Temperature- and age-dependent seizures in a mouse model of severe myoclonic epilepsy in infancy

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Heterozygous loss-of-function mutations in the α subunit of the type I voltage-gated sodium channel $Na_V1.1$ cause severe myoclonic epilepsy in infancy (SMEI), an infantile-onset epileptic encephalopathy characterized by normal development followed by treatment-refractory febrile and afebrile seizures and psychomotor decline. Mice with SMEI (mSMEI), created by heterozygous deletion of $Na_V1.1$ channels, develop seizures and ataxia. Here we investigated the temperature and age dependence of seizures and interictal epileptiform spike-and-wave activity in mSMEI. Combined video-EEG monitoring demonstrated that mSMEI had seizures induced by elevated body core temperature but wild-type mice were unaffected. In the 3 age groups tested, no postnatal day (P)17–18 mSMEI had temperature-induced seizures, but nearly all P20–22 and P30–46 mSMEI had myoclonic seizures followed by generalized seizures caused by elevated core body temperature. Spontaneous seizures were only observed in mice older than P32, suggesting that mSMEI become susceptible to temperature-induced seizures before spontaneous seizures. Interictal spike activity was seen at normal body temperature in most P30–46 mSMEI but not in P20–22 or P17–18 mSMEI, indicating that interictal epileptic activity correlates with seizure susceptibility. Most P20–22 mSMEI had interictal spike activity with elevated body temperature. Our results define a critical developmental transition for susceptibility to seizures in SMEI, demonstrate that body temperature elevation alone is sufficient to induce seizures, and reveal a close correspondence between human and mouse SMEI in the striking temperature and age dependence of seizure frequency and severity and in the temperature dependence and frequency of interictal epileptiform spike activity.

Dravet syndrome | febrile seizures | generalized tonic-clonic seizures | sodium channels | SCN1A

Advances in human genetics have led to identification of numerous mutations associated with epileptic syndromes (1). The gene most commonly mutated in epilepsy is SCN1A, with more than 200 mutations identified (2, 3). SCN1A codes for the pore-forming α subunit of the $Na_V1.1$ voltage-gated sodium channel (4), a quantitatively minor brain sodium channel subtype localized to cell bodies of central neurons, where it contributes to action potential generation (5, 6).

Mutations in SCN1A are associated with epileptic syndromes spanning a continuum of severity from the relatively mild generalized epilepsy with febrile seizures plus (GEFS+) to severe myoclonic epilepsy in infancy (SMEI) (7). GEFS+ is an autosomal-dominant epileptic syndrome associated with missense mutations in SCN1A (8). Affected individuals have febrile seizures early in life but also have both febrile and afebrile seizures that persist beyond age 6. They do not develop cognitive or motor dysfunction. In contrast, SMEI is a severe infantile-onset epilepsy associated with loss-of-function mutations in SCN1A (2, 3, 9). Development is initially normal, but at 6–12 months of age affected individuals experience seizures that are frequently associated with fever followed by more severe spontaneous seizures that are resistant to pharmacotherapy and eventually psychomotor dysfunction (10–12). The EEG is initially normal but interictal epileptic activity is observed with disease progression (13). Psychomotor dysfunction may correlate with seizure severity at an early age, and stepwise decline in psychomotor function has been described after status epilepticus (12).

It is a paradox that loss-of-function mutations in $Na_V1.1$ channels, which contribute to action potential generation, cause hyperexcitability and epilepsy. To understand SMEI in more detail, we created a mouse model of SMEI by targeted deletion of one copy of the SCN1A gene (14). Mice with SMEI (mSMEI) have spontaneous seizures, ataxia, and premature deaths. Heterozygous loss of function in $Na_V1.1$ results in reduced whole-cell sodium current in hippocampal GABAergic inhibitory interneurons but not excitatory pyramidal cells (14). A similar loss of sodium current is observed in cerebellar Purkinje cells, which are also GABAergic inhibitory neurons (15). The disinhibition caused by selective failure of excitability of GABAergic inhibitory neurons is likely the cause of hyperexcitability, epilepsy, and ataxia in mSMEI (14–16).

Because of the small number of patients and their variable clinical course, it has been difficult to precisely define the essential features of SMEI in humans. Therefore, the mouse model of SMEI offers a unique opportunity to probe its pathophysiology experimentally. Using combined video-EEG recording, we have addressed 6 fundamental questions concerning mSMEI: (*i*) Can seizures be induced by elevated body core temperature? (*ii*) Is there a critical age for development of temperature-induced seizure susceptibility? (*iii*) Can temperature-induced seizures be provoked at a time in development before spontaneous seizures occur? (*iv*) How do the frequency and severity of seizures evolve with age? (*v*) What interictal EEG activities predict seizure susceptibility and severity? (*vi*) How do these features of mSMEI compare to those of the human disease?

