



It Takes Two to Tango: Channel Interplay Leads to Paradoxical Hyperexcitability in a Loss-of-Function Epilepsy Variant

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Paradoxical hyperexcitability from NaV1.2 sodium channel loss in neocortical pyramidal cells

Spratt, PWE, Alexander, RPD, Ben-Shalom, R, Sahagun, A, Kyoung, H, Keeshen, CM, Sanders, SJ, Bender, KJ. *Cell Rep.* 2021; 36(5):109483.

Loss-of-function variants in the gene *SCN2A*, which encodes the sodium channel Na_v1.2, are strongly associated with autism spectrum disorder and intellectual disability. An estimated 20%–30% of children with these variants also suffer from epilepsy, with altered neuronal activity originating in neocortex, a region where Na_v1.2 channels are expressed predominantly in excitatory pyramidal cells. This is paradoxical, as sodium channel loss in excitatory cells would be expected to dampen neocortical activity rather than promote seizure. Here, we examined pyramidal neurons lacking Na_v1.2 channels and found that they were intrinsically hyperexcitable, firing high-frequency bursts of action potentials despite decrements in action potential size and speed. Compartmental modeling and dynamic-clamp recordings revealed that Na_v1.2 loss prevented potassium channels from properly repolarizing neurons between APs, increasing overall excitability by allowing neurons to reach threshold for subsequent APs more rapidly. This cell-intrinsic mechanism may, therefore, account for why *SCN2A* loss-of-function can paradoxically promote seizure.

Commentary

Sodium channelopathies are caused by mutations in genes that encode the subunits of voltage-gated sodium channels, which are integral to maintaining proper action potential (AP) initiation and propagation. Mutations often lead to neurodevelopmental disorders, intellectual disability, and epilepsy syndromes. In most epilepsy syndromes associated with a sodium channel mutation, epilepsy manifests from either gain-of-function (GoF) mutations of *SCN8A*, primarily affecting excitatory neurons but also in inhibitory neurons, or loss-of-function (LoF) mutations of *SCN1A*, primarily affecting inhibitory neurons, with some exceptions.¹ Interestingly, *SCN2A* epileptic encephalopathy is most often associated with GoF variants in the Na_v1.2 sodium channel, yet approximately 20–30% of patients with *SCN2A* LoF variants also develop epilepsy.²

In a recent report,³ Spratt and colleagues used mice with a conditional heterozygous deletion of *Scn2a* (*Scn2a*^{+/-}) in an attempt to recapitulate the phenotype seen in *Scn2a* LoF patients and a homozygous deletion (*Scn2a*^{-/-}) to further understand the impact of a complete loss of *Scn2a* on seizure susceptibility. The study focused on neocortical pyramidal cells since these neurons are thought to be the origin of seizures in mouse models of *SCN2A*.⁴ Using whole-cell electrophysiology recordings, the authors found that *Scn2a*^{-/-} neurons were hyperexcitable, with a pronounced burstlet of 2–3 APs at the onset of current injection. To establish if *Scn2a* deletion had any effect on AP initiation

and propagation, the authors simultaneously recorded from the soma and axonal bleb of the same neuron and showed no difference in terms of AP initiation. Elegant calcium imaging of axonal boutons confirmed AP propagation to synapses in *Scn2a*^{-/-} neurons. These findings suggest that processes mediated by *Scn8a* (Na_v1.6), such as AP initiation and propagation, remain intact even with loss of *Scn2a* (Na_v1.2).

Detailed analysis of somatic APs revealed that AP amplitude and upstroke velocity were decreased in *Scn2a*^{-/-} neurons, signifying that the somatodendritic components to the AP were affected by the partial or complete loss of Na_v1.2. Consistent with their findings, calcium transients in the dendrites were also significantly reduced. These results, along with findings that *Scn2a*^{-/-} neurons are hyperexcitable, indicate a seemingly opposing effect on axonal and dendritic function in *Scn2a*^{-/-} neurons. Dendritic function is understandably impaired due to the fact that Na_v1.2 is expressed in the dendrites and is critical for AP backpropagation to the somatodendritic region.⁵ Additionally, repolarization speed in *Scn2a*^{-/-} cells was significantly reduced and interspike afterhyperpolarization (AHP) levels were more depolarized, suggesting a reduction in repolarizing potassium (K) currents, a likely consequence of decreased AP amplitude leading to a reduction in the driving force for K⁺ ions. The authors propose a mechanism in which a more depolarized AHP allows neurons to reach the AP threshold more quickly, initiating more APs and resulting in neuronal hyperexcitability.





