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Augmented seizure susceptibility and hippocampal epileptogenesis in a translational mouse model of febrile status epilepticus

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Abstract

Objective: Prolonged fever-induced seizures (febrile status epilepticus [FSE]) during early childhood increase the risk for later epilepsy, but the underlying mechanisms are incompletely understood. Experimental FSE (eFSE) in rats successfully models human FSE, recapitulating the resulting epileptogenesis in a subset of affected individuals. However, the powerful viral and genetic tools that may enhance mechanistic insights into epileptogenesis and associated comorbidities, are better-developed for mice. Therefore, we aimed to determine if eFSE could be generated in mice and if it provoked enduring changes in hippocampal-network excitability and the development of spontaneous seizures.

Methods: We employed C57BL/6J male mice, the strain used most commonly in transgenic manipulations, and examined if early life eFSE could be sustained and if it led to hyperexcitability of hippocampal networks and to epilepsy. Outcome measures included vulnerability to the subsequent administration of the limbic convulsant kainic acid (KA) and the development of spontaneous seizures. In the first mouse cohort, adult naive and eFSE-experiencing mice were exposed to KA. A second cohort of control and eFSE-experiencing young adult mice was implanted with bilateral hippocampal electrodes and recorded using continuous video–electroencephalography (EEG) for 2 to 3 months to examine for spontaneous seizures (epileptogenesis).

Results: Induction of eFSE was feasible and eFSE increased the susceptibility of adult C57BL/6J mice to KA, thereby reducing latency to seizure onset and increasing seizure severity. Of 24 chronically recorded eFSE mice, 4 (16.5%) developed hippocampal epilepsy with a latent period of ~3 months, significantly different from the expectation by chance (P = .04). The limbic epilepsy that followed eFSE was progressive.

Significance: eFSE promotes pro-epileptogenic network changes in a majority of C57BL/6J male mice and frank "temporal lobe–like" epilepsy in one sixth of the cohort. Mouse eFSE may thus provide a useful tool for investigating molecular, cellular, and circuit changes during the development of temporal lobe epilepsy and its comorbidities.

1 | INTRODUCTION

Prolonged febrile seizures during early childhood, especially febrile status epilepticus (FSE), are associated with an increased risk for epilepsy.[1–3] In addition, adults with temporal lobe epilepsy (TLE) commonly have a history of prolonged febrile seizures during childhood.[3–5] Whereas genetic factors play a key role in epileptogenesis,[6–8] available information suggests a direct contribution of FSE to the development of subsequent hippocampal injury and

epilepsy.[6,9,10] Animal models enable testing of the causal relation among these observations and elucidating potential mechanisms by which FSE may provoke pro-epileptogenic changes that culminate in epilepsy.[11–13]

Experimental FSE (eFSE) in rats has been induced via several approaches,[11,12,14,15] which have altered cortical and hippocampal excitability.[15,16] Notably, in the hyperthermia model of eFSE, spontaneous seizures (epilepsy) developed in 30%–40% of rats, in line with several human analyses.[2,4,6] Rat models have advanced our understanding of cellular and network mechanisms of the epileptogenesis and cognitive problems that follow eFSE, including transcriptional and inflammatory changes.[11,12,14,15,17,18] These models allowed the identification of predictive imaging markers[19,20] and have uncovered early cognitive deficits.[9,21]

Yet, the large majority of novel technologies for interrogating molecular, cellular, and circuit functions and their alteration in disease have been developed for mice, not rats. For example, whereas optogenetic manipulations and transgenic models can be created in rats, the armamentarium of modern science instruments is significantly larger for mice. These rapidly evolving tools can be used to identify the circuitry and cellular signals that specify normal and pathological brain function.[22] Therefore, discovery of the complex cellular and circuit mechanisms by which eFSE might contribute to epilepsy development, and the identification of novel therapeutic targets, would benefit from an immature mouse model of FSE.

A number of mouse models of febrile seizures (FS) have been published.[23–30] Most have involved short FS and have been used extensively to probe how specific genetic mutations increase risk for FS.[23,25,26,29] Other groups have addressed the role of FS in mice on short-term changes of hippocampal structure and memory and stress response.[27,31,32] Notably, mouse models of epileptogenesis following long FS and FSE are few.[28,30] Here, we set out to examine whether eFSE in the immature mouse (a) can be reliably induced, (b) increases susceptibility to chemical convulsants, and (c) generates spontaneous seizures later in life.

2 | METHODS

2.1 | Animals

All experiments were performed in accordance with National Institutes of Health (NIH) guidelines and were approved by the University of California-Irvine Animal Care and Use Committee. Principles outlined in the Animal Research: Reporting of in Vivo Experiments (ARRIVE) guidelines and the Basel declaration including the 3R (Replacement, Reduction, Refinement) concept have been considered when planning the experiments.

Male and female mice (C57BL/6J) were originally obtained from Jackson Laboratory (ME, USA) and housed in the animal facility under a normal 12-h light-dark cycle in quiet, humidity- and temperature-controlled rooms, with free access to water and food. C57BL/6J male and female mice were then mated in-house and the resulting offspring were used for subsequent experiments. The day of birth was considered postnatal day zero (P0). On P1, litters were culled to eight pups and on P21 mice were weaned and housed four per cage. Control mice were littermates of the eFSE cohorts. Overall, the mice tested for susceptibility to kainic acid (KA) were derived from 7 litters, and mice tested for epileptogenesis were derived from 21 litters (15 in the eFSE group, 5 in the normothermic control group, and 1 in the hyperthermic control group).

