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Spotlight

The Na_Vy paradox: reducing sodium currents increases excitability

Leonard K. Kaczmarek [1,2,*]

Mutations that increase sodium currents in excitatory neurons typically produce hyperexcitability and epileptic seizures. Paradoxically, mutations that reduce $Na_V1.2$ sodium currents also have a similar effect. Two research groups (Spratt *et al.* and Zhang *et al.*) have now found that in some excitatory neurons, loss of $Na_V1.2$ increases intrinsic excitability by altering activation and/or expression of potassium channels.

Neuroscience students learn early on that action potentials result from the combined action of voltage-dependent sodium and potassium channels. While the original Nobel Prize-winning biophysical description of this, provided some 70 years ago [1], is quite detailed, the basic concepts are relatively simple. The opening of sodium channels depolarizes a neuron, leading to the firing of action potentials, while opening of potassium channels hyperpolarizes the membrane, suppresses excitability, and provides a relative refractory period after each action potential.

Since that time, a wealth of data has supported the notion that increases in sodium current promote excitation. Clearly, blockade of sodium channels eliminates neuronal firing. Nine voltage-dependent sodium channels have been identified, four of which, $Na_V1.1$, $Na_V1.2$, $Na_V1.3$, and $Na_V1.6$, provide sodium current for CNS neurons. Phosphorylation of Na_V subunits can reduce their currents, raising the threshold for action potential generation and suppressing firing [2]. Conversely, genetic mutations of Na_V subunits that increase sodium currents in excitatory neurons, often by preventing channel inactivation, can produce uncontrolled firing leading to epileptic seizures [3,4]. Finally, some sodium channels enter a mode termed persistent sodium current (INaP), that does not inactivate. Stimuli or mutations that increase INaP invariably depolarize neurons to increase firing rates, promoting hyperexcitability and epileptic seizures [5].

In the cerebral cortex, $Na_V1.2$ is expressed primarily in excitatory pyramidal neurons. Consistent with concepts outlined in the previous paragraph, mutations of $Na_V1.2$ that increase sodium current produce several types of early-onset epilepsies [6]. Surprisingly, however, a significant proportion of patients with mutations that cause loss of $Na_V1.2$ current also develop childhood-onset seizures, as well as severe intellectual disability. The seizure phenotype has also been recapitulated in animal models. Specifically, seizures occur in $Na_V1.2^{+/-}$ mice lacking one copy of the channel gene. Such seizures would be simple to explain if $Na_V1.2$ were expressed in GABAergic interneurons, preventing their ability to inhibit pyramidal cells, but interneurons primarily express $Na_V1.1$. Thus, one is left with the conundrum that a loss of sodium current in pyramidal cells increases overall cortical excitability, resulting in seizures. (In fact, a similar conundrum exists for potassium channels, with mutations that either increase or decrease current, causing seizures [7].)

Two manuscripts published recently in *Cell Reports* directly address this quandary [8,9]. Both reports come to the same counterintuitive conclusion that the intrinsic excitability of excitatory neurons is enhanced by suppressing $Na_V1.2$ current. The two studies, however, used different approaches to overcome the fact that complete knockout of $Na_V1.2$ channels is lethal, while little change in excitability is detected in $Na_V1.2^{+/-}$ mice. Spratt *et al.* [8] used a conditional knockout model in which $Na_V1.2$ expression was completely deleted after animals reached adulthood, while Zhang *et al.* [9] used gene trapping to generate mice in which $Na_V1.2$ channels were present but their levels reduced to ~20–30% of those of the wild type.

Complete elimination of $Na_V1.2$, which in adult animals is found selectively at the soma of neurons and dendrites, does not prevent firing because another channel, $Na_V1.6$, is present at the axon initial segment where action potentials are triggered (Figure 1). Recording from cortical pyramidal neurons [8,9] and medium spiny neurons of the striatum [9], both studies found, however, that loss of $Na_V1.2$ resulted in action potentials that were smaller in amplitude and wider than those of controls, and that afterhyperpolarizations following each action potential were reduced. Importantly, these changes were associated with increased rates of firing in response to depolarization. Because axonal conduction is intact, this means that the overall intrinsic excitability of these neurons is increased by loss of $Na_V1.2$.

While both groups provide data to support the notion that potassium currents are key to the increase in excitability, this is where the findings and conclusions of the two groups diverge. In voltage-clamp experiments, Spratt *et al.* found no overall change in potassium currents and only minor changes in other currents or input resistance on deletion of $Na_V1.2$. The fact that the action potentials are smaller in amplitude means, however, that they trigger less voltage-dependent potassium current. Using a combination of numerical computation and dynamic clamp experiments, they build a compelling case that this alone increases overall firing rate, because of the more depolarized membrane potential between spikes. Essentially, the reduction in $Na_V1.2$ reduces the relative refractory period. It is worth noting that

