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SCN8A: When Neurons Are So Excited, They Just Can't Hide It

Prominent role of forebrain excitatory neurons in SCN8A encephalopathy.

Bunton-Stasyshyn RKA, Wagnon JL, Wengert ER, Barker BS, Faulkner A, Wagley PK, Bhatia K, Jones JM, Maniaci MR, Parent JM, Goodkin HP, Patel MK, Meisler MH. *Brain*. 2019;142(2):362-375. doi:10.1093/brain/awy324.

De novo mutations of the sodium channel gene *SCN8A* result in an epileptic encephalopathy with refractory seizures, developmental delay, and elevated risk of sudden death. p.Arg1872Trp is a recurrent de novo SCN8A mutation reported in 14 unrelated individuals with epileptic encephalopathy that included seizure onset in the prenatal or infantile period and severe verbal and ambulatory comorbidities. The major biophysical effect of the mutation was previously shown to be impaired channel inactivation accompanied by increased current density. We have generated a conditional mouse mutation in which expression of this severe gain-of-function mutation is dependent upon Cre recombinase. Global activation of p.Arg1872Trp by EIIa-Cre resulted in convulsive seizures and lethality at 2 weeks of age. Neural activation of the p.Arg1872Trp mutation by Nestin-Cre also resulted in early-onset seizures and death. Restriction of p.Arg1872Trp expression to excitatory neurons using Emx1-Cre recapitulated seizures and juvenile lethality between 1 and 2 months of age. In contrast, activation of p.Arg1872Trp in inhibitory neurons by Gad2-Cre or Dlx5/6-Cre did not induce seizures or overt neurological dysfunction. The sodium channel modulator GS967/Prax330 prolonged survival of mice with global expression of R1872W and also modulated the activity of the mutant channel in transfected cells. Activation of the p.Arg1872Trp mutation in adult mice was sufficient to generate seizures and death, indicating that successful therapy will require lifelong treatment. These findings provide insight into the pathogenic mechanism of this gain-of-function mutation of SCN8A and identify excitatory neurons as critical targets for therapeutic intervention.

Commentary

Since its identification as an epilepsy-associated gene in 2012,[1] pathogenic *SCN8A* variants have been readily identified in patients spanning the spectrum from benign familial infantile seizures (BFIS), to an intermediate phenotype characterized by milder cognitive impairment and higher likelihood of seizure control, to the most severe developmental and epileptic encephalopathies (DEEs).[2-4] Given the preceding genotype–phenotype associations of *SCN1A, SCN2A*, and *SCN3A* with epilepsy and *SCN5A* with sudden unexpected death in epilepsy patients (SUDEP), the revelation that the *SCN8A* gene encoding $Na_v1.6$, one of the most robustly expressed voltage-gated sodium channels in the brain, is intolerant to genetic variation was not unexpected.

$Na_v1.6$ is expressed shortly after birth, in multiple neuronal classes and glia across the cortex, hippocampus, brain stem, and cerebellum, as well as in the heart and peripheral/lower motor neurons.[5-7] Localized to the axon initial segment and nodes of Ranvier, its roles in action potential generation and propagation are well-delineated.[8] Compared to other sodium channel subtypes, its near-exclusive representation at the action potential trigger zone and its relatively hyperpolarized activation voltage, suggest that $Na_v1.6$ is predominantly responsible for determining the action potential threshold.[9,10]

SCN8A pathogenic variants have been identified in a spectrum of patients with neurodevelopmental disorders and some generalizable genotype–phenotype correlations have emerged. For instance, *SCN8A* loss-of-function variants are linked to autistic-like features, developmental delay, and less severe epilepsies syndromes (e.g. BFIS, myoclonus, or absence).[11,12] Loss-of-function *SCN8A* variants associated with a single case of DEE but more commonly intellectual disability without seizures are suggested to result from impaired protein trafficking and channel stability at the membrane,[13,14] with the caveat that variability in channel biophysics measured in transformed cell lines compared to primary neurons is repeatedly reported.[11] $Na_v1.6$ in experimental mouse models is critical for survival. Med^{tg} mice, generated by nondirected transgene insertion into

Scn8a, have limited survival due to progressive muscle atrophy, early paralysis, and Purkinje cell degeneration.[15] Similarly, haploinsufficient Scn8a is protective in electroconvulsant and chemoconvulsant models of seizure susceptibility.[16] Moreover, prevention of Scn8a upregulation (using short hairpin RNAs) is also protective in animal models of seizure priming and delayed-onset epilepsy.[17]

