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Specific deletion of $Na_V1.1$ sodium channels in inhibitory interneurons causes seizures and premature death in a mouse model of Dravet syndrome

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Heterozygous loss-of-function mutations in the brain sodium channel $Na_V1.1$ cause Dravet syndrome (DS), a pharmacoresistant infantile-onset epilepsy syndrome with comorbidities of cognitive impairment and premature death. Previous studies using a mouse model of DS revealed reduced sodium currents and impaired excitability in GABAergic interneurons in the hippocampus, leading to the hypothesis that impaired excitability of GABAergic inhibitory neurons is the cause of epilepsy and premature death in DS. However, other classes of GABAergic interneurons are less impaired, so the direct cause of hyperexcitability, epilepsy, and premature death has remained unresolved. We generated a floxed *Scn1a* mouse line and used the Cre-Lox method driven by an enhancer from the *Dlx*1,2 locus for conditional deletion of *Scn1a* in forebrain GABAergic neurons. Immunocytochemical studies demonstrated selective loss of $Na_V1.1$ channels in GABAergic interneurons in cerebral cortex and hippocampus. Mice with this deletion died prematurely following generalized tonic-clonic seizures, and they were equally susceptible to thermal induction of seizures as mice with global deletion of *Scn1a*. Evidently, loss of $Na_V1.1$ channels in forebrain GABAergic neurons is both necessary and sufficient to cause epilepsy and premature death in DS.

Voltage gated sodium (Na_V) channels are composed of a 260-kDa pore-forming α subunit and one or more smaller auxiliary β subunits (1, 2). The $Na_V1.1$, $Na_V1.2$, $Na_V1.3$, and $Na_V1.6$ isoforms are highly expressed in the brain, where they initiate and propagate action potentials in neurons. $Na_V1.1$ and $Na_V1.3$ are prominently expressed in the cell soma and axon initial segment where they integrate incoming information from the dendrites, whereas $Na_V1.2$ channels are found in unmyelinated axons and dendrites, and $Na_V1.6$ channels are found in cell bodies, dendrites, and the nodes of Ranvier of myelinated axons (1, 3–6). Whereas each channel is responsible for sodium conduction through the membrane, their differential localization leads to discrete disease pathologies (7). Mutations in the pore-forming subunits of $Na_V1.1$ channels cause multiple types of epilepsy (8, 9). Heterozygous missense and truncation mutations in the *Scn1a* gene encoding $Na_V1.1$ channels lead to haploinsufficiency of the channel function and cause Dravet syndrome (DS, also known as severe myoclonic epilepsy of infancy) (10–12). This disease begins with infantile-onset febrile seizures at 6–9 mo of age and progresses to intractable afebrile, generalized seizures in later years. In addition to seizures, patients with DS suffer from several severe comorbidities including ataxia, psychomotor delay, cognitive impairment, and often premature death (13).

In mice, heterozygous deletion of exon 26 in *Scn1a* leads to global haploinsufficiency of $Na_V1.1$ and recapitulates the symptoms of DS (14). These global heterozygous mice exhibit temperature-induced and spontaneous seizures, mild ataxia, and premature death (14–16). $Na_V1.1$ channels are expressed in both excitatory and inhibitory neurons (3, 6), yet electrophysiological studies in dissociated hippocampal neurons from DS mice showed selective loss of sodium current and excitability in GABAergic interneurons (14, 17). These results led to the hypothesis that the epilepsy and comorbidities of DS are caused by selective impairment of inhibitory interneuron function. However, subsequent studies have revealed lesser impairments of sodium current and excitability in GABAergic inhibitory neurons in the cerebellum, reticular nucleus of the thalamus, and cerebral cortex (16, 18, 19), so this hypothesis requires further validation.

To target gene expression in GABAergic neurons selectively, we have taken advantage of the *Dlx* gene family, which encodes homeobox transcription factors that are required for specification, maturation, and survival of forebrain GABAergic neurons (20, 21). Deletion of one of these genes, *Dlx1*, in mice leads to selective interneuron loss and seizures (22). In the Dlx1/2-I12b-Cre transgenic mouse, an intergenic *Dlx1* and *Dlx2* enhancer drives expression of Cre recombinase specifically in GABAergic neurons in the forebrain (23). We have used this Dlx-Cre mouse line in conjunction with a floxed $Na_V1.1$ mouse line to directly test the hypothesis that deletion of $Na_V1.1$ channels in the forebrain is sufficient to recapitulate the premature death and seizure phenotypes observed in the $Na_V1.1$-deletion model of DS.

