



Cognitive and Social Impairment in Mouse Models Mirrors Dravet Syndrome

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Han S, Tai C, Westenbroek RE, Yu FH, Cheah CS, Potter GB, Rubenstein JL, Scheuer T, de la Iglesia HO, Catterall WA. Autistic-Like Behavior in *Scn1a*^{+/-} Mice and Rescue by Enhanced GABA-Mediated Neurotransmission [published online ahead of print]. *Nature* 2012;489:385–390. doi: 10.1038/nature11356.

Haploinsufficiency of the SCN1A gene encoding voltage-gated sodium channel NaV1.1 causes Dravet syndrome, a childhood neuropsychiatric disorder that includes recurrent intractable seizures, cognitive deficit, and autism-spectrum behaviors. The neural mechanisms responsible for cognitive deficit and autism-spectrum behaviors in Dravet syndrome are poorly understood. Here, the authors report that mice with SCN1A haploinsufficiency exhibit hyperactivity, stereotyped behaviors, social interaction deficits, and impaired context-dependent spatial memory. Olfactory sensitivity is retained, but novel food odors and social odors are aversive to *Scn1a*^{+/-} mice. GABAergic neurotransmission is specifically impaired by this mutation, and selective deletion of NaV1.1 channels in forebrain interneurons is sufficient to cause these behavioral and cognitive impairments. Remarkably, treatment with low-dose clonazepam, a positive allosteric modulator of GABA_A receptors, completely rescued abnormal social behaviors and deficits in fear memory in the mouse model of Dravet syndrome, demonstrating that they are caused by impaired GABAergic neurotransmission and not by neuronal damage from recurrent seizures. These results demonstrate a critical role for NaV1.1 channels in neuropsychiatric functions and provide a potential therapeutic strategy for cognitive deficit and autism-spectrum behaviors in Dravet syndrome.

Ito S, Ogiwara I, Yamada K, Miyamoto H, Hensch TK, Osawa M, Yamakawa K. Mouse with NaV 1.1 Haploinsufficiency, a Model for Dravet Syndrome, Exhibits Lowered Sociability and Learning Impairment [published online ahead of print]. *Neurobiol Dis.* 2012;49C:29–40. doi: 10.1016/j.nbd.2012.08.003.

Dravet syndrome is an intractable epileptic encephalopathy characterized by early onset epileptic seizures and followed by cognitive decline, hyperactivity, autistic behaviors, and ataxia. Most Dravet syndrome patients possess heterozygous mutations in SCN1A gene encoding voltage-gated sodium channel α_1 subunit (NaV1.1). We have previously reported that mice heterozygous for a nonsense mutation in SCN1A develop early onset epileptic seizures. However, the learning ability and sociability of the mice remained to be investigated. In the present study, we subjected heterozygous SCN1A mice to a comprehensive behavioral test battery. We found that while heterozygous SCN1A mice had lowered spontaneous motor activity in their home cage, they were hyperactive in novel environments. Moreover, the mice had low sociability and poor spatial learning ability corresponding to autistic behaviors and cognitive decline seen in Dravet syndrome patients. These results suggested that NaV1.1 haploinsufficiency intrinsically contributes not only to epileptic seizures but also lowered sociability and learning impairment in heterozygous SCN1A mutant mice, as in cases of patients with Dravet syndrome.

Commentary

Dravet syndrome is an infant-onset epileptic encephalopathy characterized by generalized clonic, tonic-clonic, or hemi-clonic seizures. Patients subsequently develop other types of seizures, including myoclonic, absence, or partial seizures. Sei-

zures are often refractory to conventional antiepileptic drugs and lack of adequate seizure control is correlated with poor outcomes. Development is normal prior to seizure onset, but once seizures begin, there is disease progression accompanied by a decline of cognitive development and often by autistic-like behaviors (1, 2). The most commonly reported behavioral co-morbidities are poor language ability, hyperactivity, and impaired social behavior (3).

Two mouse models of Dravet syndrome were recently evaluated for comorbid cognitive and behavioral deficits.

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The models are an *Scn1a*^{+/-} knockout mouse developed in the laboratory of Dr. William Catterall (4) and a mouse engineered with the Dravet syndrome mutation SCN1A-R1407X (*Scn1a*^{RX/+}) developed in the laboratory of Dr. Kazuhiro Yamakawa (5). Both models exhibit similar epilepsy-related phenotypes, with spontaneous generalized tonic-clonic seizures and lowered threshold to hyperthermia-induced seizures (4, 5, 6). In the current studies, both groups sought to determine if their models recapitulated behavioral and cognitive comorbidities associated with Dravet syndrome. To do this, they performed large batteries of neurobehavioral tests in adult mice on a C57BL/6J strain background.