Results

Temperature-Induced Seizures in mSMEI. The first seizure in infants with SMEI frequently occurs during fever or less frequently during a hot bath (10, 12, 13, 17). In light of these clinical data, we tested whether elevated temperature per se was sufficient to cause seizures in mSMEI. Because C57BL/6 mSMEI were much more susceptible to premature death than 129SvJ mSMEI (14), we studied SCN1A(+/−) mice bred into C57BL/6 for 10 generations. Core body temperature was increased by 0.5 °C every 2 min (supporting information (*SI*) Fig. S1*A*, *Top*), mimicking a typical fever. We recorded EEGs from 6 bilateral cranial sites simultaneously (Fig. 1*A* and *B*), and seizure-related behaviors were captured with video recording. Wild-type mice had no epileptic activity up to 42.5 °C at

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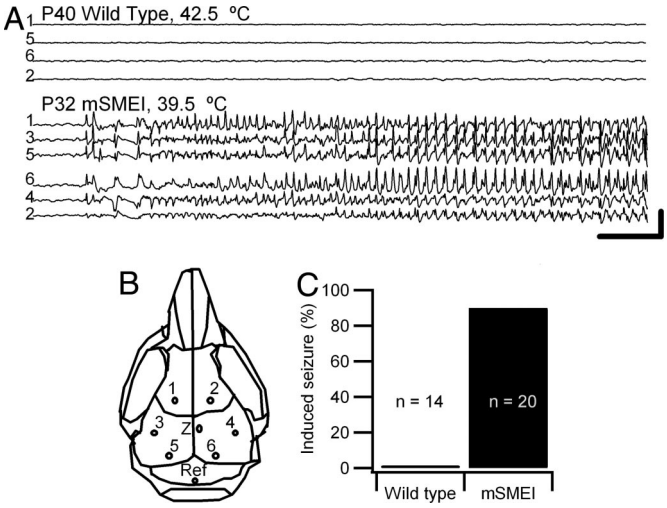

Fig. 1. Temperature-dependent seizures in mSMEI. (*A*) Representative 10-s traces of intracranial EEG activity at the temperature specified. Trace labels correspond to electrode positions in *B*. The top traces are from a representative P40 wild-type mouse. The bottom traces are from a P32 mSMEI. A seizure was provoked at 39.5 °C. Calibration: 1 s, 1000 μV. (*B*) A drawing of a mouse cranium with electrode positions indicated. (*C*) Percentage of P20–46 mSMEI having seizure induced by elevated temperature, 38.0–42.5 °C (wild type, 0%; mSMEI, 90%).

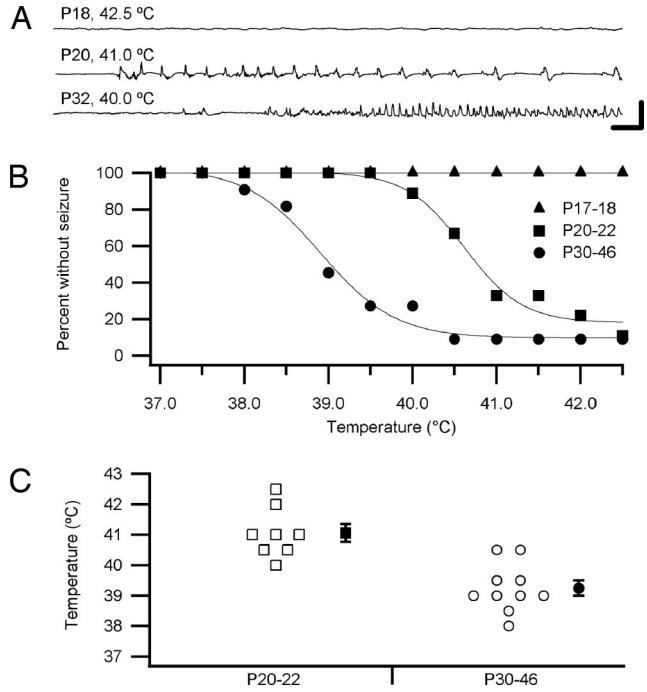

Fig. 2. Age-dependent susceptibility to temperature-induced seizures. (*A*) Representative 10-s single channel (position 1 in Fig. 1*B*) EEG traces from P18 *Top*, P20 *Middle*, and P32 *Bottom* at the temperatures indicated. Calibration: 0.5 s, 2000 μV. (*B*) Percentage of mSMEI remaining free of seizure plotted against body temperature. (*C*) Mean and distribution of seizure temperatures for P20–22 and P30–46 mSMEI.

any age tested (Fig. 1*A* [*Top*] and *C*; Fig. S1*A*; $n = 14$). In contrast, mSMEI often had temperature-induced seizures at 39.5 °C (Fig. 1*A*, *Bottom*, postnatal day (P)32, and Fig. S1*B*), and nearly all of the P20–46 mSMEI had temperature-induced seizures (Fig. 1*C*; $n = 18/20$). These results demonstrate that temperature elevation alone is sufficient to reliably induce seizures in mSMEI and therefore implicates a temperature-dependent mechanism, rather than an inflammatory mechanism, in the genesis of febrile seizures in SMEI. mSMEI also have increased sensitivity to the convulsant flurothyl (18), suggesting a general increase in susceptibility to seizure inducers.

