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# Dravet Syndrome: A Developmental and Epileptic Encephalopathy

**Selective Nav1.1 Activation Rescues Dravet Syndrome Mice From Seizures and Premature Death**

Richards KL, Milligan CJ, Richardson RJ, Jancovski N, Grunnet M, Jacobson LH, Undheim EAB, Mobli M, Chow CY, Herzig V, Csoti A, Panyi G, Reid CA, King GF, Petrou S. *PNAS*. 2018;115:E8077-E8085. doi:10.1073/pnas.18047641l5

Dravet syndrome is a catastrophic, pharmaco-resistant epileptic encephalopathy. Disease onset occurs in the first year of life, followed by developmental delay with cognitive and behavioral dysfunction and substantially elevated risk of premature death. The majority of affected individuals harbor a loss-of-function mutation in one allele of*SCN1A*, which encodes the voltage-gated sodium channel $Na_v1.1$. Brain Nav1.1 is primarily localized to fast-spiking inhibitory interneurons; thus, the mechanism of epileptogenesis in Dravet syndrome is hypothesized to be reduced inhibitory neurotransmission leading to brain hyperexcitability. We show that selective activation of $Na_v1.1$ by venom peptide Hm1a restores the function of inhibitory interneurons from Dravet syndrome mice without affecting the firing of excitatory neurons. Intracerebroventricular infusion of Hm1a rescues Dravet syndrome mice from seizures and premature death. This precision medicine approach, which specifically targets the molecular deficit in Dravet syndrome, presents an opportunity for treatment of this intractable epilepsy.

**A Transient Developmental Window of Fast-Spiking Interneuron Dysfunction in a Mouse Model of Dravet Syndrome**

Favero M, Sotuyo NP, Lopez E, Kearney JA, Goldberg EM. *J Neurosci*. 2018;38:7912-7927. doi:10.1523/JNEUROSCI.0193-18.2018

Dravet syndrome is a severe childhood-onset epilepsy largely due to heterozygous loss-of-function mutation of the gene *SCN1A*, which encodes the type 1 neuronal voltage-gated sodium ($Na^+$) channel $\alpha$ subunit $Na_v1.1$. Prior studies in mouse models of Dravet syndrome (*Scn1a*$^{+/-}$ mice) indicate that, in cerebral cortex, $Na_v1.1$ is predominantly expressed in GABAergic interneurons, in particular in parvalbumin-positive fast-spiking basket cell interneurons (PVINs). This has led to a model of Dravet syndrome pathogenesis in which Nav1.1 mutation leads to preferential dysfunction of interneurons, decreased synaptic inhibition, hyperexcitability, and epilepsy. However, such studies have been implemented at early developmental time points. Here, we performed electrophysiological recordings in acute brain slices prepared from male and female *Scn1a*$^{+/-}$mice as well as age-matched wild-type littermate controls and found that, later in development, the excitability of PVINs had normalized. Analysis of action potential waveforms indirectly suggests a reorganization of axonal $Na^+$ channels in PVINs from *Scn1a*$^{+/-}$ mice, a finding supported by immunohistochemical data showing elongation of the axon initial segment. Our results imply that transient impairment of action potential generation by PVINs may contribute to the initial appearance of epilepsy, but is not the mechanism of ongoing, chronic epilepsy in Dravet syndrome. Significance Statement: Dravet syndrome is characterized by normal early development, temperature-sensitive seizures in infancy, progression to treatment-resistant epilepsy, developmental delay, autism, and sudden unexplained death due to mutation in *SCN1A* encoding the $Na^+$ channel subunit Nav1.1. Prior work has revealed a preferential impact of $Na_v1.1$ loss on the function of GABAergic inhibitory interneurons. However, such data derive exclusively from recordings of neurons in young *Scn1a*$^{+/-}$ mice. Here, we show that impaired action potential generation observed in parvalbumin-positive fast-spiking interneurons (PVINs) in *Scn1a*$^{+/-}$ mice during early development has normalized by postnatal day 35. This work suggests that a transient impairment of PVINs contributes to epilepsy onset, but is not the mechanism of ongoing, chronic epilepsy in Dravet syndrome.

