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The voltage-gated sodium channel *Scn8a* is a genetic modifier of severe myoclonic epilepsy of infancy

Melinda S. Martin[1], Bin Tang[1], Ligia A. Papale[2], Frank H. Yu[3], William A. Catterall[3] and Andrew Escayg[1,*]

[1]Department of Human Genetics, Emory University, Atlanta, GA 30322, USA, [2]Department of Psychobiology, Universidade Federal de São Paulo, São Paulo, Brazil and [3]Department of Pharmacology, University of Washington, Seattle, WA 98195, USA

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The mammalian genome contains four voltage-gated sodium channel genes that are primarily expressed in the central nervous system: *SCN1A*, *SCN2A*, *SCN3A* and *SCN8A*. Mutations in *SCN1A* and *SCN2A* are responsible for several dominant idiopathic epilepsy disorders, including generalized epilepsy with febrile seizures plus (GEFS+) and severe myoclonic epilepsy of infancy (SMEI). Mutations in *SCN8A* are associated with cognitive deficits and neuropsychiatric illness in humans and movement disorders in mice; however, a role for *SCN8A* ($Na_v1.6$) in epilepsy has not been investigated. To determine the relationship between $Na_v1.6$ dysfunction and seizure susceptibility, we examined the thresholds of two *Scn8a* mouse mutants, $Scn8a^{med}$ and $Scn8a^{med\text{-}jo}$, to flurothyl- and kainic acid (KA)-induced seizures. Both mutants were more seizure resistant than wild-type littermates, suggesting that altered $Na_v1.6$ function reduces neuronal excitability. To determine whether impaired $Na_v1.6$ function could ameliorate seizure severity in a mouse model of SMEI, we generated $Scn1a^{+/-}$; $Scn8a^{med\text{-}jo/+}$ double heterozygous mice. Unlike $Scn1a^{+/-}$ mice that are more susceptible to flurothyl-induced seizures, $Scn1a^{+/-}$; $Scn8a^{med\text{-}jo/+}$ mice displayed thresholds that were comparable to wild-type littermates. The $Scn8a^{med\text{-}jo}$ allele was also able to rescue the premature lethality of $Scn1a^{+/-}$ mice and extend the lifespan of $Scn1a^{-/-}$ mutants. These results demonstrate that genetic interactions can alter seizure severity and support the hypothesis that genetic modifiers contribute to the clinical variability observed in SMEI and GEFS+.

INTRODUCTION

Voltage-gated sodium channels are transmembrane protein complexes that play a critical role in electrical signaling between cells. Following an initial membrane depolarization, voltage-gated sodium channels open, permitting the rapid influx of sodium ions and the initiation and propagation of an action potential. Voltage-gated sodium channels are comprised of one α subunit and one or more auxiliary β subunits (β1–β4). The 260 kDa α subunit contains several functional domains including an ion selective pore, an intracellular inactivation gate, and a positively charged voltage sensor (1).

The human genome contains 9 voltage-gated sodium channel α subunit genes, each with distinct spatial and temporal expression patterns (2). Four α subunits, $Na_v1.1$, $Na_v1.2$, $Na_v1.3$ and $Na_v1.6$, respectively encoded by *SCN1A*, *SCN2A*, *SCN3A* and *SCN8A*, are primarily expressed in the central nervous system (3–5). Mutations in *SCN1A* and *SCN2A* cause several subtypes of dominant idiopathic generalized epilepsy. Mutations in both *SCN1A* and *SCN2A* lead to generalized epilepsy with febrile seizures plus (GEFS+; MIM 604233) (6,7). *SCN2A* dysfunction also causes benign familial neonatal-infantile seizures (BFNIS; MIM 607745) (8), while *SCN1A* mutations are the main cause of severe myoclonic epilepsy of infancy (SMEI; MIM 607208) (9). SMEI is a debilitating form of childhood epilepsy characterized by complex febrile seizures, the development of afebrile seizures, and mental retardation. SMEI often results from loss of function

*To whom correspondence should be addressed at: Department of Human Genetics, Emory University, 615 Michael Street, Whitehead Building, Suite 301, Atlanta, GA 30322. Tel: +1 4047128328; Fax: +1 4047273949; Email: aescayg@genetics.emory.edu

SCN1A mutations, and represents a haploinsufficiency phenotype (10). Affected members of GEFS+ families with the same *SCN1A* mutation often present with a variety of epilepsy subtypes and severities, ranging from childhood febrile seizures to adult generalized or partial epilepsy to SMEI (11,12). Variable clinical severities are also observed among sporadic SMEI patients.

Yu *et al*. (13) constructed a mouse model of SMEI by targeted disruption of the mouse *Scn1a* gene. Heterozygous $Scn1a^{+/-}$ mutants, expressing 50% of normal $Na_v1.1$ channel levels, exhibit spontaneous seizures and a high mortality rate from 4 weeks after birth. Homozygous mutants, which completely lack $Na_v1.1$ channels, are ataxic and die 15 days after birth. Electrophysiological analysis of hippocampal pyramidal neurons from $Scn1a^{-/-}$ mice revealed normal levels of sodium currents, indicating that $Scn1a^{-/-}$ does not make a major contribution to sodium currents in these neurons. In contrast, sodium currents were significantly reduced in hippocampal interneurons that are critical for GABA-mediated neuronal inhibition. The ataxia observed in homozygous $Scn1a^{-/-}$ mutants is thought to be caused by reduced peak, persistent and resurgent sodium current in cerebellar Purkinje neurons (14). A second mouse model of SMEI was recently generated by the targeted introduction of a premature stop codon (R1407X), identified in three SMEI patients, into the mouse *Scn1a* gene (15). These heterozygous mutants also displayed decreased $Na_v1.1$ expression, spontaneous seizures, and abnormal firing of neocortical interneurons. Both studies therefore suggest that reduced neuronal inhibition contributes to the SMEI phenotype.