A Critical Age-Dependent Transition for Susceptibility to Temperature-Induced Seizures in mSMEI. Infants with SMEI do not begin to have seizures until 6 months of age, suggesting age-dependent seizure susceptibility. We tested susceptibility to temperature-induced seizures for 3 age groups: P17–18, 20–22, and P30–46. Baseline recording was performed at normal body temperature (37.5 °C) for at least 30 min, allowing the animals to explore the environment and become more relaxed. Body temperature was increased by 0.5 °C every 2 min until a seizure occurred or 42.5 °C was reached (Fig. S1*A*). No P17–18 mSMEI ($n = 5/5$) had seizures (Fig. 2*A*, *Top*), even at 42.5 °C. However, most P20–22 mSMEI ($n = 8/9$, Fig. 2*A*, *Middle*) and P30–46 mSMEI ($n = 10/11$, Fig. 2*A*, *Bottom*) had temperature-induced seizures. The seizures consisted of spike-and-wave complexes that were generalized at the onset of the seizure (Fig. 2*A*, *Bottom*, $n = 18/18$), followed by postictal suppression of the background EEG patterns. These experiments define P18–20 as a critical time point in mSMEI development at which susceptibility to temperature-induced seizures first emerges.

While P20–22 and P30–46 mSMEI both reliably have temperature-induced seizures, P30–46 mSMEI have temperature-induced seizures at a lower threshold temperature than the younger P20–22 mSMEI (Fig. 2 *B* and *C*, $P = 0.0002$). No temperature-induced seizures were seen in P20–22 mSMEI until 40 °C, whereas seizures were first seen in P30–46 mSMEI at 38.0 °C (Fig. 2*B*). Half of the P20–22 mSMEI had seizures by 41 °C (Fig. 2*B*) with temperature-induced seizures occurring at an average temperature of 41.1 ± 0.3 °C (Fig. 2*C*). In contrast, half of the P30–46 mSMEI had seizures by 39 °C (Fig. 2*B*) with temperature-induced seizures occurring at an average temperature of 39.3 ± 0.3 °C (Fig. 2*C*). Despite their difference in threshold for temperature-induced seizures, nearly equal percentages of P20–22 and P30–46 mSMEI have seizures by 42.5 °C (87.5% versus 90.9%, Fig. 2*B*). These results reveal a striking age-dependent transition in the development of seizure susceptibility in SMEI.

Age Dependence of Seizure Severity in mSMEI. A crucial feature of human SMEI is increasing severity of seizures with age. To examine whether mSMEI exhibit this feature of the human syndrome, we further evaluated the electrographic and behavioral characteristics of temperature-induced seizures by age in mSMEI. Seizure severity was graded by duration, behavior, seizure-related spike frequency, and total number of spikes. The average seizure duration for P30–46 mSMEI (25.3 ± 1.5 s) was longer than for P20–22 mSMEI (13.3 ± 3.1 s, $P = 0.002$, Fig. 3*A*). Older mSMEI also have more severe seizure-related behaviors graded by Racine score (Fig. 3*B*; P30–46, 4.6 ± 0.2 versus P20–22, 3.4 ± 0.3, $P = 0.002$). The average spike frequency for P20–22 mSMEI (2.3 ± 0.4 Hz) was lower than for older P30–46 mSMEI (7.5 ± 0.3 Hz, $P = 2.3 \times 10^{-8}$; Fig. 3*C*), and the total number of spikes during a seizure was less for P20–22 (31 ± 7 spikes) than for P30–46 mSMEI (176 ± 13 spikes, $P = 1.5 \times 10^{-7}$; Fig. 3*D*). Altogether, these results demonstrate a substantial increase in the severity of seizures with age in mSMEI.

Myoclonic Seizures in mSMEI. Myoclonic seizures are frequently observed preceding generalized tonic-clonic seizures in children with SMEI. With elevated body temperatures, myoclonic seizures were recorded in both P20–22 (Fig. 4*A*) and P30–46 mSMEI (data not shown) before generalized seizures. Myoclonic seizures were never seen in P17–18 mSMEI up to 42.5 °C

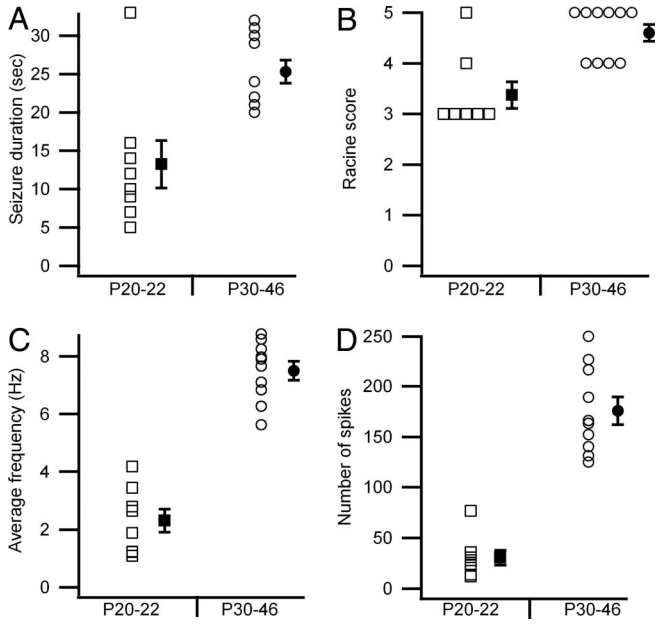

Fig. 3. Severity of age-dependent temperature-induced seizures. (*A*) Mean and distribution of seizure duration. P20–22, 13.3 ± 3.1 s; P30–46, 25.3 ± 1.5 s, $P = 0.002$. (*B*) Mean and distribution of seizure-related behavioral severity determined by the Racine score. P20–22, 3.4 ± 0.3; P30–46 mSMEI, 4.6 ± 0.2; $P = 0.002$). (*C*) Mean and distribution of average spike frequency during seizure. P20–22, 2.3 ± 0.4 Hz; P30–46, 7.5 ± 0.3 Hz; $P = 2.3 \times 10^{-8}$. (*D*) Mean and distribution of total number of spikes during seizure. P20–22, 30.6 ± 0.2 spikes; P30–46, 176 ± 13.5 spikes, $P = 1.5 \times 10^{-7}$.