2.2 | Induction of experimental febrile status epilepticus (eFSE) in mice

Prolonged experimental febrile seizures were modified from a paradigm originally designed for the rat[14,16,17,33,34] and adapted for use in immature mice.[24,25] On postnatal day 14–15 male mice (6–8 g) received a generous application of a hydrating ointment to their tails, ears, and paws to prevent potential heat-related injuries. Pups were placed two at a time in a 3-L glass beaker lined with absorbent bench paper. Hyperthermia (ie, increased core and brain temperature) was induced using a regulated stream of mildly heated air created by a Conair Pro Styler 1600-watt hairdryer set at warm and low settings to obtain a core temperature of approximately 39°C (as during high fever). Core temperatures were measured at baseline prior to the onset of experimental FSE and at 2-minute intervals during experimental FSE using a probe rectal thermometer (Physitemp RET2). Hyperthermia-induced core temperature above 38°C typically led to hyperkinesis, "face cleaning" and facial automatisms, and the onset of eFSE typically involved sudden freezing and forelimb clonus. This was followed by running, together with continued facial automatisms.[24] Hyperthermia, defined as core temperatures above 38.5°C, was induced for 30 minutes (cohort 1, n = 17) or 40 minutes (cohort 2, n = 24). This elevated core temperature was maintained, and seizure behaviors assessed throughout the hyperthermia period. If the core temperature of the mice exceeded 41.5°C, they were removed from the chamber for the next 2 minutes. Following the hyperthermia period, mice were cooled using room temperature water and were provided with Lactated Ringer's solution (intraperitoneally, body weight (g) × 0.03 ml) to counteract potential dehydration. Pups were then placed on a euthermic pad (HTP-1500) for 15–20 minutes and then returned to their home cages with littermates. Hyperthermic control mice underwent the same hyperthermia for the same period (40 minutes) after the administration of diazepam intraperitoneally to prevent hyperthermia-induced seizures. Notably, diazepam did not influence the hyperthermia-induced temperature elevation. Euthermic control mice were removed from their cages for a period of time comparable to that of the experimental group (~1 hour) on a warming pad.

2.3 | Kainic acid challenge

Control mice (n = 8) and mice that experienced eFSE (n = 17) at P14–15 were tested as adults (2–4 months of age) using the chemoconvulsant kainic acid (or KA). KA (ab120100; Abcam; stock solution 5 mg/mL) was administered i.p. at 15 mg/kg. Mice were video recorded as well as monitored by two investigators who were unaware of treatment group, and their behaviors were scored. These individuals measured latencies in a blinded fashion. The latency to the onset of seizures, time to reach each seizure stage and maximal seizure stage were quantified using a modified Racine scale as follows: normal behavior = stage 0; immobility = stage 1; head nodding = stage 2; forelimb clonus = stage 3; rearing = stage 4; rearing more than 3 times = stage 5; running/jumping = stage 6, and death = stage 7.35 One KA mouse was excluded from analysis because of an apparent brain malformation/cyst.

2.4 | Intrahippocampal electrode implantation: for tethered and telemetric recordings

Electrode placement was performed as described previously.[14,16,17,20,24] Briefly, on P60-65, mice of cohort 2 underwent surgery to implant bipolar intracranial electrodes bilaterally into the dorsal hippocampi. Mice were anesthetized with 4% isoflurane, and then placed in a

stereotaxic frame with hydrating gel for their eyes and maintained using 1%–1.5% isoflurane. Heads were shaved and cleaned three times with ethanol and iodine. A midline incision was made to expose the skull, and the skull was cleaned with 30% hydrogen peroxide. Using the coordinates (anteroposterior [A/P]:-1.9 mm, mediolateral [M/L]: ±1.6 mm, and dorsoventral [D/V]: 2.7 mm) from Bregma, holes were drilled in the skull for the two electrodes as well as above the frontal cortex (for a reference electrode) and the cerebellum (for a ground electrode) (Plastics One). Ground and reference electrodes and small anchoring screws (Antrin Miniature Specialties Inc.) were secured to increase stability of the head cap. Stainless steel bipolar polyimide coated electrodes (Plastics One) were cut to 2.7 mm and lowered slowly into the hippocampus. Electrodes were inserted into a 6-channel pedestal (Plastics One) and secured, together with the anchoring screws, using dental cement. Mice were monitored recovered for 5–7 days postsurgery and then connected via cables to the tethered system (PowerLab data acquisition hardware, Bio Amplifiers, and LabChart 7/8 software, AD Instruments).

For mice recorded via remote Bluetooth DSI receivers, the receiver was inserted into a pocket created from an incision from below the scalp to the posterior left scapular region. Using the same coordinates, we implanted two hippocampal electrodes (left orange, right blue) as well as frontal cortex and cerebellar electrodes (Plastics One). As per the tethered system, reference and ground electrodes and small anchoring screws (Antrin Miniature Specialties Inc.) were secured to increase stability of the head cap.