Results

Generation of a Floxed $Na_V1.1$ Mouse Line. Homologous recombination was used to replace exon 25 of the endogenous *Scn1a* gene using a targeting vector containing the exon flanked by LoxP sites and a neomycin-selection cassette, which itself was flanked by flippase recognition target (FRT) sites (Fig. 1*A*). Before breeding animals for experiments, the neomycin-selection cassette was removed to avoid possible impairment of *Scn1a* function by mating a heterozygous flox mouse with a flippase-expressing mouse. This genetic cross excises the neomycin-selection cassette and leaves only a single FRT site (Fig. 1*A*). Flox heterozygous (F/+) and heterozygous mice maintained on a C57BL/6 background are indistinguishable from WT littermates and breed, thrive, and survive normally. Breeding with a globally deleting Meox2-Cre strain (Jackson Labs) excised exon 25 and resulted in the expected truncated PCR product (Fig. 1*B*) in DNA samples from F/+:Meox2-Cre^+ and F/F:Meox2-Cre^+ mice at postnatal day (P) 14. Immunoblotting of protein samples from these mice showed a substantial reduction in $Na_V1.1$ protein levels in F/+:Meox2-Cre^+ mice and no detectable $Na_V1.1$ protein in F/F:

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The authors declare no conflict of interest.

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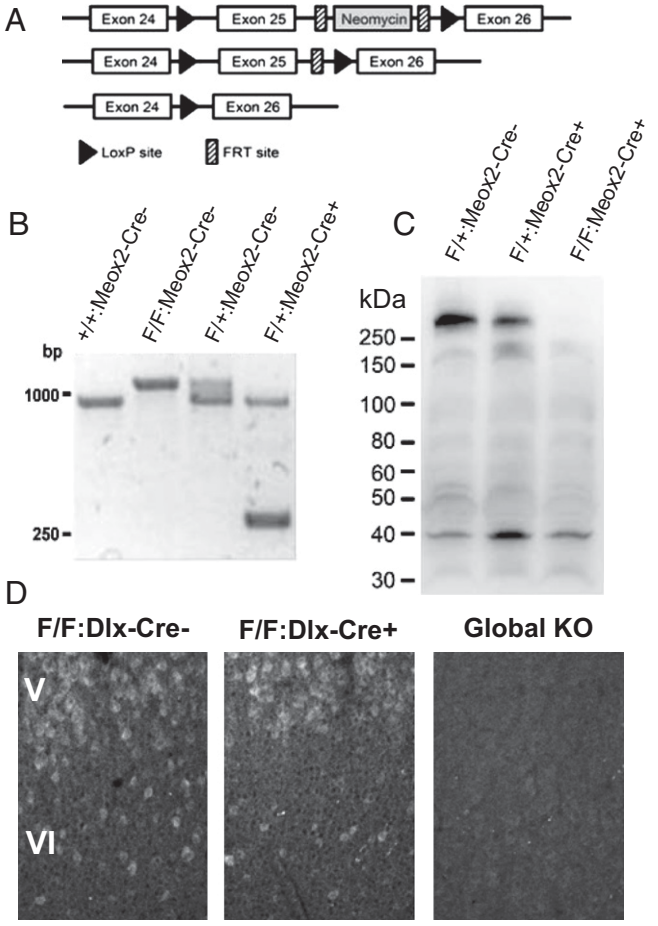

Fig. 1. Floxed *Scn1a* mouse has reduced $Na_V1.1$ expression in presence of Cre. (*A*) Schematic representation of the targeting construct used to generate our floxed *Scn1a* mouse. Exon 25 is flanked on the 5′ and 3′ ends by LoxP sites in combination with an FRT flanked neomycin selection marker (*Top*). The neomycin cassette was removed in breeding cross of heterozygous Flox animals with a flippase-expressing animal, leaving behind a single FRT site (*Middle*). In the presence of Cre recombinase exon 25, the FRT site and one LoxP site are excised leaving behind a single LoxP site (*Bottom*). (*B*) Representative genomic DNA gel of PCR products from +/+, F/F, and F/+ tail DNA in the absence of Meox2-Cre and F/+:Meox2-Cre$^+$, demonstrating an excision of exon 25 and a corresponding decrease in PCR product size. WT allele, 846 bp; Flox allele, 1019 bp; and excised allele, 258 bp. (*C*) Western blot for $Na_V1.1$ protein expression in whole brain membrane proteins (150 μg) from P14 F/+:Meox-Cre$^-$, F/+:Meox2-Cre$^+$, and F/F:Meox2-Cre$^+$, demonstrating a stepwise decline in $Na_V1.1$ protein levels with the successive removal of exon 25 in the presence of Meox2-Cre. The F/F:Meox2-Cre$^+$ sample contains no $Na_V1.1$ protein and recreates the global knockout expression profile as expected. (*D*) Following mating a F/F female with a Dlx-Cre$^+$ male, a visually detectable decrease in $Na_V1.1$ immunoreactive staining in deep somatosensory cortex at P14 can be seen. *Left,* F/F:Dlx-Cre$^-$. Immunostaining of neuronal cell bodies for $Na_V1.1$ channels. *Center,* F/F:Dlx-Cre$^+$. Immunostaining of $Na_V1.1$ channels in layer V pyramidal cells appears intact, but decreases in immunostaining for $Na_V1.1$ channels in layer IV and VI cells can be detected. *Right,* global $Na_V1.1$ knockout, included as a negative control for antibody specificity. $Na_V1.1$ protein expression is not observed in an age- and gain-matched global knockout at P14.

