons transfected with the L858H IEM mutant channel (gold trace) was significantly reduced compared with that in neurons transfected with wild-type $Na_v1.7$ (blue trace). The action potential overshoot was restored to wild-type levels when $Na_v1.8$ was coexpressed with L858H (green trace). Modified from Rush et al. (2006).

activation (Cummins et al., 2007; Rush et al., 2007). Importantly, $Na_v1.7$ is characterized by slow closed-state inactivation, which permits the channel to respond to small slow depolarizations (ramp stimulus). This property suggests that $Na_v1.7$ might act as a "threshold" channel, amplifying generator potentials, and thus setting the gain in nociceptors (Cummins et al., 2007; Rush et al., 2007).

Symptoms of IEM can start as early as 1 year old (early-onset) or in adults (adult-onset), and both types have been described in families and in sporadic cases (Dib-Hajj et al., 2007; Drenth and Waxman, 2007). Recently, a familial case from Taiwan has been reported with symptoms first appearing in the feet of affected teenagers and with almost a decade delay in the involvement of hands (Dib-Hajj et al., 2007). Although early- and delayed-onset IEM have been linked to mutations in $Na_v1.7$, the etiology of adult-onset IEM remains an enigma. The mutations in $Na_v1.7$ from patients with early- and delayed-onset IEM generally shift voltage dependence of activation in a hyperpolarized direction, increase ramp current, and slow deactivation, which allows the channel to activate in response to weaker stimuli (Dib-Hajj et al., 2007). These changes in the gating properties of mutant $Na_v1.7$ have been shown to lower threshold for single action potentials and increase frequency of firing in DRG neurons (Fig. 4). Treatment for IEM, even with sodium channel blockers such as lidocaine or mexiletine, has been primarily ineffective (Dib-Hajj et al., 2007; Drenth and Waxman, 2007). In one case, the ineffectiveness of these drugs may be the result of a reduced affinity of the mutant channel for drug binding (Sheets et al., 2007).

Although many cases of PEPD have been directly linked to mutations in $Na_v1.7$, several other cases do not carry mutations in this channel, indicating the involvement of another target (Fertleman et al., 2006). Severe pain in PEPD patients along with redness in the lower body can start in infancy and possibly *in utero* (Fertleman et al., 2007). It is not known whether the seizures and cardiac symptoms that sometimes accompany pain are caused by autonomic dysfunction, which might be induced by $Na_v1.7$ mutant channels in sympathetic neurons. Pain progresses with age to ocular and maxillary/mandibular areas and is triggered by cold, eating, or emotional state (Fertleman et al., 2007). Unlike IEM, the anticonvulsant sodium channel blocker carbamazepine has been effective in relieving PEPD symptoms (Fertleman et al., 2006).

PEPD mutations in $Na_v1.7$ (Fertleman et al., 2006) change residues that have been implicated in fast inactivation of sodium channels (Catterall, 2002). The voltage dependence of steady-state fast inactivation of PEPD mutant channels is shifted by ~20 mV in a depolarizing direction, and inactivation is incomplete, resulting in a persistent current (Fertleman et al., 2006). Impaired channel fast inactivation and the persistent current produced by the mutant channels would be expected to increase frequency of action potential firing (Catterall and Yu, 2006). Indeed, expression of PEPD mutant $Na_v1.7$ channels renders DRG neurons hyperexcitable (Dib-Hajj et al., 2008; Estacion et al., 2008). The favorable response of the patients to carbamazepine, which is a use-dependent inhibitor of sodium channels, is consistent with the impaired inactivation of the mutant channels.

Loss-of-function mutations invariably truncate the channel protein, resulting in $Na_v1.7$-related CIP with impaired sense of smell (Cox et al., 2006). These mutations do not produce functional $Na_v1.7$ channels when expressed in mammalian expression systems (Cox et al., 2006). Patients do not experience pain from normally painful acts, such as puncture wounds, bone fracture, tongue and lip biting, or walking on hot surfaces (including burning coals), but do not suffer from other sensory, motor, or cognitive deficits. Heterozygous parents are asymptomatic, indicating that a null mutation on one allele does not lead to haploinsufficiency.