Hyperactivity is a frequently documented behavioral characteristic in children with Dravet syndrome. To examine this feature in mice, the authors used open field tests with mice placed in a novel chamber and allowed to explore. Using this assay, both groups demonstrated that the Dravet mouse models exhibited hyperactivity compared to wild-type littermates. Ito and colleagues performed an additional experiment in which they documented home cage behavior and demonstrated that *Scn1a*^{RX/+} mice are not hyperactive in that environment, suggesting that the hyperactivity is context-dependent and may be exacerbated by unfamiliar situations. In both studies, the authors observed that the Dravet mice exhibited increased levels of anxiety-like behavior and excessive self-grooming, which may mimic repetitive behaviors seen in autism. Additionally, both groups reported that the Dravet mice exhibited inflexibility or avoidance of environmental change. This is reminiscent of the negative reaction apparent in autism spectrum disorder patients in response to a change in their routine.

To examine social behavior, the authors used a variety of tests to interrogate interest in other mice. Mice are innately social animals and will exhibit characteristic behaviors when introduced to unfamiliar mice, including active interaction with novel mice. In a series of tests to leverage this innate sociability, both groups demonstrated that the Dravet mouse models exhibited deficits in social behavior. Given the opportunity to interact with an unfamiliar mouse, wild-type animals showed a strong preference and spent the majority of time interacting with the other mouse. In contrast, the Dravet mouse models showed no preference for interaction with the unfamiliar mouse. Further, given the choice between a familiar mouse and an unfamiliar mouse, wild-type mice spent more time interacting with the novel mouse, while Dravet mouse models showed no preference. The authors addressed several factors that might contribute to these behaviors: First, mice need to be able to recognize unfamiliar versus familiar. Both groups used a novel object recognition test, which is similar to the social interaction test except that it measures preference for familiar versus unfamiliar inanimate objects. The Dravet mouse models performed similar to wild-type in these tests, indicating that they are able to recognize familiar versus novel. Second, social interactions in mice are highly dependent on olfactory cues. Both groups performed tests to assess whether the mouse models have olfactory deficits, but the results were inconsistent. Ito and colleagues performed a single test of olfaction and demonstrated that the *Scn1a*^{RX/+} mice had reduced olfactory ability. However, Han and col-

leagues performed a more extensive series of olfactory tests and determined that the *Scn1a*^{+/-} mice are able to perceive odors but are disinterested or avoid unfamiliar odors. Thus, both studies conclude that the Dravet mouse models have low sociability, consistent with reports in Dravet syndrome patients.

Dravet syndrome patients exhibit profound cognitive deficits with particularly poor visuoperceptual abilities. To assess cognitive function of Dravet mouse models, both groups used a spatial learning and memory task. They demonstrated that the Dravet mouse models exhibited deficits in spatial learning and memory. Han and colleagues further demonstrated cognitive deficits in *Scn1a*^{+/-} mice in context-dependent fear-conditioning, a robust emotional learning and memory test. It is possible that seizures experienced by Dravet mouse models may contribute to the observed cognitive deficits. Neither group reported any seizures occurring during behavioral testing. Additionally, Ito and colleagues performed EEG monitoring and reported no seizures and no abnormalities in interictal EEG activity in adult *Scn1a*^{RX/+} mice. This finding is consistent with previous reports of rare spontaneous seizures in adult *Scn1a*^{+/-} mice (4, 5). However, this result does not exclude the possibility that seizures experienced during critical stages of brain development contribute to later deficits.

Interestingly, Han and colleagues demonstrated that administration of a low dose of clonazepam rescued the social behavior and some cognitive deficits. Clonazepam is a benzodiazepine that acts as an allosteric modulator of GABA_A receptors and potentiates neurotransmission in the presence of GABA released at the synapse. It has both anxiolytic and antiseizure properties, but the low dose used in this study was 20-fold lower than the typical therapeutic anxiolytic dose. The authors demonstrated that the mice did not exhibit any sedation or anxiolytic effects at this low dose but did show improvement in social behaviors and cognition. These results have two important implications: First, rescue of behavioral and cognitive deficits by medication suggests that these are primary deficits resulting from dysfunction in GABAergic signaling rather than secondary effects of seizures. Second, pharmacological enhancement of GABAergic neurotransmission can ameliorate some behavioral and cognitive deficits. First-line treatment for Dravet syndrome includes polytherapy with valproate, a benzodiazepine (typically clobazam), and stiripentol (7). This polytherapy regime includes two positive allosteric modulators of GABA_A receptors: a benzodiazepine and stiripentol. The current study by Han and colleagues suggests that in addition to preventing seizures, these agents may also alleviate some of the cognitive and behavioral comorbidities.

Overall, these studies demonstrate that the Dravet syndrome mouse models exhibit features reminiscent of the cognitive and behavioral comorbidities observed in Dravet syndrome patients. Further, they suggest that these comorbid deficits result directly from dysfunction in GABAergic signaling and not as a secondary effect of seizures. Thus, these mouse models may be useful tools for examining the biological basis of comorbidities and evaluating therapeutic strategies.

by Jennifer A. Kearney, PhD



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