## Commentary

Dravet syndrome (DS) is a catastrophic developmental and epileptic encephalopathy with cognitive, behavioral, and motor impairments, as well as a high risk of sudden unexpected death in epilepsy (SUDEP).[1,2] Most DS patients have *de novo* mutations in *SCN1A*, encoding the $\alpha$ subunit of the voltage-gated

sodium channel $Na_v1.1$, resulting in haploinsufficiency.[3] Exciting new research shows evidence for therapeutic rescue of seizures and SUDEP in a DS mouse model following selective activation of $Na_v1.1$ channels by a spider venom peptide, providing hope for patients with DS and their families. However, other recent work demonstrates that there may be only a transient, developmental window of fast-spiking interneuron dysfunction in DS mouse brain, suggesting that drugs targeting $Na_v1.1$ activation may be effective only during a defined time window in early postnatal brain development, while potentially producing adverse effects at later time points.

Catterall and colleagues were the first to develop a mouse model of DS, demonstrating that *Scn1a* haploinsufficiency in mice results in seizures.[4] They showed that postnatal day (P) 14-16 $Scn1a^{+/-}$ hippocampal bipolar neurons, but not pyramidal neurons, had significantly reduced sodium current density, resulting in reduced interneuron firing frequency. Further work showed that *Dlx1/2*Cre-mediated inactivation of *Scn1a* in parvalbumin-positive (PV+) fast-spiking interneurons phenocopied the severe seizures and SUDEP.[5] This work led to the hypothesis that pathophysiology in DS could be explained by disinhibition, or dysfunctional inhibitory circuits, that involved PV+ fast-spiking interneurons.

Dravet syndrome is pharmacoresistant.[6] Moreover, traditional antiepileptic drugs that inhibit the activity of pan sodium channels in brain, for example, carbamazepine or phenytoin, can exacerbate seizures in patients with DS. Taking a novel approach to drug discovery in DS, Petrou and colleagues turned to natural products to test a selective $Na_v1.1$ activating agent. Venom from the tarantula *Heteroscodra maculate* contains a toxin called $\delta$-theraphotoxin-Hm1a ("Hm1a").[7] Previous work from other investigators, and confirmed in the Petrou study, showed that Hm1a administration to heterologous cells or primary neurons selectively activates $Na_v1.1$ via a mechanism that delays fast inactivation and enhances the level of persistent sodium current.[7] The Petrou group showed that application of Hm1a to mouse brain slices rescued hippocampal CA1 PV+ fast-spiking interneuron function in $Scn1a^{+/-}$ DS mice but, surprisingly, had no effect on CA1 inhibitory neuron firing in WT littermate mice or on excitatory CA1 pyramidal neurons in DS mice. Importantly, acute introcerebroventricular (ICV) infusion of Hm1a into the brains of $Scn1a^{+/-}$ DS mice that showed epileptiform activity at P19, prior to infusion, reduced whole-brain hyperexcitability, measured as mean spike frequency by electrocorticography, postadministration. A 4-day ICV infusion of Hm1a beginning at P18 into the brains of $Scn1a^{+/-}$ DS mice in which 2 or more seizures had been recorded prior to infusion resulted in significantly reduced or abolished seizure frequency and significantly reduced mortality. Remarkably, 6 of 9 DS mice were rescued after 3 days of Hm1a infusion. In contrast, by day 3, all vehicle-treated DS mice were dead. Hm1a-treated $Scn1a^{+/-}$ animals were not monitored longer than 4 days, thus it is not yet known whether the beneficial effects of Hm1a persisted. The authors did not test the safety profile of Hm1a infusion into the brains of WT mice or $Scn1a^{+/-}$ that did not exhibit seizures. A potential caveat to the development of Hm1a as a therapeutic agent is that activation of $Na_v1.1$ channels by this toxin is not selective to the brain. If delivered systemically, the toxin can activate $Na_v1.1$ channels all over the body, for example, mechanosensitive nociceptors in the gut, resulting in acute pain and mechanical allodynia,[7] illustrating the tissue-specific complexity of sodium channel gene expression and sodium channel pharmacology.