2.5 | Digital video–electroencephalography (EEG) recording and analyses

Continuous video-EEG monitoring was initiated a week after surgery as described.[14,17,20] Digital EEG recordings were examined visually by two experienced investigators unaware of treatment group. The investigators visually scanned the coded EEG recordings for seizures or spike series.[14] Any suspicious activity led to viewing of the concurrent video recordings for behavioral manifestations of seizures. Only events with both EEG and behavioral changes and that lasted over 10 s were classified as seizures. We evaluated typical behaviors associated with limbic-onset seizures, including sudden cessation of activity, facial automatisms, head-bobbing, prolonged immobility with staring. These progressed to alternating or bilateral clonus, rearing, and falling.[35] Mice were considered epileptic if they had at least one documented seizure as defined earlier. As a measure of network hyperexcitability, we recorded and quantified interictal spikes and spike series. [14,17,34] Criteria for spikes were 20–70 ms duration and amplitude at least twice that of baseline recorded for 3 minutes during exploration. Concurrent video-monitoring was used to exclude chewing movement and electrical noise.[36] We used a tethered system (AD Instruments; PowerLab data acquisition hardware, Bio Amplifiers, and LabChart 7/8 software) for EEG acquisition and we employed the same software for seizure detection and analysis. LabChart 7 EEG recording required manual synchronization with Logitech Dynex (DX-NW080) video webcam. LabChart 8 directly synchronized our 1.0 Megapixel USB camera with EEG recording via a video capture plug-in. For the remote Bluetooth DSI receiver system, Neuroscore Version 3.0 with directly synchronized video data acquired with Dataquest A.R.T or Ponemah was used for seizure detection and analysis.

2.6 | End points and tissue validation

Upon completion of the experiments (or upon loss of the electrode cap), mice were killed, and brains were carefully removed, sectioned, and examined. We assessed electrode placement and the presence of infarcts, brain malformations, or hemorrhages.

2.7 | Analyses and statistical considerations

All analyses were performed blindly, without knowledge of assigned group. In the KA groups, a mouse with an apparent brain cyst was excluded. In the long-term EEG groups, one each of eFSE and control mice were excluded because of an electrode-related infarct, and one control had a misplaced electrode. All experimental groups were tested for normal distribution of data via the Kolmogorov-Smirnov test. All statistical analyses employed the GraphPad Prism Software Package (San Diego, CA). Descriptions of individual statistical tests can be found in the Results section.

3 | RESULTS

3.1 | Effects of eFSE on severity of KA-induced seizures

All mice exposed to hyperthermia developed behavioral seizures (eFSE), which have been correlated previously with electrographic seizures.[24,33] To assess the enduring effects of eFSE on subsequent susceptibility to limbic convulsants, adult control mice and mice that had experienced eFSE on P14–15 were administered a dose of KA (15 mg/kg, i.p.). Initial assessment of the maximal seizure stage (defined by the Racine scale) within 2 hours following KA administration did not reveal a significant difference between eFSE and control mice (Figure 1A; Mann-Whitney U test, P = .18, Mann Whitney $U = 42.50$, control median = 3, eFSE median = 5). Yet, a higher proportion of eFSE mice (56%) than controls (25%) reached stage 5 seizures, (Figure 1B; chi-square = 2.098, df = 1, $P = .147$). Further analysis demonstrated that, whereas the population of control mice was normally distributed (Kolmogorov-Smirnov test, $P = .27$), the population of eFSE mice was not normally distributed (Kolmogorov-Smirnov test, $P = .0007$), with two distinct sample distributions (Figure 1C). Because in both humans and rats, FSE may result in epileptogenesis only in a subset of individuals, we categorized eFSE mice into eFSE responders (eFSE-R) and eFSE nonresponders (eFSE-NR). The nonresponders (eFSE-NR, n = 7) resembled controls with a median maximal seizure stage of 2, not statistically different from control mice (Figure 1D, Kruskal-Wallis with Dunn correction for multiple comparisons, control vs eFSE-NR, P > .99, mean rank diff = 3.31). By contrast, eFSE-R (n = 9; 56% of the cohort), reached a significantly higher median maximal seizure stage of 6, and differed significantly from both the control animals and the eFSE-NR (Figure 1D, Kruskal-Wallis with Dunn correction for multiple comparisons, control vs eFSE-R, P = .009, mean rank diff = −9.74; eFSE-R vs eFSENR, P = .0004, mean rank diff = 13.06). Thus following eFSE, a majority subset of mice developed more severe seizures than controls upon exposure to a limbic convulsant (KA). Latencies to seizure onset in both responders and nonresponders as well as seizure propagation also distinguished eFSE from control mice: The latency to seizure stage 1 was significantly shorter in eFSE mice compared with controls (Figure 2A, unpaired t test, $t = 2.302$, df = 22, $P = .031$; control mean = 244.6 ± 40.11 s; eFSE mean = 161 ± 16.42 s).