Meox2-Cre$^+$ mice at P14 (Fig. 1*C*). Immunocytochemical studies with specific antibodies against $Na_V1.1$ protein revealed a visually evident reduction in staining of the cell bodies of GABAergic inhibitory neurons in layers IV and VI in somatosensory cortex, which contain primarily interneurons, whereas there was no evident decrease in immunostaining of neuronal cell bodies in layer V, where the cell bodies of excitatory pyramidal neurons are located (Fig. 1*D*). Further quantitative analysis of the selective deletion of $Na_V1.1$ channels is presented below.

Specific Deletion of $Na_V1.1$ Protein in GABAergic Neurons in Dlx-I12b-Cre Mice. The *Dlx* gene family encodes homeobox transcription factors that are essential for specification, maturation, and survival of interneuron progenitors (20). Mating with the Dlx1/2-I12b-Cre line (Dlx-Cre) (23) mouse targets Cre recombinase expression to forebrain interneurons, and deletion of *Dlx1* leads to interneuron loss and seizures (22). Our C57BL/6 F/F mice were crossed with Dlx1/2-I12b-Cre$^+$ (Dlx-Cre$^+$) mice to produce heterozygous loss of $Na_V1.1$ channels in interneurons of the cerebral cortex and hippocampus on a C57BL/6:CD1 background. This genetic cross was consistent through all experiments and all comparisons were made between Dlx-Cre$^-$ and Dlx-Cre$^+$ littermates.

Double labeling with antibodies recognizing $Na_V1.1$ and GABA revealed a specific decrease in immunostaining for $Na_V1.1$ in GABA-expressing inhibitory interneurons, as illustrated in the dentate gyrus from F/F:Dlx-Cre$^+$ and F/F:Dlx-Cre$^-$ mice (Fig. 2 *A–F*). The arrowheads indicate individual examples of neurons that are double labeled in F/F:Dlx-Cre$^-$ mice (Fig. 2 *A–C*) but only labeled with GABA in F/F:Dlx-Cre$^+$ mice (Fig. 2 *D–F*). Arrows indicate clusters of two to four neurons that are double-labeled in F/F:Dlx-Cre$^-$ mice but not in F/F:Dlx-Cre$^+$ mice (Fig. 2 *A–F*). Similar images were obtained for the visual and somatosensory cortex (Fig. S1). Quantification of this double-labeled immunostaining showed a significant reduction in the fraction of $Na_V1.1$/GABA double-labeled interneurons in F/F:Dlx-Cre$^+$ brain sections compared with F/F:Dlx-Cre$^-$ controls across several regions of the cerebral cortex and hippocampus (Fig. 2*G*). As expected, F/F:Dlx-Cre$^+$ brain slices retained normal numbers of $Na_V1.1$-expressing layer V pyramidal cells in visual cortex and somatosensory cortex at P14 compared with F/F:Dlx-Cre$^-$ slices (Fig. 2*H*). GABA$^+$ neurons do not all contain $Na_V1.1$, and Cre expression is not expected to result in complete loss of $Na_V1.1$ expression in all cells because of differences in timing and extent of activation by the *Dlx* enhancer. We determined the average pixel intensity of $Na_V1.1$ staining in GABA$^+$ and GABA$^-$ staining cells and observed that the intensity of immunostaining of $Na_V1.1$ in remaining $Na_V1.1$/GABA double-labeled interneurons in F/F:Dlx-Cre$^+$ mice was reduced compared with F/F:Dlx-Cre$^-$ littermates, but no change in $Na_V1.1$ staining intensity was observed in $Na_V1.1^+$, GABA$^-$, excitatory pyramidal cells in layer V of the cerebral cortex, or in dentate granule cells (Fig. 2*I*). These results demonstrate selective deletion of $Na_V1.1$ channels in GABAergic interneurons in the hippocampus and cerebral cortex.