Recently, it has been shown that the phenotype of neurons expressing mutant $Na_v1.7$ is dependent on the ion channel repertoire of these cells. The expression of the same mutant $Na_v1.7$ channel, L858H, renders DRG neurons hyperexcitable but sympathetic neurons [superior cervical ganglion (SCG)] hypoexcitable, and this phenomenon can be explained by the expression of another sodium channel, $Na_v1.8$, in DRG but not SCG (Fig. 4). This role of $Na_v1.8$ in sensory neuron hyperexcitability is supported by its ability to rescue firing properties of SCG neurons when it is coexpressed with the L858H mutant $Na_v1.7$ channel. Sympathetic neuron dysfunction may contribute to attenuated cutaneous vasoconstriction and skin flushing, which is observed in patients with IEM or PEPD. However, it is not clear why patients with the gain-of-function mutations in $Na_v1.7$ do not show symptoms of global sympathetic dysfunction.

The elucidation of the role of $Na_v1.7$ in different forms of inherited painful neuropathies has identified a potential target for treatment. Because total lack of $Na_v1.7$ does not cause cognitive, motor, and sensory deficits, it suggests that $Na_v1.7$-specific blockers might be free from significant side effects. Together with the recent demonstration of a specific $Na_v1.8$ blocker (Jarvis et al., 2007), the pursuit of small molecule blockers of these peripheral sodium channels holds hope for better pharmacotherapy in the foreseeable future.

Calcium channels and inherited migraine

Like sodium channels, voltage-gated calcium channels are the molecular targets for mutations that cause several inherited diseases, including migraine, periodic paralysis, cardiac arrhythmia, and an autism-spectrum disorder. The pore-forming $\alpha 1$ subunits of voltage-gated calcium channels resemble the α subunits of sodium channels in structure with four internally repeated domains (I–IV), which each consists of six α-helical transmembrane segments (S1–S6) and a pore loop connecting S5 and S6 (Catterall, 2000b). They are associated with a distinct set of auxiliary subunits: $Ca_V\beta$, $Ca_V\alpha_2\delta$, and $Ca_V\gamma$. Ten calcium channel subtypes can be divided into three closely related subfamilies based on amino acid sequence. The Ca_V2 subfamily ($Ca_V2.1$, $Ca_V2.2$, and $Ca_V2.3$) are the primary calcium channels initiating neurotransmitter release at fast conventional synapses (Olivera et al., 1994; Dunlap et al., 1995), and mutations in $Ca_V2.1$ channels are implicated in inherited migraine as described below. $Ca_V2.1$ channels are located in somatodendritic membranes and in high density in presynaptic terminals throughout the CNS (Westenbroek et al., 1995), in which they play a key role in initiating action potential-evoked neurotransmitter release (Pietrobon, 2005).

Migraine is a common, disabling brain disorder of episodic headache pain of unknown etiology that affects >10% of the population (Pietrobon and Striessnig, 2003). The World Health Organization ranks migraine as one of the 20 most disabling diseases. Despite the great societal and personal costs, drug therapy for preventing and treating migraine remains unsatisfactory for many patients. Our poor understanding of the nature and mechanisms of the primary brain dysfunction(s) causing migraine slows the development of new therapeutic approaches. Unique insights into the pathophysiology of migraine can be gained by studying the molecular and cellular mechanisms of FHM, a monogenic type of inherited migraine with aura in which

otherwise typical migraine attacks are associated with transient hemiparesis.