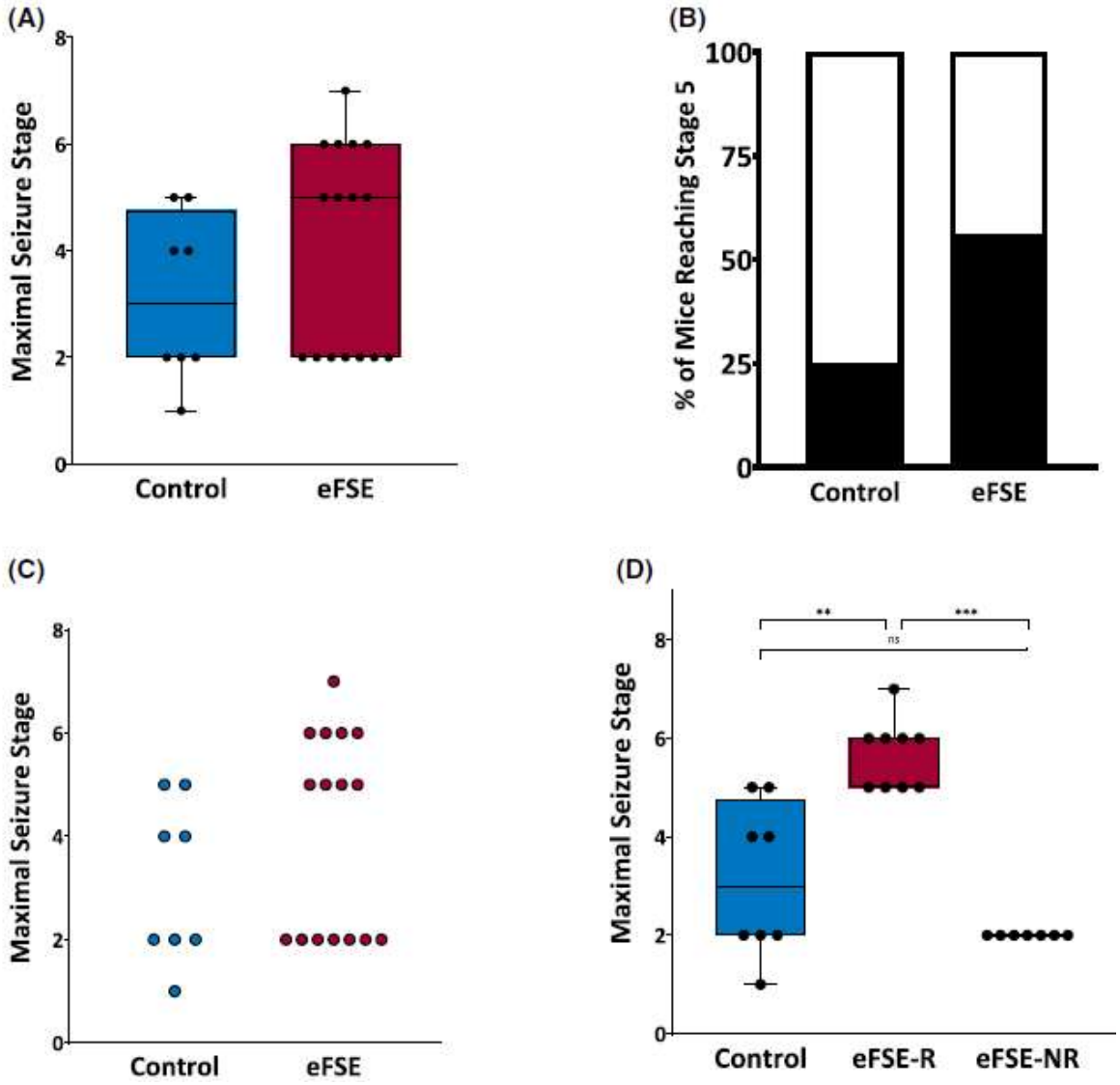

FIGURE 1 Experimental febrile status epilepticus (eFSE) increases susceptibility to kainic acid (KA)–induced seizures later in life. A, The likelihood of developing severe seizures (measured by the Racine scale) after a given dose of KA, was compared in adult mice that had experienced eFSE (n = 16) and in control mice (n = 8). A trend for more severe seizures (median stage: control = 3; eFSE = 5) was not significant (Mann-Whitney U test, P = .18, Mann Whitney U = 42.50, 5). B, eFSE mice had a greater likelihood (56%) than control mice (25%) of reaching Racine stage 5 seizures (chi-square = 2.098, df = 1, $P = .148$) C, The Kolmogorov-Smirnov test excluded a normal distribution of the eFSE group, istinguishing mice affected by eFSE (responders; eFSE-R) and nonresponders (eFSE-NR), as described for rats. (Kolmogorov-Smirnov test, $P = .0007$). D, When analyzed for reaching seizure stage 5, eFSE-R differed significantly from both eFSE-NR and control mice (Kruskal-Wallis with Dunn correction for multiple comparisons, control vs eFSE-NR, P > .99, mean rank diff = 3.31; control vs eFSE-R, P = .009, mean rank diff = −9.74; eFSE-R vs eFSE-NR, $P = .0004$, mean rank diff = 13.06). $^{**}P < .01$, $^{***}P < .001$. A and D are whisker-plots showing min-max as well as individual mouse values