The first mutation in the human *SCN8A* gene, encoding $Na_v1.6$ channels, was recently reported in a proband with juvenile-onset ataxia and mental retardation. Additional family members with the same dominant mutation displayed mild cognitive deficits and a variety of behavioral abnormalities (16). In the mouse, recessive mutations in *Scn8a* result in neuromuscular disorders. Mice homozygous for a point mutation in *Scn8a* ($Scn8a^{med\text{-}jo}$) display cerebellar ataxia and a tremor involving the head and body (17–19). Homozygosity for null mutations of *Scn8a* ($Scn8a^{med}$ or $Scn8a^{med\text{-}tg}$) results in progressive paralysis, muscular atrophy of the hind limbs, and premature death between 3 and 4 weeks of age (20–23). In addition, mice that lack *Scn8a* only in cerebellar Purkinje neurons exhibit impaired learning when examined using the Morris water maze (24).

A number of studies have examined the neuromuscular phenotypes of the *Scn8a* mutant mice; however, the effect of $Na_v1.6$ dysfunction on seizure thresholds has not been investigated. To determine if $Na_v1.6$ dysfunction leads to altered seizure thresholds, we examined the thresholds of heterozygous $Scn8a^{med/+}$ and $Scn8a^{med\text{-}jo/+}$ mice to seizures induced by the chemiconvulsants flurothyl and KA. In addition, we explored possible genetic interactions between *Scn1a* and *Scn8a* by analyzing the seizure thresholds and lifespan of $Scn1a^{+/-}$; $Scn8a^{med\text{-}jo/+}$ double heterozygous mutants.

RESULTS

$Na_v1.6$ dysfunction increases seizure thresholds

To examine the effect of $Na_v1.6$ dysfunction on seizure thresholds, $Scn8a^{med/+}$, $Scn8a^{med\text{-}jo/+}$, and wild-type littermates were exposed to flurothyl, a chemiconvulsant thought to inhibit GABA-mediated neurotransmission (Fig. 1) (25). The latency to the myoclonic jerk (MJ), the first observable behavioral response, and the latency to the generalized tonic-clonic seizure (GTCS) were measured. Latencies to the MJ and the GTCS were increased by 25% ($P = 2.0 \times 10^{-4}$) and 58% ($P = 8.1 \times 10^{-7}$), respectively, in $Scn8a^{med/+}$ mutants when compared to wild-type littermates (Fig. 1A). Similarly, $Scn8a^{med\text{-}jo/+}$ mice showed a 31% increase ($P = 4.4 \times 10^{-7}$) in the latency to the MJ and a 55% increase ($P = 1.4 \times 10^{-6}$) in the latency to the GTCS (Fig. 1B), indicating that the *Scn8a* mutants are more resistant to flurothyl-induced seizures.

Seizure thresholds in response to KA, a kainate glutamate receptor agonist, were also assessed in $Scn8a^{med/+}$ and $Scn8a^{med\text{-}jo/+}$ mice (Fig. 2). Seizure severity was measured using a modified Racine scale in which a score of 0 indicates no response and a score of 6 indicates death (26). At a dose of 20 mg/kg KA, $Scn8a^{med/+}$ mice progressed to an average seizure severity of 3.0, and no GTCS was observed. In contrast, wild-type littermates progressed to an average seizure severity of 5.0 (P=0.009), with 80% exhibiting a GTCS ($P = 7.1 \times 10^{-4}$) (Fig. 2A). $Scn8a^{med/+}$ mice also displayed a trend toward increased seizure resistance at a dose of 30 mg/kg KA; however, the measured parameters were not statistically different when compared to wild-type littermates (data not shown). At a dose of 20 mg/kg KA, $Scn8a^{med\text{-}jo/+}$ mutants progressed to an average seizure severity of 4.5, with 50% exhibiting a GTCS. Wild-type littermates reached an average seizure severity of 5.4 ($P = 0.37$), with 80% exhibiting a GTCS ($P = 0.20$) (Fig. 2B). The average seizure severity score of the $Scn8a^{med\text{-}jo/+}$ mutants reflected a similar trend ($P = 0.19$) consistent with increased seizure resistance at a dose of 15 mg/kg KA (data not shown).

$Na_v1.6$ dysfunction restores normal seizure thresholds in a mouse model of SMEI

A mouse model of SMEI was recently generated by targeted deletion of the mouse *Scn1a* gene (13). $Scn1a^{+/-}$ mutants exhibit spontaneous tonic-clonic seizures and reduced lifespan. In order to investigate whether the increased seizure resistance associated with $Na_v1.6$ dysfunction could ameliorate the SMEI phenotype, we compared the thresholds to flurothyl-induced seizures in $Scn1a^{+/-}$ mice to $Scn1a^{+/-}$; $Scn8a^{med\text{-}jo/+}$ double heterozygous mutants.