FHM1 is caused by mutations in the *CACNA1A* gene, encoding the pore-forming subunit of $Ca_V2.1$ channels that conduct P/Q-type Ca^{2+} current (Fig. 5*A*) (Ophoff et al., 1996). FHM1 mutations shift channel activation to lower voltages and increase Ca^{2+} influx through single recombinant human $Ca_V2.1$ channels (Fig. 5*B*) (Tottene et al., 2002, 2005; Pietrobon, 2007). Accordingly, the P/Q-type Ca^{2+} current density in cerebellar and cortical neurons of mutant mice carrying human FHM1 mutations was larger than in neurons of wild-type mice in a broad range of mild depolarizations (van den Maagdenberg et al., 2004; Pietrobon, 2007). Interestingly, these FHM mutant mice showed a reduced threshold for and increased velocity of cortical spreading depression (CSD), the phenomenon that underlies migraine aura and may activate migraine headache mechanisms (Pietrobon and Striessnig, 2003; van den Maagdenberg et al., 2004; Pietrobon 2007). Gain-of-function of Ca^{2+} influx at low voltages and facilitation of CSD induction and propagation were even larger in mice carrying mutation S218L, which causes unusually severe FHM with seizures, coma, and severe cerebral edema after mild head trauma (as well as cerebellar ataxia and atrophy). Interestingly, these S218L mutant mice showed a unique propensity to multiple CSD events, a feature that may underlie some of the severe clinical consequences of the S218L mutation (Pietrobon, 2007) (T. Pizzorusso, L. Gherardini, M. D. Ferrari, A. M. van den Maagdenberg, and D. Pietrobon, unpublished observations).

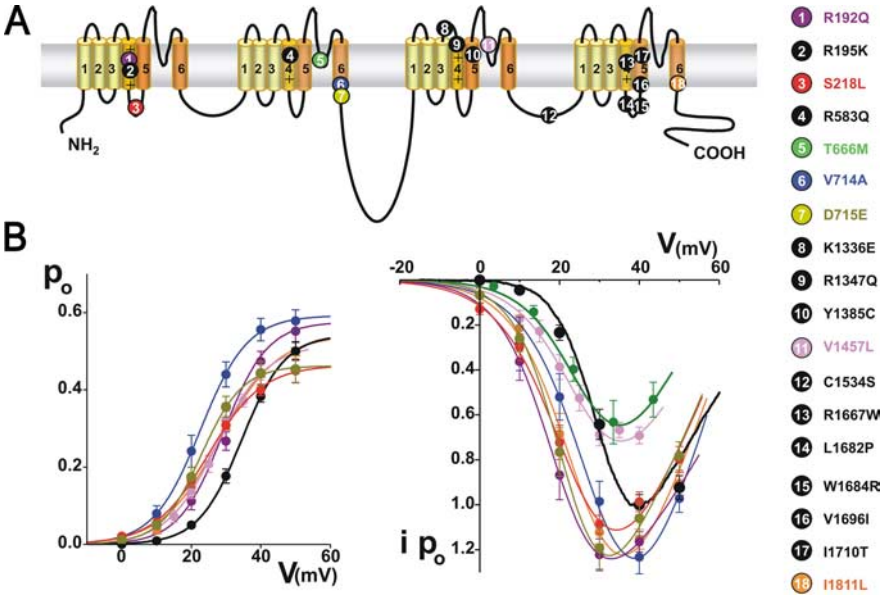

Figure 5. Mutations of $Ca_V2.1$ channels in patients with FHM1. ***A***, The positions of mutations causing FHM1 in the $\alpha 1$ subunit of CaV2.1 channels are illustrate by numbered circles, and the specific mutations are listed at the right. Mutations whose functional consequences on the single-channel properties of recombinant human $Ca_V2.1$ channels have been studied are shown in color (Hans et al., 1999; Tottene et al., 2002, 2005) (D. Pietrobon, unpublished observations). ***B***, Single-channel analysis (with 90 mM Ba^{2+}) revealed an increase of the open probability, p_o, and a corresponding increase in the single-channel Ca^{2+} influx as measured by the product of the unitary current, *i*, and p_o, over a broad voltage range, resulting in a shift to more negative voltages for activation of mutant channels. The thick black line represents the normalized whole-cell current–voltage curve (5 mM Ba^{2+}, shifted 26 mV toward more positive voltages to account for the difference in surface potential caused by 90 mM Ba^{2+}) (cf. Tottene et al., 2002).