Heterozygous Conditional Deletion of $Na_V1.1$ Leads to Premature Death Following Spontaneous Seizures. F/+:Dlx-Cre$^+$ mice died prematurely compared with F/+:Dlx-Cre$^-$ littermates, with initial deaths beginning late in the third postnatal week and only 30% of animals remaining at 90 d (Fig. 3*A*). Video recordings in a separate population of animals captured spontaneous seizures in approximately one-third of the F/+:Dlx-Cre$^+$ mice, which were scored 1–5 using the Racine scale (n = 10/35) (24). Seizures and death in these animals followed a stereotyped progression. Seizures began with forelimb clonus (Racine 3) and progressed in both frequency and severity through forelimb clonus with rearing (Racine 4), eventually culminating in generalized tonic-clonic seizure (GTC, Racine 5), and finally death 4–48 h after initial seizure onset. This time course of seizures and death is illustrated for a typical animal in Fig. 3*B*. Age at death ranged from P18 to P22 and all deaths were observed immediately following a Racine 5 GTC seizure (Fig. 3 *B* and *C*). Animals died an average of 17.9 h after seizure onset, during which time they experienced an average of nine seizures (Fig. 3 *D* and *E*). This timing of death

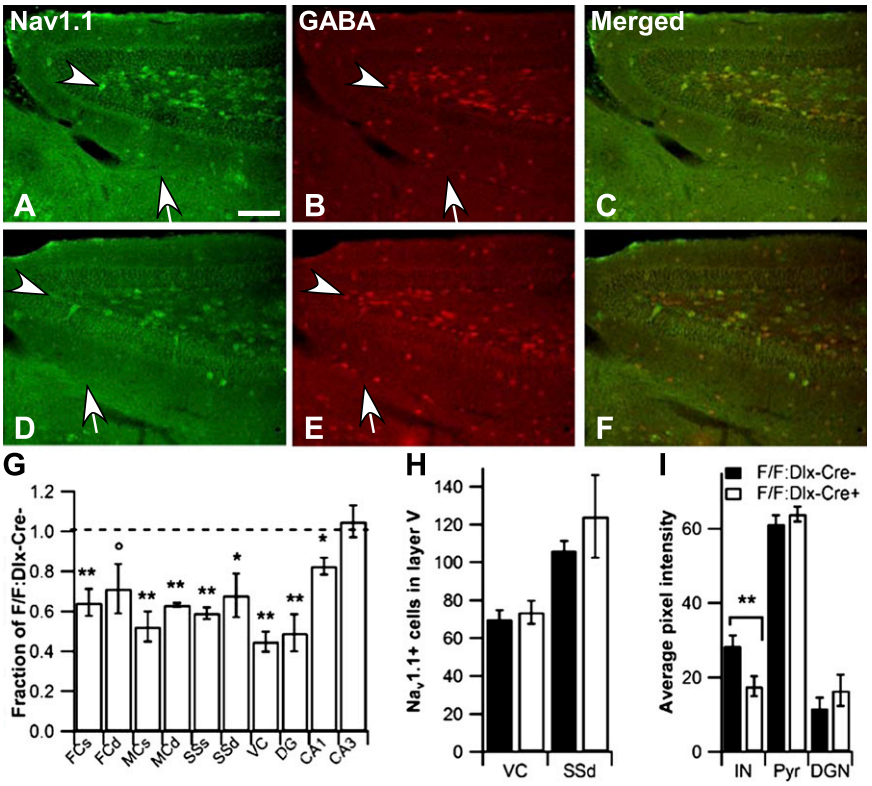

Fig. 2. Dlx1/2-I12b-Cre preferentially decreases Na$_V$1.1 expression in interneurons. (*A–F*) Representative immunostaining for Na$_V$1.1 (green), GABA (red), and co-localized (yellow) in the dentate gyrus from F/F:Dlx-Cre$^-$ (*A–C*) and F/F:Dlx-Cre$^+$ (*D–F*) demonstrate a selective loss of cell body Na$_V$1.1 staining in F/F:Dlx-Cre$^+$ slices in GABA$^+$ cells of the hilus (arrowheads) and molecular layer (arrows). (*G*) Fraction of neurons in F/F:Dlx-Cre$^+$ tissue coimmunostained for GABA and Na$_V$1.1 channels quantified across a range of brain regions relative to GABA and Na$_V$1.1 coimmunostained cells from F/F:Dlx-Cre$^-$ tissue. FC, frontal cortex; MC, motor cortex; SS, somatosensory cortex; VC, visual cortex; s, superficial layers; d, deep layers; DG, dentate gyrus; CA1, hippocampal CA1; and CA3, hippocampal CA3 (*$P < 0.05$, **$P < 0.01$, °$P = 0.08$). (*H*) Number of excitatory pyramidal neurons immunostained for Na$_V$1.1 channels but not GABA in the pyramidal layers of SS and VC. (*I*) Decreased Na$_V$1.1 immunostaining intensity is observed only in F/F:Dlx-Cre$^+$ interneurons also coexpressing GABA (IN) and not in pyramidal cells (Pyr) or dentate granule neurons (DGN) expressing Na$_V$1.1 alone. (Scale bar, 100 μm.)

in the population of mice monitored for spontaneous seizures correlated with the time of highest incidence of death in the total population, suggesting seizures precede death in the general population. Throughout this time period, no F/+:Dlx-Cre$^-$ animals were observed to have seizures or die ($n = 28$). These results show that the premature death observed in F/+:Dlx-Cre$^+$ mice is comparable to that in Na$_V$1.1 global heterozygotes (14) and therefore provide support for the conclusion that heterozygous deletion of Na$_V$1.1 channels in forebrain GABAergic inhibitory neurons is sufficient to cause premature death in the mouse model of DS.