The latency to the MJ in $Scn1a^{+/-}$ mutants was not statistically different from wild-type littermates (Fig. 3A). In contrast, the latency to the GTCS was reduced by 27% ($Scn1a^{+/-}$, 4.46 ± 1.19 min; wild-type, 6.13 ± 0.69 min; $P = 0.0049$) (Fig. 3B). $Scn1a^{+/-}$ mice also displayed a more severe response with 62% of $Scn1a^{+/-}$ mutants progressing to tonic hind-limb extension following the GTCS, compared to only 9% of the wild-type littermates ($P = 0.043$) (Table 1). The latency to the MJ and the GTCS were increased by 39% ($P = 0.0034$) and 45% ($P = 0.0017$) in $Scn1a^{+/-}$; $Scn8a^{med\text{-}jo/+}$ mutants when compared to $Scn1a^{+/-}$ littermates (Fig. 3A and B). Thresholds to the GTCS in the double heterozygous mutants were comparable to that of wild-type littermates ($Scn1a^{+/-}$; $Scn8a^{med\text{-}jo/+}$, 6.46 ± 0.65 min; wild-type, 6.12 ± 0.41 min; $P = 0.31$) (Fig. 3B). The percentage of mice progressing to tonic hind-limb extension following the

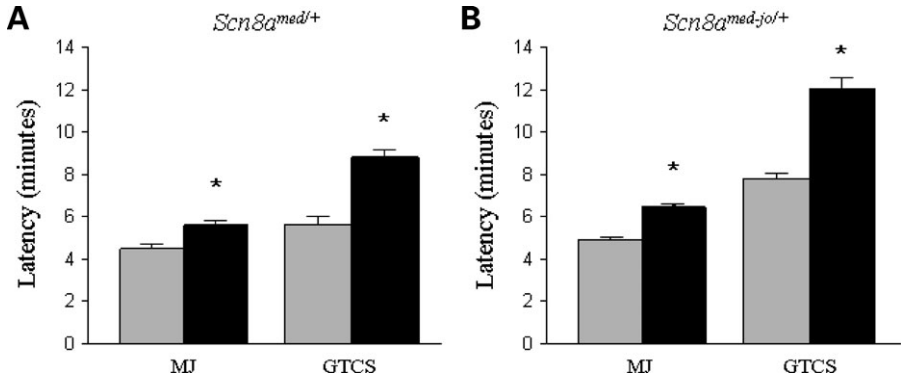

Figure 1. Elevated thresholds to flurothyl-induced seizures in the *Scn8a* mutants. The average latency in minutes to the MJ and the GTCS is shown for (**A**) *Scn8a*$^{med/+}$ and (**B**) *Scn8a*$^{med\text{-}jo/+}$ mutants. Gray bars, wild-type; black bars, mutant; $n = 12–14$ per group. Error bars represent SEM. Asterisk indicates $P < 0.05$ when compared to wild-type littermates.

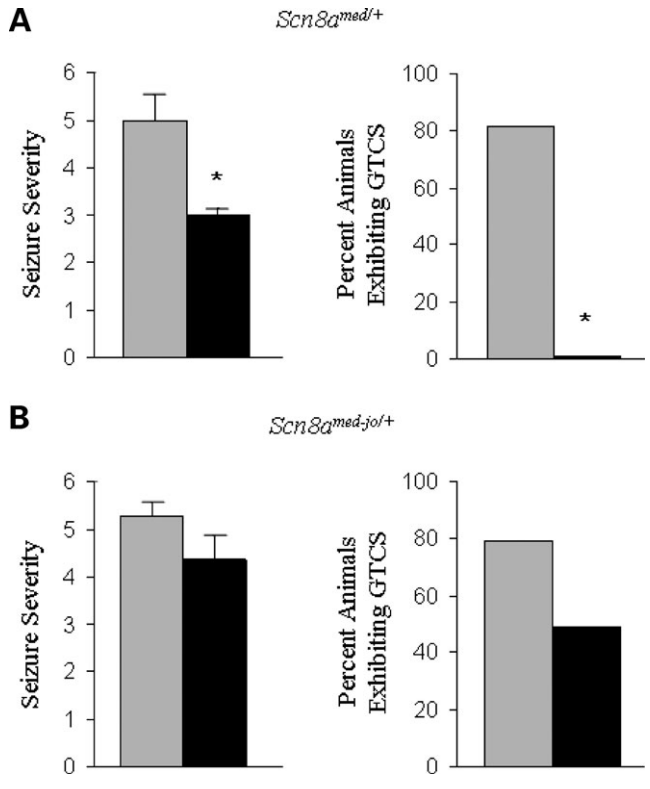

Figure 2. Elevated thresholds to KA-induced seizures in the *Scn8a* mutants. The average seizure severity and the percentage of mice progressing to GTCS are shown for (**A**) *Scn8a*$^{med/+}$ and (**B**) *Scn8a*$^{med\text{-}jo/+}$ mutants after administration of 20 mg/kg KA. Gray bars, wild-type; black bars, mutant; $n = 10–12$ per group. Error bars represent SEM. Asterisk indicates $P < 0.05$ when compared to wild-type littermates.

GTCS was reduced from 63% in *Scn1a*$^{+/-}$ mice to 0% in *Scn1a*$^{+/-}$; *Scn8a*$^{med\text{-}jo/+}$ mice ($P = 0.026$), which was similar to the incidence in wild-type littermates (Table 1). The increased threshold to flurothyl-induced GTCS and the reduced severity of the seizure response demonstrates that the *Scn8a*$^{med\text{-}jo/+}$ mutation can rescue the seizure susceptibility of *Scn1a*$^{+/-}$ mice.