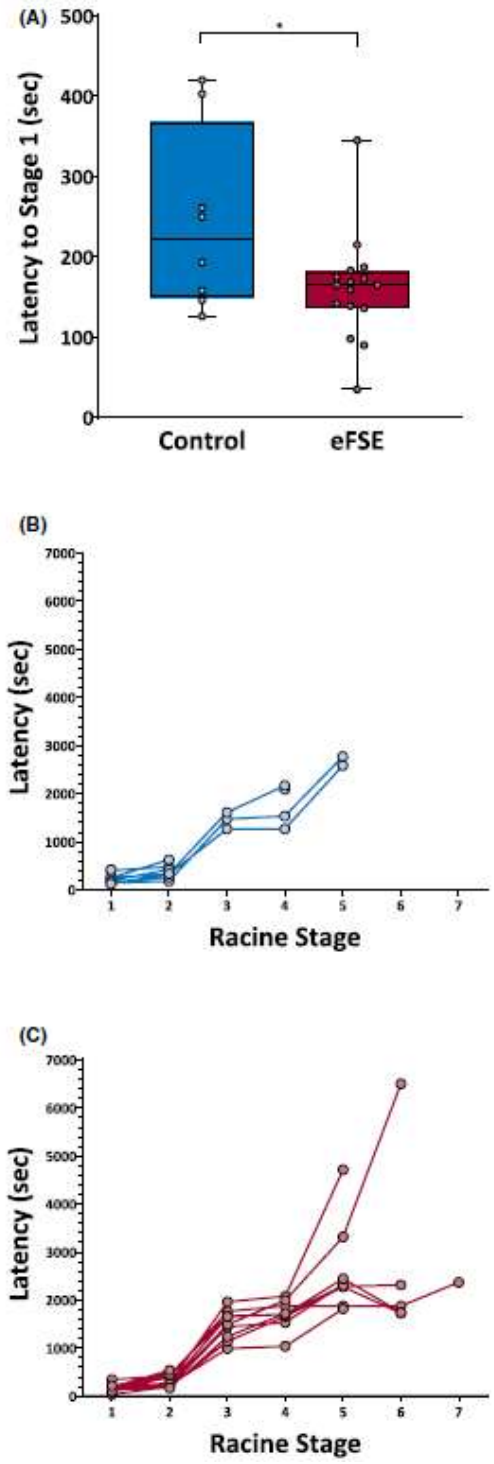

FIGURE 2 Mice that experience experimental febrile status epilepticus (eFSE) have decreased latencies to kainic acid (KA)–induced seizures and augmented seizure propagation compared to controls. A, Latency to Racine stage 1 following KA administration was lower in eFSE mice vs controls (unpaired t test, t = 2.302, df = 22, P = .031; control mean = 244.6 ± 40.11 s; eFSE mean = 161 ± 16.42 s). B and C, compared with controls, eFSE mice were more likely to reach Racine stage 5 (see Figure 1A for statistical comparison), and no control mice reached stage 6 or 7

Indeed, the latencies to progressive seizure stages were shorter in the eFSE group compared to controls for each seizure stage, with only eFSE rates reaching stages 6 and 7 (Figure 2B,C). Together, these findings suggest that eFSE rendered mice more sensitive to limbic seizure generation, with reduction of latency in all, and increase of seizure severity in a large subset, compared with controls.

3.2 | Development of spontaneous seizures

The preceding results indicated that eFSE was pro-epileptogenic in C57BL/6 mice in that it increased vulnerability to subsequent seizures. We next assessed whether eFSE was sufficient to initiate recurrent, spontaneous seizures, that is, frank epilepsy. We further queried if eFSE-induced epilepsy involved the hippocampal circuit, and whether it progressed. C57BL/6 mice were assigned to hyperthermia induced eFSE (n = 24), hyperthermia alone (seizures prevented using diazepam; n = 3), or to serve as euthermic controls (n = 7). At ~P60, mice underwent bilateral intrahippocampal electrode implantation followed by digital video-EEG monitoring (Figure 3A), aiming for 60 days,of continuous recording. Four of the 24 mice (16.5%) developed epilepsy following a latent period of ~3 months (Figure 3B). The onset of the spontaneous seizures was on days 101, 101, 115, and 119 following eFSE, respectively (109 ± 4.7 days; Figure 3B). Control mice were recorded to a mean age of 206 days (206 ± 40.2; range 146–436 days) and no seizures or spike series were detected (Figures 3B and 4). The number of mice developing spontaneous seizures in the eFSE group was significantly higher than,zero (P = .043; one-sample t test).

Examples of the seizures recorded from bipolar intrahippocampal electrodes in the four epileptic mice are shown in Figure 4, in comparison to a hippocampal EEG from a control mouse (Figure 4A). Hippocampal seizures were often preceded by or interspersed with spike series and consisting of the typical electrographic progression of increasing amplitude, followed by a period of background suppression (Figure 4B). Thus following eFSE, a minority of C57BL/6 mice developed spontaneous seizures that involved the hippocampus, analogous to temporal lobe epilepsy in humans.

3.3 | Characteristics and progression of eFSE-induced epilepsy in mice

To determine whether the eFSE-provoked epilepsy in mice was progressive, we examined quantitatively the frequency and duration of the spontaneous seizures. After the onset of their first seizure, the four epileptic mice were recorded for a mean 29 ± 9.4 days (range 5–52), a duration constrained by the loss of EEG caps (Figures 3B, 5A). The four epileptic eFSE mice had a mean 60.75 ± 44.4 seizures (range 2–193), and their cumulative seizure burden is shown in Figure 5A. The three mice recorded for over a week from the onset of their first seizure had 80.33 seizures each, on average. Notably, the cumulative burden of the recurrent spontaneous seizures of these chronically recorded epileptic mice was best fit with exponential growth curves, suggesting epilepsy progression (Figure 5B, eFSE-5: $R2 = .963$, $df = 12$; eFSE-6: $R2 = .991$, $df = 31$; eFSE-21: $R2 = .932$, $df = 16$).