Heterozygous Conditional Deletion of Na$_V$1.1 Leads to Thermal Sensitivity to Seizure. Patients with DS (13) and our mouse model of this disease (15) are susceptible to seizures evoked by elevation of body temperature. When the core body temperature of our F/+:Dlx-Cre$^+$ animals was increased, seizures were induced in all animals tested with mean temperature for seizure induction of 39.6 °C (Fig. 4*A*) (F/+:Dlx-Cre$^+$, $n = 17$). Conversely, no F/+:Dlx-Cre$^-$ animals exhibited thermally induced seizures (Fig. 4*A*; F/+:Dlx-Cre$^-$, $n = 10$). Seizures provoked by thermal induction ranged in severity from simple forelimb clonus (Racine 3) to full GTC (Racine 5), but the majority of animals had Racine 4 seizures (Fig. 4*B*). The two animals that progressed to GTC died soon thereafter. These results show that the sensitivity to thermal induction of seizures in our F/+:Dlx-Cre$^+$ is indistinguishable from global Na$_V$1.1 heterozygotes, supporting the conclusion that heterozygous deletion of Na$_V$1.1 channels in forebrain GABAergic inhibitory neurons is sufficient to cause the susceptibility to thermally induced seizures observed in our mouse model of DS.

A subset of animals was implanted with four EEG leads at P24, and EEGs were recorded during thermal induction on the following day after the mice recovered from surgery and were free from the effects of anesthesia. In each F/+:Dlx-Cre$^+$ animal tested, electrographic seizures and behavioral correlates were closely linked (F/+:Dlx-Cre$^+$, $n = 4$) (Fig. 4*C*). In contrast, none of the F/+:Dlx-Cre$^-$ mice experienced either electrographic seizures or behavioral correlates (F/+:Dlx-Cre$^-$, $n = 5$) (Fig. 4*C*). The pattern of epileptiform electrical activity preceding and during the seizures induced in F/+:Dlx-Cre$^+$ mice was similar to those characterized previously in global Na$_V$1.1 heterozygotes (14, 15) and in children with DS (25).

Lack of Effect of Heterozygous Conditional Deletion of Na$_V$1.1 in the Heart. Na$_V$1.1 channels are expressed in the mouse heart (26); therefore, it is possible that a direct effect of our gene deletion on cardiac function could contribute to seizures or premature death. In an extensive study using sensitive detection methods, Potter et al. demonstrated that Dlx-I12b-Cre expression is restricted to the forebrain and no expression was observed in the peripheral nervous system or heart (23). Our findings that deletion of Na$_V$1.1 channels is specific to inhibitory neurons and not excitatory neurons further demonstrates the specificity of this gene deletion and indicates that no leaky Cre expression has occurred in our F/+:Dlx-Cre$^+$ mice. Consistent with these studies, ECG recordings at rest revealed no significant differences between F/+:Dlx-Cre$^-$ ($n = 5$) and F/+:Dlx-Cre$^+$ ($n = 6$)