The *Scn8a*$^{med\text{-}jo}$ mutation rescues the premature lethality of heterozygous *Scn1a*$^{+/-}$ mice

To determine if the *Scn8a*$^{med\text{-}jo}$ mutation could also improve the survival of *Scn1a*$^{+/-}$ mice, we compared the lifespan of *Scn1a*$^{+/-}$ mice to *Scn1a*$^{+/-}$; *Scn8a*$^{med\text{-}jo/+}$ littermates (Fig. 4A). Twelve *Scn1a*$^{+/-}$ and 10 *Scn1a*$^{+/-}$; *Scn8a*$^{med\text{-}jo/+}$ mutants were examined for 150 days from birth. During this time period we observed frequent spontaneous generalized seizures in the *Scn1a*$^{+/-}$ mice. A mortality rate of 40 and 100% at postnatal days 50 (P50) and 125 (P125) were noted for the *Scn1a*$^{+/-}$ mice. In contrast, no visible seizures or death was observed in *Scn1a*$^{+/-}$; *Scn8a*$^{med\text{-}jo/+}$ mice during the 150 day observation period (Fig. 4A).

To confirm that the increased lifespan of the double heterozygous mutants was due to reduced seizure activity, we examined the electrocorticogram (ECoG) pattern of wild-type, *Scn1a*$^{+/-}$, and *Scn1a*$^{+/-}$; *Scn8a*$^{med\text{-}jo/+}$ littermates (Fig. 5). ECoGs were recorded from 8 to 10 week-old freely moving mice. *Scn1a*$^{+/-}$ mice display ictal polyspike activity accompanied by stereotypic seizure behaviors with a typical duration of 15–30 s (Fig. 5B) (13). *Scn1a*$^{+/-}$; *Scn8a*$^{med\text{-}jo/+}$ mutants displayed frequent generalized spike and wave discharges that were associated with behavioral arrest. The average occurrences of the discharges in the *Scn1a*$^{+/-}$; *Scn8a*$^{med\text{-}jo/+}$ mutants were 35 and 50 per hour during the light and dark cycles, respectively. The frequencies of the discharges ranged from 2.3 to 12 Hz, with the majority of discharges occurring between 5–6 Hz. Unlike the ictal polyspike activity observed in the *Scn1a*$^{+/-}$ mice, the spike and wave discharges in the double heterozygous mutants were typically much shorter in duration (2–3 s) and did not involve a behavioral component (Fig. 5C). These brief spike and wave discharges were not observed in *Scn1a*$^{+/-}$ mice (13).

$Na_v1.6$ dysfunction prolongs the lifespan of homozygous *Scn1a*$^{-/-}$ mutants

We also investigated the survival of *Scn1a*$^{-/-}$ mice carrying the *Scn8a*$^{med\text{-}jo}$ mutation. Unlike homozygous *Scn1a*$^{-/-}$ mice that do not survive beyond P15, six of the nine *Scn1a*$^{-/-}$; *Scn8a*$^{med\text{-}jo/+}$ mutants observed survived beyond

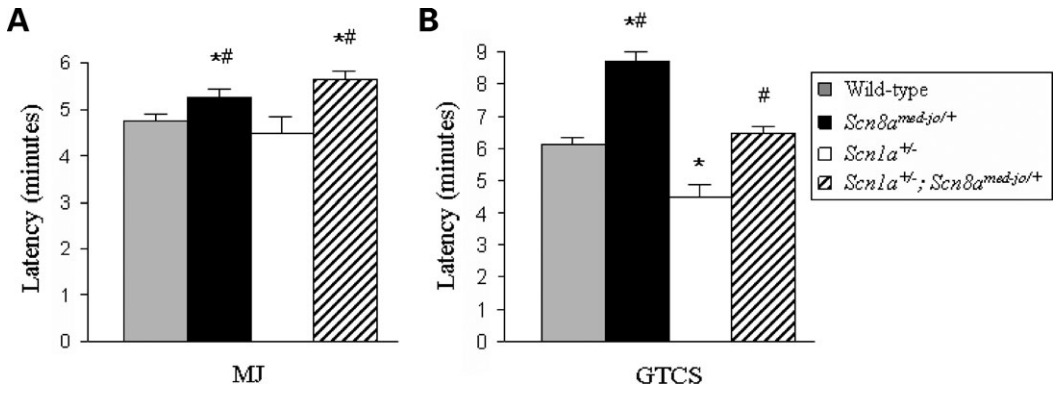

Figure 3. Restoration of normal thresholds to flurothyl-induced seizures in *Scn1a*$^{+/-}$; *Scn8a*$^{med\text{-}jo/+}$ mutants. The average latency in minutes to the (**A**) MJ and (**B**) GTCS is shown. Gray bars, wild-type; black bars, *Scn8a*$^{med\text{-}jo/+}$; white bars, *Scn1a*$^{+/-}$; stripped bars, *Scn1a*$^{+/-}$; *Scn8a*$^{med\text{-}jo/+}$; $n = 8-11$ per group. Error bars represent SEM. Asterisk indicates $P < 0.05$ when compared to wild-type littermates. Hash mark indicates $P < 0.05$ when compared to *Scn1a*$^{+/-}$ littermates.