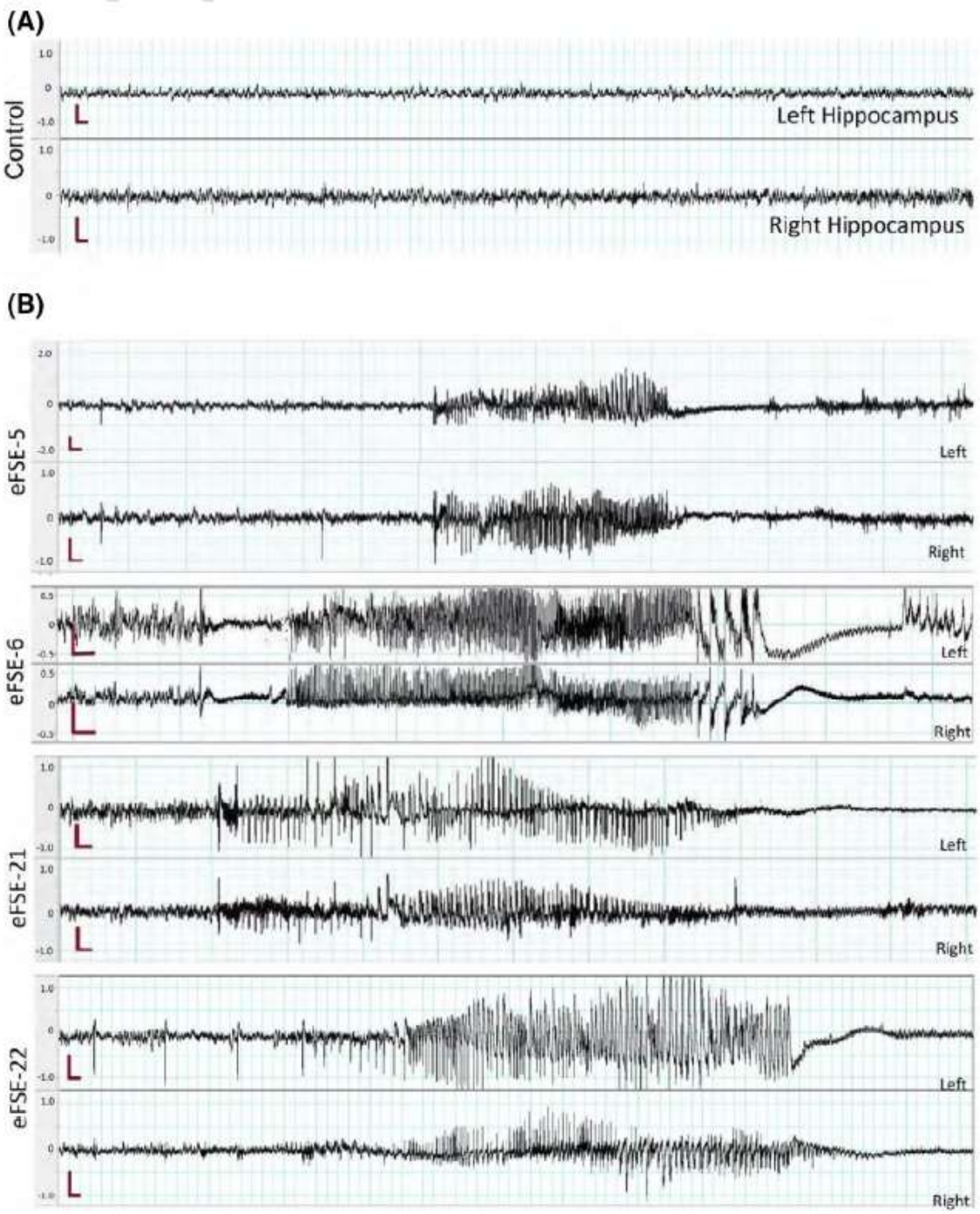

FIGURE 4 Representative electroencephalography (EEG) recordings from a control mouse and four epileptic mice. A, Bilateral intrahippocampal EEG recordings from a control mouse. B, Representative EEG examples of seizures from the epileptic eFSE mice. The typical progression of electrographic hippocampal seizures is apparent, followed by a postictal suppression of the background. For all EEG traces, the scale on the left is in millivolts. The scale bar for each tracing is denoted in red, with the vertical bar representing 0.5 mV, and the horizontal bar representing 1 s

The number of seizures per day increased with time for the chronically recorded epileptic mice (Figure 5C). In addition, the average seizure duration per day ranged from 17 to 59 s (Figure 5D) and progressed over time. The use of linear regression indicated a significant, non-zero slope for seizure duration in animals that were recorded for more than a week after the first seizure (Figure 5D; eFSE-5: F = 24.31, Degrees of Freedom in the numerator (DFn) = 1, Degrees of Freedom in the denominator (DFd) = 10, P = .0006; eFSE-6: F = 31.13, DFn = 1, DFd = 25, P = <.0001; eFSE-21: F = 16.51, DFn = 1, DFd = 13, P = .0013). The increases in both seizure number per day and average seizure duration led to increased time spent in seizures per day, a measure of epilepsy progression (Figure 5F, linear regression eFSE-5: F = 5.25, DFn = 1, DFd = 10, P = .044; eFSE-6: F = 55.79, DFn = 1, DFd = 25, P = <.0001; eFSE-21: F = 8.14, DFn = 1, DFd = 13, P = .0014).