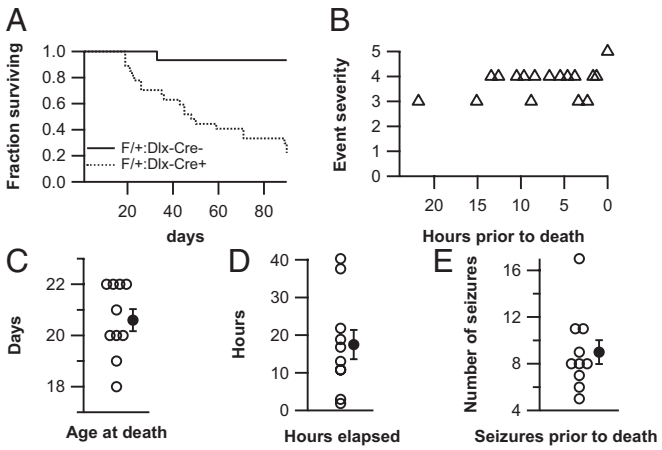

Fig. 3. Conditional heterozygotes experience premature death and spontaneous seizures. (*A*) Survival of F/+:Dlx-Cre$^-$ and F/+:Dlx-Cre$^+$ mice. The fraction of each genotype surviving is plotted versus postnatal day (F/+:Dlx-Cre$^-$, n = 45; F/+:Dlx-Cre$^+$, n = 27). (*B*) Representative example of spontaneous seizure progression in a F/+:Dlx-Cre$^+$ mouse. Racine score of each seizure is plotted as a function of the time before death (t = 0 at time of death). (*C*) Graphical representation of the age of death in animals monitored for spontaneous seizure. Average age of death: 20.6 ± 0.4 d (n = 10). (*D*) Graphical representation of time elapsed from initial seizure onset to death in animals monitored for spontaneous seizures. Average latency to death: 17.9 ± 3.9 h (n = 10). (*E*) Graphical representation of the number of seizures preceding death in animals monitored for spontaneous seizures. Average number of seizures before death: 9 ± 1 seizures (n = 10).

animals in heart rate (F/+:Dlx-Cre$^-$, 672.2 ± 24; F/+:Dlx-Cre$^+$, 658 ± 57), PR interval (F/+:Dlx-Cre$^-$, 33 ± 2; F/+:Dlx-Cre$^+$, 32 ± 2), QRS interval (F/+:Dlx-Cre$^-$, 9.4 ± 1; F/+:Dlx-Cre$^+$, 8.3 ± 1), or QT interval (F/+:Dlx-Cre$^-$, 23 ± 5; F/+:Dlx-Cre$^+$, 20 ± 1.5) (Fig. 4*D*, $P > 0.05$). These results demonstrate that selective deletion of $Na_V1.1$ channels in forebrain neurons has no effect on intrinsic action potential generation and conduction in the heart and therefore support the conclusion that premature deaths in our conditional deletion mouse model of DS are initiated by seizures.

Discussion

Dysfunction of GABAergic interneurons is known to produce seizures in mice (22). Moreover, GABAergic interneurons in the hippocampus have a profound and selective loss of sodium current and electrical excitability in global loss-of-function mouse models of DS (14, 17). However, studies of other GABAergic inhibitory neurons in the cerebellum, reticular nucleus of the thalamus, and cerebral cortex have revealed smaller losses of sodium currents and lesser impairments of excitability in our mouse model of DS (16, 18, 19). Therefore, these previous observations did not establish a causal relationship between the loss of sodium current and action potential firing in GABAergic interneurons and the hyperexcitability, seizure, and premature death in DS. Our experiments using the Cre-Lox method of conditional gene deletion clearly demonstrate that loss of $Na_V1.1$ only in GABAergic inhibitory Dlx1/2-I12b-Cre$^+$ interneurons in the forebrain replicates the severe spontaneous and temperature-induced GTC seizures observed in a global DS model. Furthermore, our data reveal that premature death in this animal model is precipitated by spontaneous seizures caused by deletion of $Na_V1.1$ channels in forebrain inhibitory neurons. These results show directly that hyperexcitability and seizures in mouse DS are caused by loss of GABAergic interneuron excitability.

There are many different classes of interneurons in the forebrain; therefore, our results do not provide insight into the specific classes of forebrain interneurons whose loss of excitability leads to seizures and premature death. In light of the very large loss of sodium current in GABAergic inhibitory neurons in the hippocampus in our mouse model of DS (50% loss of sodium current in dissociated inhibitory neurons in heterozygotes), our working model is that failure of firing of GABAergic interneurons in the hippocampus is one important component of the functional impairment in this disease. In contrast, similar studies of the entire dissociated GABAergic interneuron pool in the cerebral cortex did not reveal a significant loss of sodium current (19). These results illustrate a profound difference between the role of $Na_V1.1$ channels in GABAergic interneurons in the hippocampus and cerebral cortex. The lack of detectable effect of deletion of $Na_V1.1$ channels on sodium currents measured in whole-cell voltage clamp studies of cortical GABAergic interneurons might