Table 1. Number of mice exhibiting tonic hind-limb extension following flurothyl-induced GTCS

Genotype	No. of mice examined	No. of mice exhibiting hind-limb extension	Percentage
Wild-type	11	1	9
Scn8a$^{med\text{-}jo/+}$	9	0	0
Scn1a$^{+/-}$	8	5	63
Scn1a$^{+/-}$;*Scn8a*$^{med\text{-}jo/+}$	8	0	0

P15 (P=0.003). Three mutants died at P15, two at P16, three at P17, and one at P18 (Fig. 4B). Although the presence of the *Scn8a*$^{med\text{-}jo}$ allele was unable to rescue the premature lethality of *Scn1a*$^{-/-}$ mice, it was able to modestly improve lifespan.

DISCUSSION

Mutations in *SCN1A* cause several dominant idiopathic generalized epilepsy syndromes including GEFS+ and SMEI. A prominent feature of both GEFS+ and SMEI is the variable severity of the disease phenotype. Family members carrying the same GEFS+ mutation display phenotypes ranging from childhood febrile seizures to mild or severe adult afebrile epilepsy to SMEI (11,12). This variable clinical presentation within a family suggests that genetic and/or environmental factors may modify the severity of the GEFS+ and SMEI phenotypes.

Although it is widely accepted that the clinical presentation of a disease can be influenced by genetic modifiers, only a small number of human modifier genes have been identified (27–29). Studies conducted in mice with sodium channel mutations, including the *Scn2a*^{Q54}, *Scn8a*^{medJ} and *Scn1a* loss of function mutants, have demonstrated that their phenotypes can be influenced by genetic modifiers (15,30–32). Genetic modifiers that influence the seizure phenotype of the *Scn2a*^{Q54} mutant have been localized to chromosomes 11 and 19 (30). The phenotype of *Scn2a*^{Q54} mice can also be altered by the *Szt1* or *Nmf134* mutant alleles of the potassium channel *Kcnq2*. Double heterozygous mutants carrying the *Scn2a*^{Q54} transgene and either *Kcnq2* mutation display a more severe phenotype characterized by early onset generalized epilepsy and juvenile lethality at 3 weeks of age (33). A dramatic example of genetic modification is provided by homozygous *Scn8a*^{medJ} mutants. When maintained on a C57BL/6J background, homozygous *Scn8a*^{medJ} mutants exhibit premature lethality by 4 weeks of age. In contrast, on genetic backgrounds that carry a functional copy of the dominant genetic modifier *Scnm1*, *Scn8a*^{medJ} mutants exhibit a normal lifespan (31,32). Here, we demonstrate that *Scn8a* can function as a modifier in a mouse model of SMEI by restoring normal seizure thresholds and improving survival. These results provide evidence for genetic modification as a mechanism by which SMEI and possibly GEFS+ phenotypes can be altered, and further supports the notion that genetic variants in one ion channel can modify the phenotype caused by a deleterious mutation in another ion channel.

Mutations in the mouse *Scn8a* gene result in a variety of recessive neuromuscular phenotypes, including tremor, cerebellar ataxia, dystonia and paralysis. The tremor and ataxia are believed to result from the altered biophysical properties of Purkinje cells (34,35). Purkinje cells of homozygous *Scn8a* mutants display reduced persistent current, reduced resurgent current and diminished spontaneous simple spikes (34,36,37). In addition, prefrontal cortex pyramidal cells and retinal ganglion cells of the homozygous *Scn8a*^{med} mutants also show reduced excitability (38,39).

Scn8a$^{med\text{-}jo/+}$ and *Scn8a*$^{med/+}$ mutants exhibited comparable increases in thresholds to flurothyl-induced seizures, while *Scn8a*$^{med/+}$ mice appeared to exhibit higher thresholds to KA-induced seizures. Electrophysiological analysis of Purkinje cells from homozygous *Scn8a*^{med} and *Scn8a*$^{med\text{-}jo}$ mice revealed more pronounced defects in persistent, transient and resurgent sodium currents in the *Scn8a*^{med} mutants (37). The higher threshold to KA-induced seizures observed in *Scn8a*$^{med/+}$ mice may therefore reflect a larger effect of this mutation on neuronal excitability. However, we cannot rule out that the possibility that differences in genetic backgrounds may have contributed to this observation, since the *Scn8a*^{med} and *Scn8a*$^{med\text{-}jo/+}$ mice are maintained on the C3HeB/FeJ and C57BL/6J genetic backgrounds, respectively. In addition, loss of function *Scn8a* mutations, such as *Scn8a*^{med}, result in