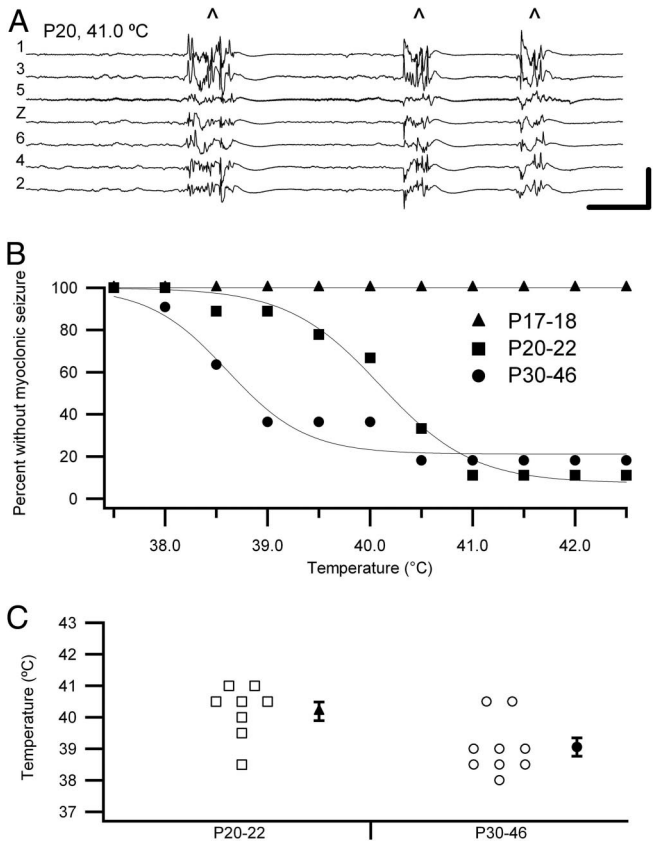

Fig. 4. Temperature dependence of myoclonic seizures in mSMEI. (*A*) Representative EEG traces in P20 mSMEI during temperature induction of seizures (myoclonic seizures indicated by ^). Trace labels correspond to electrode positions in Fig. 1*B*. Calibration: 1 s, 1000 μV. (*B*) Percentage of mSMEI remaining free of myoclonic seizures plotted against temperature. (*C*) Mean and distribution of the temperatures at which myoclonic seizures are first seen. P20–22, 40.2 ± 0.3 °C; P30–46, 39.1 ± 0.3 °C; $P = 0.02$.

($n = 5/5$, data not shown). Temperature-induced myoclonic seizures consisted of brief (< 1 s) bursts of generalized fast spikes followed by a large slow wave and brief suppression of the background EEG activity (Fig. 4*A*, Fig. S2*B*, [^]). Behaviorally, the myoclonic seizures consisted of single or occasionally multiple sharp, rapid, irregular, brief (< 1-s) jerks of forelimb or axial musculature. In the example shown in Fig. 4*A*, brief bursts of multiple spike and slow wave are seen in P20 mSMEI. The slow wave is prominent and the EEG is suppressed briefly following the complex.

Temperature-induced myoclonic seizures are age dependent. The average temperature difference between the first myoclonic seizure and the temperature-induced generalized tonic-clonic seizure was 0.88 ± 0.25 °C in P20–22 mSMEI, and all (8/8) temperature-induced seizures were preceded by myoclonic seizures. In contrast, myoclonic seizures preceded temperature-induced generalized seizures in only 70% (7/10) P30–46 mSMEI, and myoclonic seizures occurred at the same temperature as the temperature-induced seizure in 6/7 of those mice. The average temperature difference between the first myoclonic seizure and the temperature-induced generalized seizure was significantly greater for P20–22 than for P30–46 mSMEI (0.88 ± 0.25 °C versus 0.05 ± 0.05 °C, $P = 0.002$).

No P17–18 mSMEI had temperature-induced myoclonic seizures at temperatures up to 42.5 °C (Fig. 4*B*). Myoclonic seizures first occurred in P20–22 mSMEI at 38.5 °C, and 88.9% had myoclonic seizures by 42.5 °C (Fig. 4*B*). In contrast, myoclonic seizures first occurred at 38 °C in P30-P46 mSMEI and 81.8% had myoclonic seizures by 42.5 °C. The mean temperature at which temperature induced myoclonic seizures were first observed was significantly lower for P30–46 than for P20–22 mSMEI (Fig. 4*C*, 39.1 ± 0.3 °C versus 40.2 ± 0.3 °C, $P = 0.02$). These data provide evidence that the mechanism underlying myoclonic seizures has both age-dependent and temperature-dependent components.