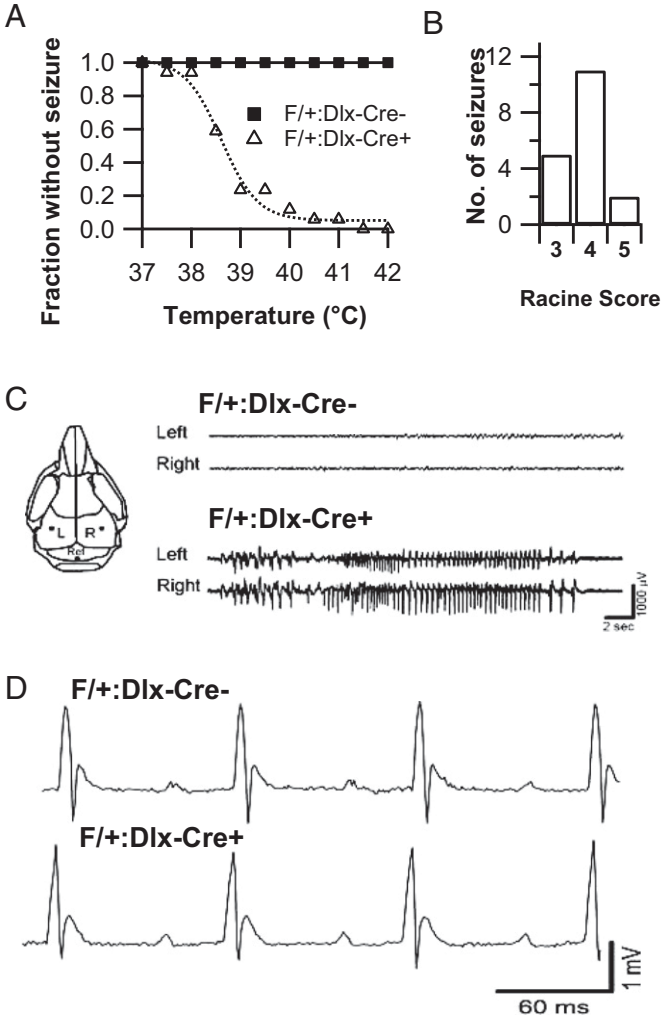

Fig. 4. Conditional heterozygotes experience evoked behavioral and electrographic seizures. (*A*) Thermally induced seizures were evoked in all F/+:Dlx-Cre$^+$ animals with a mean temperature of 39 °C. No F/+:Dlx-Cre$^-$ mice presented with seizure (F/+:Dlx-Cre$^-$, n = 10; F/+:Dlx-Cre$^+$, n = 17). (*B*) Distribution of thermally induced seizure severity in F/+:Dlx-Cre$^+$ mice. (*C*) Representative EEG traces of F/+:Dlx-Cre$^-$ (n = 5) and F/+:Dlx-Cre$^+$ (n = 4) mice during thermal induction at P24. *Top*, F/+:Dlx-Cre$^-$ mouse at 39.5 °C. *Bottom*, F/+:Dlx-Cre$^+$ mouse during GTC seizure at 39.5 °C. (*D*) Representative ECG traces of F/+:Dlx-Cre$^-$ (n = 5) and F/+:Dlx-Cre$^+$ (n = 6) mice at rest. *Top*, F/+:Dlx-Cre$^-$ mouse: heart rate, 672.2 ± 24; PR interval, 32.8 ± 2; QRS interval, 9.4 ± 1; and QT interval, 23.5 ± 5.5. *Bottom*, F/+:Dlx-Cre$^+$ mouse: heart rate, 658.4 ± 57; PR interval, 32.3 ± 2; QRS interval, 8.3 ± 1; and QT interval, 19.7 ± 1.5 ($P > 0.05$ for all parameters).

indicate that these channels comprise only a small fraction of the sodium current in the cell bodies in the major classes of these neurons. Alternatively, other Na_V channels may be up-regulated to compensate for the loss of $Na_V1.1$ channels, or the relevant $Na_V1.1$ channels in these cells may be located in the axon initial segment and therefore are lost in dissociated cells as prepared for whole-cell voltage clamp. In any case, further studies with more selective gene-knockout methods and more detailed physiological recordings of GABAergic interneurons in situ in brain slices will be necessary to identify the specific neuron types in the cerebral cortex whose loss of $Na_V1.1$ channels contributes to seizures and premature death in our mouse model of DS.

Premature death in our mouse model parallels the high percentage of sudden unexplained death in epilepsy (SUDEP) in DS patients (27, 28). Careful review of our video records showed that premature deaths occur following Racine 5 seizures of short duration (<2 min), rather than during extended periods of status epilepticus (>30 min), and therefore would be classified as SUDEP as generally defined in human epilepsy. $Na_V1.1$ channels are expressed throughout the central nervous system and in the sinoatrial node, atria, and ventricles in the mouse heart (26, 29). Our results show that loss of $Na_V1.1$ channels in GABAergic interneurons in the forebrain is sufficient to cause premature death that is similar to global heterozygotes. These results eliminate loss of $Na_V1.1$ channels per se in the spinal cord, peripheral nervous system, or heart as a requirement for premature death and show that fatal dysfunction originates with failure of firing of forebrain GABAergic interneurons and the resulting Racine 5 seizures. Further studies in which EEG, ECG, and respiratory recordings are captured during spontaneous seizures and deaths will be required to determine how the seizures caused by loss of $Na_V1.1$ channels in GABAergic interneurons in the forebrain actually cause premature death, which could result from primary brain damage, respiratory failure, or heart failure caused by seizures.