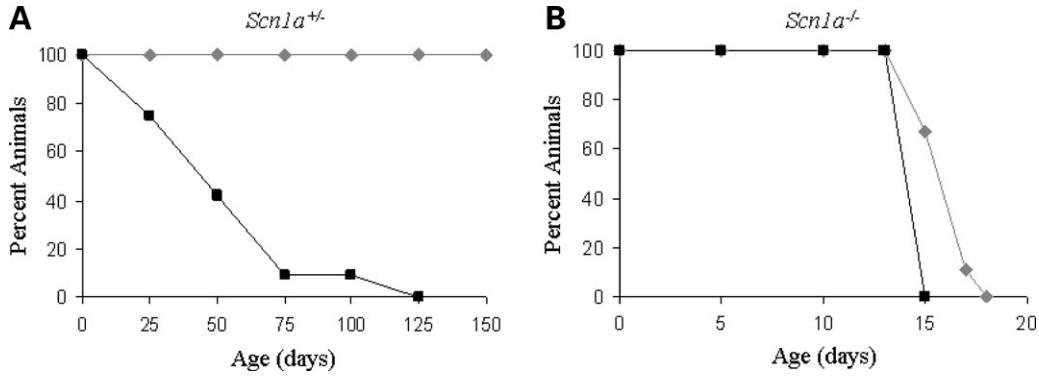

Figure 4. Improved survival of *Scn1a* mutants with the $Scn8a^{med\text{-}jo}$ allele. (**A**) $Scn1a^{+/-}$; $Scn8a^{med\text{-}jo/+}$ mutants. Black squares, $Scn1a^{+/-}$; gray diamonds, $Scn1a^{+/-}$; $Scn8a^{med\text{-}jo/+}$; $n = 9–13$ per group. (**B**) $Scn1a^{-/-}$; $Scn8a^{med\text{-}jo/+}$ mutants. Black squares, $Scn1a^{-/-}$; gray diamonds, $Scn1a^{-/-}$; $Scn8a^{med\text{-}jo/+}$; $n = 9–13$ per group.

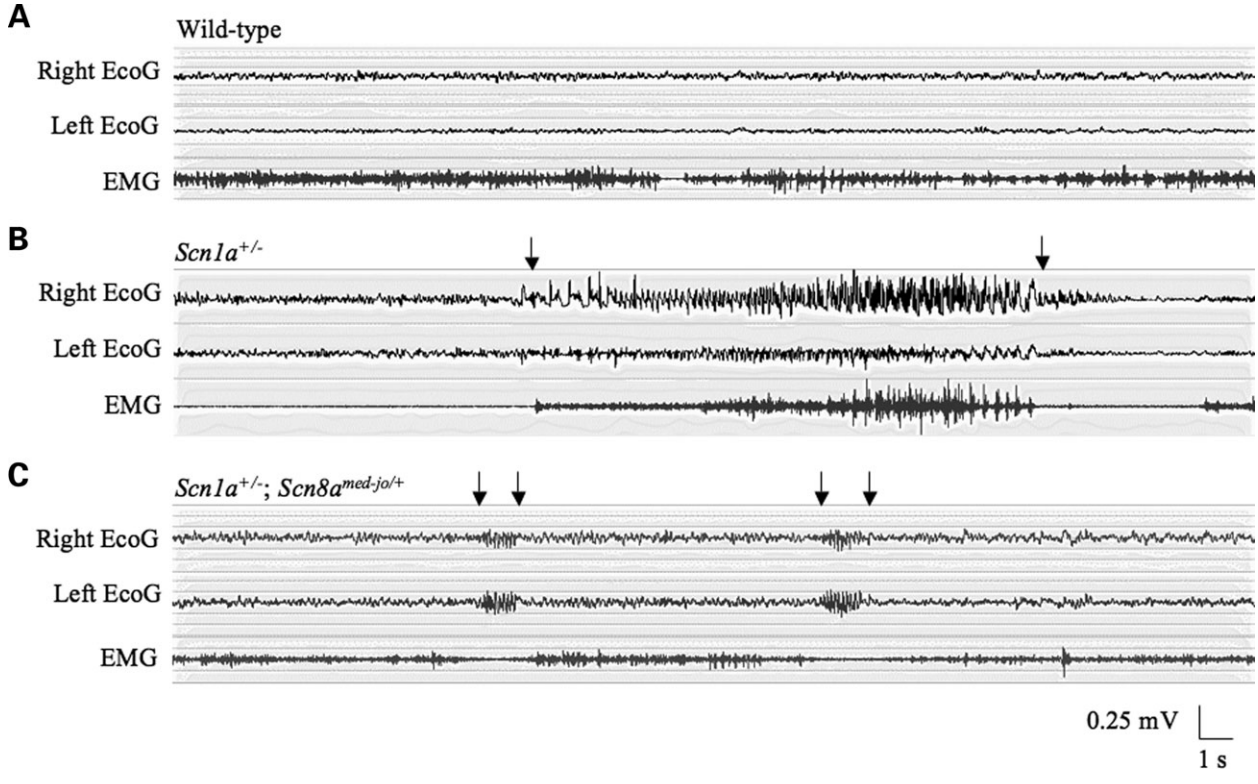

Figure 5. Decreased seizure severity in $Scn1a^{+/-}$; $Scn8a^{med\text{-}jo/+}$ mutants. Representative ECoG recordings from wild-type ($n = 3$), $Scn1a^{+/-}$ ($n = 1$), and $Scn1a^{+/-}$; $Scn8a^{med\text{-}jo/+}$ ($n = 3$) mice. (**A**) Normal ECoG pattern in an awake wild-type littermate. (**B**) ECoG pattern from an $Scn1a^{+/-}$ mutant during an ictal episode. Arrows indicate a 25 s polyspike discharge in both hemispheres accompanied by muscle activity, as indicated by the EMG channel. (**C**) ECoG pattern from $Scn1a^{+/-}$; $Scn8a^{med\text{-}jo/+}$ double heterozygous mutants. Arrows indicate brief 2–3 s spike and wave discharges in both hemispheres not associated muscle movement, as indicated by the EMG channel. Right ECoG, channel recording from the right hemisphere; Left ECoG, channel recording from the left hemisphere; EMG, channel recording from neck muscle.

cell-specific compensatory changes in which $Na_v1.1$ or $Na_v1.2$ channels accumulate at the nodes of Ranvier and initial segments of the cerebellum, optic nerve, cortex, and hippocampus (39). As a result, redistribution or up-regulation of sodium channel subunits in the $Scn8a^{med/+}$ mutant may have also contributed to the higher threshold to KA-induced seizures.