The findings that CSD is facilitated in FHM1 mutant mouse models *in vivo* and that CSD facilitation correlates with the severity of the clinical phenotype of different FHM1 mutations support the idea that CSD plays a key role in migraine pathogenesis and suggest that FHM1 mutant mice may be very valuable tools to investigate the mechanisms underlying migraine and associated disorders. In particular, investigation of the cortical mechanisms that produce facilitation of CSD in FHM1 mouse models may provide unique insights into the unknown mechanisms that lead to CSD susceptibility and initiate migraine attacks in patients, as well as into the mechanisms underlying their hypersensitivity to intense, repetitive sensory stimulation in the interictal period (Pietrobon and Striessnig, 2003). To accomplish this aim, cortical synaptic transmission was investigated in R192Q mutant mice at synapses of cortical pyramidal cells in microculture and in connected pairs of layer 2/3 pyramidal cells and fast-spiking interneurons in acute thalamocortical slices. The results show increased strength of excitatory neurotransmission attributable to enhanced action potential-evoked Ca^{2+} influx through synaptic $Ca_V2.1$ channels and increased probability of glutamate release at pyramidal cell synapses of FHM1 mutant mice. At the same synapses, short-term depression during trains of action potentials was enhanced. There was no evidence of homeostatic compensatory mechanisms at excitatory synapses onto pyramidal cells (A. Tottene, R. Conti, and D. Pietrobon, unpublished). To investigate possible alterations of the cortical excitation–inhibition balance in FHM1, inhibitory neurotransmission was measured in connected pairs of multipolar fast-spiking interneurons and pyramidal cells in thalamocortical slices. Total inhibitory synaptic drive onto layer 2/3 pyramidal cells (in the absence of afferent stimulation) was also studied by measuring spontaneous inhibitory synaptic currents in the presence of ongoing network activity. Preliminary data from these experiments show unaltered strength of inhibitory neurotransmission in FHM1 mutant mice (R. Conti, A. Fabbro, and D. Pietrobon, unpublished).

These findings may explain CSD facilitation in FHM1 mice and point to tipping the finely tuned dynamic balance between excitation and inhibition toward excitation as the basis for increased propensity for CSD and abnormal processing of sensory information in migraine. Novel therapeutic strategies that consider CSD and cortical hyperexcitability as key targets of preventive migraine treatment promise to improve the lives of the large number of migraine sufferers worldwide.

Neuronal ion channelopathies and neurological disease

Nongenetic epilepsy, chronic pain, and migraine are among the most common neurological disorders, impairing the lives of millions of patients and placing a substantial burden on families, caregivers, and healthcare systems. Hyperexcitability is common to these diseases, but the alterations of excitability at the molecular, cellular, and systems levels that underlie their pathophysiology are not well understood. In contrast, the genetic forms of epilepsy, chronic pain, and migraine discussed here are rare diseases, but the well established genetic basis of their pathophysiology provides unique opportunities for studies of the mechanisms underlying their hyperexcitability at the molecular, cellular, and systems levels. A common theme from the research on these ge-

netic ion channelopathies reviewed here is that imbalance between excitability and inhibition is a key element in each case. Such imbalance can arise from loss-of-function mutations that impair action potential firing in inhibitory neurons in SMEI and possibly in GEFS+ epilepsy, gain-of-function mutations that enhance action potential firing in peripheral sensory neurons in chronic pain, and gain-of-function mutations that enhance synaptic transmission in cortical neurons in inherited migraine. Although these studies are still at an early stage, we are optimistic that additional work on these rare neuronal ion channelopathies will yield key insights that lead to deeper mechanistic understanding of the pathophysiology and eventually to novel and effective therapies for the more widespread nongenetic forms of epilepsy, chronic pain, and migraine.

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