Age Dependence of Spontaneous Generalized Seizures in mSMEI. Infants with SMEI frequently have febrile seizures before developing spontaneous seizures. This suggests a developmentally regulated seizure susceptibility in which initial seizures are usually realized only with an additional provoking factor such as elevated temperature. We previously reported spontaneous seizures in mSMEI beginning on P21–27 (14). These seizures were noted from behavioral observations during daily routine handling of many cages of animals, so the frequency of spontaneous seizures per animal was very low and could not be accurately quantified. In our current experiments, video-EEG monitoring lasting 0.5–6 h was performed on younger (P17–22) and older (P32–57) mice. No spontaneous seizures were captured in younger mSMEI (28.2 h, $n = 17$ mice, average recording duration 1.7 ± 0.3 h). Seven spontaneous seizures were captured in older mSMEI (97 h, $n = 16$ mice, average recording duration 1.9 ± 0.2 h) corresponding to a rate of 1 spontaneous seizure per 13.9 h of recording. Fig. 5*A* shows a typical example of a spontaneous seizure in P44 mSMEI. The EEG shows abrupt-onset, bilaterally synchronous generalized spike-and-wave activity consistent with a generalized onset seizure and is similar to the generalized seizure pattern induced by elevated temperature (Fig. 1*A*, *Bottom*). Following the seizure, a period of suppressed EEG is seen consistent with a postictal state (Fig. 5*A*). All of the spontaneous seizures had bilaterally synchronous spike-and-wave activity at the onset. Spontaneous seizures were somewhat longer than temperature-induced seizures (compare Fig. 3*B* and

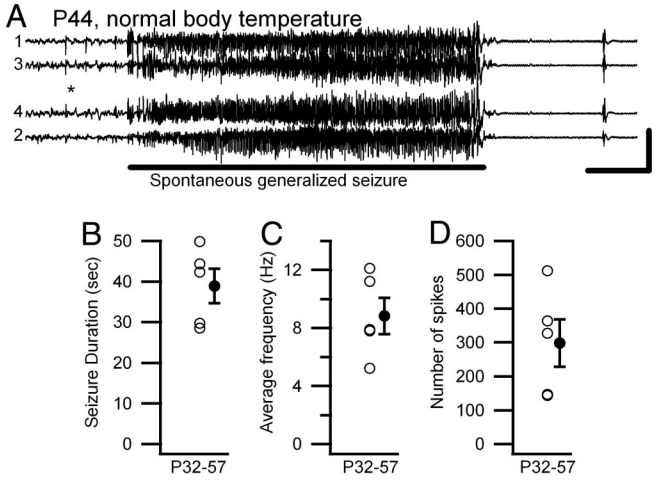

Fig. 5. Spontaneous seizures in SMEI. (*A*) Representative EEG traces from P44 mSMEI showing a spontaneous seizure occurring at normal body temperature. Trace labels correspond to electrode positions in Fig. 1*B*. Calibration: 5 s, 1000 μV. (*B*) Mean and distribution of spontaneous seizure duration (38.9 $\pm$ 4.2 s). (*C*) Mean and distribution of average spike frequency during spontaneous seizures (8.9 $\pm$ 1.2 Hz). (*D*) Mean and distribution of total number of spikes during spontaneous seizure (298 $\pm$ 70 spikes).

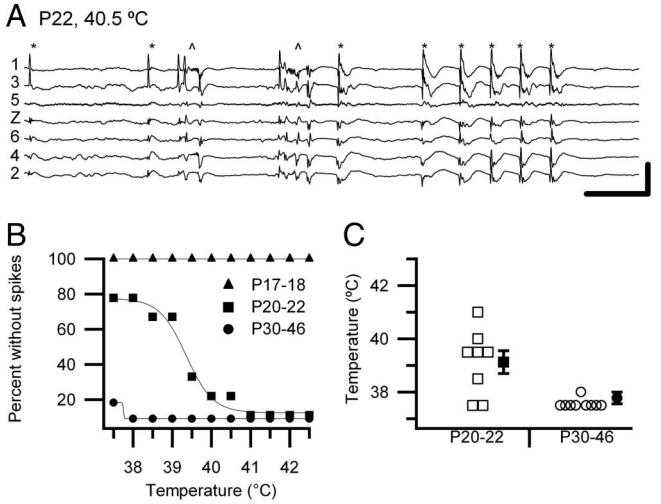

Fig. 6. Interictal spikes in mSMEI. (*A*) Representative EEG traces in a P22 mSMEI during the temperature-elevation protocol. As temperature is increased, spikes (indicated by *) and at slightly higher temperatures, myoclonic seizures (indicated by ^) are observed. Calibration: 1 s, 1000 μV. (*B*) Percentage of mSMEI of each age range remaining free of spikes plotted against body temperature. (*C*) Mean and distribution of body temperatures at which spikes are first observed. P20–22, 39.1 $\pm$ 0.4 °C; P30–46 mSMEI, 37.6 $\pm$ 0.1 °C; P = 0.0009. All mSMEI were included in the analysis in *B*, whereas only mSMEI having spikes (P20–22, 8/9; P30–46, 10/11) were included in *C*.