Lower seizure thresholds and spontaneous seizures are observed in mice with reduced *Scn1a* expression. In contrast, $Scn8a^{med/+}$mutants with reduced *Scn8a* expression display elevated seizure thresholds. We hypothesize that the opposing effects of $Na_v1.1$ and $Na_v1.6$ dysfunction on seizure thresholds are due to differences in the cell types that are influenced by these sodium channel subtypes. Support for this hypothesis is derived from the observation that GABAergic interneurons of the hippocampus and cortex, but not excitatory pyramidal cells, have reduced sodium currents in $Scn1a^{+/-}$ and $Scn1a^{-/-}$

mutants (13,15). In addition, $Na_v1.1$ channels preferentially localize to the soma and axons of parvalbumin-positive interneurons in the hippocampus and neocortex (15). These findings suggest that the reduced excitability of interneurons in the cortex and hippocampus contribute to seizure generation in SMEI. In contrast, excitatory neuronal cell types, such as Purkinje cells, pyramidal cells of the cortex and retinal ganglion cells, are affected in *Scn8a* mutants. Therefore, reduced excitability of pyramidal cells in the hippocampus and cortex in *Scn8a* mutants may underlie the elevated seizure resistance of these mice.

The *Scn8a*$^{med\text{-}jo}$ allele was also capable of restoring normal thresholds to flurothyl-induced seizures in *Scn1a*$^{+/-}$; *Scn8a*$^{med\text{-}jo/+}$ double heterozygous mice. Since the mechanism of action of flurothyl is not fully understood, we also evaluated the double heterozygous mutants for more physiologically relevant indicators of improved neuronal excitability. Unlike *Scn1a*$^{+/-}$ mice that display frequent spontaneous seizures with stereotypic motor behaviors and a reduced lifespan, no mortality or seizures were observed in the double heterozygous mutants. *Scn1a*$^{+/-}$ mutants also display excessive jumping and erratic behavior in response to mild startle. Whether this hyper-responsive behavior reflects seizure activity is unclear; however, this behavioral abnormality was not observed in the double heterozygous mutants.

In the presence of the *Scn8a*$^{med\text{-}jo}$ allele, a modest improvement in the lifespan of *Scn1a*$^{-/-}$ mice was also observed. Interestingly, the increase in lifespan is similar to that achieved by manual feeding of *Scn1a*$^{-/-}$ mutants (13). The incomplete rescue of the premature lethality of *Scn1a*$^{-/-}$ mice suggests that there is a limit to the magnitude of the abnormal neuronal excitability that can be corrected by altered $Na_v1.6$ function. It is also possible that the level of expression of *Scn8a* in neonatal pups may be insufficient to restore normal neuronal excitability in pups that lack $Na_v1.1$ channels. Alternatively, $Na_v1.1$ channels may play a unique role in one or more biological processes that are necessary for survival. Consistent with this possibility is the observation that *SCN1A* mutations in humans are dominant and individuals with two mutant *SCN1A* alleles have not been identified.

The findings of this study indicate that *Scn8a* can function as a genetic modifier of SMEI. Together with previous studies showing genetic interactions between *Scn2a* and *Kcnq2* in mouse epilepsy (32), our results support the idea that genetic testing for mutations and single nucleotide polymorphisms in a panel of selected ion channel genes might provide useful clinical information to guide treatment of generalized epilepsy. Furthermore, our results suggest that blocking $Na_v1.6$ channels may be one of the therapeutic benefits of currently used non-selective sodium channel-blocking anti-epileptic drugs, and raises the possibility that selective blocking of $Na_v1.6$ channels may improve efficacy in patients with epilepsy. Although pharmacological agents that can selectively target sodium channel subtypes are currently unavailable, new methodologies, such as the development of siRNAs against *SCN8A*, may enable the selective targeting of $Na_v1.6$ channels in the future. Additional studies using mice with different forms of epilepsy will also provide the opportunity to evaluate the ability of *Scn8a* to ameliorate seizure severity in more common types of epilepsy.

MATERIALS AND METHODS

Animals

C3HeB/FeJ-*Scn8a*^{med}/J and C57BL/6J-*Scn8a*$^{med\text{-}jo}$/J male mice were purchased from the Jackson Laboratory, Bar Harbor and maintained on C3HeB/FeJ and C57BL/6J backgrounds, respectively. *Scn1a*$^{+/-}$ mice were maintained on a FVB/NJ background. To generate *Scn1a*$^{+/-}$; *Scn8a*$^{med\text{-}jo/+}$ double heterozygous mutants, *Scn8a*$^{med\text{-}jo/+}$ females were crossed to *Scn1a*$^{+/-}$ males. Expected Mendelian ratios were observed (23% *Scn1a*$^{+/-}$; *Scn8a*$^{med\text{-}jo/+}$, 27% *Scn1a*$^{+/-}$, 27% *Scn8a*$^{med\text{-}jo/+}$, 23% wild-type). To generate *Scn1a*$^{-/-}$; *Scn8a*$^{med\text{-}jo/+}$ mice, *Scn1a*$^{+/-}$; *Scn8a*$^{med\text{-}jo/+}$ females were crossed to *Scn1a*$^{+/-}$ males. Expected Mendelian ratios were observed (29% *Scn1a*$^{+/-}$; *Scn8a*$^{med\text{-}jo/+}$, 24% *Scn1a*$^{+/-}$, 16% *Scn1a*$^{-/-}$; *Scn8a*$^{med\text{-}jo/+}$, 12% *Scn1a*$^{-/-}$, 8% *Scn8a*$^{med\text{-}jo/+}$, 12% wild-type). Littermates were used for all experiments to minimize variation due to differences in genetic backgrounds. Mice were reared in a pathogen-free mouse facility with a 12 h light/dark cycle. Food and water were available *ad libitum*. All experimental protocols involving mice were approved by the Emory University IACUC committee.