Using compartmental modeling, Spratt and colleagues recapitulated neuronal hyperexcitability and AP morphology changes associated with *Scn2a* reduction and deletion. They also assessed the potential impact of K current activity on neuronal excitability and found an exponential relationship between $\text{Na}_v1.2$ expression and K current, somewhat validating their results from electrophysiological recordings, as *Scn2a*^{+/-} neurons more closely resembled wild-type neurons than *Scn2a*^{-/-} neurons. Increasing K channel density rescued hyperexcitability of *Scn2a*^{-/-} cells, while decreasing K channel density increased excitability in wild-type cells. Considering the ability of increased K channel density to rescue *Scn2a*^{-/-} hyperexcitability in a compartmental model, the authors then applied this to whole-cell recordings using a dynamic clamp, allowing them to inject conductances to resemble those from $\text{Na}_v1.2$ and K channels. This approach rescued the hyperexcitability and burst-firing phenotype seen in the *Scn2a*^{-/-} cells.

In summary, Spratt and colleagues provide a novel mechanism behind the somewhat contradictory phenomenon that a loss-of-function *SCN2A* mutation leads to hyperexcitability. Their proposed mechanism identifies a potential role for the interplay between sodium channels and K channels in *Scn2a* conditional knockout mice. The authors show that hyperexcitability is likely not due to any effects on action potential initiation and is specific to somatodendritic defects, indicating a sort of independence between $\text{Na}_v1.2$ and $\text{Na}_v1.6$ channels, and identifying a new piece to the puzzle, namely, K channels. Genetic mosaicism would likely result in patient phenotypes more similar to *Scn2a*^{+/-} rather than *Scn2a*^{-/-}, but an exponential relationship between $\text{Na}_v1.2$ and K channel currents provides a plausible explanation for the incidence of seizures in 20–30% of *SCN2A* LoF patients. Additionally, loss-of-function *SCN2A* patients are often not diagnosed until about 12 months of age, which corresponds with a developmental switch in the patterning of $\text{Na}_v1.2$ and $\text{Na}_v1.6$.⁵ This raises questions about the interplay between both types of sodium channels along with any interaction they may have with K channels to coincide with the change in patterning.

While this study introduces K channels as a potential solution to this paradoxical concept, the potential contribution of other ion channels may also be important. Here, the complete loss of *Scn2a* led to an increase in $\text{Na}_v1.6$ axon initial segment staining and persistent sodium current, similar to previous work showing $\text{Na}_v1.2$ compensation for loss of $\text{Na}_v1.6$.⁶ Recordings of whole-cell sodium channel currents, either from isolated neurons or somatic nucleated patches, would have provided more insight into the relative contributions of *Scn2a* and *Scn8a* to somatic sodium channel currents, especially considering the reductions in AP amplitude and upstroke velocity observed. Moreover, their data show AP burstlets in *Scn2a*^{-/-} neurons that their compartmental model fails to reproduce. AP burstlets seen at the onset of current injection in *Scn2a*^{-/-} mice could be a consequence of increased $\text{Na}_v1.6$ activity⁷ or of other ion channels such as T-type calcium currents that are known to facilitate AP bursting.⁸

Seizure treatment in *SCN2A* epileptic encephalopathy currently varies greatly due to a large phenotypic spectrum. However, previous studies have shown striking differences in response to different anti-epileptic drugs (AEDs) based on age of seizure onset, particularly that patients with later onset of seizures (> 3 months), which may correspond somewhat to patients with LoF mutations, respond poorly to sodium channel blockers such as phenytoin.⁹ This study by Spratt et al offers an important step in demonstrating interactions between various channel types as a consequence for the loss of a specific channel function and opens the door for future studies to explore pharmacological mediation and novel therapeutics in *SCN2A* loss-of-function epilepsy. Based on these results showing an important role for K channels in facilitating neuronal hyperexcitability in *Scn2a*^{-/-} neurons, an interesting potential target for treatment may be the potentiation of dendritically expressed K channels, such as $\text{K}_v4.2$ channels, which are known to modulate A-type currents and mediate action potential back-propagation.¹⁰ Overall, to develop more mechanistically precise therapeutics for *SCN2A* and other epileptic encephalopathies, it will be critical to further understand the interplay between various ion channels and how this affects the neuronal network as a whole.

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