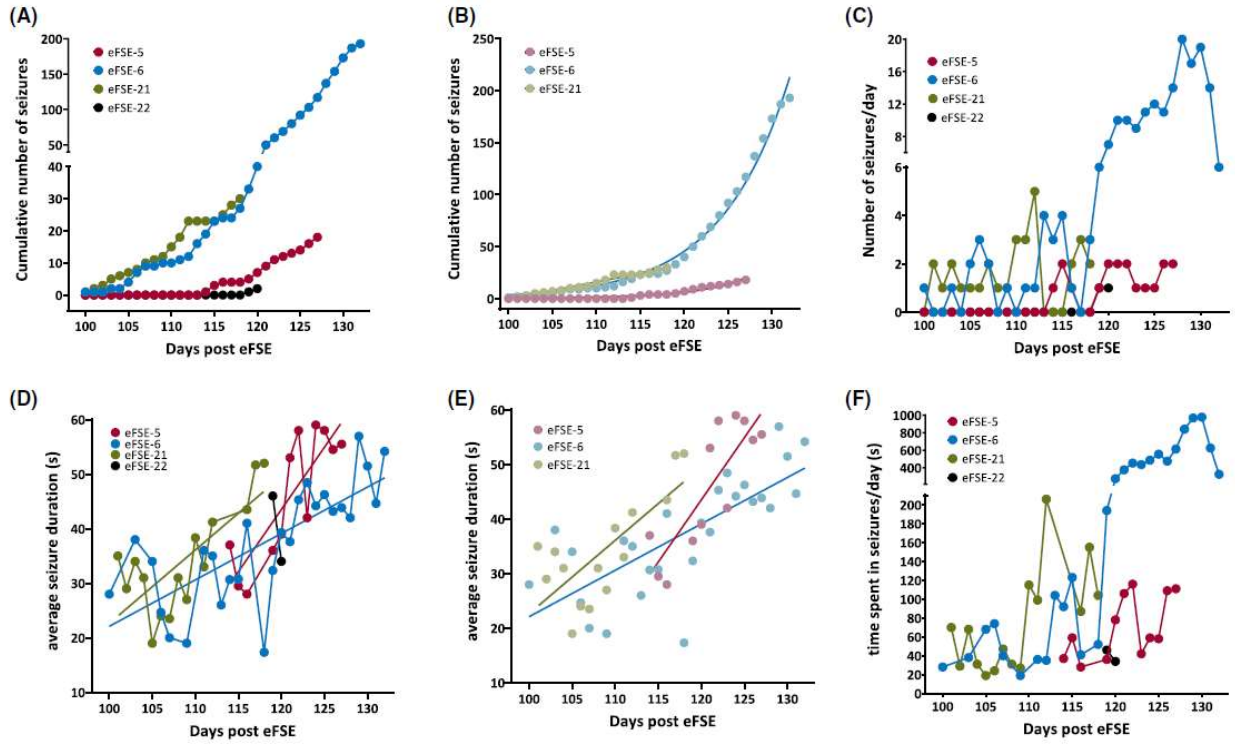

FIGURE 5 Quantitative characterization of the hippocampal epilepsy resulting from experimental febrile status epilepticus (eFSE) in mice. A, The cumulative number of seizures for each of the four epileptic eFSE mice. B, The cumulative seizure burden for the three mice recorded for more than a week after their first spontaneous seizure fits an exponential growth curve (eFSE-5: R2 = .963, df = 12; eFSE-6: R2 = .991, df = 31; eFSE-21: R2 = .932, df = 16). C, The total number of seizures per day for each epileptic mouse. D, The average daily seizure duration for each epileptic mouse. E, The average seizure duration per day fit with a linear regression for each mouse had a non-zero slope, consistent with increased duration over time eFSE-5: F = 24.31, DFn = 1, DFd = 10, P = .0006; eFSE-6: F = 31.13, DFn = 1, DFd = 25, P = <.0001; eFSE-21: F = 16.51, DFn = 1, DFd = 13, P = .0013). F, The total daily time spent in seizures for each epileptic mouse increased progressively with time. This is apparent using linear regression (linear regression eFSE-5: F = 5.25, DFn = 1, DFd = 10, P = .044; eFSE-6: F = 55.79, DFn = 1, DFd = 25, P = <.0001; eFSE-21: F = 8.14, DFn = 1, DFd = 13, P = .0014)

4 | DISCUSSION

The principal findings of this series of experiments are: (a) mouse eFSE enduringly increases the susceptibility to a subsequent limbic convulsant, indicative of persistent alterations in hippocampal network excitability; (b) mouse eFSE provokes spontaneous recurrent hippocampal seizures in a minority of C57BL/6 mice, and (c) this TLE-like epilepsy is likely progressive. Because of the predominant use of C57BL/6 mice in transgenic and viral-genetic modern technologies, the mouse eFSE paradigm characterized here may provide a novel and robust tool for studies of the mechanisms of network, cellular, and molecular changes that lead to temporal lobe epilepsy.

4.1 | Susceptibility to acute seizure inductionin the mouse—role of genetic background

In aiming for a mouse model for eFSE-induced epileptogenesis, we chose to employ the C57BL/6 strain because it is a common inbred strain of laboratory mouse that has been used for numerous models of human disease. It is important to note that this strain has been used widely as a genetic background for congenic and mutant mouse research paradigms. Compared with other mouse strains, the C57BL/6 mouse has a distinct profile of resilience and susceptibility as applied to (a) induction of acute seizures by chemoconvulsants or "febrile seizures," (b) the development of spontaneous seizures,
that is, epileptogenesis.

Susceptibility to chemically or electrically induced seizures has been studied extensively in diverse mouse strains.[37–42] These studies have shown that genetic background plays a considerable role in affecting seizure thresholds in mice.[37,38] The C57BL/6 strain, in comparison to others such as ICR, FVB/N, and BALB/c was resistant to seizures induced by systemic administration of KA.37 Genetic analyses have suggested a contribution of specific chromosomal loci and a role for differences in inhibitory interneurons in some of these strain diferences,[39,40] and differences in sensitivity to chemoconvulsants have been pointed out also for substrains within C57BL/6 mice.[37,41]

Sensitivity to seizure induction by hyperthermia (eFSE) has been used to assess the contribution of specific genes or mutations to febrile seizures.[23,25,26] In addition, some authors have reported that C57BL/6J mice are among the most susceptible to febrile seizures, despite their relative resistance to chemical and electrical convulsants, suggesting distinct susceptibility genes.