Genotyping of mice

Scn1a mutants were genotyped using primers pairs specific to the neomycin cassette; NF (AGGATCTCCTGTCATCT CACCTTGCTCCTG), NR (AAGAACTCGTCAAGAAGGC GATAGAAGGCG) and to *Scn1a* exon 26; 1aF (CGAATC CAGATGGAAGAGCGGTTCATGGCT), 1aR (ACAAGCTG CTATGGACATTGTCAGGTCAGT). A 290 bp PCR product was generated from the wild-type allele and a 490 bp PCR product was generated from the mutant allele using the primer pairs 1aF, 1aR and NF, NR, respectively. PCR amplification was carried out with one cycle at 94°C for 2 min and 32 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 45 s. Genotyping of C3HeB/FeJ-*Scn8a*^{med}/J mice was performed with PCR primers and conditions suggested by the Jackson Laboratory (http://jaxmice.jax.org/pub-cgi/protocols/protocols.sh?objtype=protocol&protocol_id=454). Genotyping of C57BL/6J-*Scn8a*$^{med\text{-}jo}$/J mice was performed using primer pair, 8aF (ATGCCACAGAAGTGTCATTCC) and 8aR (GGTATTTCCCAGCAAACAGGT). PCR amplification was performed with one cycle at 94°C for 2 min and 32 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 30 s. The 204 bp PCR product was digested with *Msl*I to produce a 129 bp fragment from the wild-type allele and a 102 bp fragment from the mutant allele.

Flurothyl seizure induction

Mice between 5 and 12 weeks of age were placed in a clear plexiglass chamber, and flurothyl (2,2,2-trifluroethylether) (Sigma-Aldrich) was slowly introduced into the chamber via a syringe pump at a rate of 20 μl/min and allowed to volatilize. Seizure thresholds were determined by measuring the latency to the first MJ and to the GTCS. The MJ is the first observable behavioral response, and is characterized by a brief jerk of the shoulders and/or neck. The GTCS is

characterized by convulsions of the entire body and a loss of posture. The mice were also scored for progression of the GTCS to tonic hind-limb extension. Data from males and females were analyzed separately. No sex differences in the data were observed; therefore, the data from both sexes were combined.

KA seizure induction

Mice between 3 and 4 months of age were injected intraperitoneally (i.p.) with 15, 20 or 30 mg/kg KA (Ocean Produce International). KA was dissolved in 0.9% saline to a concentration of 2.5 mg/ml in order to obtain an appropriate injection volume. All mice were injected between 12 noon and 4 p.m. to minimize behavioral variation due to the circadian rhythm. After the injection of KA, mice were observed for 2 h and scored according to a modified Racine scale (26). This modified scale is based on the following criteria: stage 0 – no response; stage 1 – staring; stage 2 – head nodding; stage 3 – forelimb clonus; stage 4 – rearing and falling; stage 5 – GTCS; stage 6 – death. Data from males and females were analyzed separately. No sex differences in the data were observed; therefore, the data from both sexes were combined.

Statistical analysis

For parametric data sets, statistical analysis between genotypes was performed using the Student *t*-test. Dichotomous data sets (the number of mice exhibiting a GTCS and the mortality rates) were analyzed for statistical significance using the Fisher Exact test, while non-parametric data (the KA seizure stage) was analyzed using a Mann–Whitney Rank Sum test.

Electrocorticography

Wild-type, *Scn1a*$^{+/-}$, and *Scn1a*$^{+/-}$; *Scn8a*$^{\text{med-jo}}$ mice were implanted for ECoG recordings. Briefly, mice were anesthetized with isoflurane gas and placed onto a stereotaxic frame (Cartesian Research, OR, USA). Two pairs of bipolar stainless steel screws were placed ipsilaterally for ECoG monitoring. One pair on the right hemisphere (anteroposterior (AP) = + 2.00 mm from bregma, mediolateral (ML) = + 1.00 mm from midline; AP = − 1.5 mm from bregma, ML = + 1.0 mm from midline) and the other pair on the left hemisphere (AP = − 0.5 mm from bregma, ML = − 2.2 mm from midline; AP = − 3.5 mm from bregma, ML = − 2.2 mm from midline). In addition, two fine wires (Cooner AS632, CA, USA) were inserted into the dorsal neck muscles to facilitate recording of muscle activity. ECoG and electromyogram (EMG) electrodes were attached to a micro-connector (VMS, OH, USA), and dental ceramic compound was poured over the screws, wires and base of the skullcap.

After 3 days of recovery, each mouse was placed into a Plexiglas box (15 cm × 15 cm × 15 cm) and was attached to a series of bioelectric amplifiers (Embla A10, Iceland) via a small counterbalanced commutator (Dragonfly Research, WV, USA). Following 24 h of acclimatization, ECoG and EMG data were collected for 96 h. Amplified ECoG and EMG signals were digitally collected, processed and viewed in real-time with the Somnologica Science rodent sleep-recording software package (Iceland). Epileptiform activity was manually scored.

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