5*B*, 25.3 $\pm$ 1.5 s versus 38.9 $\pm$ 4.2 s, $P = 0.002$) and had a greater total number of spikes (Fig. 3*D* and 5*D*, 176 $\pm$ 13 spikes versus 298 $\pm$ 70 spikes, $P = 0.03$). The average spike frequency was not significantly different between temperature-induced and spontaneous seizures (compare Fig. 3*C* and 5*C*, 7.5 $\pm$ 0.3 Hz versus 8.9 $\pm$ 1.2 Hz, $P = 0.18$). All of the spontaneous seizures had a behavioral correlate consisting of Racine 5 activity, which was not significantly different from the average age-matched, temperature-induced seizure behavioral severity ($P = 0.08$).

Age- and Temperature-Dependent Interictal Epileptic Activity in mSMEI. Epileptic activity between seizures (interictal activity) is used clinically to help confirm the diagnosis of epilepsy and to help characterize the epilepsy syndrome. In SMEI, the severity of interictal EEG abnormalities correlates with epilepsy severity (12, 19). Interictal EEG recordings in mSMEI were examined for epileptic abnormalities. Epileptic spikes were defined as abrupt onset, sharply contoured waveforms that were greater than twice the background EEG amplitude, briefly disrupting the ongoing EEG background activity, and not associated with movement on review of the video recording. Spontaneous interictal spikes were not seen at normal body temperature in P17–18 and P20–22 mSMEI (not shown). In contrast, spontaneous interictal spikes were observed in P32–57 mSMEI during prolonged (1- to 6-h) recordings at baseline temperature (37.5 °C; Fig. 5*A* and Fig. S2*A* [*]). Typically the spikes were generalized, but occasionally focal spikes occurred as well (Fig. S2*A*[+]).

The temperature at which interictal spikes were first seen was age-dependent. Most P20–22 mSMEI had interictal spikes at elevated temperatures, as illustrated for 40.5 °C in Fig. 6*A* (*) and in Fig. S2*B*. In contrast, P17–18 mSMEI did not have interictal spikes up to 42.5 °C (Fig. 6*B*). A small number of P20–22 mSMEI had interictal spikes at the baseline temperature of 37.5 °C, whereas most of the P30–46 mSMEI had spikes at 37.5 °C (Fig. 6 *B* and *C*). The percentage of mSMEI having spikes in both of these age groups increased with temperature, such that nearly equal percentages of P20–22 and P30–46 mSMEI had spikes induced by 40.5 °C (Fig. 6*B*, Fig. S2; 88.9% versus 90.9%). The average temperature at which spikes were first observed was higher in P20–22 mSMEI than in P30–46 mSMEI (39.1 $\pm$ 0.4 °C versus 37.6 $\pm$ 0.1 °C, $P = 0.0009$, Fig. 6*C*).

The difference between the temperature at which interictal spikes were first seen and the temperature at which an induced seizure occurred was not different between P20–22 and P30–46 age groups (1.9 $\pm$ 0.6 °C versus 1.7 $\pm$ 0.2 °C, $P = 0.7$).

Discussion

Temperature-Induced Seizures in mSMEI. Infants with SMEI are indistinguishable from unaffected infants until they have their first seizure, typically at 6 to 9 months of age. This first seizure usually occurs with fever (12, 19–21). There has been debate regarding the role of elevated temperature versus the role of underlying infection or inflammation in the genesis of febrile seizures. Excess febrile seizures have been associated with HHV6 infections in some studies (22) but not all (23). There is evidence that hot baths alone are sufficient to provoke febrile seizures in SMEI (12, 24), but only 7 individuals of restricted ethnic background (Japanese) were tested (25). Is temperature the key factor that is responsible for induction of seizures in the earliest stages of SMEI or do additional pathophysiological aspects of fever also contribute? Here we demonstrated that elevated temperature alone, in the absence of infection, is sufficient to provoke seizures in mSMEI, suggesting that temperature elevation alone is responsible for seizure provocation. Our results show that elevated temperature is both necessary and sufficient for induction of seizures with high frequency in young mSMEI, implicating a temperature-sensitive mechanism in the genesis of seizures in this disease. Spontaneous seizures are very infrequent in P20–22 mSMEI, but elevated temperature induces seizures in nearly every animal within a few minutes. These results indicate that thermal sensitivity is a fundamental feature of hyperexcitability caused by loss of $Na_V1.1$ channels in the early phase of SMEI.

Myoclonic seizures are a defining characteristic of SMEI but frequently do not occur in children with SMEI until after the age of 2, making early diagnosis difficult in some cases (12). In one study of 7 patients, myoclonic seizures were seen with increasing frequency with elevated body core temperature from a hot bath (12, 25). We also observed temperature-induced myoclonic

seizures in mSMEI. Myoclonic seizures precede 100% of generalized tonic-clonic seizures in mSMEI from P20−22 and 70% of generalized tonic-clonic seizures from P30–46. We speculate that these myoclonic seizures reflect aborted generalized tonic-clonic seizures in which the competition between excitation and inhibition is won by inhibition. According to this idea, myoclonic seizures would recur until either the episode of hyperexcitability subsides or excitation prevails and a generalized tonic-clonic seizure occurs.