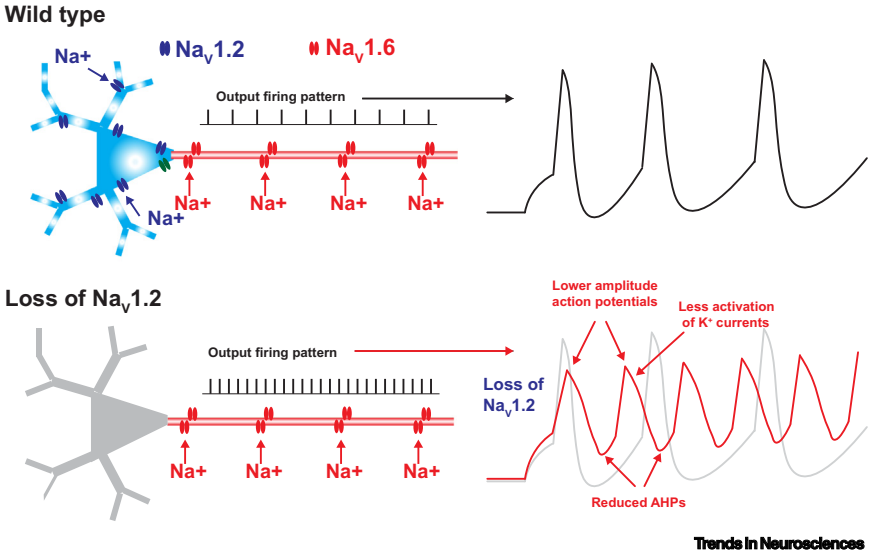

Figure 1. Loss of $Na_V1.2$ channels at the soma and dendrites of excitatory neurons increases neuronal output. Top panel shows relative distribution of $Na_V1.2$ and $Na_V1.6$ subunits and sites of sodium influx through the two types of channel, depicted in blue and red, respectively. The bottom panel shows that loss of $Na_V1.2$ subunits abolishes sodium currents at the soma and dendrites (essentially reducing the overall excitability of the somato-dendritic compartment). Nevertheless, as demonstrated in two recent studies [8,9], because the truncated action potentials at the soma activate less potassium conductance, the membrane potential between spikes becomes more positive, triggering a higher rate of firing along the axon. Abbreviation: AHP, afterhyperpolarization.

this is not a universal effect of reduced sodium current but requires specific combinations of channel kinetics that apply in these neurons.

By contrast, Zhang *et al*. found a clear reduction in potassium currents measured in voltage-clamp experiments on neurons with reduced levels of $Na_V1.2$. Moreover, in RNA-seq studies, they found significant decreases in expression of several potassium channel subunits. Intriguingly, although some changes were detected in the well-characterized and ubiquitous $K_V1.1$ and $K_V1.2$ delayed-rectifier channel subunits, the largest changes detected were in a channel modifier subunit MirP1 and in so called 'silent subunits' such as $K_V6.4$ and $K_V8.1$. The latter do not form functional channels by themselves but co-assemble with other K_V subunits to alter or suppress their currents. The biological role of these modifier/subunits is poorly understood and it is not yet known how they contribute to altered excitability. Convincingly, however, both the changes in potassium channel mRNAs and normal excitability were rescued when $Na_V1.2$ was partially restored using adenovirus-mediated transduction [9].

How does one reconcile these two apparently different findings about changes in potassium channels following loss of $Na_V1.2$ channels? Among many possibilities, an intriguing one is that $Na_V1.2$ subunits, which are still retained in the gene-trap mice, are themselves a component of the signaling pathway that alters expression of potassium channels. This would be consistent with evidence that sodium channels have biochemical functions independent of ion conduction [10].

The discrepancy between the two groups also raises a fundamental, but as yet unanswered, question in neuroscience. How do neurons coordinate the expression of hundreds of channel genes to prevent pathological firing patterns while allowing modulation of excitability for adaptation to sensory stimuli and for certain forms of learning and memory? Studies like the present ones, which probe this question with naturally occurring and investigator-designed mutations, may eventually provide an answer.

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Declaration of interests

The author declares no competing interests in relation to this work.

[1]Department of Pharmacology, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06520, USA
[2]Department of Cellular and Molecular Physiology, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06520, USA

*Correspondence:
leonard.kaczmarek@yale.edu (L.K. Kaczmarek).

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References

1. Hodgkin, A.L. and Huxley, A.F. (1952) A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol.* 117, 500–544
2. Scheuer, T. (2011) Regulation of sodium channel activity by phosphorylation. *Semin. Cell Dev. Biol.* 22, 160–165
3. Catterall, W.A. (2014) Sodium channels, inherited epilepsy, and antiepileptic drugs. *Annu. Rev. Pharmacol. Toxicol.* 54, 317–338
4. Kaplan, D.I. *et al.* (2016) Role of sodium channels in epilepsy. *Cold Spring Harb. Perspect. Med.* 6, a022814
5. Wengert, E.R. and Patel, M.K. (2021) The role of the persistent sodium current in epilepsy. *Epilepsy Curr.* 21, 40–47
6. Sanders, S.J. *et al.* (2018) Progress in understanding and treating SCN2A-mediated disorders. *Trends Neurosci.* 41, 442–456
7. Niday, Z. and Tzingounis, A.V. (2018) Potassium channel gain of function in epilepsy: an unresolved paradox. *Neuroscientist* 24, 368–380
8. Spratt, P.W.E. *et al.* (2021) Paradoxical hyperexcitability from NaV1.2 sodium channel loss in neocortical pyramidal cells. *Cell Rep.* 36, 109483
9. Zhang, J. *et al.* (2021) Severe deficiency of voltage-gated sodium channel NaV1.2 elevates neuronal excitability in adult mice. *Cell Rep.* 36, 109495
10. Kruger, L.C. and Isom, L.L. (2016) Voltage-gated Na+ channels: not just for conduction. *Cold Spring Harb. Perspect. Biol.* 8, a029264