While the work from the Petrou group is encouraging, growing evidence suggests that $Na_v1.1$ activating drugs may be beneficial in the brain only during a short developmental window of time, at least in mice. The Catterall group showed selective reduction of interneuron sodium current and firing rate at P14 in $Scn1a^{+/-}$mouse hippocampus.[4] However, in subsequent work, George, Kearney, and colleagues demonstrated that sodium current density was increased in $Scn1a^{+/-}$ hippocampal pyramidal neurons, but not in GABAergic interneurons, at P21-24.[8] In addition, $Scn1a^{+/-}$P21-24 pyramidal neurons exhibited spontaneous firing and hyperexcitability. This work suggested that compensatory overexpression of one or more sodium channel genes may occur in DS brain in response to *Scn1a* haploinsufficiency by P21, contributing to the mechanism of epileptogenesis. To add to this complexity, new work by Goldberg and colleagues examines fast-spiking PV+ interneuron excitability over the life time of $Scn1a^{+/-}$ animals. They show that during early postnatal development (P10), there are no differences in excitability between $Scn1a^{+/+}$ and $Scn1a^{+/-}$ fast-spiking PV+ interneurons in layer 2/3 of the somatosensory cortex. At P18-20, they observed similar hypoexcitability of $Scn1a^{+/-}$ layer 2/3 fast-spiking PV+ interneurons as reported by the Catterall group in the hippocampus of P14-16 mice. Surprisingly however, in adult mice (P35-56), no changes in layer 2/3 fast-spiking PV+ interneuron excitability between genotypes could be detected, suggesting that interneuron excitability normalizes in DS mouse brain with development. A caveat to the Goldberg et al work is that only $\sim$50% of $Scn1a^{+/-}$ mice on the (C57BL/6J $\times$ 129S6/SvEvTac)F1 strain develop the DS phenotype.[9] By P35, a large percentage of $Scn1a^{+/-}$ mice that have developed seizures have died. It is likely that the cohort of mice that showed seizure activity by P18-19, as defined in the Petrou et al study, would have died by P35 and thus are not represented in the Goldberg et al study. Thus, the 2 research studies may not have analyzed similar subpopulations of $Scn1a^{+/-}$ DS mice, in spite of similar breeding strategies. Nevertheless, it would be interesting to test whether ICV infusion of Hm1a in DS brains beginning at P21 would normalize the compensatory sodium channel overexpression and hyperexcitability reported by George and Kearny[8] or whether this treatment would exacerbate hyperexcitability and worsen the disease.

Finally, mice and humans develop on very different time scales. In patients with DS, symptoms typically present toward the end of the first year of life, which for mice is approximately equivalent to the time of weaning ($\sim$P21-P28). According to the work of George and Kearney,[8] compensatory overexpression of other sodium channel genes has occurred by this time point. Is it too late to administer $Na_v1.1$ activating drugs or to

perform genetic manipulations to increase $Na_v1.1$ expression? Could this type of pharmacological intervention in pediatric patients with DS older than 1 year be detrimental? An essential experiment that is missing from the literature is to reactivate *Scn1a* gene expression after the critical period of brain development in $Scn1a^{+/-}$ DS mice. Can the pathophysiology be rescued? Are compensatory changes that occur in the brain in response to *Scn1a* haploinsufficiency reversible? A problem with this proposed experimental strategy is that the short time span from disease onset to SUDEP in the $Scn1a^{+/-}$mouse model on the (C57BL/6J × 129S6/SvEvTac)F1 strain may not allow sufficient time to reactivate *Scn1a* expression between disease onset and death. New DS models will be needed to test this hypothesis and to gain this critical information for effective therapeutic discovery in DS.

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