The physiological mechanisms that underlie reduced seizure threshold with elevated body temperature are not well understood (26). Temperature-dependent changes in GABA receptor trafficking (27), cytokine release (28), temperature-induced hyperventilation (29), and temperature-dependent modulation of temperature-sensitive channels (30) have been suggested to contribute to febrile seizures. In humans, mutations in both voltage-gated and ligand-gated ion channels (31–34) have been associated with susceptibility to febrile seizures. Febrile seizures are a defining characteristic of genetic epilepsies associated with mutations in SCN1A encoding $Na_V1.1$ channels (7, 35), in SCN1B encoding the auxiliary $Na_V1.1$ β subunit (34, 36), and in the $\gamma 2$ subunit of the GABA-A receptor (32, 37), suggesting a unique role for these channels in the genesis of febrile seizures. As we have shown here for mSMEI, temperature may be the controlling factor in febrile seizures in this larger set of seizure syndromes.

A Critical Transition for Development of Seizure Susceptibility and Severity in mSMEI. Seizures may arise stochastically on the basis of random variations in the level of excitability and/or activity in the brains of seizure-prone mSMEI, or the frequency and severity of seizures may be strongly influenced by developmental and/or environmental factors. Children with SMEI develop normally for several months before the beginning of their seizures. Is there a strong age dependence of seizure frequency and severity in mSMEI? Our results reveal an all-or-none transition in seizure susceptibility during P18–20 in mSMEI. By recreating a typical fever temperature profile, we found that mSMEI first become susceptible to thermal induction of seizures between P18 and P20. This initial susceptibility occurs at an age when we were unable to capture spontaneous seizures during recording sessions of similar duration suggesting that, as in humans, seizures are initially realized only with an additional provoking factor. This finding suggests that there is a developmentally regulated process that occurs in 2 steps during this critical transition period, resulting first in susceptibility to thermally induced seizures and then in spontaneous seizures.

While a myriad of developmental changes occur during this time, there is a specific change in sodium channel expression that may in part explain the development of seizure susceptibility. $Na_V1.1$ and $Na_V1.3$ channels are preferentially localized in the cell bodies of neurons (6, 38). Using quantitative analysis of mRNA expression, it was shown that rat central neurons express $Na_V1.3$ during prenatal and early postnatal development, and the adult sodium channel subtype $Na_V1.1$ cannot be detected (39). Between the second and third postnatal weeks, $Na_v1.3$ expression declines and $Na_V1.1$ increases such that $Na_V1.1$ should contribute most significantly to neuronal function after day P21. A similar time course of appearance of $Na_V1.1$ protein was also observed in immunoblotting experiments (40). Therefore, it is possible that susceptibility to seizures is the result of loss of GABAergic inhibition because of reduced expression of $Na_V1.1$ in inhibitory interneurons as previously shown (14, 15), and the loss of inhibition occurs during the third postnatal week when the $Na_V1.1$ sodium channel normally reaches full level of expression and $Na_V1.3$ expression has declined to near basal levels. In agreement with this time dependence, we observed frequent spontaneous seizures at ages older than P30, and a striking age-dependent increase in sensitivity to temperature induction of seizures and in the severity of seizures on the basis of behavioral scoring, spike frequency, and duration.

Interictal Events in mSMEI. The interictal EEG in infants with SMEI is typically normal early in the disease with interictal spikes becoming apparent only at older ages (12). Interictal spikes in humans are associated with an increased risk of unprovoked seizures and are thus correlated with the onset of spontaneous seizure susceptibility. The appearance of interictal spikes is also correlated with spontaneous seizures in mSMEI. Interictal spikes are not seen during prolonged recordings at normal body temperature in P17–18 or P20–22 mSMEI, but are consistently observed at elevated temperature for P20–22 mice during temperature-induction of seizures. Similarly, interictal spikes are also seen in P30–46 mSMEI at the time when spontaneous seizures at normal body temperature are captured. Thus, interictal spikes may be an indicator of susceptibility to spontaneous seizures in mSMEI. The increased frequency of interictal spikes and spontaneous seizures in older mice may reflect the decrease and eventual complete loss of $Na_V1.3$ channels, which support action potentials in inhibitory neurons in younger animals but decline in development (39).

Comparison of Human and Mouse SMEI. Our mouse model of SMEI recapitulates the human disease with surprising fidelity. Infants with SMEI are indistinguishable from unaffected infants until they have their first seizure, typically around 6 to 9 months of age, at or just before the time of normal weaning. mSMEI develop susceptibility to seizures on P18 to P20, just before weaning. The first human seizures in SMEI usually occur with elevated body temperature, most often with a fever or occasionally during a hot bath. P20–22 mice are also susceptible to temperature-induced seizures, but spontaneous seizures are very rare at that age. Following an initial phase of febrile seizures, humans with SMEI have spontaneous seizures of increasing frequency and severity. Mice older than P30 with mSMEI also have increased frequency and severity of spontaneous seizures. In humans, more severe seizures, additional seizure types, and a transition from a normal to an epileptic interictal EEG are seen at older ages, culminating in a period between ages 2 and 8 when seizure severity reaches a peak, status epilepticus is common, and multiple seizure types including generalized tonic-clonic, atypical absence, complex partial, and myoclonic seizures can all occur (13). In mSMEI, epileptic interictal EEG patterns are also observed after P30. The further evolution of seizure types and seizure severity after this age is an interesting area for further study.