Materials and Methods

Generation and Maintenance of Floxed Mouse Line. All animal procedures were approved by the Institutional Animal Care and Use Committee of the University of Washington, Seattle. Exon 25, the second-to-last coding exon of *Scn1a*, was cloned into plasmid 4517D (a gift from G. Stanley McKnight, University of Washington, Seattle) containing a single LoxP site, FRT-flanked neomycin-selection cassette, and multiple cloning sites. The *NheI/SalI* sites were used to insert a 6-kb long arm containing exon 26 (DIV S3-CT) and the *XhoI/NotI* sites were used to insert a 1.5-kb short arm containing exon 25 (DIV S1-S3) and second LoxP site. The plasmid was linearized at the *AscI* site, electroporated into embryonic stem cells, and used to generate chimeric mice. Animals were genotyped using FHY311 (5′-CTTGATGTGTTGAAATTCAC-3′) and FHY314 (5′-TATAGAGTGTTTAATCTCAAC-3′): WT allele, 846 bp; floxed allele, 1019 bp; and excised allele, 258 bp. The neomycin-selection cassette was removed following mating with a flippase-expressing mouse (B6.SJL-Tg(ACTFLPe)9205Dym/J; Jackson Labs), and the resulting neomycin-excised animals were backcrossed with C57BL/6J (Jackson Labs) for 10 generations. Dlx-I12b-Cre mice (obtained from John L. Rubenstein, University of California, San Francisco) were received and maintained on a CD1 (Jackson Labs) background. Mouse lines were maintained independently and mated together to generate F_1, F/+:Dlx-Cre$^+$ and F/+:Dlx-Cre$^-$ littermates. All experimental comparisons were made between F_1 littermates.

Immunohistochemistry. Animals were perfused at P14 with 4% (wt/vol) paraformaldehyde in PB, brains were removed and saturated in 30% sucrose, sliced to 50 μm, and double labeled as free-floating slices (30). Primary antibodies were affinity-purified rabbit anti-$Na_V1.1$ (1:150, SP11A) (3) and guinea pig anti-GABA (1:600; Abcam). Secondary antibodies were goat anti–guinea-pig IgG labeled with Alexa 555 (1:400; Invitrogen), goat anti-rabbit IgG labeled with Alexa 488 (1:400; Invitrogen). Tissue samples from F/F:Dlx-Cre$^-$, F/F:Dlx-Cre$^+$ and global *Scn1a* knockout animals were processed simultaneously. Gain- and offset-matched images were collected on a Leica SL confocal microscope at the Keck Imaging Facility of the University of Washington. Sections stained in the absence of primary antibody showed no detectable labeling, similar to the global $Na_V1.1$-knockout tissue stained with $Na_V1.1$ antibodies.

Spontaneous Seizure Recording and Analysis. Animals were continuously video monitored from P19 to P27. The resulting video files were reviewed at ~8× speed. Suspected seizure events were reviewed at 2× speed and scored from 1 to 5 for seizure severity on the basis of the Racine scoring system: 1, mouth and facial movements; 2, head nodding; 3, forelimb clonus, usually one limb; 4, forelimb clonus with rearing; and 5, generalized tonic-clonic seizure (GTC), rearing, clonus, and falling over (24).

Thermal Induction and EEG. Each animal's core body temperature was continuously monitored by a rectal temperature probe and controlled by a feedback circuit in line with a heat lamp. Body temperature was increased in 0.5 °C steps at 2-min intervals until a seizure occurred or a temperature of 42 °C was reached. The animal was then cooled and returned to its home cage. Thermal induction was performed on P22, and the induction process and resulting seizures were recorded by video monitoring. Seizures were scored on the Racine scale using the same criteria as in spontaneous seizure monitoring. EEG surgeries were performed at P23 with the animals under ketamine/xylazine anesthesia and aseptic conditions. Animals were implanted with four to six small platinum wire electrodes and allowed to recover for 24 h before referential EEG recording during thermal induction (15).

Electrocardiogram Recordings. Surgeries to implant ECG electrodes were performed under ketamine/xylazine (130/8.8 mg/kg) anesthesia and aseptic conditions. A small midline skin incision was made above the skull and a second incision above the thorax. Two silver electrodes were tunneled s.c. from the head incision to the thoracic incision site and anchored in place with suture. One electrode was positioned near the heart apex and the other near the right forelimb. Mice were allowed to recover from surgery overnight. On experiment day, mice were transferred to the recording chamber and allowed to acclimate for 30 min before recording. ECG recordings were collected in conscious mice in daytime on a PowerLab 35 data acquisition unit using LabChart software (ADInstruments), at 1-kHz sampling rate and processed off-line with a 3-Hz highpass filter.

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