4.2 | Effect of experimental FSE on susceptibility to subsequent seizures

Here, employing C57BL/6J mice, we induced long FS and then queried if the experimental FSE influenced susceptibility to KA 2 to 3 months later. We found that, as a group, latency to KA-induced seizure onset was reduced in FSE vs control mice. Maximal seizure stage on the Racine scale and the proportion of mice that reached stage 5 were increased in 9 of 16 eFSE mice (56%), whereas 7 of 16 were similar to controls. The basis of this dichotomy is not fully understood. We specifically failed to identify any differences in the characteristics of the original inciting FSE: Its duration or maximal body temperature did not separate the groups. Individual mouse characteristics such as weight were also not different in the KA-susceptible vs

the more resistant group. Of interest, a similar dichotomy, leading to "FS-responsive" and FS-nonresponsive subsets was identified in immature rats,[19,34] in which the mechanism for the vulnerability vs resistance to eFSE was individual variation in the synthesis of pro-inflammatory molecules including cytokines. Of note, the FS-responsive rats developed MRI changes when imaged 2–6 hours after the eFSE, and these changes predicted the development of hippocampal epilepsy in the same rats.[20] We speculate that similar processes might take place in mice.

4.3 | A minority of C57BL/6 mice develop hippocampal epilepsy following eFSE

In the current studies, 4 of 24 mice (16.5%) developed spontaneous seizures, and the duration of these seizures as well as their frequency progressed with time. Whereas only a minority of mice developed epilepsy, this was significantly different than chance, as a robust body of work has demonstrated that spontaneous epileptic activity is not found in control C57BL/6 mice.[43–47] Furthermore, the augmented susceptibility to KA in 56% of mice suggests that pro-epileptogenic changes were initiated by eFSE in a larger proportion of mice. Because we employed the C57BL/6 strain, these results are not surprising: It has long been recognized that C57BL/6 mice are resistant to the development of spontaneous seizures after an initial insult such as chemoconvulsant-induced status epilepticus.[38,42] The mechanisms underlying this resistance are not well understood.

In the context of eFSE-induced epileptogenesis, human and rat studies provide clues to the potential mechanisms for the resistance of C57BL/6 mice to epileptogenesis: In humans[48] and in several rat models, FSE promotes a dramatic activation of immune cascades in both neurons and glia, including transport of danger sensing molecules,[20,21,49] release of cytokines,[14,15,17,24,34] and activation of cyclooxygenase 2 and prostaglandin pathways.[50] These mediators of innate immunity processes likely contribute to reduced seizure threshold51 and an ensuing epileptogenesis.[17,52,53] In mice, there are significant strain differences in immune responses, and C57BL/6 mice specifically have defects in neutrophil recruitment to inflammatory sites, associated with a missense mutation in the gene Nlrp12.[54] Whether neuro-inflammatory responses to eFSE are lower in C57BL/6 mice compared with other strains will be a topic of future investigation.

4.4 | Context, strengths, and limitations of the current work

Cognizant of the significant challenge of employing C57BL/6 mice as a model for epileptogenesis, we tested here whether long-duration FS (eFSE) could be reliably induced in this strain, whether it altered network excitability, and finally, whether it provoked spontaneous seizures. All mice had behavioral seizures at temperatures that fell within the high-fever range in children. As found in rats, a large subgroup of eFSE mice (9/16; 56%) developed a significant vulnerability to limbic convulsants, which endured to adulthood. Finally, following a latent period of roughly 3 months, a minority of mice developed spontaneous recurrent seizures.

The presence of increased susceptibility to chemoconvulsants suggests that hippocampal networks were enduringly altered by the eFSE in a "pro-epileptogenic" manner. Whereas the current work has not examined memory deficits in eFSE mice, cognitive problems have been robustly identified in eFSE rats, including those who did not develop frank epilepsy.[19,55] Hence, eFSE mice should provide a useful instrument to study hippocampal changes that compromise cognitive functions, a problem now emerging in eFSE-experiencing children.[9]

Interictal spike series and recurrent spontaneous seizures developed in 4 of 24 chronically recorded eFSE-experiencing mice, and no interictal spike series or seizures were identified in euthermic control mice or in mice that had hyperthermia of the same degree and duration but in which eFSE was prevented using diazepam. Notably, these two sets of control mice were recorded to comparable ages as the eFSE mice, and beyond the observed latency period of roughly 3 months (Figure 3B). The duration of the recording without development of seizures, coupled with previous prolonged monitoring of adult control mice, suggests that the lack of detection of seizures in these mice was not a result of a failure to record them for a sufficient period of time.

In conclusion, we describe an FSE model in C57BL/6 mice, which promotes hippocampal network hyperexcitability in a majority, and epileptogenesis culminating in spontaneous seizures in a minority. This mouse model significantly advances the field by enabling the use of genetic models and transgenic and viral-genetic technologies to identify the circuitry and cellular signals underlying epilepsy.

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CONFLICTS OF INTEREST

The authors do not have potential conflicts of interest to report.

AUTHOR CONTRIBUTIONS

AMH, MMGC, and TZB conceived and designed the study.
KC, AMH, MMGC, GAS, JD, and RL performed the experiments.
AMH, MMGC, and TZB carried out the analyses.
AMH, MMGC, and TZB wrote the manuscript.

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