In contrast, missense variants with gain-of-function channel biophysics when heterologously expressed are associated with DEE, hypotonia, and paroxysmal dyskinesia. DEE–associated variants clustering in the transmembrane domains, inactivation gate, and C-terminus overwhelmingly induce a hyperpolarizing shift in activation voltage and a shortened or destabilized inactivated state, manifesting primarily as increased persistent current. The first de novo pathogenic variant reported, p.Asn1768Asp (N1768D), was identified in a patient with a DEE and SUDEP and demonstrated enhanced persistent current in transfected hippocampal neurons.[1] A mouse model with the homologous N1768D variant displays 50% penetrant epilepsy, mild deficits in motor coordination and social tasks, and SUDEP.[18] In sum, multiple lines of evidence converge on SCN8A as a "Goldilocks" gene whereby too much or too little impacts proper neuronal function. However, until now, the principal cell types responsible for *SCN8A*-associated epilepsy in animal models represented a gap in our knowledge.

With the goal of dissecting the key pathogenic cell type in *SCN8A*-associated DEE, Bunton-Stasyshyn et al generated a conditional *Scn8a* epilepsy mouse model with TALEN-targeted messenger RNAs based upon the recurrent variant, p.Arg1872Trp (R1872W), impacting a key residue in the C-terminus responsible for stabilization of the inactivation gate.[19] Pathogenic variants at this position result in a DEE with comparably earlier seizure onset, higher risk of status epilepticus, and more profound changes in muscle tone and difficulty ambulating.[20] Insertion of a final exon containing R1872W with flanking loxP sites upstream of a synonymous exon without R1872W was used to target Cre-mediated recombination to defined cellular subpopulations. In contrast to the Scn8a-N1768D mouse, global Scn8a-R1872 W expression (using EIIa-Cre) resulted in fully penetrant seizures and mortality at 2 to 3 weeks, consistent with a comparably more severe phenotype in mice, just as in humans. Electrophysiologic recordings of excitatory neurons in the hippocampus and cortex both demonstrated intrinsic hyperexcitability. Recombination of the conditional allele in neurons alone (using Nestin-Cre) resulted in similarly early status epilepticus and death, though a $\sim$1 week delay suggests a non-neuronal (and likely cardiac) contribution to early mortality. Most interestingly, recombination of the conditional allele in primarily excitatory neurons (using Emx1-Cre) resulted in multiple seizure types around 6 weeks of age, while recombination in inhibitory neurons using either the Gad2- or Dlx5-Cre did not produce a phenotype. As the dogma of epilepsy-associated neuronal hypersynchrony is that seizures arise from an excitatory—inhibitory imbalance due to either excess excitation or insufficient inhibition, the results of these conditional recombination experiments convincingly implicate excitatory neurons as the essential effector of *SCN8A*-associated epilepsy. However, it should be noted that Emx1-Cre also drives expression in some glia[21] and that this confounding factor cannot be eliminated in this model.

Though expression of *SCN8A* occurs early in postnatal development,[5] dissecting the circuit-level consequences of early seizures from the impact of persistent hyperexcitation is not easily attainable in a patient or a conventional knock-in transgenic mouse model. This question has some clinical relevance in predicting whether patients who successfully achieve seizure freedom early in life can wean anticonvulsants, such as in *SCN2A*-associated benign familial neonatal and infantile seizures versus patients with juvenile myoclonic or absence epilepsy who often require lifelong medication. Using tamoxifen-inducible global recombination driven by a CAG-Cre, adult mice fed tamoxifen at 8 weeks old developed severe epilepsy and SUDEP approximately 2 weeks later. From these data, it appears that *SCN8A* is critical not only to proper neuronal development but also to maintenance of normal neuronal circuit activity.

Overall, these studies evidence a dichotomy of the pathogenic mechanisms that may underlie DEEs. Here, Bunton-Stasyshyn and colleagues have demonstrated that a gain-of-function pathogenic variant in $Na_v1.6$ leads to hyperactivity primarily in excitatory neurons. This is in contrast to *SCN1A*-associated DEE (primarily, Dravet syndrome) caused by $Na_v1.1$ haploinsufficiency where impaired function of inhibitory neurons is proposed to underlie hyperexcitability.[22] These early studies in mice, as well as similar observations in other genetic and acquired models (e.g. see the study by Price et al[23]), have led to the prevailing "interneuronopathy hypothesis" as the pathogenic mechanism in epilepsy. However, proof that either maladapted excitatory or inhibitory neurons may serve as drivers of epilepsy pathogenesis has now been demonstrated in a number of cellular and animal models,[24,25] warranting careful consideration of activity-modulating anticonvulsants. This current study provides conclusive evidence that excitatory neurons play an important role in *SCN8A*-associated DEE, a finding that will likely hold true for a broader spectrum of epilepsies. These results have important implications for novel therapies moving forward, particularly as the field considers new treatments on the horizon to target the "channelome" of specific neuronal subtypes.

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