The generally close correspondence between human and mouse SMEI observed in these experiments and in our previous work suggests that mSMEI provide a useful model for quantitative experimental studies of this disease. Documentation of the similarities of seizure generation in the early phase of mouse and human SMEI in this work supports the relevance for human SMEI of our previous findings in mice (14, 15) that loss of firing of GABAergic interneurons is the precipitating factor for hyperexcitability and epilepsy and for comorbidities such as ataxia. Further studies of this epilepsy model may shed additional light on the pathophysiology of this devastating childhood epilepsy syndrome.

Materials and Methods

All experiments were performed in accordance with animal protocols approved by the Institutional Animal Care and Use Committee of the University of Washington.

Generation of $Na_V1.1$ Mutant Mice. Mutant mice were generated as previously described (14, 15). Briefly, mutant mice were generated by targeted deletion of the last exon encoding domain IV from the S3 to S6 segment and the entire

C-terminal tail of the $Na_V1.1$ channel. All experiments with $Na_V1.1(+/-)$ (called mouse SMEI [mSMEI] for convenience) and $Na_V1.1(+/+)$ mice were done in the C57BL/6 genetic background. Study animals were generated by breeding $Na_V1.1(+/-)$ males with $Na_V1.1$ $(+/+)$ females. $Na_V1.1(+/-)$, and $Na_V1.1(+/+)$ mice were determined by genotyping using a 4-oligonucleotide multiplex PCR of genomic DNA samples isolated from mouse tails (41). The wild-type (WT) band (291 bp) was amplified by FHY209 (5′-CGAATCCAGATGGAAGAGCGGTTCATGGCT-3′) and FHY210 (5′-ACAAGCTGCTATGGACATTGTCAGGTCAGT-3′), and the mutant band (493 bp) was amplified by Neo5 (5′-AGGATCTCCTGTCATCTCACCTTGCTCCTG-3′) and Neo3 (5′-AAGAACTCGTCAAGAAGGCGATAGAAGGCG-3′). The 2 primer sets were mixed in a single PCR using the protocol: 2-min denaturation at 94 °C followed by 30 cycles of 20 s at 95 °C, 20 s at 66 °C, and 30 s at 71 °C. PCR products were analyzed by agarose gel electrophoresis.

EEG Recording. P17–57 mice underwent survival surgery for implantation of EEG electrodes. The mice were anesthetized with ketamine/xylazine (130/8.8 mg/kg). Using aseptic technique, a midline incision was made anterior to posterior to expose the cranium. Fine platinum wires were placed through small cranial holes created with a fine cutting needle and fixed in place with cyanoacrylite glue. Recording electrodes were placed at visually identified locations: bilateral frontal, bilateral central, bilateral posterior, and in some experiments at the vertex. A reference electrode was placed at the midline cerebellum and a ground electrode was placed s.c. over the back. Electrode impedances were typically <25 kΩ. After killing, the cranium was removed and the cortical surface was inspected in 5 mice to look for electrode-related damage. At most sites no evidence of damage was identified. At a few locations, a faint red blush was seen suggesting trace hemorrhage. After electrode placement, the skin was closed with suture and the mice were allowed to recover for 3 h to overnight. EEG patterns were evaluated before recording to determine whether anesthesia-related changes were still present.

A digital video-EEG recording system (DEEG, Telefactor) was used to record EEGs from freely moving mice. The low-frequency filter was set at 1 Hz, the high-frequency filter was set at 70 Hz and a 60-Hz trap filter was used to reduce line noise if necessary. Simultaneous time-locked digital video recording was also obtained. Seizure-related spikes were identified by their large amplitude above baseline. A computer-based algorithm was used to determine EEG amplitudes crossing a set threshold defined a priori by visual inspection of spike amplitudes relative to background amplitude for each seizure (IGOR PRO 5.0, Wavemetrics, Lake Oswego, OR). The peak amplitude of the spike was marked and subsequently visually confirmed.

Seizure behaviors were graded from 1 to 5 using the Racine score (42): 1, mouth and facial movement; 2, head nodding; 3, forelimb clonus; 4, rearing with forelimb clonus; and 5, rearing and falling with forelimb clonus.

Temperature Induction. In P17–46 mice, after EEG electrode implantation, a rectal temperature probe was placed and connected to a rodent temperature controller (TCAT 2DF, Physitemp). The mouse body core temperature was maintained at a command temperature ± 0.3 °C by controlling a heat lamp positioned above the Plexiglas box. Average mouse body core temperature is 36.9 °C (http://www.informatics.jax.org/mgihome/other/mouse_facts1.shtml). Mice were held at 37.5 °C for at least 10 min to become accustomed to the chamber. Body temperature was then elevated by 0.5 °C every 2 min (Fig. S1*A*, *Top*) until a seizure occurred or 42.5 °C was reached. The temperature dependence of seizure induction is illustrated in the figures with sigmoid functions, which assumes a Gaussian distribution of seizure susceptibility among individual animals that is centered on the temperature for 50% induction.

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